

Methods of Soil Analysis
Part 5—Mineralogical Methods

Methods of Soil Analysis

Part 5—Mineralogical Methods

APRIL L. ULERY & L. RICHARD DREES, Co-editors

Book and Multimedia Publishing Committee

David Baltensperger, Chair
Kenneth Barbarick, ASA Editor-in-Chief
Craig Roberts, CSSA Editor-in-Chief
Sally Logsdon, SSSA Editor-in-Chief
Mary Savin, ASA Representative
Hari Krishnan, CSSA Representative
April Ulery, SSSA Representative
Managing Editor: Lisa Al-Amoodi

Number 5 in the Soil Science Society of America Book Series

Published by Soil Science Society of America, Inc., Madison, Wisconsin, USA



Copyright © 2008 by the Soil Science Society of America, Inc.

ALL RIGHTS RESERVED. No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher.

The views expressed in this publication represent those of the individual Editors and Authors. These views do not necessarily reflect endorsement by the Publisher(s). In addition, trade names are sometimes mentioned in this publication. No endorsement of these products by the Publisher is intended, nor is any criticism implied of similar products not mentioned.

Soil Science Society of America, Inc.
677 South Segoe Road, Madison, WI 53711-1086 USA

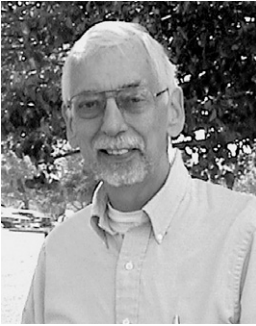
ISBN: 978-0-89118-846-9

Library of Congress Control Number: 2008922682

Cover: Selenite crystals exposed in a deflational zone. Inset: A secondary electron image of kaolinite vermiforms. Back: Thin section of exfoliating selenite. Photos courtesy of Curtis Monger, New Mexico State University and G. Norman White, Texas A&M and Texas Commission on Environmental Quality.

Printed in the United States of America.

Dedication



This monograph is dedicated to L. Richard Drees, the first editor of this project. Unfortunately, he fell ill and had to relinquish his editorial duties before this book was complete. Richard was instrumental in getting this project approved, lining up chapter authors, and establishing the aim and scope of the book. Although it was generally agreed upon that the 1986 *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods* was in need of updating, no one else stepped forward to lead the effort.

Richard was born and raised in Ohio and earned B.S. and M.S. degrees from The Ohio State University. In 1977, he joined the Soil and Crop Sciences Department at Texas A&M University as a research technician working with Dr.

Larry Wilding. In the 1980s he began work, part-time, toward a Ph.D. degree in soil science, which he received in 1986. He remained at Texas A&M as a research scientist working in soil mineralogy and soil genesis. He was the author or co-author of more than 50 scientific publications and became internationally recognized in the area of soil micromorphology. Richard served as an associate editor for the *Soil Science Society of America Journal*, chaired the SSSA Soil Mineralogy Division, and was active in many professional organizations.

Dr. Richard Drees was widely recognized for the number and quality of his research publications published in prestigious outlets. The results of his research were timely, generated through meticulous investigations, creative, and widely cited in the literature. Richard's assistance was sought by graduate students and faculty that needed his expertise in X-ray analysis, X-ray spectroscopy, thin section microfabrics, spatial soil variability, sampling theory, and image analysis procedures. He was a very unassuming individual and commonly gave credit to his peers and students rather than claiming his rightful recognition for seminal contributions. He received numerous awards for excellence in research, teaching, and service.

Richard's family was the single most important thing in his life, although he spent many hours helping students complete their degrees and assisting colleagues with their research. He died in October 2005 surrounded by his family.

April Ulery
Mike Vepraskas
Larry Wilding

Contents

Foreword	ix
Preface	xi
Contributors	xiii
Conversion Factors for SI and Non-SI Units	xv
Chapter 1	
Sampling Soils for Mineralogical Analyses	1
D.A. Soukup	
L.R. Drees	
W.C. Lynn	
Chapter 2	
Preparing Soils for Mineralogical Analyses	13
D.A. Soukup	
B.J. Buck	
W. Harris	
Chapter 3	
Selective Dissolution Techniques for Mineral Analysis of Soils and Sediments	33
Chao Shang	
Lucian W. Zelazny	
Chapter 4	
X-ray Diffraction Techniques for Soil Mineral Identification	81
Willie Harris	
G. Norman White	
Chapter 5	
Thermal Analysis of Soil Minerals	117
A.D. Karathanasis	
Chapter 6	
Petrographic Microscope Techniques for Identifying Soil Minerals in Grain Mounts	161
Warren Lynn	
J.E. Thomas	
L.E. Moody	
Chapter 7	
Soil Micromorphology: Concepts, Techniques, and Applications	191
M.J. Vepraskas	
M.A. Wilson	

Chapter 8

Digital Image Analysis of Soil Micromorphology 227

A. J. VandenBygaart

R. Protz

C. Duke

Chapter 9

Transmission Electron Microscopy for Soil Samples: Preparation Methods and Use 235

Françoise Elsass

Claire Chenu

Daniel Tessier

Chapter 10

Scanning Electron Microscopy 269

G. Norman White

Chapter 11

Use of Atomic Force Microscopy to Study Soil Particle Properties and Interactions 299

Patricia A. Maurice

Steven K. Lower

Chapter 12

Electron Microprobe Techniques 335

Renald N. Guillemette

Chapter 13

Diffuse Reflectance Spectroscopy 367

José Torrent

Vidal Barrón

Chapter 14

Analysis of Soils and Minerals Using X-ray Absorption Spectroscopy 387

S.D. Kelly

D. Hesterberg

B. Ravel

Chapter 15

Structural Allocation of Clay Mineral Elemental Components 465

A.D. Karathanasis

Chapter 16

Analysis of Layer Charge, Cation and Anion Exchange Capacities, and Synthesis of Reduced Charge Clays 485

David Laird

Pierce Fleming

Subject Index 509

Foreword

The Soil Science Society of America is extremely pleased to publish this comprehensive compilation of modern mineralogical methods. Co-editors April L. Ulery and L. Richard Drees have done an outstanding job of assembling this volume. This valuable work began with the vision of Dr. L. Richard Drees, who unfortunately fell ill during the early stages of the monograph and was unable to complete the task. Co-editor Dr. April Ulery has done a great job of bringing it to completion.

The authors contributing to this new installment in *Methods of Soil Analysis* are scientists at the forefront of research in mineralogy, and their expertise brings great credibility to this work. Research scientists and students from a broad range of disciplines will benefit greatly from this assemblage of proven and practical methodology.

The Soil Science Society of America certainly appreciates the efforts of the editors, Drs. Ulery and Drees, who skillfully guided the development of the book. Thank you also to the Members of the Editorial Committee, which included Will Gates, David Laird, William “Billy” Kingery, Mark Elless, and Michael Vepraskas.

The editors, along with the highly qualified authors, provide a truly excellent book, one of which our Society can be justly proud. We hope you will find this book to be a highly valued resource for your laboratories.

Gary A. Peterson, President of the Soil Science Society of America

Preface

Soil mineralogy has a profound influence on the chemical characteristics and dynamic behavior of soils and the environment. An understanding of the mineralogical composition provides insight into the fundamental behavior of soils and their response to environmental conditions and management. This book is intended to give students and scientists proven methods for measurement of soil mineralogical properties.

In this volume we have updated *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods* (1986) to reflect improvements in techniques, new techniques, newer instruments, and more quantitative analysis. There has been a proliferation of instrumentation and the interface of computer manipulation, data acquisition, and data analysis with new and old methodologies. Several methodologies and instruments were not even on the minds of soil scientists just a few years ago, e.g., atomic force microscopy and X-ray absorption spectroscopy. While this volume does not represent a comprehensive treatise of all mineralogical methods, we present a range of valuable techniques and subjects that will enable researchers to analyze mineralogy for a wide variety of applications—from soil classification to environmental remediation. Highlights include extensive coverage of new techniques, such as X-ray absorption and diffuse reflectance spectroscopy, and updated chapters on thermal analysis and selective dissolution methodologies.

Our target audience is researchers and advanced students in soils, geology, mineralogy, environmental engineering, and environmental sciences. The text was written with soil minerals in mind, not geological specimens or ideal endmembers that have perfect crystallinity or morphology. Soils are dynamic and diverse, and so are soil minerals. The emphasis of this volume is on the analysis and interpretation of data to aid in identifying soil minerals and understanding their impact on soils and the environment. The goal of each chapter is to equip the reader with an understanding of the basic principles and theory of the analytical method, guide the reader through the method itself, and finally assist in the interpretation and analysis of results collected.

This title in the *Methods of Soil Analysis* series began with the vision of my co-editor, Richard Drees, to whom I am personally grateful and this book is dedicated. The authors are acknowledged for their cooperation in updating all of their chapters when I came on as co-editor. All of the chapters were reviewed by at least two scientists familiar with the method and I sincerely appreciate all of their efforts and professionalism. Special recognition and thanks go to Drs. Will Gates and David Laird for their constructive reviews and revisions of several chapters each. Members of the Editorial Committee also included William “Billy” Kingery, Mark Elless, and Michael Vepraskas. I also want to acknowledge the Managing Editor, Lisa Al-Amoodi. She and the SSSA Headquarters Staff were always professional, extremely competent, and a pleasure to work with.

April Ulery, Co-editor, New Mexico State University, Las Cruces

Contributors

Barrón, V.	Dep. de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Edificio C4, Campus de Rabanales, 14071 Córdoba, Spain (vidal@uco.ed)
Buck, B.J.	Dep. of Geosciences, University of Nevada, 4505 Maryland Pkwy., Las Vegas, NV 89154 (buckb@unlv.nevada.edu)
Chenu, C.	AgroParisTech, BIOEMCO, Bâtiment EGER, F-78850 Thiverval Grignon, France (chenu@grignon.inra.fr)
Drees, L.R.	deceased, formerly Soil and Crop Sciences Dep., Texas A&M University, College Station
Duke, C.	Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada
Elsass, F.	Institut National de la Recherche Agronomique, Centre de Géochimie de Surface, 1 rue Blessig, F-67084 Strasbourg, France (elsass@versailles.inra.fr)
Fleming, P.	USDA-ARS, National Soil Tilth Laboratory, 2150 Pammel Drive, Ames, IA 50011
Guillemette, R.N.	Dep. of Geology and Geophysics 3115, Texas A&M University, College Station, TX 77843-3115 (guillemette@ geo.tamu.edu)
Harris, W.G.	Soil and Water Science Dep. 2169 McCarty Hall, University of Florida, Gainesville, FL 32611 (apatite@ufl.edu)
Hesterberg, D.	North Carolina State University, College of Agriculture and Life Sciences, Dep. of Soil Science, Box 7619, Raleigh, NC 27695 (dean_hesterberg@ncsu.edu)
Karathanasis, A.D.	Dep. of Plant & Soil Sciences, N-122K Ag. Science-North, University of Kentucky, Lexington, KY 40546 (akaratha@ uky.edu)
Kelly, S.D.	Argonne National Laboratory, Bioscience Division, 9700 South Cass Avenue, Argonne, IL 60439 (SKelly@anl.gov)
Laird, D.A.	USDA-ARS, National Soil Tilth Laboratory, 2150 Pammel Drive, Ames, IA 50011 (laird@nsl.gov)
Lower, S.K.	Dep. of Geological Sciences and The School of Natural Resources, The Ohio State University, Columbus, OH 43210
Lynn, W.	retired, formerly USDA-NRCS, National Soil Survey Center, Lincoln, NE

Maurice, P.A.	Dep. of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, IN 46556 (pmaurice@nd.edu)
Moody, L.E.	Earth & Soil Sciences Dep., California Polytechnic State University, San Luis Obispo, CA
Protz, R.	deceased, formerly University of Guelph, Ontario, Canada
B. Ravel	Argonne National Laboratory, Bioscience Division, 9700 South Cass Ave., Argonne, IL 60439; currently at National Institute of Standards and Technology, Ceramics Division, 100 Bureau Drive, Gaithersburg, MD 20899-8520 (bravel@bnl.gov)
Shang, C.	Dep. of Crop and Soil Environmental Sciences Virginia Polytechnic and State University, Blacksburg, VA
Soukup, D.A.	Geoscience Dep., University of Nevada, 4505 Maryland Pkwy., Las Vegas, NV 89154 (gsoukup@bak.rr.com)
Tessier, D.	Institut National de la Recherche Agronomique, PESSAC Unit RD 10, Route de Saint-Cyr, F-78026 Versailles, France (tessier@versailles.inra.fr)
Thomas, J.E.	USDA-NRCS, National Soil Survey Center, Lincoln, NE
Torrent, J.	Dep. de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Edificio C4, Campus de Rabanales, 14071 Córdoba, Spain (torrent@uco.es)
Ulery, A.L.	New Mexico State University, Plant and Environmental Sciences, P.O. Box 30003, MSC 3Q, Las Cruces, NM 88003-8003 (aulery@nmsu.edu)
VandenBygaart, A.J.	Agriculture & Agri-food Canada, 960 Carling Avenue/960 Carling Avenue, Ottawa, Ontario, K1A 0C6 (VandenBygaart@AGR.GC.CA)
Vepraskas, M.J.	Soil Science Dep., Box 7619, North Carolina State University, Raleigh, 27695-7619 (Michael_Vepraskas@ncsu.edu)
White, G.N.	formerly Dep. of Soil and Crop Science, Texas A&M University, College Station, TX; currently Texas Commission on Environmental Quality, MC-150, P.O. Box 13087, Austin, TX 78711-3087 (gn-white@austin.rr.com)
Wilson, M.A.	USDA-Natural Resources Conservation Service, 100 Centennial Mall N., Rm. 152, MS 41, Lincoln, NE 68508-3866 (mike.wilson@lin.usda.gov)
Zelazny, L.W.	Dep. of Crop and Soil Environmental Sciences Virginia Polytechnic and State University, Blacksburg, VA (zelazny@vt.edu)

Conversion Factors for SI and Non-SI Units

To convert Column 1 into Column 2 multiply by	Column 1 SI unit	Column 2 non-SI unit	To convert Column 2 into Column 1 multiply by
Length			
0.621	kilometer, km (10^3 m)	mile, mi	1.609
1.094	meter, m	yard, yd	0.914
3.28	meter, m	foot, ft	0.304
1.0	micrometer, μm (10^{-6} m)	micron, μ	1.0
3.94×10^{-2}	millimeter, mm (10^{-3} m)	inch, in	25.4
10	nanometer, nm (10^{-9} m)	Angstrom, $\text{\AA}$	0.1
Area			
2.47	hectare, ha	acre	0.405
247	square kilometer, km^2 (10^3 m) ²	acre	4.05×10^{-3}
0.386	square kilometer, km^2 (10^3 m) ²	square mile, mi^2	2.590
2.47×10^{-4}	square meter, m^2	acre	4.05×10^3
10.76	square meter, m^2	square foot, ft^2	9.29×10^{-2}
1.55×10^{-3}	square millimeter, mm^2 (10^{-3} m) ²	square inch, in^2	645
Volume			
9.73×10^{-3}	cubic meter, m^3	acre-inch	102.8
35.3	cubic meter, m^3	cubic foot, ft^3	2.83×10^{-2}
6.10×10^4	cubic meter, m^3	cubic inch, in^3	1.64×10^{-5}
2.84×10^{-2}	liter, L (10^{-3} m ³)	bushel, bu	35.24
1.057	liter, L (10^{-3} m ³)	quart (liquid), qt	0.946
3.53×10^{-2}	liter, L (10^{-3} m ³)	cubic foot, ft^3	28.3
0.265	liter, L (10^{-3} m ³)	gallon	3.78
33.78	liter, L (10^{-3} m ³)	ounce (fluid), oz	2.96×10^{-2}
2.11	liter, L (10^{-3} m ³)	pint (fluid), pt	0.473
Mass			
2.20×10^{-3}	gram, g (10^{-3} kg)	pound, lb	454
3.52×10^{-2}	gram, g (10^{-3} kg)	ounce (avdp), oz	28.4
2.205	kilogram, kg	pound, lb	0.454
0.01	kilogram, kg	quintal (metric), q	100

continued

To convert Column 1 into Column 2 multiply by	Column 1 SI unit	Column 2 non-SI unit	To convert Column 2 into Column 1 multiply by
1.10 × 10 ⁻³	kilogram, kg	ton (2000 lb), ton	907
1.102	megagram, Mg (tonne)	ton (U.S.), ton	0.907
1.102	tonne, t	ton (U.S.), ton	0.907
Yield and Rate			
0.893	kilogram per hectare, kg ha ⁻¹	pound per acre, lb acre ⁻¹	1.12
7.77 × 10 ⁻²	kilogram per cubic meter, kg m ⁻³	pound per bushel, lb bu ⁻¹	12.87
1.49 × 10 ⁻²	kilogram per hectare, kg ha ⁻¹	bushel per acre, 60 lb	67.19
1.59 × 10 ⁻²	kilogram per hectare, kg ha ⁻¹	bushel per acre, 56 lb	62.71
1.86 × 10 ⁻²	kilogram per hectare, kg ha ⁻¹	bushel per acre, 48 lb	53.75
0.107	liter per hectare, L ha ⁻¹	gallon per acre	9.35
893	tonne per hectare, t ha ⁻¹	pound per acre, lb acre ⁻¹	1.12 × 10 ⁻³
893	megagram per hectare, Mg ha ⁻¹	pound per acre, lb acre ⁻¹	1.12 × 10 ⁻³
0.446	megagram per hectare, Mg ha ⁻¹	ton (2000 lb) per acre, ton acre ⁻¹	2.24
2.24	meter per second, m s ⁻¹	mile per hour	0.447
Specific Surface			
10	square meter per kilogram, m ² kg ⁻¹	square centimeter per gram, cm ² g ⁻¹	0.1
1000	square meter per kilogram, m ² kg ⁻¹	square millimeter per gram, mm ² g ⁻¹	0.001
Density			
1.00	megagram per cubic meter, Mg m ⁻³	gram per cubic centimeter, g cm ⁻³	1.00
Pressure			
9.90	megapascal, MPa (10 ⁶ Pa)	atmosphere	0.101
10	megapascal, MPa (10 ⁶ Pa)	bar	0.1
2.09 × 10 ⁻²	pascal, Pa	pound per square foot, lb ft ⁻²	47.9
1.45 × 10 ⁻⁴	pascal, Pa	pound per square inch, lb in ⁻²	6.90 × 10 ³
Temperature			
1.00 (K – 273)	kelvin, K	Celsius, °C	1.00 (°C + 273)
(9/5 °C) + 32	Celsius, °C	Fahrenheit, °F	5/9 (°F – 32)
Energy, Work, Quantity of Heat			
9.52 × 10 ⁻⁴	joule, J	British thermal unit, Btu	1.05 × 10 ³
0.239	joule, J	calorie, cal	4.19
10 ⁷	joule, J	erg	10 ⁻⁷
0.735	joule, J	foot-pound	1.36
2.387 × 10 ⁻⁵	joule per square meter, J m ⁻²	calorie per square centimeter (langley)	4.19 × 10 ⁴
10 ⁵	newton, N	dyne	10 ⁻⁵
1.43 × 10 ⁻³	watt per square meter, W m ⁻²	calorie per square centimeter minute (irradiance), cal cm ⁻² min ⁻¹	698

continued

To convert Column 1 into Column 2 multiply by	Column 1 SI unit	Column 2 non-SI unit	To convert Column 2 into Column 1 multiply by
Transpiration and Photosynthesis			
3.60×10^{-2}	milligram per square meter second, $\text{mg m}^{-2} \text{s}^{-1}$	gram per square decimeter hour, $\text{g dm}^{-2} \text{h}^{-1}$	27.8
5.56×10^{-3}	milligram (H_2O) per square meter second, $\text{mg m}^{-2} \text{s}^{-1}$	micromole (H_2O) per square centimeter second, $\mu\text{mol cm}^{-2} \text{s}^{-1}$	180
10^{-4}	milligram per square meter second, $\text{mg m}^{-2} \text{s}^{-1}$	milligram per square centimeter second, $\text{mg cm}^{-2} \text{s}^{-1}$	10^4
35.97	milligram per square meter second, $\text{mg m}^{-2} \text{s}^{-1}$	milligram per square decimeter hour, $\text{mg dm}^{-2} \text{h}^{-1}$	2.78×10^{-2}
Plane Angle			
57.3	radian, rad	degrees (angle), $^\circ$	1.75×10^{-2}
Electrical Conductivity, Electricity, and Magnetism			
10	siemen per meter, S m^{-1}	millimho per centimeter, mmho cm^{-1}	0.1
10^4	tesla, T	gauss, G	10^{-4}
Water Measurement			
9.73×10^{-3}	cubic meter, m^3	acre-inch, acre-in	102.8
9.81×10^{-3}	cubic meter per hour, $\text{m}^3 \text{h}^{-1}$	cubic foot per second, $\text{ft}^3 \text{s}^{-1}$	101.9
4.40	cubic meter per hour, $\text{m}^3 \text{h}^{-1}$	U.S. gallon per minute, gal min^{-1}	0.227
8.11	hectare meter, ha m	acre-foot, acre-ft	0.123
97.28	hectare meter, ha m	acre-inch, acre-in	1.03×10^{-2}
8.1×10^{-2}	hectare centimeter, ha cm	acre-foot, acre-ft	12.33
Concentration			
1	centimole per kilogram, cmol kg^{-1}	milliequivalent per 100 grams, $\text{meq } 100 \text{ g}^{-1}$	1
0.1	gram per kilogram, g kg^{-1}	percent, %	10
1	milligram per kilogram, mg kg^{-1}	parts per million, ppm	1
Radioactivity			
2.7×10^{-11}	becquerel, Bq	curie, Ci	3.7×10^{10}
2.7×10^{-2}	becquerel per kilogram, Bq kg^{-1}	picoCurie per gram, pCi g^{-1}	37
100	gray, Gy (absorbed dose)	rad, rd	0.01
100	sievert, Sv (equivalent dose)	rem (roentgen equivalent man)	0.01
Plant Nutrient Conversion			
	Elemental	Oxide	
2.29	P	P_2O_5	0.437
1.20	K	K_2O	0.830
1.39	Ca	CaO	0.715
1.66	Mg	MgO	0.602

Sampling Soils for Mineralogical Analyses

D. A. SOUKUP, University of Nevada, Las Vegas

L. R. DREES, deceased, formerly Texas A&M University, College Station

W. C. LYNN, USDA-NRCS, National Soil Survey Center, Lincoln, Nebraska

Soil mineralogy has a profound influence on the chemical characteristics and dynamic behavior of soils. The chemical composition of the soil solution is often maintained by weathering and/or precipitation of minerals. The reactive nature of the surface of many minerals and the ability to form chemical bonds with soluble constituents also serves to maintain the chemical composition of the soil solution. If soils were not capable of binding with chemical elements and compounds, the fertilizers, pesticides, herbicides, and other constituents applied to soils may be leached and eventually transported to groundwater or discharged to surface water bodies. Increasing recognition of the significance of soil mineralogy and its relationship to environmental quality has resulted in the performance of numerous soil mineralogical studies during recent years. Although soil mineralogical studies are primarily performed in the laboratory, it should be emphasized that mineralogical analysis typically begins in the field. Therefore, it is critical that the soil samples collected in the field are representative of the soils being studied. This chapter summarizes general procedures that will ensure the collected samples are appropriate for the specific objectives of the soil mineralogical study.

PURPOSE OF SAMPLING

Soil samples may be collected for a variety of different objectives. The number and types of samples ultimately collected from a site will be determined by the objectives of the study. The following sections discuss some potential study objectives, the role of soil variability in achieving those objectives, and some considerations when selecting a site.

Objectives

There are three general types of projects for which sampling objectives are developed. These include reference, characterization, and geomorphic and/or stratigraphic projects (Natural Resources Conservation Service [NRCS], 1996). *Reference projects* are those projects that are designed to answer specific questions or for collection of calibration standards. A reference project, for example, may focus on establishing the variation in clay mineralogy in soils in a given mapping unit. In this situation, samples may be collected from specific horizons in three to five locations that are representative of the map unit. A

limited number of analyses that address the variation in clay mineralogy between these samples would be performed.

Characterization projects are designed to obtain comprehensive characterization data for a representative pedon of a map unit or a pedon included in a research study. A characterization project would typically include detailed analysis of the physical, chemical, and mineralogical characteristics of the sampled pedon.

Geomorphic and/or stratigraphic projects are designed to study relationships between soils, landforms, and/or the stratigraphy of parent materials. For example, a project may be undertaken to study how redoximorphic features and hydrologic regime vary in soils along a catena. Representative pedons occupying different landscape positions would be sampled and their redoximorphic features, often expressed as specific soil minerals, and their hydrologic regimes compared.

Soil Variability

Pedologists have long been aware of the spatial variability of soils in a landscape (Wilding and Drees, 1983). Soils as landscape bodies often exhibit wide ranges of physical, chemical, morphological, and mineralogical characteristics, both laterally and vertically. Many characteristics, such as hydraulic conductivity and water retention, also vary with transient soil features, do not vary randomly in space, and therefore are not normally distributed (Wilding and Drees, 1983).

Spatial variability in soils can be grouped into two general categories, systematic and random. Systematic variability is a gradual or marked change in soil properties occurring as a function of:

- Landforms—mountains, plateaus, basins, plains, terraces, fans, valleys, etc.
- Geomorphic position—summit, shoulder, backslope, or toeslope
- Soil-forming factors—climate, parent material, time, biota, topography
- Interactions of the above factors.

Random variability refers to those changes in soil properties that cannot be related to a known cause (Wilding and Drees, 1983). Systematic and random variability in soil properties may occur concurrently and in association with one another. Causes of random variability include:

- Differential lithology
- Differential intensity of weathering
- Differential erosion and deposition
- Differences in biological factors, including anthropogenic activities
- Differences in hydrology
- Sampling and analytical errors

These causes of random variability, with the exception of sampling and analytical errors, may also result in systematic variability.

Site Selection

Selecting the proper site or study area is critical to the successful completion of any investigation, and the specific objectives of the study should be considered during site selection. If possible, uncultivated and undisturbed sites should be utilized for sampling, unless one of the study objectives is to characterize cultivated or disturbed soils (Buol et al., 2003). Similarly, sites selected for sampling should be located away from roads, fence

lines, utility locations, and any other anthropogenic features that may have altered soil characteristics, unless the study objective is to characterize or quantify the degree of alteration. Historical aerial photographs, topographic maps, and other relevant information may also be obtained from government agencies, libraries, private companies, and potentially other sources to document the history of a site, which may be useful in selecting and characterizing a study site.

Information about geomorphology, geologic and hydrogeologic conditions, and dominant soil types at the site or its vicinity may be available from the USGS, the Bureau of Land Management (BLM), the USDA-NRCS, other government agencies, and published literature. Many USDA-NRCS soil surveys have been digitized and are available electronically. These and other reports can be combined with geographical information systems (GIS) tools to identify potential study sites. Review of readily available site information from a variety of sources is recommended to facilitate selection of specific sites and/or pedons for detailed study.

The selection of a specific site or pedon(s) is determined by the objectives of a study but may often be selected to represent typical landform segments. If a transect is to be sampled, it is important that the variable to be studied can be adequately evaluated after considering the other variables in the transect. The distances between pedons included in the study may vary from a few meters to kilometers, depending on the objectives of the study. Reid et al. (1996) studied smectite mineralogy and charge characteristics along an arid geomorphic transect to evaluate the relationship between smectite properties and geologic and pedologic factors. Wilcke and Amelung (1996) investigated heterogeneity of Al and other heavy metals in soil aggregates along a climatic transect from Saskatchewan in Canada to Texas.

DEVELOPING A SAMPLING STRATEGY

Detailed planning is critical for successful execution of field activities and project completion. Mobilizing equipment and personnel to the project site is expensive and time-consuming. Therefore, it is important to ensure that the field activities performed provide the information, documentation, and samples needed to achieve the project objectives. The following sections describe some of the site-specific and other factors to be considered when developing a sampling plan.

Specific-Site Characteristics and Considerations

There are several site characteristics that should be considered when developing a sampling plan or strategy. These include both pre-field and field activities. Pre-field activities to consider, and questions to ask, include:

Site Accessibility

Do you need to secure permission from the owner (including the state or federal government), operator, or some other entity to enter a site and perform sampling activities? Can personnel and equipment be transported easily to the site?

Obtaining Required Permits and Insurance

Are any permits or other paperwork required from any governmental agency or other entity to perform the planned sampling activities? Does a Certificate of Insurance naming the owner or some other entity as Additional Insured need to be obtained before the field activities?

Clearance of Underground Utility Locations

Have the proposed sampling locations been marked and utility-marking companies contacted to “clear” locations? In California, for example, Underground Service Alert (USA) must be notified a minimum of 48 h before the start of work to mark utility locations within the proposed work area. If USA clearance is not obtained, the company and/or the individual(s) performing excavation activities are liable for any damage to subsurface utilities. Whenever possible, it is also recommended that the owner or operator or their representative be contacted to clear locations where other underground utilities and/or irrigation system components are located. Many land owners with drip-line and/or other subsurface irrigation systems may be unwilling to allow sampling because of the potential for irrigation system damage.

Resources Required for Field Activities

What resources (i.e., personnel and equipment) are required to complete the field sampling activities? Is any specialized equipment required for the sampling and transport to the laboratory? Does any equipment need to be rented or purchased?

Types of Samples

There are several different methods available for sample collection. The method(s) selected depend on the research objectives, time, and resources available for the sampling effort. The following sections describe some of the different methods of sampling.

Soil Pits or Trenches

A large backhoe pit, approximately 2 m long, 1 to 2 m wide, and 2 m or more in depth, is recommended for describing and sampling a soil pedon. One of the major advantages to excavating a large pit or trench is that it allows for observation and sampling of horizontal as well as vertical variability in the landscape. Such variability may not be apparent in smaller exposures. One disadvantage is that excavation of large pits or trenches requires access for heavy equipment, including backhoes or excavators. The rental costs for equipment and operator may also be substantial.

Soil pits may also be dug by hand, but this is more labor intensive, and the pits are not usually as large or as deep as those excavated by heavy equipment.

Regardless of the digging and sampling methodologies employed, personal safety should be the primary consideration during field work. Special precautions should be taken whenever work is performed in close proximity to machinery such as backhoes, drilling rigs, and hydraulic probes. Soil pits that are deeper than 1.25 m (5 feet) are required to be shored in accordance with U.S. Department of Labor Occupational Safety and Health Administration (OSHA) requirements. In many states, shoring is also required for Vertisols and soils with vertic properties.

Road Cuts or Other Exposures

Road cuts or other exposures, such as stream banks, may also expose a large horizontal and vertical area of soil. However, road cuts and other exposures are not satisfactory for sampling unless they have been freshly exposed and there has been no disturbance of topsoil (Buol et al., 2003). Wetting–drying cycles, oxidizing conditions, plant root and animal activity, and cross contamination from dust or other atmospheric pollutants at these sites may result in modification of the soil structure and other physical and chemical characteristics. As a result, samples obtained from such sites may not be representative of undisturbed

soils in the area. Extreme caution must be taken when parking and working on roadsides, which also may be illegal on many highways.

Auger Borings, Probes, or Tube Samples

A wide variety of equipment is available to advance soil boreholes and obtain soil samples. Borings may be advanced with a hand auger, power tools, or a truck-mounted drill or direct-push rig. Depending on the type of equipment used, the sample diameter may range from approximately 2 to 12 cm. Larger diameter cores up to 1.5 m in length may also be collected using a truck-mounted drill or coring rig. These cores may be split apart for description and sampling. One advantage of this type of sampling is that numerous intact cores and/or samples from boreholes may be collected across the landscape in a relatively short period of time. However, samples collected in this manner do not allow for delineation and sampling of the various horizons as they occur in the pedon because of the small surface area available for observation (Buol et al., 2003). There is also a risk of cross contamination due to sloughing of the borehole walls as samples are lifted from the hole. Additionally, it is not always possible to retrieve intact cores or samples because of incomplete recovery (i.e., the sample tubes or core barrel are not full of soil). In this situation, it is not known at which depth the material in the tubes or core barrel was obtained. Incomplete recovery is fairly common in gravelly soils or soils with abundant coarse fragments. Compaction of soil when using a push probe also occurs and may further complicate identifying the sample depth.

Composite Samples

In some soil investigations, laboratory analyses may be performed on a composite of the samples rather than on each individual sample collected in the field. The composite sample consists of a number of samples that have been mixed together in equal proportions. All of the samples that constitute the composite sample must be from the population being studied, and each sample must contribute the same amount to the composite. The assumption when samples are composited is that a valid estimate of the mean of some characteristic of the population may be obtained from the analysis of the composite sample (Petersen and Calvin, 1986). This assumption may or may not be true.

It should be noted that the composite samples only provide an estimate of the mean of the population from which the samples making up the composite were collected. No estimate of the variance of the mean and the precision with which the mean is estimated can be obtained from a single composite sample. The accuracy with which a population mean is estimated from a composite sample is dependent on the variability among sampling units within the population and the number of sampling units included in the composite (Petersen and Calvin, 1986).

Composite samples are commonly used in agricultural nutrient testing and in certain environmental characterization studies. For example, composite samples may be collected from soil stockpiles generated during excavation of contaminants to evaluate potential off-site disposal options for the stockpiled material. Composite sampling may also be used to evaluate the effectiveness of bioremediation of soils impacted by petroleum hydrocarbons that are being mitigated by aboveground land treatment.

One of the advantages of compositing is that the analytical costs and time required for analyses to be performed are reduced. However, Petersen and Calvin (1986) advised, "If no estimate of variability is available, compositing should be avoided if possible." If environmental sampling is being performed, the regulatory agency with site oversight should

be contacted before sample collection to ensure that the results of composite sampling are acceptable to their agency.

Number of Samples

The number of samples collected depends on the specific objective(s) and the desired accuracy for measuring a particular characteristic of the soil population being studied. The true value of each characteristic in the population is called a parameter (Petersen and Calvin, 1986). The purpose of sampling is to estimate the parameter of interest with the desired accuracy at the lowest possible cost. If the population of interest is relatively homogeneous, collection of a few samples may be adequate to characterize the population. However, heterogeneity is common in soils, and additional sample collection will be necessary for parameter estimation and population characterization. Additional information concerning sampling and statistical analysis of data is provided in Petersen and Calvin (1986). If possible, consultation with a statistician may facilitate development of an appropriate sampling plan.

SAMPLE COLLECTION

The importance of selecting pedons that are representative of the study area for sampling purposes cannot be overemphasized. Buol et al. (2003) cautioned, “The time, effort, and expense invested in lab analyses of the profile samples will be wasted if the samples are not representative of the soil.” These authors also noted that data generated from a given study may be used by numerous investigators and other individuals for different purposes. Additionally, any interpretations and extrapolations made by individuals using the data will likely be erroneous if the samples are not representative. Buol et al. (2003) concluded with this recommendation: “Soil profile samples should be taken from a pedon determined by field studies and observations to be truly representative of the area under study.” The following sections summarize some of the important site and pedon observations that should be documented during field activities.

Field Observations

There are several field observations that are important to document for any study or investigation. These include:

- **Location.** The geographical location of the area of interest needs to be recorded as precisely as possible. Latitude and longitude recorded in degrees, minutes, seconds, direction, and associated datum is the preferred method for documenting location (Schoeneberger et al., 2002). Avoid using constructed landmarks such as roads and buildings to locate a sampling area, as these will invariably change with time.
- **Topographic quadrangles/geologic maps.** The topographic quadrangle, typically a USGS topographic map, for the study area should be documented. Similarly, existing geologic maps of the study area that may be available from the USGS and possibly other sources should be recorded.
- **Soil survey area.** If the study area is covered by an existing soil survey, the survey information should be used to document the soil series and map units within the study area. This information may also be useful for interpreting the results of the research.
- **Geomorphic information.** If available, the physiographic location including division, province, section, and state physiographic area for the study area should be

noted. The researchers should record the landscape position, landform, microfeatures, and surface morphometry, including slope gradient, complexity, and shape.

- **Water status.** The water status, including drainage, flooding, ponding, observed soil water status, and depth to water table should be recorded or estimated based on field observations.
- **Vegetation and land cover.** The dominant land cover at the site should be recorded.
- **Parent material.** The parent material and kinds of unconsolidated material within the study area should be documented.
- **Erosion and runoff.** Evidence of erosion and surface runoff should be described.
- **Site-specific information.** The slope, aspect, soil surface conditions (i.e., native or under cultivation) and an estimate of exposed materials >2 mm within the surface horizon should be recorded. If Vertisols are present, the size, depth, and distribution of gilgai features should be recorded. Any other surface feature(s) that may affect horizonation within the soil profile should also be measured, described, and recorded.

Additional information and details concerning field observations that should be documented were provided by Schoeneberger et al. (2002).

Pedon Descriptions

The methodology for describing soils has been developed and refined by soil scientists since the Soil Survey Program began in the United States more than a century ago. The USDA first published booklets entitled *Instructions to Field Parties* in 1902–1904, 1906, and 1914 (Schoeneberger et al., 2002). These booklets included soil descriptions. The first USDA guide for identification and description of soil horizons was published in 1937 and has since been revised several times (Schoeneberger et al., 2002). Soil science is a dynamic and evolving field, and changes to the existing methodologies for describing and sampling soils will occur in the future. The information provided in the following sections is intended to emphasize some important points about pedon description and sampling. It should not be considered as a comprehensive guide to pedon description and sampling. The reader is referred to Schoeneberger et al. (2002) for additional details and recommendations regarding description and sampling of soils in the field, which may be helpful before performing field study and soil sampling.

Soil macromorphology refers to field observations that are made with the unaided eye and is best evaluated from the in situ examination of a freshly exposed soil profile (Buol et al., 2003). Old exposures should only be used for preliminary observations, because soil characteristics and mineralogy are often altered after exposure to the elements. Once a profile has been exposed, it can be prepared for examination, description, photographing, and sampling using the following procedures:

1. One side of the soil pit should be selected for description, sampling, and photographing. The soil surface on this side of the pit should not be walked on or stood on to minimize any topsoil disturbance. Access into, and exit from the pit should be on one of the other three sides of the pit that will not be described or sampled. A ramp is often constructed on one side of the pit to facilitate access.
2. Probe the exposed profile by hand using a small knife to remove alterations resulting from the digging equipment. The objective of this step is to expose the natural condition of the soil. The probing may also assist in the designation of soil horizon boundaries, because textural and structural changes may be readily apparent during probing.

3. A measuring tape should be placed on the exposed profile to allow references to depth during description. The tape also provides depth references for photographs of the soil, which we recommend taking at this time. Photographs may also be taken after description and sampling have been completed, but the profile will need to be probed again to expose the natural condition of the soil. Excess soil that may have sloughed off the walls during sampling may also need to be removed from the bottom of the profile before photographing.
4. Mark the depths in the profile where differences in color, structure, or texture are observed as an initial approximation of soil horizon boundaries. Nails or golf tees work well for marking horizon boundaries. For soils that have noncontiguous horizons, string may be used for marking the horizons. This allows for a complete description and ensures sampling of all genetic horizons. Some pedologists prefer to prepare detailed drawings of the pedon or polypedons while in the field.
5. Begin the detailed examination of the profile, describing horizons according to their color, texture, structure, consistence, root quantity and size, boundary characteristics, horizon continuity, nodules or concretions, mottles, redoximorphic features, voids, pores, pH (field method) and effervescence on treatment with a ~1 M hydrochloric acid (HCl) solution. The approximate soil horizon boundaries may be adjusted, as needed, as the detailed examination progresses.

Sampling Procedures and Equipment

The sampling methodology and the location of the soil material collected for analysis will ultimately be determined by the objective(s) of the study (Schoeneberger et al., 2002). General information and some recommendations concerning soil sampling procedures are provided below. Additional details regarding soil sampling are provided in Soil Survey Investigations Rep. 42 (Soil Survey Staff, 1996) and Schoeneberger et al. (2002).

1. As described above, all sampling locations should be documented as precisely as possible. Buol et al. (2003) summarized the consequence of not doing so: "A soil sample without complete documentation of the location is merely a handful of dirt."
2. The sampling procedure is the same for both hand-dug and backhoe pits. Once the horizons or zones from which samples will be collected are marked, the profile may be sampled from top to bottom or from bottom to top. Bottom-to-top sampling is generally recommended to reduce the potential for cross-contamination during sampling. If top to bottom sampling is performed, care should be taken to ensure that material sloughing off the upper horizons is removed before deeper horizons are sampled. Top-to-bottom sampling is recommended if a large number of undisturbed cores will be collected and the soil is dry and hard (Buol et al., 2003).
3. Each horizon should be sampled vertically from boundary to boundary and laterally to include observable short-range variability. The sample should be collected in a sampling tray, mixed thoroughly, placed into a sampling bag or other collection vessel and labeled. The amount of sample collected will vary depending on the purposes of the study. Collecting additional sample is recommended to ensure that sufficient sample is available for future, unanticipated analyses. If a horizon is especially thick, it may be split for sampling if desired by the sampling personnel. For horizons with two distinct materials, such as tongues of an E horizon into a Bt horizon, separate samples of each material should be collected, noting the relative volume percentage of each.

Care should be taken not to sample krotovina material. This may be difficult if a large portion of the pedon or horizons being sampled have been mixed by burrowing

animals. This is especially difficult for soils that are used for crawfish production. Under these circumstances, a different location may have to be used for sampling.

Special sampling techniques are also required for organic soils, permafrost-affected soils and Vertisols (Soil Survey Staff, 1996). Collecting samples from organic soils may be difficult because of the high water contents characteristic of these soils. Permafrost-affected soils may be resistant to excavation and commonly exhibit cryoturbation that disrupts horizon morphology. In this case, hand drawing and photographing the pit face before sample collection is recommended. The shear failure that forms slickensides in Vertisols disrupts the soil so that conventional horizon designations do not adequately describe the morphology. The gilgai surface topography characteristic of many Vertisols is reflected in the subsurface by bowl-shaped lows and highs across a distance of several meters. Both the high and low areas are commonly sampled since these areas represent the extremes in the cyclic morphology of Vertisols. Additional details regarding sampling procedures for organic soils, permafrost-affected soils, and Vertisols are provided in Soil Survey Investigations Rep. 42 (Soil Survey Staff, 1996) and Schoeneberger et al. (2002).

4. We recommend using indelible ink or paint pens for marking so that the sample identification labels do not smear, smudge, or fade with time. It is also recommended that both the outside and inside of the sample container be marked with the sample identification information.
5. Soil clods or blocks of undisturbed soil approximately 100 to 500 cm³ may also be collected from each horizon for micromorphology studies, determination of bulk density and coefficient of linear extensibility (COLE), and possibly other physical and chemical characteristics. The orientation of the clods or blocks should be marked to show which was the upper side within the profile or sediments sampled. We have found that brightly colored nail polish works well for marking the upper side of the clods or blocks. Once the upper side of the clods or blocks has been marked, they should be wrapped in paper towels or packing material and placed into a paper can or some other protective container. The containers should be labeled both inside and out with the sample identification and other relevant information. Soil Survey Staff (1996) recommended collecting three clods for bulk density analysis and two additional clods for preparation of thin sections and micromorphological study. The actual number of clods collected will depend on the specific objectives of the study.

SAMPLE PRESERVATION

Equilibrium conditions change as soon as a sample is collected and removed from its native environment. Some of these changes may result in alteration of the mineralogy and other physical and chemical characteristics of the sample. As a result, procedures should be followed during collecting and transporting and/or shipping samples to the laboratory to ensure that cross contamination does not occur and the condition of the samples is not compromised. Some general procedures to be followed to preserve sample integrity and some special handling procedures that may be required are described in the following sections.

Transporting Samples from the Field to the Laboratory

Bulk soil samples may be placed into prelabeled plastic bags or other collection vessels for transport to the laboratory. Using plastic bags with a minimum thickness of 8-mil and/or double bagging is recommended to prevent breakage of the bags and cross contamination of samples during transport.

Once the upper side of clods or blocks have been marked, they should be wrapped in paper towels and placed into a paper can or some other container that protects them from physical damage during transport and before preparation for analysis. The containers should be labeled both inside and out with the sample identification and other relevant information.

Special handling procedures may also be required for samples collected for microbial, redox, and evaporite mineralogy studies. Samples for microbial studies, for example, may need to be placed on ice and/or protected from sunlight and air. Several soil minerals also have specific hydration and oxidation states (e.g., halloysite and iron oxides) that may change irrevocably if not stored or handled properly.

Initial Preparation of Samples for Analyses

Once the samples have been transported to the laboratory, the samples are prepared for further analyses or archived (discussed below). Preparation of samples for subsequent mineralogical and other chemical analyses typically involves the following:

1. In most cases, the field-moist samples should initially be air dried. This is typically accomplished by placing the samples on brown paper on a bench in the greenhouse or in the laboratory. The paper that the samples are dried on should be labeled with the sample identification and any other relevant information.

It should be noted that volcanic ash soils high in amorphous clay-sized materials are very resistant to dispersion, particularly after air or oven-drying (Gee and Bauder, 1986). Kubota (1972) reported that clay contents for one volcanic ash soil ranged from 1 to 56% (w/w) depending on pretreatment. The maximum clay content was determined when the soil was retained at field moisture before dispersion. Warkentin and Maeda (1980) recommended that volcanic ash soils be maintained at field moisture and dispersed at either pH 3 or above pH 9.

Organic and continually saturated soils, especially clayey saturated soils, also dry irreversibly like many volcanic ash soils. These soils should not be air dried or allowed to change temperature, particularly if Fe, Mn, and other redox sensitive elements are present. Gypsum-containing and gypsiferous soils may also be altered by air drying when exposed directly to sunlight or temperatures exceeding 30°C.

2. Once the samples have been air dried, the entire sample should be passed through a 2-mm sieve. Larger soil aggregates may be gently crushed using a wooden rolling pin or mortar and pestle. Care should be taken when crushing the aggregates to prevent disintegration of coarse-fragments larger than 2 mm or sand-sized particles, including quartz, feldspar, mica, and nodules. Samples should not be ground using any mechanical grinding equipment if particle-size analyses are to be performed. After the entire sample has been crushed, it should be thoroughly mixed. A sample splitter, if available, may be used if the sample is to be divided.
3. The sieved samples should be segregated from the coarse fragments (i.e., particles >2 mm) and placed into labeled plastic bags, paper cans, or some other container for storage.

Sample Archiving

If sufficient quantities of samples remain, archiving bulk and ground soil material, soil clods, coarse fragments, and other materials is recommended. Careful records must

be kept and sample identities preserved. The results of current investigations may suggest the need for additional studies. It is also possible that temporal changes in soil characteristics may be of scientific interest. Graham and Wood (1991) were able to measure the time and conditions required to form an argillic horizon in uniformly packed lysimeter soils in southern California by comparing archived soil samples from the 1930s to the soil profiles that developed in the lysimeters over the next 41 yr. Perhaps the most famous use of archived samples is from the plots of Rothamsted, England where soil samples collected and carefully preserved since the 1800s have allowed quantitative assessment of changes in a variety of soil properties, including nutrients, heavy metals, and organic chemicals, with time and management (e.g., Green et al., 2001). Other examples of important comparative studies that were made possible because of archived samples are provided in Ulery et al. (1995) and De Clerck et al. (2003).

REFERENCES

- Buol, S.W., R.J. Southard, R.C. Graham, and P.A. McDaniel. 2003. Soil genesis and classification. 5th ed. Iowa State Press, Ames.
- De Clerck, F., M.J. Singer, and P. Lindert. 2003. A 60-year history of California soil quality using paired samples. *Geoderma* 114:215–230.
- Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. p. 383–411. *In* A. Klute (ed.) *Methods of soil analysis*. Part 1. Physical and mineralogical methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Graham, R.C., and H.B. Wood. 1991. Morphologic development and clay redistribution in lysimeter soils under chaparral and pine. *Soil Sci. Soc. Am. J.* 55:1638–1646.
- Green, N.J.L., J.L. Jones, A.E. Johnston, and K.C. Jones. 2001. Further evidence for the existence of PCDD/Fs in the environment prior to 1900. *Environ. Sci. Technol.* 35:1974–1981.
- Kubota, T. 1972. Aggregate formation of allophonic soils: Effects of drying on the dispersion of the soils. *Soil Sci. Plant Nutr.* 18:79–87.
- Petersen, R.G., and L.D. Calvin. 1986. Sampling. p. 33–51. *In* A. Klute (ed.) *Methods of soil analysis*. Part 1. Physical and mineralogical methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Reid, D.A., R.C. Graham, L.A. Douglas, and C. Amrhein. 1996. Smectite mineralogy and charge characteristics along an arid geomorphic transect. *Soil Sci. Soc. Am. J.* 60:1602–1611.
- Schoeneberger, P.J., D.A. Wysocki, E.C. Benham, and W.D. Broderson (ed.). 2002. Field book for describing and sampling soils. Version 2.0. USDA-NRCS, National Soil Survey Center, Lincoln, NE.
- Soil Survey Staff. 1996. Soil survey laboratory methods manual. Soil Survey Investigations Rep. 42. U.S. Gov. Print. Office, Washington, DC.
- Ulery, A.L., R.C. Graham, O.A. Chadwick, and H.B. Wood. 1995. Decade-scale changes in soil carbon, nitrogen, and exchangeable cations under chaparral and pine. *Geoderma* 65:121–134.
- Warkentin, B.P., and T. Maeda. 1980. Physical and mechanical characteristics of Andisols. p. 281–301. *In* B.K.G. Theng (ed.) *Soils with variable charge*. New Zealand Society of Soil Science, Lower Hutt, New Zealand.
- Wilcke, W., and W. Amelung. 1996. Small-scale heterogeneity of aluminum and heavy metals in aggregates along a climatic transect. *Soil Sci. Soc. Am. J.* 60:1490–1495.
- Wilding, L.P., and L.R. Drees. 1983. Spatial variability and pedology. p. 83–116. *In* L.P. Wilding et al. (ed.) *Pedogenesis and soil taxonomy*. I. Concepts and interactions. Elsevier Science Publishers, B.V., Amsterdam, The Netherlands.

Preparing Soils for Mineralogical Analyses

D. A. SOUKUP, University of Nevada, Las Vegas

B. J. BUCK, University of Nevada, Las Vegas

W. HARRIS, University of Florida, Gainesville

Sample preparation is an important aspect of soil mineralogical analysis. The use of pretreatments is often necessary to facilitate sample dispersion and/or to concentrate a particular size fraction for subsequent analyses. However, pretreatments may alter, or even destroy, certain fractions of the soil (Kunze and Dixon, 1986). The pretreatments described in the following sections of this chapter are designed to have a minimum effect on constituents other than those being eliminated. In spite of this, the analyst must still be careful to perform only those pretreatments that are essential to accomplish the study objectives. Additionally, the analyst should have a clear understanding of the possible consequences for data interpretation. The reagents used for the various pretreatments do not need to be prepared with a high degree of precision. For example, the reagents may be mixed in beakers rather than in a volumetric flask.

SOIL PREPARATION

As discussed in Chapter 1 (Soukup et al., 2008, this volume), special field sampling and preservation techniques are required for organic soils, permafrost-affected soils, and Vertisols (Soil Survey Staff, 1996; Schoeneberger et al., 2002). To preserve organic soils, Blevins et al. (1968) recommended freezing blocks of soil in the field with liquid N₂ and transporting them to the laboratory in a frozen state. Other soils, including those affected by salts, reducing conditions, or high water contents, may also require special sampling, transportation, and preparation techniques before mineralogical analysis. Once collected, it is critical that such samples be transported and stored under conditions similar to those existing at the field site because many of the minerals in such soils may hydrate, dehydrate, oxidize, or dissolve during transport and storage. We strongly recommend that when working with potentially “problematic” soils researchers consult the literature, local NRCS personnel and other investigators regarding sampling, transport, and preparation techniques that have been employed previously on similar soils. The relative merits of each technique must be evaluated on a soil by soil basis to determine the most appropriate method(s). We also recommend that when there is the potential for mineralogical and other changes, samples should be analyzed as quickly as possible after sampling.

The amount of soil required for mineralogical analyses will vary depending on the study objectives and the sample characteristics. Generally, a sufficient amount of sample

should be utilized to yield approximately 5 to 10 g of clay (<2- μ m fraction) following the necessary pretreatments and subsequent fractionation procedures.

REMOVAL OF SOLUBLE SALTS, GYPSUM, AND CARBONATES

The presence of soluble salts and/or gypsum in soil samples may make it difficult or impossible to disperse the soil samples before fractionation procedures, because of the flocculating action of the salts (Kunze and Dixon, 1986). Soluble salts may also prevent saturation of the exchange complex with a specific cation for determination of cation exchange capacity. As will be discussed below, it is possible to first identify and quantify the soluble salts and gypsum present in the sample using X-ray diffraction (XRD) in combination with other mineralogical analyses. This is especially important as the soluble salt or gypsum content of some soils can be >80% (Buck et al., 2006a, 2006b). After this has been achieved, removing the soluble salts and gypsum is recommended because it simplifies other mineralogical analyses (such as XRD and differential thermal analyses [DTA]) (Kunze and Dixon, 1986). If done in this order, then identification and quantification of all soil constituents is possible.

Carbonates may also make it difficult or impossible to fractionate the sample and effectively separate the silt and clay fractions. Moreover, the presence of carbonates may result in poor X-ray diffractograms, because the degree of orientation of clay-sized particles (i.e., <2 μ m) is decreased in the presence of finely divided carbonates (Kunze and Dixon, 1986). As with soluble salts and gypsum, carbonates may be identified and quantified using a variety of mineralogical and chemical analyses. This is important because the carbonate contents in some calcareous soil may comprise more than 80% of the soil mass (Loeppert and Suarez, 1996).

Test for Presence of Soluble Salts and Gypsum

Soluble Salts

The presence of soluble salts in a sample is indicated by elevated electrical conductivity (EC) measurements in the soil solution. Pure water is a poor conductor of electricity, but conductivity increases as more salt is dissolved in the water. Thus, the EC of the soil solution provides an indirect measurement of the salt content. As an initial test for soluble salts, a soil sample is saturated with distilled water and mixed into a paste. The EC of the water-saturated soil paste may be measured. Alternatively, the water may be extracted from the soil paste, and the EC of the saturated paste extract measured (Soil Survey Staff, 1996). It is difficult to suggest a lower limit of EC below which removal is not necessary, because of the variability in the properties of the soluble salts occurring in soils (Kunze and Dixon, 1986). However, if the EC of the saturated paste or saturated paste extract is in excess of 2 to 4 dS m⁻¹, then treatment to remove excess soluble salts is recommended.

Gypsum

The presence of gypsum in a soil sample can be determined qualitatively using the following procedure (Soil Survey Staff, 1996).

Reagents

- Acetone, reagent grade

Equipment and Materials

- 250-mL bottle or Erlenmeyer flask
- Mechanical shaker
- Funnel
- Filter paper with medium porosity
- Test tube

Procedure

1. Place 10 to 20 g of air-dried soil into a 250-mL bottle or flask and add 100 to 150 mL of distilled water.
2. Place a lid or stopper on the container and place on in a mechanical shaker for 15 min.
3. Filter the extract through the filter paper.
4. Place approximately 5 mL of the extract in a test tube and add an approximately equal volume of acetone. Mix the solutions. The formation of a white precipitate indicates the presence of gypsum in the soil.

Removal of Soluble Salts and Gypsum

Soluble salts and gypsum are typically removed from soils and sediments by one or more rinses with distilled water.

Equipment and Materials

- 250-mL plastic centrifuge bottles with lids
- Ultrasonic probe or vortex mixer effective at mixing and dispersing the sample
- Centrifuge with head for 250-mL bottles

Note: The centrifuge used in all of the procedures outlined in this chapter should be equipped with swing out buckets (i.e., the walls of the containers swing to a position perpendicular to the rotation axis when spinning). Additional details concerning centrifuge equipment are discussed in Section VII.C of this chapter.

Procedure

1. After rinsing the soil with distilled water at least once, transfer enough soil to provide a thickness of approximately 2 to 3 cm to a 250-mL centrifuge bottle labeled with the sample identification number.
2. Add 100 mL of distilled water to the centrifuge bottle and mix. Effective dispersion and mixing of the sample may be accomplished quickly using an ultrasonic probe. If an ultrasonic probe is not available, it is critical to ensure that the sample has been effectively mixed. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Centrifugation speeds will be given in revolutions per minute (rpm) throughout this chapter, rather than as relative centrifugal force or units of gravity (g). Most centrifuges only have settings in rpm. If the centrifuge you are using only measures a g force, then refer to the owner's manual or a variety of nomographs available to obtain the conversion factor based on the radius of the centrifuge rotor. It is important to make sure that the centrifuge is balanced. Decant the clear supernatant and check for the presence of gypsum or salts by treating an aliquot with acetone or measuring the EC. If the EC is 1 dS m^{-1} or less, the soluble salts and gypsum have been removed from the sample.
3. Repeat treatment with water until the salts and gypsum are dissolved. If the soil is dispersed, centrifuge again or add a few drops of saturated NaCl or 0.5 M MgCl_2 solution to induce flocculation (Kunze and Dixon, 1986).

Caution: Never centrifuge bottles with rubber stoppers in place or the stopper may end up inside the bottle. Always use a screw on lid; the top of the centrifuge bottle may be wrapped with Teflon tape to prevent leakage during mixing.

Test for the Presence of Carbonates

The most common carbonate minerals occurring in soils include calcite (CaCO_3), dolomite [$\text{CaMg}(\text{CO}_3)_2$], and magnesium-calcite [$\text{Ca}_x\text{Mg}_{1-x}(\text{CO}_3)$] (Doner and Grossl, 2002). The presence of carbonates may be determined by the addition of dilute hydrochloric acid (~1 M HCl) to the soil (Schoeneberger et al., 2002). The HCl should be added using a medicine dropper; only one or two drops are necessary for the reaction of carbonates with the acid to occur. If effervescence or bubbling occurs with the addition of HCl, this indicates that CaCO_3 or other carbonate minerals are present. Calcite reacts faster than dolomite when acid is added to the soil (Doner and Grossl, 2002). In fact, dolomite may show no effervescence with acid unless it is powdered.

Removal of Carbonates

Carbonates are commonly removed from soils and sediments by treatment with Na-acetate adjusted to a pH of 5. Soluble salts may also be removed as part of this treatment (Kunze and Dixon, 1986). Other methods of removing carbonates are discussed in the chapter on selective dissolution techniques for mineral analysis of soils and sediments (Shang and Zelazny, 2008, this volume).

Reagents

- Sodium acetate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$), 1 M adjusted to pH 5 with glacial acetic acid
- Sodium hydroxide (NaOH), 1 M

Equipment

- 250-mL plastic centrifuge bottles with lids
- Stirring rods
- 100-mL graduated cylinder
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles

Procedure

1. Weigh out a sufficient amount of sample to provide the desired amount (5–10 g) of clay ($<2\ \mu\text{m}$), and transfer it to a 250-mL centrifuge bottle labeled with the sample identification number. The sample should make a layer in the bottom of the centrifuge bottle that is no more than 1.5 cm thick.
2. Add 50 mL of the pH 5 acetate solution to the centrifuge bottle, mix, and place in the water bath. Check for effervescence to confirm destruction of carbonates. After the bubbling stops, place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid.

Caution: It is important that the sample not be allowed to evaporate to dryness on the water bath during any of the pretreatment procedures utilized.

3. Repeat Step 2.
4. Add 15 mL of distilled water to the centrifuge bottle, mix, and adjust to a pH of 9 to 10 with 1 M NaOH. The pH may be checked using pH indicator paper. Place the

bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid. If the suspension is not flocculated, add a few drops of saturated NaCl to the centrifuge bottle, mix, and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.

Techniques for Analysis of Soluble Salts, Gypsum, and Carbonates

When studying soils with accumulations of soluble salts, gypsum, and/or carbonates, it is possible to identify and quantify these constituents using XRD and other analyses. When these mineral constituents are visible in the soil profile, they can be physically extracted using dental tools and analyzed separately (Buck and Van Hoesen, 2002; Buck et al., 2006b). Once collected, the samples should be transported and stored under conditions similar to those existing at the field site because many of these minerals can hydrate, dehydrate, dissolve, and/or precipitate during storage. Therefore, it is also recommended that the samples be analyzed as quickly as possible after sampling.

Before XRD analyses, the physically separated salt minerals are crushed dry using a mortar and pestle and prepared as a powder mount. Scanning electron microscopy–energy dispersive spectrometer (SEM–EDS) analyses are recommended in conjunction with XRD analyses. Important salt mineral phases within the soil may not be detected by XRD analysis alone because many of these minerals have peaks similar to or overlapping common soil minerals such as feldspar and quartz. Additionally, the identification of accessory minerals (those that constitute <5% of the soil) is difficult because the high-intensity peaks of accessory minerals are often overprinted by the numerous low-intensity peaks of the dominant minerals.

For SEM–EDS analyses, the physically separated salt minerals are mounted using carbon tape or carbon glue and coated with either gold or carbon. Individual Stage I snowballs (0.5–3.0 mm powdery spheres of pedogenic gypsum) can be placed directly on aluminum or brass mounts as small peds to facilitate accurate assessment of the physical relationship of different minerals with the soil. If small peds are mounted, sketching a map showing the locations of the salt minerals before coating with gold or carbon is recommended. This will assist in finding the desired locations during the SEM–EDS analyses. Thin sections of soils containing salt minerals can also be used for petrographic and/or SEM–EDS analyses. However, all thin-section preparation techniques need to be carefully performed without using heat or water to prevent the loss or alteration of the minerals in question. Therefore epoxy materials must not contain water, nor should heat be used to cure the epoxy. Direct cutting of the soil samples (when indurated) and/or of the epoxied samples (when not indurated) should only be performed dry or with oil-based saws. After a sample has been prepared for SEM–EDS analyses, it must be coated with a conductive material. Coating with gold should be used if carbonate minerals are believed to be present because of overlapping peaks during EDS analyses; carbon coating should be used if analyses of phosphorus-containing minerals is desired. Separate subsamples must be coated, one with gold and one with carbon, if analyses of both carbonate and phosphate minerals are needed. The amount of gold or carbon coating utilized for salt mineral analyses is important and also varies between samples. Charging, the accumulation of negative charge on a nonconductive sample or sample that is not properly grounded, during SEM analyses is a common problem when studying salt minerals. Charging may interfere with image formation and analysis because of beam deflection, but may be minimized by careful mounting and coating to ensure effective contact for the electrons across the sample surface. This often requires additional gold or carbon coating or additional carbon glue be added to the

base or sides of the sample. However, excess coating can result in high intensity gold or carbon peaks, rendering the EDS analyses useless. Therefore, careful sample preparation is critical to obtain quality SEM images and EDS analyses.

In addition to timely analysis of the collected samples, we recommend that all the necessary SEM–EDS data be collected during the first analysis. As stated above, these minerals may hydrate, dehydrate, dissolve, or precipitate during storage. Furthermore, the gold or carbon coating on the mounted samples often fractures, and samples may become detached from their mounts, making additional analyses impossible.

The XRD and SEM–EDS analyses described above require that salt minerals are present in visible accumulations within the soil, most commonly as surficial salt crusts, Stage I snowballs, Stage II nodules, or as Stage III indurated horizons (Buck and Van Hoesen, 2002; Buck et al., 2006b). These different morphologies of pedogenic gypsum are very similar to morphologies formed by pedogenic calcium carbonate and described by Gile et al. (1966) as Stage I, II, and III (Reheis, 1987; Buck and Van Hoesen, 2002). Additionally, in some instances, salt minerals may only occur as individual crystals within the soil matrix making identification by XRD much more difficult. In such cases, SEM–EDS analyses should enable determination of mineral habit and elemental weight percentage so that a reasonable interpretation of the mineralogy can be made. However, these analyses may be time-consuming because of the difficulty inherent in trying to visually locate salt minerals in the midst of the more common minerals during SEM analyses. Backscattered electron microscopic analysis is particularly useful if the minerals contain high atomic number elements such as barium. Water soluble elemental analyses may also provide additional data for interpretation of possible mineral phases.

REMOVAL OF ORGANIC MATTER

Once the soluble salts, gypsum, and carbonates have been removed from a sample, if necessary, the next step is to remove the organic matter. If soil and sediment samples contain organic matter, they are typically treated to remove it before clay mineralogical analyses. The presence of organic matter may result in aggregation of soil particles, and its removal is necessary if subsequent analyses require sample dispersion (Kunze and Dixon, 1986). Organic matter may also cause an exothermic reaction between 250 and 500°C in DTA studies. Additionally, organic matter may cause background interference and prevent parallel orientation of clay minerals during preparation of slides for XRD analysis (Douglas and Fiessinger, 1971).

Removal of organic matter from soils is generally accomplished using either hydrogen peroxide (H_2O_2) or sodium hypochlorite. These two methods are described in the following sections. See Mikutta et al. (2005) for a comprehensive review and history of these methods.

Hydrogen Peroxide (H_2O_2) Method

The effective use of H_2O_2 for removal of organic matter requires acidic conditions. Therefore, soluble salts and carbonates that add alkalinity to the soil are removed before removal of organic matter. Artificial formation of calcium oxalate is common when carbonates are present during H_2O_2 oxidation of organic matter (Farmer and Mitchell, 1963; Mikutta et al., 2005). A small amount of glacial acetic acid may be added to control the oxidizing effects of manganese oxides, if present, in the sample (Kunze and Dixon, 1986).

Manganese oxides are very strong oxidizing agents with oxidizing potentials higher than O_2 (Dixon and White, 2002).

Reagents

- Hydrogen peroxide (H_2O_2), 30%
- Acetic acid or acetone, reagent grade
- Sodium chloride (NaCl), 1 M
- Sodium chloride (NaCl) saturated solution: Shake 80 g of reagent-grade NaCl in 200 mL of water.

Equipment

- 250-mL plastic centrifuge bottles
- Stirring rods
- Ultrasonic probe or vortex mixer
- 5-mL pipette (graduated)
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles

Procedure

1. Add approximately 20 mL of water to the soil sample remaining in the centrifuge bottle after soluble salts, gypsum, and carbonates have been removed. Heat the bottle to 80°C in a water bath or on a steam table.
2. Add approximately 1 mL of H_2O_2 to the centrifuge bottle. When the oxidation of organic matter begins, as indicated by frothing, add a few drops of acetic acid or acetone and stir constantly.

Caution: Severe chemical burns can result if H_2O_2 comes in contact with the skin. Affected areas should be rinsed thoroughly with water immediately after contact.

3. When the frothing has subsided, additional H_2O_2 should be added to the centrifuge bottle in approximately 5-mL increments. The solution should be stirred constantly, and drops of acetic acid or acetone added to control frothing.
4. Repeat Step 3 until a total of 25 mL of H_2O_2 has been added.

Note: The rate at which the H_2O_2 is added depends on the violence of the frothing that occurs. Soils with high organic matter contents will froth more than those with low organic matter contents. Some soil may be lost if the solution overflows the bottle during the reaction between H_2O_2 and organic matter. If Mn oxides are present, the H_2O_2 is decomposed so that it is less effective (or ineffective) at oxidizing the soil organic matter. Fizzing, rather than frothing, results during the exothermic reaction of Mn oxides, and H_2O_2 and may cause the suspension to overflow.

5. The oxidation of organic matter is assumed to be complete when a total of 25 mL of the 30% H_2O_2 has been added to the centrifuge bottle, and the frothing reaction subsides to moderate bubbling. The bubbling is the result of the thermal decomposition of H_2O_2 . This will usually take several hours.
6. After H_2O_2 is decomposed and bubbling has subsided, place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid. If the sample disperses, add 1 or 2 mL of saturated NaCl solution and centrifuge again.
7. Add 50 mL of 1 M NaCl solution to the centrifuge bottle, disperse the soil, and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
8. Repeat Step 7.

9. The sample is now ready for removal of oxides or dispersion and particle-size fractionation.

Sodium Hypochlorite Method

Sodium hypochlorite may also be used to remove organic matter from soils and sediments before clay mineralogical analyses (Anderson, 1963). The use of sodium hypochlorite is generally preferred because degradation of clay minerals by H_2O_2 treatments has previously been documented (Douglas and Fiessinger, 1971).

Reagents

- Sodium hypochlorite (unbuffered household bleach), adjusted to pH 9.5 or less with 0.2 M hydrochloric acid (HCl) shortly before use. Determine whether the bleach is buffered or not by attempting to adjust the pH. If it is buffered, a large volume of HCl will need to be added to change the pH, and the pH will drift back to the original value (~11). Several brands of bleach may need to be tested to find one that is unbuffered.
- Hydrochloric acid (HCl) 0.2 M
- Sodium hydroxide (NaOH) 1 M
- Sodium chloride (NaCl) 0.1 M
- 95% Ethanol

Equipment

- 250-mL plastic centrifuge bottles
- Stirring rods
- 100-mL graduated cylinder
- Ultrasonic probe or vortex mixer
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles

Procedure

1. Add 50 mL of sodium hypochlorite (bleach) adjusted to pH 9.5 or less to the soil in the 250-mL centrifuge bottle, mix, and disperse the sample. Place the bottle in an 80°C water bath for 15 min.
2. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid.
3. Repeat Step 2 twice for a total of three treatments with bleach, or until organic matter has been completely removed as indicated by a clear, uncolored solution.
Note: A pink color may result if the soil contains manganese oxides. The pink coloration should be disregarded in terms of organic matter removal.
4. Add 50 mL of 0.1 M NaCl to the centrifuge bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant solution.
5. Repeat Step 4 one more time. The rinse with 0.1 M NaCl serves to wash out the products of organic matter decomposition. If the sample disperses, add a few drops of saturated NaCl solution.
6. Add 25 mL of distilled water to the centrifuge bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at 2000 rpm for 10 min. Check the supernatant for suspended clay. If the supernatant contains suspended clay, the sample is now ready for removal of oxides or dispersion and particle-size fractionation.

7. If the supernatant was clear, decant it and add 25 mL of ethanol. Mix and disperse the sample. Place the bottle in the centrifuge and centrifuge at 2000 rpm for 10 min. Check the supernatant for suspended clay. If the supernatant contains suspended clay, the sample is now ready for removal of oxides or dispersion and particle-size fractionation. If the solution is clear, decant and discard it and then proceed with additional pretreatments and/or dispersion and fractionation.

Note: During any of the pretreatment procedures described in this chapter, a high-speed centrifuge (>10,000 rpm) may be used to bring down dispersed clays when the other flocculating methods are not working.

REMOVAL OF IRON OXIDES

The presence of free oxides and hydrous oxides of Al, Fe, Mn, Si, and Ti may make it difficult or impossible to disperse soil samples before fractionation procedures because these compounds may act as cementing agents. These compounds may exist in crystalline, poorly crystalline (short-range order), and/or noncrystalline (amorphous) forms in soils as a result of pedogenic processes. In particular, the removal of free iron oxides enhances the parallel orientation of layer silicate clays and allows for the detection of X-ray diffraction peaks that might otherwise be obscured (Mehra and Jackson, 1960).

Most of our existing information on Al, Fe, Mn, Si, and Ti oxides and hydroxides in soils has been derived from selective chemical dissolution (SCD) analyses. Selective chemical dissolution analyses may be performed either on the untreated <2-mm soil fraction or the clay (<2- μ m) fraction. Detailed information concerning SCD techniques and their applicability to Al, Fe, Mn, Si, and Ti oxides and hydroxides are provided by Shang and Zelazny (2008, this volume).

Reagents

- Citrate buffer solution, comprised of the following two reagents:
 - Sodium citrate, 0.3 M $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ (88 g L^{-1})
 - Sodium bicarbonate, 1 M NaHCO_3 (84 g L^{-1})Premix these reagents at a ratio of 8:1 citrate/bicarbonate solutions (i.e., 40 mL of 0.3 M sodium citrate to 5 mL of 1 M sodium bicarbonate).
- Sodium dithionite powder ($\text{Na}_2\text{S}_2\text{O}_4$)
- Sodium chloride (NaCl) 1 M
- Saturated NaCl solution
- Acetone, reagent grade

Equipment

- 250-mL plastic centrifuge bottles
- Stirring rods
- 100-mL graduated cylinder
- Ultrasonic probe or vortex mixer
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles
- Analytical balance

Procedure

Note: This procedure produces a strong “rotten egg” smell with irritating fumes and should be conducted under a ventilated fume hood.

1. Centrifuge and discard the clear supernatant from the previous treatment for removal of organic matter or soluble salts.

2. Add 10 to 15 mL of saturated NaCl solution and 150 mL of distilled water to the centrifuge bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid.
3. Add 100 ± 5 mL of citrate buffer solution and heat the bottle to 75 to 80°C in a water bath.
4. When the sample reaches 75 to 80°C, add approximately 1 g of $\text{Na}_2\text{S}_2\text{O}_4$ to the bottle. Stir continuously for 1 min, and then occasionally for 5 min.
5. Add a second 1-g portion of $\text{Na}_2\text{S}_2\text{O}_4$ to the bottle and stir as described above.
6. Add a 2-g portion of $\text{Na}_2\text{S}_2\text{O}_4$ and stir as described above.
7. After 10 to 15 min, remove the bottle from the water bath and cool. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
8. Add 10 mL of saturated NaCl solution, 10 mL of acetone, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
9. Add 100 mL of 1 M NaCl solution to the bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
10. Repeat Step 9.

REMOVAL OF SHORT-RANGE ORDER ALUMINOSILICATE MATERIALS

The identification of short-range-order aluminosilicate materials, such as allophane and imogolite, in a variety of soils has resulted in the development of several different techniques that attempt to quantify the abundance and physical and chemical characteristics of these materials. Characterization and quantification of these materials is important because of their large specific surface areas and high proportion of reactive sites, which influence the surface chemistry of soils (Dahlgren, 1994).

Most of our existing information on short-range order aluminosilicates in soils has been derived from SCD analyses. For example, allophane and imogolite are differentiated from other poorly ordered inorganic phases and from organically bound Al based on their relative resistance to dissolution by various chemical reagents (Dahlgren, 1994). Selective chemical dissolution analyses may be performed either on the untreated <2-mm soil fraction or the clay (<2- μm) fraction. For additional information concerning SCD techniques and their applicability to short-range order materials, please refer to Shang and Zelazny (2008, this volume) and Dahlgren (1994).

PARTICLE-SIZE FRACTIONATION

Particle-size fractionation is an integral component of soil characterization. It is one of a number of concentration techniques applicable to soil mineralogical analysis and is by far the most routinely used (Laird and Dowdy, 1994). Fractionation of particle sizes increases detection sensitivity because specific minerals tend to be more prevalent in specific size ranges. Furthermore, mineralogical assessment of the clay fraction is often the sole objective of the analyst. Other concentration techniques, including density or magnetic fractionation, are also typically performed on specific particle-size fractions.

The increments of particle size in the fractionation procedure are discretionary. A common practice is to fractionate the <2-mm soil material into sand (2–0.05 mm), silt

(0.05–0.002 mm [50–2 μm]), and clay (<0.002-mm [<2- μm]). These particle-size ranges are specified by the USDA, but other standard size ranges may also be used. Further fractionation may be necessary for some specialized mineralogical assessments. A method will be presented below for particle-size fractionation using USDA specifications for sand, silt, and clay, as well as methods for further fractionation of these three major divisions.

Principles

All particle-size fractionation procedures are designed to prevent, or more realistically, minimize particle aggregation so that discrete particles behave independently. Soils tend to have aggregated particles due to the presence of organic matter, metal oxides, carbonates, and salts—these components can bind primary particles together. The procedures described at the beginning of this chapter are designed to remove these aggregating agents before particle-size fractionation and facilitate dispersion of the sample.

The first step in the particle-size fractionation procedure is generally to separate the clay (<2 μm) from the silt (0.05–0.002 mm [50–2 μm]) and the sand (2–0.05 mm [2000–50 μm]). Fractionation is accomplished by differential settling of the particles in a liquid. Centrifugation is routinely used to increase the settling rates. Sedimentation in large beakers is possible, but is much slower for separating clay from silt. Several variables, including particle size, affect the rate of particle settling in viscous media and must be accounted for or controlled when performing any fractionation procedure.

The relationship between the settling velocity of a particle within a liquid and the variables affecting it is expressed by Stokes' Law (Stokes, 1851),

$$V = g(s_p - s_l)D^2/1.8\eta \quad [1]$$

where V is terminal particle velocity (cm s^{-1}) in a liquid medium of density (s_l), g is acceleration due to gravity (980 cm s^{-2}), s_p is particle density (g cm^{-3}), D is the “equivalent spherical diameter” of the particle (cm), and η is the viscosity of the liquid (Pascal seconds [$\text{Pa}\cdot\text{s}$]). This equation shows that settling velocity is directly proportional to force applied, difference between particle density and liquid density, and particle diameter; and inversely proportional to liquid viscosity.

The time in seconds (t_{sec}) for a particle of diameter D (cm) to settle a distance h (cm) is given by the following equation (Jackson, 1979):

$$t_{\text{sec}} = 1.8\eta h/[g(s_p - s_l)D^2] \quad [2]$$

This relation can be used to calculate settling times for specific particle sizes and settling distances of interest to the analyst, using a computer spreadsheet (Table 2–1).

Settling times under centrifugal acceleration must be calculated using an integrated form of Stokes' Law (Svedberg and Nichols, 1923; modified by Jackson, 1979), since acceleration increases as a particle settles and the rotation axis increases,

$$t_{\text{min}} = 6.3 \times 10^8 \eta \log_{10}(R/S)/[(\text{rpm})^2(D_m)^2(s_p - s_l)] \quad [3]$$

where t_{min} is settling time (min), R is the distance (cm) from the rotation axis (i.e., the center point of the centrifuge head) to the top of the sediment in the tube when the walls of the containers are perpendicular to the rotation axis, S is the distance (cm) from the rotation axis (i.e., the center point of the centrifuge head) to the top of the suspension, rpm is revolutions per minute, and D_m is particle diameter (μm). The R and S distances may be different for different centrifuges and should be measured prior to calculating settling times. This equation can also be set up in a spreadsheet to generate a table of times and corresponding rpm values (Table 2–2).

Table 2–1. Settling times (*t*) at specified temperatures (*T*, °C) for particles with specific gravity of 2.65 g cm⁻³ and equivalent spherical diameters of 20, 5, and 2 μm. Settling distance used in calculations was 10 cm. Values were calculated from the equation $t_{\text{set}} = 1.8\eta h/[g(s_p - s_l)D^2]$ (Jackson, 1979), where η is viscosity (Pa·s) of the liquid, *h* is distance (cm) from top of suspension to top of sediment, *g* is acceleration due to gravity, *s_p* is particle density, *s_l* is liquid density, and *D* is particle diameter (cm). The main *T* effect is through η . Changes in *s_l* are minimal over the range in *T* specified. Tables such as the following can be easily generated over any range of *T* or *D* of interest to the analyst using a computer spreadsheet and the equation given. Wider ranges in *T* may require an additional correction for (*s_p* - *s_l*) variation (Jackson, 1979).

<i>T</i>	Viscosity	<i>t</i>					
		20-μm particle		5-μm particle		2-μm particle	
	Pa·s	s	min	s	min	s	min
20	0.101	281	4.7	4497	74.9	28,103	468.4
21	0.098	273	4.5	4363	72.7	27,269	454.5
22	0.096	267	4.5	4274	71.2	26,712	445.2
23	0.094	262	4.4	4185	69.7	26,156	435.9
24	0.091	253	4.2	4051	67.5	25,321	422.0
25	0.089	248	4.1	3962	66.0	24,764	412.7

Table 2–2. Settling times (*t*) at 25°C and specified rpm for particles with specific gravity (*s_p*) of 2.65 or 2.50 g cm⁻³ and equivalent spherical diameters of 2, 0.2, and 0.08 μm. Values were calculated from: $t_{\text{min}} = 6.3 \times 10^8 \eta \log_{10} [(R/S)/[(\text{rpm})^2(D_m)^2(s_p - s_l)]]$ (Svedberg and Nichols, 1923, modified by Jackson, 1979), where η is viscosity (Pa·s) of the liquid, *R* is the distance (cm) from the rotation axis to the top of the sediment, *S* is the distance (cm) from the rotation axis to the top of the suspension, *s_p* is particle density, *s_l* is liquid density, and *D_m* is particle diameter (μm). Values for *R* and *S* used for calculations were 25 and 16 cm, respectively. Tables such as the following can be easily generated over any range of rpm or *D* of interest to the analyst using a computer spreadsheet and the equation given. Values of rpm at different temperatures (*T*) would be required to account for *T* effects on η (Table 2–1). Wider ranges in *T* may require an additional correction for (*s_p* - *s_l*) variation (Jackson, 1979).

rpm	<i>t</i>							
	2-μm particle density of 2.65		2-μm particle density of 2.50		0.2-μm particle density of 2.50		0.08-μm particle density of 2.50	
	min	s	min	s	min	s	min	s
500	6.6	396	7.2	434	724.0	43,440	4,520.0	271,200
750	2.9	176	3.2	193	321.8	19,307	2,008.9	120,533
1000	1.7	99	1.8	109	181.0	10,860	1,130.0	67,800
1250	1.1	63	1.2	70	115.8	6,950	723.2	43,392
1500	0.7	44	0.8	48	80.4	4,827	502.2	30,133
1750	0.5	32	0.6	35	59.1	3,546	369.0	22,139
2000	0.4	25	0.5	27	45.3	2,715	282.5	16,950
2250	0.3	20	0.4	21	35.8	2,145	223.2	13,393
2500	0.3	16	0.3	17	29.0	1,738	180.8	10,848
2750	0.2	13	0.2	14	23.9	1,436	149.4	8,965
3000	0.2	11	0.2	12	20.1	1,207	125.6	7,533
3250	0.2	9	0.2	10	17.1	1,028	107.0	6,419
3500	0.1	8	0.1	9	14.8	887	92.2	5,535
3750	0.1	7	0.1	8	12.9	772	80.4	4,821
4000	0.1	6	0.1	7	11.3	679	70.6	4,238

Some variables affecting particle settling rate can be controlled for the purpose of fractionation, while others cannot. The variable *g* can be maintained as constant or accounted for mathematically, as can the variables *s_l* and η if temperature is controlled. However, the particle density can only be set at some reasonably representative value, which is commonly taken to be the density of quartz (2.65 g cm⁻³). Obviously, minerals with different densities occur in soils, and fixing the density at a single value precludes precisely defined particle-size fractions.

Another unavoidable simplification of the Stokes model is the assumption that particles are spheres. Many soil mineral particles in the fine size fractions are platy rather than spherical, and settle at a slower rate than a sphere of equivalent volume. There is a “hidden” temperature effect on settling time, since η and (to a lesser extent) $(s_p - s_i)$ vary with temperature. However, concern about small temperature variations (e.g., $<3^\circ\text{C}$) in light of the uncertainties of particle densities and shapes is probably unwarranted. There is also a slight error resulting from the time needed to bring the centrifuge up to speed and for it to slow down at the end. Particle-size fractions separated using the principles of Stokes’ Law are therefore unavoidably nominal and operationally defined. Nonetheless, particle-size fractionation is a vitally useful procedure in soil mineralogical analysis, assumptions and simplifications notwithstanding.

Pretreatments and Dispersion

Pretreatments to selectively remove aggregating agents such as soluble salts, carbonates, oxides, and organic matter were described above. Tradeoffs in using pretreatments are (i) the removal of components that are themselves mineral constituents of the soil (Jones and Malik, 1994; Buck et al., 2006b) and (ii) the risk of altering other minerals (e.g., Harward and Theisen, 1962; Harward et al., 1962; Douglas and Fiessinger, 1971; Brewster, 1980). The decision to use a given pretreatment depends on whether or not the minerals of primary interest for a particular soil can be adequately assessed without the selective removal of an aggregating component. Ultrasonic techniques (Edwards and Bremner, 1967; Busacca et al., 1984) have been used as an alternative to chemical pretreatments in promoting dispersion.

Particle-size fractionation requires that particles be dispersed and free to settle independently in a liquid medium. “Particles” ideally are individual minerals, but they can also be strongly cemented aggregates. For example, rock fragments (e.g., shale) and oxide-cemented nodules can occur in the sand and silt fractions. Thus, fractionation of particles can be operationally dependent on the severity of the dispersion approach.

Electrostatic attraction among fine particles is another phenomenon that can impede effective dispersion. The degree of attraction is inversely related to the magnitude of charge for mineral surfaces, as well as to the valence and hydrated radius of counterions. Dispersion of soils having a significant amount of mineral surfaces with pH-dependent charge can be promoted by adjusting the pH away from the “point of zero net charge” of the prevalent pH-dependent components. Such an adjustment increases surface charge, and hence electrostatic repulsion and dispersion. Dispersion of negatively charged minerals can also be promoted, for both permanent- and variable-charge minerals, by saturating with monovalent cations with large hydrated radii (e.g., Na^+). Low ionic strength is a requirement for effective dispersion, since thickness of the diffuse double layer (counterion “cloud” that electrostatically maintains distance between particles) diminishes with increasing ionic strength.

Methodology for Particle-Size Fractionation

The methods for particle-size fractionation must be tailored to the available equipment and the particle-size ranges of interest. The analyst must also make decisions regarding pretreatment from what is known about the sample at the outset. There is no “one size fits all” methodology for particle-size fractionation. The method described in this section involves fractionation within three nominal particle-size ranges (2–0.05 mm, 50–2 μm , and $<2 \mu\text{m}$), but analysts can customize ranges for their objectives using a computer

spreadsheet and the equations given above (e.g., Tables 2–1 and 2–2). Modern programmable centrifuges may also facilitate the targeting of different size ranges. This method requires use of a centrifuge for which the walls of the containers swing to a position perpendicular to the rotation axis. The tube used would be filled to the 10-cm mark, allowing 1 cm for sediment thickness in the bottom of the tube. Centrifugation speeds and times shown in Tables 2–1 and 2–2 are based on an R value (see Eq. [3]) of 25 cm and an S value of 16 cm, but other dimensions of R and S can be specified, depending on the centrifuge model. Angle-head centrifuges require an adjustment to the S measurement because of the (i) shorter distance from the top of the suspension to the top of sediment and (ii) the turbulent and convective flow that occurs when the walls of the container are oblique to the rotation axis (Jackson, 1979). Angle-head centrifuges require careful removal of the centrifuge tube from the centrifuge. The sloping surface of the sediment may result in instability and slumping, and hence some resuspension of the sediments.

Readers that have a centrifuge with different dimensions from those specified for the procedure presented in this chapter can modify the times and/or rpm values according to their equipment specifications or to other particle-size ranges of interest. The time that is calculated for a given rpm value and particle diameter is the time it takes (ideally) for all particles of that diameter to settle, such that only particles less than that diameter would still be in suspension. For example, 2- μm diameter spherical particles of 2.65 g cm^{-3} would settle 9 cm (allowing for 1 cm of sediment from a 10-cm suspension) in 1.7 min at 1000 rpm and the other conditions specified in Table 2–2, after which the suspension could be siphoned off and labeled as “<2 μm .” Greater initial rpm values times are specified in this chapter to remove the bulk of the clay particles, most of which are much smaller than 2 μm . Repeated spins at 1000 rpm are performed to remove clay close to the 2- μm limit. The repetition is necessary because for any given spin, most particles (those not at the top of the suspension) have a travel path of less than 9 cm to the bottom of the container.

Reagents

- “pH-10 water,” sodium carbonate (Na_2CO_3) solution, pH 9.5 ($\sim 0.1 \text{ g Na}_2\text{CO}_3 \text{ L}^{-1}$)
- Sodium chloride (NaCl), 1 M

Note: If your samples are in the neutral to alkaline pH range, distilled or deionized water may be used in place of the pH-10 water for sample dispersion. The pH-10 water is used to facilitate dispersion of acidic soil samples. Also, ultrasonic techniques (Edwards and Bremner, 1967; Busacca et al., 1984) can be used for dispersion if there is concern about mineral destabilization at elevated pH.

Equipment and Materials

- Centrifuge
- pH meter
- Balance
- 53- μm sieve (300 mesh)
- Large funnel
- Ring stand
- Centrifuge containers (60-mL tubes or 250-mL bottles) with caps
- 1000- and 2000-mL beakers
- Sample fraction storage containers

Dispersion Procedure

Sodium Saturation

This step may be eliminated if Na-based pretreatments such as those described in previous sections of this chapter are used:

1. Place a layer of soil (<2 mm) approximately 1.5 cm thick in the centrifuge container.
2. Add 1 M NaCl solution to a 9-cm height (or height appropriate for the equipment being used) in the containers. Mark the containers for this height.
3. Centrifuge for 5 min at 2000 rpm.
4. Decant and discard the clear supernatant; then repeat Steps 3 and 4 twice more (i.e., a total of three washes with NaCl).
5. Decant the last NaCl wash supernatant.
6. Add deionized water to the 9-cm mark, and centrifuge as before (2000 rpm for 5 min).
7. Repeat the deionized water rinse until the supernatant is no longer clear (appears turbid) after centrifugation. Save this turbid suspension by siphoning it into a 2000-mL beaker labeled as " <2 μm " along with sample identification. You will add to this clay suspension as you perform particle-size fractionation. Save the Na-saturated sediment for the next fractionation step.

Separation of Clay (<2 μm)

The following procedure is based on the use of a 250-mL centrifuge bottle, no more than 1.5 cm of sediment, and 9 cm to the top of the suspension in the bottle. As stated above, the analyst needs to check the equipment being utilized for fractionation purposes and adjust the equation for calculating centrifuging times as appropriate.

1. Make a mark on the centrifuge bottle at 9 and 3 cm from the bottom of the bottle. The sediment in the bottle should be approximately 1.5 cm in thickness.
2. Add deionized water up to the 9-cm mark on the bottle.
3. Mix thoroughly and then centrifuge at 500 rpm for 6 min.
4. Siphon the <2 - μm fraction in suspension from 3 cm above the bottom of the bottle into a 1000- or 2000-mL beaker. If the sample is dispersed, the suspension should be cloudy or turbid. If the sample is not dispersed, as indicated by a clear suspension, the clear supernatant can be poured off and discarded. If the sample fails to disperse, add one drop of saturated NaCl solution, remix the sample, and centrifuge. Care should be taken to ensure that no portion of the sediment in the bottom of the bottle, or below the 3-cm mark, is siphoned off or discarded during this procedure.
5. Repeat Steps 2 through 4 several times until the suspension is clear or only slightly cloudy following centrifugation. In our experience, eight repetitions are generally adequate to effectively separate the <2 - μm fraction. Excessive repetitions may produce clay mechanically.
6. The suspension in the beaker now contains the Na-saturated clay (<2 - μm) fraction from your sample.

Flocculation of the Clay

The clay fraction is suspended in a rather large volume of water following fractionation. The water content should be reduced for storage and subsequent analyses. Flocculation

(chemically induced aggregation) of clay in suspension greatly increases the rate of particle settling, and produces a clear supernatant that can be siphoned off to achieve the volume reduction. There are several procedures to flocculate clay in suspension. The best procedure depends somewhat on the characteristics of the sample. The easiest method is to add saturated NaCl to the suspension, stir, cover, and allow to settle for several hours. The increase in ionic strength with the addition of the saturated NaCl reduces double layer thickness, allowing particles to approach closely enough so that forces of attraction can induce flocculation. However, some samples do not readily flocculate with NaCl. Another method is to adjust the pH close to the zero point of net charge for the major mineral components anticipated in the sample. For example, adjusting the pH to about 4.0 is sometimes effective in inducing flocculation for clay from acidic soils dominated by kaolinite. The pH should be adjusted back to a range suitable for stable storage once flocculation has been achieved to prevent mineralogical and other sample changes caused by the acid used for flocculation.

Separation of the Sand (2000–50 μm) and Silt (50–2 μm)

The silt fraction may be separated from the sand using centrifugation, gravitational settling, or wet sieving. The settling depths and times may be calculated using nomographs and equations provided in Jackson (1979) or Tanner and Jackson (1947), or by developing a spreadsheet to produce a table similar to Table 2–1 using the appropriate diameters and other variables.

Centrifugation

1. If using centrifugation, take the bottle containing the sand and silt and add pH-10 water to the 9-cm mark, shake, and centrifuge at the calculated rpm and time. The pH-10 water is used to suspend the silt in solution.
2. Following centrifugation, siphon the suspended silt off into a 1000- or 2000-mL beaker.
3. Repeat Steps 1 and 2 until the supernatant liquid is nearly clear. In our experience, three to eight repetitions are required to adequately separate the silt from the sand. Use deionized water instead of the pH-10 water for the last two to three centrifugations to avoid leaving a Na_2CO_3 residue in the sand and the silt.
4. Allow the silt in the beaker to settle until the supernatant is clear. The clear supernatant can be siphoned off and discarded, leaving the wet silt, which should be stored wet in an appropriate container.
5. Following removal of the silt, the sand and excess water remaining in the centrifuge bottle can be transferred to a beaker. The excess water can be evaporated on a steam bath. If abundant mica is present, the moist sand should be stirred after most of the free water has evaporated. This stirring helps prevent a mat of mica from forming. The sand should then be oven-dried at 105°C for about 2 h. After drying, the sand can be stored dry in a glass vial or other storage container.

Gravitational Settling

1. If using gravitational settling, transfer the sand and silt to a 1000-mL beaker. After the sand and silt are in the beaker, add pH-10 water to suspend the silt in solution. Mix and allow the suspension to stand for the calculated time for the depth used.
2. Siphon off the suspended silt and collect it another beaker.
3. Repeat Steps 1 and 2 until the supernatant is nearly clear. Use deionized water instead of the pH-10 water for the last two to three settlings to avoid leaving a Na_2CO_3 residue in the sand and the silt.

4. Allow the silt in the beaker to settle until the supernatant is clear. The clear supernatant can be siphoned off and discarded, leaving the wet silt, which should be stored wet in an appropriate container.
5. Following removal of the silt, the sand and excess water remaining in the beaker can be evaporated on a steam bath. If abundant mica is present, the moist sand should be stirred after most of the free water has evaporated. This mixing helps prevent a mat of mica from forming. The sand should then be oven-dried at 105°C for about 2 h. After drying, the sand can be stored dry in a glass vial or other storage container.

Wet Sieving

It is also possible to separate the sand from the silt by wet sieving following the removal of the clay. A 53-, 56-, 44- μm (270-, 300-, or 325-mesh) sieve is commonly used to collect the sand.

1. Set up a funnel on a ring stand and place a 1000-mL beaker beneath the funnel. Place a 53-, 56-, or 44- μm sieve in the funnel.
2. Add pH-10 water to the centrifuge bottle containing the sand and silt, cap the bottle and mix thoroughly. Pour the suspension onto the sieve. Transfer the remaining material in the centrifuge bottle, including any material adhering to the lid, to the sieve using jets of pH-10 water from a wash bottle. If you are working with a very sandy soil, you may want to add the sand gradually so that the sieve does not become clogged.
3. After all the material has been transferred to the sieve, continue to wash the dispersed soil through the sieve using jets of pH-10 water from a wash bottle. When the effluent begins to clear, switch to deionized water and continue washing until the effluent is clear. A pliable rubber policeman may be used to gently crush any aggregates, using caution not to damage the sieve.
4. Remove the beaker containing the silt from under the sieve. The silt can be concentrated and stored as described above. Quantitatively transfer the sand on the sieve to a beaker and dry at 105°C for approximately 2 h. After drying, the sand can be stored dry in a glass vial or other storage container.

FREEZE-DRYING

After completing the necessary pretreatments and particle-size fractionation, freeze-drying of the clay suspensions is recommended for preservation. Freeze-drying is used routinely in the biological sciences to preserve naturally occurring organic and inorganic substances, and it is also effective for the preservation of clays (Malcolm, 1968). Freeze-drying involves drying frozen matter under high vacuum so that the solidified solvent or suspending medium sublimates, and a residue, typically solid, remains (Malcolm, 1968). Many organisms are killed during the freezing and subsequent freeze-drying process. Those that are still viable after the treatment are dormant in the freeze-dried residue, which contains no free moisture. Freeze-dried organic materials, therefore, may be stored for future use in a desiccator without danger of microbial degradation or physicochemical changes (Malcolm, 1968).

Clays are often preserved by air drying from aqueous suspension. During air drying, hard flakes or cakes are typically formed that are difficult to crush into a fine powder. The major advantages of freeze-dried clays are related to the fluffy, powderlike consistency that results from the freeze-drying process. Freeze-dried clays can be resuspended as col-

loidal clay instantaneously (Malcolm, 1968). Resuspension of air dried clays, in contrast, is a much more time consuming process and may require considerable effort. Additionally, freeze dried clays may be used for DTA, XRD, and for infrared (IR) analysis with minimal, if any, grinding.

Freeze-drying is not recommended for soils containing soluble salts, if salt mineralogy analyses are desired. Freeze-drying and eventual thawing can drastically alter salt mineralogy.

Reagents

- Silver nitrate (AgNO_3), 0.5 M
- Slurry of dry ice and ethanol for quick freezing clay suspensions

Equipment and Materials

- 1- to 2-L glass beakers
- Dialysis tubing
- Freeze-drying flasks (various sizes depending on volume) and adaptors
- Freeze-dryer

Procedure

1. After fractionation, transfer approximately one-half of the Na-saturated clay into dialysis tubing, securely attach a sample identification label, and place the tubing in a large beaker of distilled water.
2. Replace the distilled water in the beaker at least twice a day until the washing solution is free of chloride. You can test the completeness of washes for chloride removal by adding one drop of AgNO_3 solution to a separate beaker containing a sample of the rinse water. Check for the formation of a white (cloudy) AgCl precipitate. Washing is complete when no cloudy precipitate forms.

Note: Always handle the clay-filled dialysis tubing carefully to avoid breakage and loss of sample.

3. After dialysis has been completed, transfer the Na-saturated clay from the dialysis tubes to labeled freeze-drying flasks. The size of the flask will vary depending on the volume of clay to be freeze-dried.
4. Freeze the Na-clay in the freeze-drying flasks in a dry ice–ethanol slurry.
5. Attach the frozen flasks to the freeze-dryer in accordance with the manufacturer's instructions.

REFERENCES

- Anderson, J.U. 1963. An improved pretreatment for mineralogical analyses of samples containing organic matter. *Clays Clay Miner.* 10:380–388.
- Blevins, R.L., G.M. Aubertin, and N. Holowaychuk. 1968. A technique for obtaining undisturbed soil samples by freezing in situ. *Soil Sci. Soc. Am. Proc.* 32:741–742.
- Brewster, G.R. 1980. Effects of chemical pretreatments on X-ray powder diffraction characteristics of clay minerals derived from volcanic ash. *Clays Clay Miner.* 28:303–310.
- Buck, B.J., J. Rech, M. Howell, J. Prellwitz, and A. Brock. 2006a. A new formation process for patterned ground, Atacama Desert, Chile. *Geological Society of America Abstracts with Program* 38(7):520.
- Buck, B.J., and J. Van Hoesen. 2002. Snowball morphology and SEM analysis of pedogenic gypsum, southern New Mexico, USA. *J. Arid Environ.* 51:469–487.
- Buck, B.J., K. Wolff, D. Merkler, and N. McMillan. 2006b. Salt mineralogy of Las Vegas Wash, Nevada: Morphology and subsurface evaporation. *Soil Sci. Soc. Am. J.* 70:1639–1651.
- Busacca, A.J., J.R. Aniku, and M.J. Singer. 1984. Dispersion of soils by an ultrasonic method that eliminates probe contact. *Soil Sci. Soc. Am. J.* 48:1125–1129.
- Dahlgren, R.A. 1994. Quantification of allophane and imogolite. p. 430–451. *In* J.E. Amonette and L.W. Zelazny (ed.) *Quantitative methods in soil mineralogy*. SSSA, Madison, WI.

- Dixon, J.B., and G.N. White. 2002. Manganese oxides. p. 367–388. *In* J.B. Dixon and D.G. Schulze (ed.) Soil mineralogy with environmental applications. SSSA Book Ser. 7. SSSA, Madison, WI.
- Doner, H.E., and P.R. Grossl. 2002. Carbonates and evaporates. p. 199–228. *In* J.B. Dixon and D.G. Schulze (ed.) Soil mineralogy with environmental applications. SSSA Book Ser. 7. SSSA, Madison, WI.
- Douglas, L.A., and F. Fiessinger. 1971. Degradation of clay minerals by H_2O_2 treatments to oxidize organic matter. *Clays Clay Miner.* 19:67–68.
- Edwards, A.P., and J.M. Bremner. 1967. Dispersion of soil particles by sonic vibration. *J. Soil Sci.* 18:47–63.
- Farmer, V.C., and B.D. Mitchell. 1963. Occurrence of oxalates in soil clays following hydrogen peroxide treatment. *Soil Sci.* 96:221–229.
- Gile, L.H., F.F. Peterson, and R.B. Grossman. 1966. Morphological and genetic sequences of carbonate accumulation in desert soils. *Soil Sci.* 101:347–360.
- Harward, M.E., and A.A. Theisen. 1962. Problems in clay mineral identification by X-ray diffraction. *Soil Sci. Soc. Am. Proc.* 26:335–341.
- Harward, M.E., A.A. Theisen, and D.D. Evans. 1962. Effect of iron removal and dispersion methods on clay mineral identification by X-ray diffraction. *Soil Sci. Soc. Am. Proc.* 26:535–541.
- Jackson, M.L. 1979. Soil chemical analysis. Advanced course. 2nd ed. M.L. Jackson, Madison, WI.
- Jones, R.C., and H.U. Malik. 1994. Analysis of minerals in oxide-rich soils by X-ray diffraction. p. 296–329. *In* J.E. Amonette and L.W. Zelazny (ed.) Quantitative methods in soil mineralogy. SSSA, Madison, WI.
- Kunze, G.W., and J.B. Dixon. 1986. Pretreatment for mineralogical analysis. p. 91–100. *In* A. Klute (ed.) Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Laird, D.A., and R.H. Dowdy. 1994. Preconcentration techniques in soil mineralogical analyses. p. 236–266. *In* J.E. Amonette and L.W. Zelazny (ed.) Quantitative methods in soil mineralogy. SSSA, Madison, WI.
- Loeppert, R.H., and D.L. Suarez. 1996. Carbonate and gypsum. p. 437–474. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Malcolm, R.L. 1968. Freeze-drying of organic matter, clays, and other earth materials. USGS Professional Paper 600-C:C211–C216.
- Mehra, O.P., and M.L. Jackson. 1960. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. p. 317–327. *In* Clays and clay minerals. Proc. 7th Conf. Natl. Acad. Sci. Natl. Res. Council Publ., Washington, DC.
- Mikutta, R., M. Kleber, K. Kaiser, and R. Jahn. 2005. Organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate. *Soil Sci. Soc. Am. J.* 69:120–135.
- Reheis, M.C. 1987. Gypsic soils on the Kane alluvial fans, Big Horn Country, Wyoming. USGS Bull. Rep. B 1590-C:C1–C39.
- Schoeneberger, P.J., D.A. Wysocki, E.C. Benham, and W.D. Broderson (ed.) 2002. Field book for describing and sampling soils. Version 2.0. USDA-NRCS, National Soil Survey Center, Lincoln, NE.
- Shang, C., and L.W. Zelazny. 2008. Selective dissolution techniques for mineral analysis of soils and sediments. p. 33–80. *In* A.L. Ulery and L.R. Drees (ed.) Methods of soil analysis. Part 5. Mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Soil Survey Staff. 1996. Soil survey laboratory methods manual. Soil Survey Investigations Rep. 42. U.S. Gov. Print. Office, Washington, DC.
- Soukup, D.A., L.R. Drees, and W.C. Lynn. 2008. Sampling soils for mineralogical analyses. p. 1–12. *In* A.L. Ulery and L.R. Drees (ed.) Methods of soil analysis. Part 5. Mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Stokes, G.G. 1851. On the effect of the lateral friction of fluids on the motion of pendulums. *Trans. Cambridge Phil. Soc.* 9:8–108.
- Svedberg, T., and J.B. Nichols. 1923. Determination of size and distribution of size by centrifugal methods. *J. Am. Chem. Soc.* 45:2910–2917.
- Tanner, C.B., and M.L. Jackson. 1947. Nomographs of sedimentation times for soil particles under gravity or centrifugal acceleration. *Soil Sci. Soc. Am. Proc.* 12:60–65.

Selective Dissolution Techniques for Mineral Analysis of Soils and Sediments

CHAO SHANG & LUCIAN W. ZELAZNY, Virginia Polytechnic and State University, Blacksburg

Selective chemical dissolution (SCD) is a commonly employed means to use a range of chemical reagents to selectively extract mineral components from soils or sediments for the purposes of quantifying, separating, concentrating, or pre-cleaning of materials for analysis. It is often essential to remove cementing agents (e.g., carbonates, Al and Fe oxides, poorly crystalline aluminosilicates, organic matter) present within soil aggregates before a soil is dispersed for size fractionation (Jackson, 1979). Isolating clays is the primary step for studying layer aluminosilicates by physical methods such as X-ray diffraction (XRD), infrared spectroscopy (IR), thermal analysis, and others (Amonette and Zelazny, 1994). Better resolution can often be obtained if surface coatings and selected mineral components or impurities are removed from samples (Zelazny and Qureshi, 1972; Wang et al., 1981). Although the soil components removed by SCD are often minor in terms of mass distribution (except carbonates in calcareous soils), they may control or dominate a property or properties of a soil. Selective chemical dissolution may be a better choice for quantification of a component when physical methods are either not available, too expensive, or have poor resolution due to interferences or interpretive difficulties. Selective chemical dissolution can also be used together with physical methods for quantification (e.g., determination of soil ferrihydrite by differential X-ray diffraction) or for the confirmation of other physical methods (e.g., determining soil allophane or imogolite by infrared spectroscopy). In this chapter, we emphasize the SCD methods that are used for quantitative determination of selectively dissolved components in soils and sediments.

The assumption underlying the SCD approach is that a targeted soil fraction, which may contain one or more compounds (e.g., gypsum is a single compound, while soluble salts contain multiple compounds), has a unique, distinctive solubility and can be selectively dissolved by an appropriate solvent at a set of desirable experimental conditions (i.e., reagent concentration, pH, temperature, reaction time, solution/solid ratio). This assumption largely holds true in extracting gypsum and soluble salts—both fractions are soluble in water, but gypsum has a lower solubility with limited dissolution at a low solution/soil ratio whereas soluble salts are highly extractable. Carbonates can be dissolved stoichiometrically in an acid. Amorphous and poorly crystalline oxides and hydroxides of Al, Fe and Si, because of larger specific surfaces, have higher solubility than their crystalline counterparts. However, the specificity of SCD is not as satisfactory when the fraction to be extracted does not have a unique, distinctive solubility, which is common for polymineralic samples like soils and sediments. In a polymineralic sample, several minerals or mineral

components may have the same or similar solubility (e.g., Mg-calcite and dolomite), and extracted cations and anions may come from various sources (e.g., Mg^{2+} from both carbonates and trioctahedral chlorites). In addition, the dissolution rate of a mineral varies with its crystallinity and particle size, with poorly crystalline phases and small particles being more soluble. Furthermore, the extractability of some mineral components (e.g., hydroxy Al) depends on their association with other soil constituents in the soil matrix (mineral surface coating, complexes with organic matter, or interlayers), and interlayer materials have proved most difficult to remove (Iyengar et al., 1981). Aggregation is another important factor affecting selective dissolution; the amorphous forms of oxides occluded within aggregates, for instance, could be more resistant to dissolution than free crystals. All these factors result in the limitations of SCD—that separation between poorly crystalline and crystalline fractions of metal oxides is not absolute, that minerals differing in solubility by several orders (e.g., calcite vs. dolomite) may not be quantitatively distinguishable in soils and sediments, and that a theoretically sound extraction could be incomplete in practice. Furthermore, the researcher must be confident that other mineral constituents of interest are not altered due to the treatment.

Several SCD techniques have been combined in a sequential manner in an attempt to determine P and trace metal distributions from various physicochemical forms or compartments in soil and sediments. This involves treating a sample with a series of progressively harsher reagents in an attempt to dissolve increasingly refractory forms of the element of interest. The selective sequential extraction (SSE) procedure must consider reagent specificity and selectivity, and extraction efficiency as in SCD, but also potential redistribution of extracted elements and compatibility of reagents in the series. Thus, these extracts are “operationally defined” for the specific chemical extract employed (e.g., water, magnesium chloride, sodium acetate-pH 5, sodium hypochlorite-pH 9, ammonium oxalate in darkness, citrate-bicarbonate-dithionite, and nitric-hydrochloric acid) or for the intended dissolved constituents (e.g., soluble, exchangeable, carbonate sorbed, organic or oxidizable, noncrystalline sorbed, iron sorbed or reducible, and residual, respectively). Despite concerns over the integrity of the method and indicated associations in a sample, SSE results have been useful in determining elemental partitioning patterns in natural systems, as altered with contaminant additions and influenced by a change in environment conditions. Selective sequential extraction results have also influenced our understanding of biogeochemical availability to organisms. Thus, we also provide an overview of the most widely used SSE techniques employed for soil and sediment analyses of P and trace metals.

HALIDES AND SULFATES

Water-Soluble Minerals

Water-soluble salts are the inorganic constituents in soil that are readily dissolved in water and consist mostly of Ca^{2+} , Mg^{2+} , Na^+ , K^+ , CO_3^{2-} , HCO_3^- , SO_4^{2-} , and Cl^- . The information on this soluble fraction of soil is essential to soil salinity/sodicity management, plant nutrition, and mineralogical analysis. Soluble salts as minerals are identifiable by X-ray diffraction and are present only in salt efflorescence in regions with high evaporation and low rainfall (Doner and Lynn, 1989). Dominant readily soluble minerals in soils identified thus far include the following chloride, sulfate, and carbonate species: halite (NaCl), tachyhydrite ($\text{Mg}_2\text{CaCl}_6 \cdot 12\text{H}_2\text{O}$), and carnallite ($\text{KMgCl}_3 \cdot 5\text{H}_2\text{O}$), epsomite ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), hexahydrite ($\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$), pentahydrite ($\text{MgSO}_4 \cdot 5\text{H}_2\text{O}$), starkeyite ($\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$), kieserite ($\text{MgSO}_4 \cdot \text{H}_2\text{O}$), loewite [$\text{Na}_{12}\text{Mg}_7(\text{SO}_4)_{13}$], konyaite [$\text{Na}_2\text{Mg}(\text{SO}_4)_2 \cdot 5\text{H}_2\text{O}$], bloedite [$\text{Na}_2\text{Mg}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$], mirabilite ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$), thenardite

(Na_2SO_4), tychite [$\text{Na}_6\text{Mg}_2(\text{CO}_3)_4\text{SO}_4$], burkeite [$\text{Na}_6\text{CO}_3(\text{SO}_4)_2$], trona [$\text{Na}_3\text{H}(\text{CO}_3)_2 \cdot 2\text{H}_2\text{O}$], soda [$\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$], and nahcolite (NaHCO_3). Identification of specific mineral species has been shown to be difficult because changes in temperature and water vapor can transform one species to another (Keller et al., 1986a, 1986b; Doner and Lynn, 1989). The dynamic change of mineral species could be daily under some circumstances and definitely seasonal. Because soil soluble salts have similar solubility, selective dissolution is not suitable for the identification and/or quantification of soluble mineral species. The exception is gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), which has a lower solubility ($K_s = 2.44 \times 10^{-5}$) than most other salts. Extraction and quantification of soil soluble salts were described by Rhoades (1996).

Gypsum

Principles

Gypsum has a lower solubility (14 mmol L^{-1} at 20°C) than other soluble minerals, and this difference in solubility has been used to estimate the amount of gypsum in gypsumiferous soils (Nelson, 1982; Loeppert and Suarez, 1996). The sulfate concentration in the saturation extract is assumed to represent nongypsic sulfate in soil because the dissolution of gypsum is suppressed by the low water content used in this extraction. With the sulfate concentration in a separate soil extract that is obtained at higher water content, a correction can be made to find soil gypsum contents (Loeppert and Suarez, 1996). The latter extract is assumed to contain both gypsum and nongypsic sulfate. Porta (1998) recommended washing soil samples with ethanol to remove soluble salts before gypsum dissolution. Berigari and Al-Any (1994) reported that gypsum solubility in 50% ethanol ranges from 1.0×10^{-2} to $2.5 \times 10^{-2} \text{ g L}^{-1}$ depending on the ionic composition of solution, and this solubility results in negligible errors in gypsum determinations. Since the dissolution of soil gypsum is dependent on reaction time, solution composition, sulfate concentration, and particle size, extraction of gypsum with water always leaves the question of incomplete dissolution of gypsum in soil samples. It has been shown that using a Ca^{2+} (and/or sulfate) sink by adding Na (Cl) resin to remove Ca^{2+} (sulfate or carbonate) can enhance the dissolution and result in higher estimates of gypsum content (Stern et al., 1989; Berigari and Al-Any, 1994). Either Ca^{2+} or sulfate concentration in soil extracts can be used for deriving gypsum content (Skarie et al., 1987). Porta (1998) thoroughly reviewed the methodologies for gypsum determination.

Quantitative (multiple-dilution procedure) and qualitative (acetone precipitation) methods for soil gypsum determination were described by Loeppert and Suarez (1996); here we give a modified procedure of one-step extraction with prior washing and sulfate analysis.

Equipment

- Centrifuge and 50-mL polycarbonate centrifuge tubes with stoppers.
- Whatman no. 42 filter paper.
- Ultrasound homogenizer.

Reagents

- **50% ethanol.** Mix 526 mL of 95% ethanol (laboratory grade) with 474 mL of distilled-deionized water.
- **Sodium carbonate solution ($\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$), 0.5 M.** Dissolve 62 g $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$ in 800 mL of distilled-deionized water in a 1-L volumetric flask, and bring to volume.

Procedure (Berigari and Al-Any, 1994)

1. Weigh 2 g (0.0001 g) of air-dried, <2-mm soil sample into a 50-mL centrifuge tube, add 25 mL of 50% ethanol, shake the stoppered contents for 1 h, and then centrifuge to discard the supernatant.
2. To the sediment, add 25 mL of 0.5 M Na_2CO_3 and resuspend the sediment by applying a 30-s sonification. The sonification will help to disturb formation of stable CaCO_3 coating on gypsum, enhancing the quantitative dissolution of gypsum.
3. The sample is centrifuged at 1000 g for 10 min, and the supernatant is filtered through Whatman no. 42 filter paper and the filtrates collected in a 100-mL volumetric flask.
4. This extraction procedure is repeated until a negative test for sulfate with BaCl_2 crystals is obtained and the extracts are combined for sulfate analysis.

Sulfate in solution can be analyzed by colorimetric, gravimetric, chromatographic or atomic spectroscopic methods. If the interference and sensitivity factors in sulfate determination are considered, inductively coupled plasma spectroscopy and ion chromatography are better choices, and these methods are described in Sparks (1996).

Calculation of Results

$$\text{Gypsum (mg/g)} = \frac{\text{S concentration } (\mu\text{g mL}^{-1}) \times \text{dilution factor}}{\text{sample weight (g)} \times 100} \times 5.364$$

where 5.364 is the molecular weight of gypsum divided by atomic weight of S.

Comments

Dominant moderately soluble sulfate species in soils are gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), anhydrite (CaSO_4), and hemihydrate (bassanite, $\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$), and of the three minerals, gypsum is most common. The solubility of gypsum is 2.41 g L^{-1} , and that of bassanite is 3.0 g L^{-1} at 20°C , which is considerably lower than that of soluble sulfate minerals (e.g., mirabilite, 927 g L^{-1} at 30°C).

Heating samples at 105°C enhances gypsum removal and increases the disaggregation of soil particles (Rivers et al., 1982) because heating converts gypsum into bassanite ($\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$), which is more soluble. This measure can be used in the pretreatment of gypiferous soils for particle-size and mineralogical analyses.

Addition of a Na- or Cl-resin is also effective in removing gypsum, but the recovery of fine-sand size resin is troublesome, so this size fraction of sand has to be derived indirectly (Stern et al., 1989).

CARBONATES

Solid-phase carbonates are important soil constituents that influence soil pH, the availability of plant nutrients (e.g., Zn, Fe, Mn, P), and the retention and movement of heavy metals (e.g., Cd, Ni). Determination of soil carbonates has therefore become a routine soil analysis in arid and semiarid regions. In some calcareous soils, carbonate contents may make up more than 80% of soil mass (Loeppert and Suarez, 1996), and the majority of carbonates are present as well-defined crystalline minerals. Predominant carbonate minerals in soils include calcite (CaCO_3), dolomite [$\text{CaMg}(\text{CO}_3)_2$], and Mg-calcite [$\text{Ca}_x\text{Mg}_{1-x}(\text{CO}_3)$] (Doner and Lynn, 1989). Aragonite (orthorhombic CaCO_3), vaterite ($\alpha\text{-CaCO}_3$), and

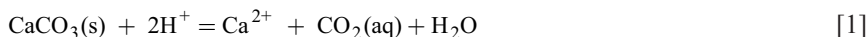
siderite (FeCO_3) are only occasionally reported. Compared with soluble carbonates (e.g., Na_2CO_3), Ca and Mg carbonates are sparingly soluble in water; however, they are much more soluble than aluminosilicates and other soil minerals ($\text{p}K_{\text{sp, calcite}} = 8.4$, $\text{p}K_{\text{sp, dolomite}} = 17.2$) (Plummer and Busenberg, 1982; Sherman and Barak, 2000). This moderate solubility of carbonate minerals allows for their quantification by selective dissolution (Loeppert and Suarez, 1996).

Quantification of total carbonates can be achieved by three general approaches: (i) quantifying CO_2 production when a strong acid is added to samples, (ii) determining the amount of acid consumed with excess acid added, and (iii) determining the amount of Ca^{2+} and Mg^{2+} after complete dissolution. The related principles and laboratory procedures for carbonate determination have been described in several monographs and manuals (U.S. Salinity Laboratory Staff, 1954; Allison and Moodie, 1965; Raad, 1978; Nelson, 1982; USDA-SCS, 1984; Goh et al., 1993; Loeppert and Suarez, 1996). Loeppert and Suarez (1996) prepared a list of determination methods and related references. In this chapter, we describe one method by measuring dissolved Ca^{2+} and Mg^{2+} and two other procedures that are used for quantitative removal of carbonates for determining associated elements (i.e., P and metals).

Removal of Carbonates by Hydrochloric Acid and Acetate Buffer

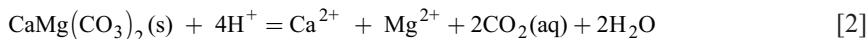
Principles

Calcite, the salt of a weak acid, dissolves in an acid medium according to the reaction:



In strong acids, this reaction can be considered irreversible and is independent of CO_2 partial pressure and Ca^{2+} concentration (Lund et al., 1975; Morse and Arvidson, 2002). The rate-controlling mechanisms of calcite dissolution can be recognized as three regimes in terms of solution pH: H^+ dependent at $\text{pH} < 4.0$, H^+ and P_{CO_2} dependent at intermediate pH, and H^+ independent at higher pH (Plummer et al., 1979; Sjöberg and Rickard, 1984). The H^+ -dependent dissolution of calcite in acid media is the basis for various selective dissolution methods that have been developed to determine carbonates in soil and sediments. It is a common practice to add drops of 10% HCl into a soil sample to test for the presence of carbonates by observing whether the soil effervesces. The dissolution rate at low pH does not seem to vary with types of inorganic acids and electrolytes used (HCl, HNO_3 , or H_2SO_4 ; NaCl, KCl, or CaCl_2) (Plummer et al., 1979).

The reaction stoichiometry of dolomite dissolving in acid is:



The dissolution rate for dolomite is about 650 times smaller than that of calcite at the solid-surface interface at 25°C in 1 M HCl (Lund et al., 1973, 1975). Experimental conditions such as high acid concentration, high reaction temperature, small particle size (i.e., larger surface area), and agitation would increase the dissolution rate of carbonates. The dissolution rate was also found to vary with the source of dolomites that have the same stability constant (Chou et al., 1989).

Selective dissolution of soil carbonates was often conducted in hydrochloric acid of 0.5 to 6 M at room temperature (Loeppert and Suarez, 1996), although sulfuric acid was used at earlier times (Allison and Moodie, 1965; Zwarich and Mills, 1971). Because of

smaller particle size of soil carbonates and higher acid concentrations used in dissolution, which both favor a high dissolution rate, heating is not necessary. The dissolution of calcite in soil or sediment is usually complete from 30 s to a few minutes if ground samples are used, but the dissolution of dolomites or dolomite-containing samples may take 2 to 4 h or longer depending on experimental conditions such as agitation, sample size, and particle size (Williams, 1948; Martin and Reeve, 1955; Skinner et al., 1959; Dreimanis, 1962; Evangelou et al., 1984).

Above pH 4, the diffusion of CO_2 and $\text{Ca}^{2+}/\text{Mg}^{2+}$ from the particle surface may become the rate-limiting step (Lund et al., 1973). This is the pH region encountered in the selective dissolution methods using organic buffers (Petersen et al., 1966; Loeppert et al., 1984; Bloom et al., 1985), where the dissolution rate is slower (Nierode and Williams, 1971). Organic anions (acetate and citrate) are believed to be relatively mild to other soil components such as aluminosilicates, and therefore they serve better as dissolution agents in disaggregating soil particles and elemental fractionation analysis (Petersen and Chesters, 1966; Zwarich and Mills, 1971; Jackson, 1979; Loeppert et al., 1984; Bloom et al., 1985; Han and Banin, 1995). Continuing agitation, heating, and gas purging would increase the dissolution rate and shorten the experimental time (Nommik, 1974; Bloom et al., 1985). One important factor that needs to be considered in using an organic buffer to remove carbonates is the molar ratio of acid to carbonates. At pH 5, a molar ratio of acid to calcite equal to 3.4 (rather than 2.0 in theory) was required for a complete neutralization within 6 h, and the ratio rose to 4.4 at pH 5.5 (Han and Banin, 1995). Han and Banin (1995) found that in 1 M NaOAc and at room temperature, the neutralization is incomplete when the final pH rose to 5.9 or higher. Magnesium-calcite and dolomite were not completely dissolved in these buffers. In the investigation of Bloom et al. (1985), the critical molar ratio was 7.6 to ensure a complete dissolution of clay carbonates within 0.5 h. Ashworth (1997) recommended a molar ratio of 10 in a 0.4-M acetic acid system for a reliable determination of soil carbonates. The requirement for higher acid/carbonate ratios is due to the weak acidity of acetic acid.

Equipment

- Centrifuge and 50- and 100-mL plastic centrifuge tubes
- 50- and 125-mL plastic bottles, or equivalent as needed
- Whatman no. 42 filter paper or equivalent

Reagents

- **Hydrochloric acid (HCl) solution, 1 M.** Transfer 83.3 mL of concentrated HCl into 800 mL distilled-deionized water and dilute to 1 L.
- **Acetate buffer ($\text{CH}_3\text{COONa}/\text{CH}_3\text{COOH}$), pH 5, 1 M.** Dissolve 82 g CH_3COONa in 800 mL of distilled-deionized water in 1-L volumetric flask. Transfer 27 mL of glacial $\text{C}_2\text{H}_3\text{COOH}$ into the acetate solution and adjust to pH 5 with acetic acid. Bring to volume with distilled-deionized water.

Procedure

Hydrochloric acid (Hedley et al., 1982). Weigh 1 to 5 g of air-dried, <2-mm soil or sediment sample into a 100-mL centrifuge tube or 125-mL plastic bottle depending on applications, add 50 to 250 mL of 1 M HCl (solution/solid = 50), and shake the content for 16 h. Centrifuge and collect supernatant in a container for elemental analysis. Alternatively, filter the content through Whatman no. 42 and collect the filtrates for elemental analysis.

pH-5 acetate buffer (Tessier et al., 1979). Weigh 1 g of air-dried, <2-mm soil or sediment sample into a 50-mL centrifuge tube or 50-mL plastic bottle, add 8 mL of 1 M

acetate buffer at pH 5, and shake the content for 5 h. Centrifuge and collect supernatant in a container for elemental analysis. Alternatively, filter the content through Whatman no. 42 and collect the filtrates for elemental analysis.

Comments

Use of dilute HCl solutions to remove carbonates is often seen in P extraction or fractionation (see Tables 3–2 and 3–3 later in this chapter); and quantification of carbonate phases is not necessary in such applications. Solutions of 0.5 M HCl or 0.25 M H₂SO₄ are also used. Higher solution/solid ratios (60–100) are seen in some procedures. Shaking time ranges from 1 to 16 h. For carbonate removal, 1-h reaction in strong acid solutions is sufficient; however, extended extraction can enhance the extraction of associated elements. For calcareous soils containing Mg-chlorites, a prolonged strong-acid treatment may result in the decomposition of trioctahedral chlorites (Wilson, 1987; Carnicelli et al., 1997).

Use of pH-5 acetate buffer for carbonate removal was first used for pretreatment in particle-size and clay concentration operations (Jackson, 1979). Readers are referred to the chapter on pretreatment by Soukup et al. (2008, this publication) for the application. The procedure described above is commonly used in extracting carbonate-associated metals (see Tables 3–4 and 3–5 later in this chapter). As mentioned, this treatment may suffer from incomplete dissolution of all carbonates, particularly dolomite, in samples. For better removal, longer shaking time (e.g., 16 h), higher solution/solid ratios (e.g., 40), or stronger acidity (0.11 to 0.4 M acetic acid) will be helpful. The general guideline is that the higher the pH of the buffer solution, the higher the molar ratio of acid to carbonates (i.e., higher solution/solid ratio). It is recommended that acetate buffer or acetic acid solutions should be only used for samples with <5% carbonate according to Ashworth (1997).

Citrate-Dithionite

Principles

The dissolution of calcite in 1 M ammonium citrate at pH 5.8 or 0.33 M sodium citrate plus 0.14 M sodium dithionite, used by Petersen and his colleagues (Petersen and Chesters, 1966; Petersen et al., 1966), has little dependency on H⁺ activity, and the driving force is the removal of reaction products Ca²⁺ and CO₂. In this method, calcite is selectively removed by complexation between citrate and Ca²⁺, and dolomite content is obtained by subtracting calcite content from the total carbonate content that is determined separately. The procedure includes heating at 75 to 80°C to facilitate the reaction and addition of dithionite to destroy Fe-oxide coating for complete calcite dissolution (Petersen et al., 1966; Raad, 1978). Because citrate also complexes with Ca²⁺ and Mg²⁺ from dolomite, a correction for dissolved dolomite using extracted Mg²⁺ is needed assuming an ideal dolomite formula. Petersen and Chesters (1966) reported that the dissolution of dolomite is approximately 25% in pure minerals and some dolomitic limestone samples.

A common practice required in this chemical method is to correct for soluble and exchangeable Ca²⁺ and Mg²⁺. Either a numerical correction with separate determinations of soluble salts or a pretreatment to remove soluble and exchangeable Ca²⁺ and Mg²⁺ can be used. Holtzclaw et al. (1986) extracted soil samples four times successively with alcoholic sodium acetate/sodium chloride mixture (0.4 M NaOAc + 0.1 M NaCl in 60% ethanol at pH 7.0). The quantity of Mg determined in each extractant solution is plotted against the extracting sequence. A straight line is drawn through the data for the last three steps and extrapolated to the y axis. The amount of Mg indicated at the y intercept is subtracted from the quantity of Mg extracted from the first extraction, and this difference is

assumed to equal exchangeable Mg. Exchangeable Ca can then be calculated from CEC and exchangeable K and Na data. The method of Misopolinos and Kalovoulos (1984) for exchangeable cations in nonsaline calcareous soils also can be used. El Mahi et al. (1987) used 1 M NH_4OAc buffered at pH 8.2 to remove soluble and exchangeable cations before acid neutralization. If a salt solution is used (e.g., NH_4OAc or NaOAc), it is necessary to buffer the pH at 8.2, because up to 1% CaCO_3 could be extracted by 1 M NH_4OAc at pH 7 and a solution/soil ratio of 15 (Wada and Furumura, 1994).

Equipment

- Water bath
- Centrifuge and 50-mL centrifuge tubes
- Vortex mixer

Reagents

- **Citrate buffer, 0.33 M, pH 5.85.** Dissolve 64 g of citric acid ($\text{C}_6\text{H}_8\text{O}_7$) in 800 mL of deionized water, titrate to pH 5.85 with concentrated NaOH, and dilute to 1 L.
- **Sodium chloride-ethanol, 1 M NaCl-30% $\text{C}_2\text{H}_5\text{OH}$.** Dissolve 58.5 g of NaCl in 800 mL of 30% (v/v) ethanol, and bring to 1 L with 30% (v/v) ethanol.
- **Sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$).** Reagent grade.

Procedure (Petersen et al., 1966; Raad, 1978)

1. Weigh 50 to 500 mg (0.1 mg) of air-dried and finely ground soil (passing through a 100-mesh sieve) into a 50-mL centrifuge tube.
2. Add 25 mL of sodium chloride-ethanol solution to the centrifuge tube, mix the contents thoroughly by using a Vortex stirrer, and leave standing for 30 min before another mixing followed by centrifugation. Discard the supernatant, and repeat the washing.
3. Add 25 mL of citrate buffer solution to the centrifuge tube and heat in a water bath at 80°C. Add approximately 0.5 g of sodium dithionite and continue heating while stirring for 15 min.
4. Centrifuge and collect supernatant in a 250-mL volumetric flask. Wash the residue once with 25 mL of citrate buffer, centrifuge, and combine the supernatants. Bring to volume with deionized water.
5. Determine Ca and Mg in the extracts by atomic absorption spectroscopy or inductively coupled plasma spectroscopy (Sparks, 1996). The standards, samples and sample blanks should have the same matrix composition. Determine the total carbonate content (i.e., % CaCO_3 equivalent of the soil) by one of the methods given by Loeppert and Suarez (1996).

Calculation of Results

$$\text{Citrate-extractable Ca (mmol)} = \frac{\text{Ca concentration } (\mu\text{g mL}^{-1}) \times 250 \text{ mL} \times \text{dilution factor}}{40.078 \text{ (mg mmol}^{-1}) \times 1000 \text{ } (\mu\text{g mg}^{-1})}$$

$$\text{Citrate-extractable Mg (mmol)} = \frac{\text{Mg concentration } (\mu\text{g mL}^{-1}) \times 250 \text{ mL} \times \text{dilution factor}}{24.305 \text{ (mg mmol}^{-1}) \times 1000 \text{ } (\mu\text{g mg}^{-1})}$$

$$\% \text{ calcite in soil} = \frac{\text{citrate-extractable Ca (mmol)} - \text{citrate-extractable Mg (mmol)}}{\text{oven-dry sample weight (mg)}} \times 10^4$$

$$\% \text{ dolomite in soil} = 0.92 \times (\% \text{ CaCO}_3 \text{ equivalent of the soil} - \% \text{ calcite in the soil})$$

Comments

Calculating the soil calcite content requires an ideal dolomite composition of 1:1 $\text{CaCO}_3/\text{MgCO}_3$. It has been shown that CaCO_3 is slightly in excess over MgCO_3 in soil dolomite. Since experimental errors are additive in subtraction operations, the dolomite value suffers more uncertainty.

This method does not differentiate between Mg-calcite and dolomite, or between aragonite and calcite. If the analyzed soils are suspected to contain these mineral species, other methods such as X-ray diffraction and thermal analysis should be used along with the chemical method. The empirical method by St. Arnaud and Herbillon (1973) may also be used to estimate Mg-calcite content.

An acetate buffer with a lower pH may be used to replace the citrate buffer, as long as the calcite in the sample is completely recovered.

This approach overcomes the problem encountered in the CO_2 -production kinetic method (Skinner et al., 1959; Dreimanis, 1962; Evangelou et al., 1984) for samples containing clay-sized dolomite, in which the dissolution kinetics of clay-sized dolomite overlaps with that of calcite in the same sample. The CO_2 -production kinetic method is based on the differentiation in dissolution rate between calcite and dolomite.

Use of citrate-extractable Mg to calculate dolomite content as suggested by Goh et al. (1993) is questionable because only about one-third of the dolomite can be dissolved in this buffer (Petersen et al., 1966). This is the reason that a separately determined total carbonate equivalent is required in the calculation.

If one of the correction methods for soluble and exchangeable Ca and Mg is used, the washing step using sodium chloride-ethanol is not needed.

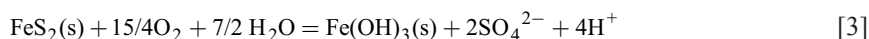
PEROXIDE-SOLUBLE SULFIDES

Dissolution of Sulfides in Hydrogen Peroxide

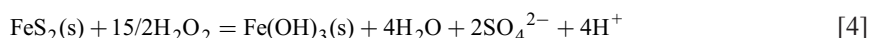
Sulfides occur mainly in mine soils and overburden, coastal acid sulfate soils, and sediments deposited in coastal environments (Doner and Lynn, 1989; Fanning and Burch, 2000; Sobek et al., 2000). Dissolution of sulfide minerals is accompanied by the oxidation of sulfide to sulfate and oxygenation of dissolved ferrous Fe to ferric Fe, thereby releasing acidity to the environment (Stumm and Morgan, 1981). This acidification process is detrimental to natural environments and agricultural production; therefore, various reclamation measures and regulations have been enacted (Plass, 2000; Zipper, 2000). Of all the sulfide minerals that may be present, the predominant ones are iron-containing minerals such as pyrite (FeS_2) or marcasite (FeS_2). Non-iron sulfides are generally far less reactive than iron sulfides. The rate of pyritic S oxidation under field conditions depends on many factors, such as reactive surface area (or particle size), ferric ion concentration, catalytic

agents (e.g., MnO_2), oxygen abundance, solution pH, forms of pyritic S, and presence of S-oxidizing microbes (Sobek et al., 2000).

Under natural conditions, iron sulfide is oxidized according to the reaction:



In the presence of H_2O_2 , the reaction is accelerated so that a complete reaction can be achieved within hours:



In theory, the resulting acidity of sulfide oxidation can be quantified by either a stoichiometric calculation using experimentally determined total sulfide S and Eq. [4] or titrating the proton concentration with a base (Sobek et al., 2000). However, some testing materials may contain carbonates that are capable of neutralizing acidity (Paktunc, 1999), and other acid-producing sulfates whose weathering does not follow the above stoichiometric relationship (Jennings et al., 2000). All these factors need to be considered in developing a method for estimating the potential acidity of a sample.

Maximum potential acidity (MPA) of a sample is the acidity that would be produced if all acid-producing components or compounds in the sample weathered completely, resulting in natural stable products at the surface of the earth. In practice, MPA is treated as the acidity produced by the oxidation of sulfides in a sample, and thus can be estimated from the total S content (Sobek et al., 2000). Neutralization potential (NP) is the acid-neutralizing capacity of a sample (i.e., alkalinity) (O'Shay et al., 1990) and can be approximated by its carbonate content (Loeppert and Suarez, 1996). The net potential acidity (NPA) is the balance between MPA (acidity) and NP (alkalinity). A related approach is termed *acid-base accounting* (ABA) (i.e., $\text{ABA} = \text{MPA} - \text{NP}$) (Knabe, 1964; Grube et al., 1973; Sobek et al., 1978). Sobek et al. (2000) recommended a modified fractionation scheme allowing for a separate determination of sulfate S, pyritic S, and organic S in a sample. The fractionation scheme improves the MPA accuracy of the total-S method since organic S and sulfate S do not produce significant amounts of acids and are treated separately.

Titration of the amount of acid produced from the reaction of a sample with H_2O_2 has evolved into another routine method for estimating the NPA (also called the potential peroxide acidity, or PPA) or MPA for nonalkaline samples ($\text{NP} = 0$). If the samples contain nonsulfidic acidity (free acidity), the titration will give the total potential acidity (TPA) (i.e., the sum of free acidity and PPA) (Barnhisel and Harrison, 1976). The method was first introduced by Yoneda (1961) and later modified by various researchers (Barnhisel and Harrison, 1976; Finkelman and Giffin, 1986; O'Shay et al., 1990). It is assumed that all pyrite is consumed by H_2O_2 added, excess H_2O_2 is completely decomposed, and neutralization reactions occur similarly in the field as under laboratory conditions. To avoid situations where the above assumptions may not hold, O'Shay et al. (1990) recommended using finely ground samples ($<149\text{ }\mu\text{m}$), increasing the initial amount of H_2O_2 , and applying heat and/or using a catalyst (Cu^{2+}) to remove excess H_2O_2 . There is no consensus on whether HCl-leaching pretreatment should be included to remove sulfate S and carbonates. O'Shay et al. (1990) reported an improved accuracy with inclusion of the acid pretreatment. Orndorff (2001), however, noticed that the leaching treatment might also remove some acid-producing sulfate minerals such as jarosite, resulting in an underestimation of potential acidity. Removing carbonates before H_2O_2 addition is based on the concern that

carbonates may react with H_2O_2 , a weak acid, and thus unpredictably influence the amount of acidity being generated. The PPA can also be estimated from the increase in SO_4^{2-} concentration caused by the peroxide oxidation, but the retention of SO_4^{2-} by soil or sediment residues was found to lead to an underestimation of PPA compared with the total-S method (Lin et al., 1996).

Total Potential Acidity (Barnhisel and Harrison, 1976)

Equipment

- pH meter and electrodes
- Hot water bath

Reagents

- **Hydrogen peroxide (H_2O_2), 30%**
- **Sodium hydroxide (NaOH), 0.025 M.** Dissolve 1 g of NaOH in 800 mL of distilled-deionized water to a volume of 1 L. Standardize the solution against an acid standard (daily), and store under CO_2 -free environment.

Procedure

1. Grind samples to pass a 100-mesh sieve ($<149 \mu\text{m}$). Care must be exercised during grinding to ensure sample homogeneity.
2. Weigh 5 g of sample (to 0.01 g) into a 500-mL beaker and cover with a ribbed watch glass (for smaller sample sizes with higher sulfide contents, see “Comments” below).
3. Place the sample beaker in a hot water bath at 50°C , and add 10 mL of H_2O_2 . Wait until reaction stops, and then add another 10 mL of H_2O_2 . Repeat this process until 120 mL of H_2O_2 have been added. Keep sample beakers at 50°C for the remainder of the day and overnight.
4. Remove sample beakers from the water bath and place on a hotplate at 95°C . Heat the sample until no fizzing occurs. Add water if volume gets low during heating; do not allow sample to dry out.
5. Cool samples to room temperature, clean inside of beakers with spatula while rinsing with water, and make to a total volume of 150 mL with distilled-deionized water.
6. Titrate samples and blanks (water with reagents only) with the standardized NaOH solution while stirring to a pH 7.00, and record the NaOH solution volumes consumed for samples (V_{sample} , mL) and blanks (V_{blank} , mL).

Calculation of Results

$$\text{TPA (mmol H}^+ \text{ kg}^{-1}) = \frac{[\text{NaOH } V_{\text{sample}} \text{ (mL)} - \text{NaOH } V_{\text{blank}} \text{ (mL)}] \times \text{M of NaOH (mmol mL}^{-1})}{\text{sample weight (g) on an oven-dry basis}}$$

where $1 \text{ mmol H}^+ \text{ kg}^{-1} = 1 \text{ mg H}^+ \text{ kg}^{-1}$.

Comments

Use 1 g of sample if $\text{TPA} > 400 \text{ mg H}^+ \text{ kg}^{-1}$, and 0.2 g of sample if $\text{TPA} > 2000 \text{ mg H}^+ \text{ kg}^{-1}$. One milligram of H^+ in theory can neutralize 50 mg CaCO_3 .

An effervescence test is suggested for samples that may have carbonates. If carbonate contents are low, ignore them, and the TPA would approximate the NPA. The resulting

errors are small. Otherwise, the amounts of carbonates should be determined separately or removed with dilute HCl. The TPA would then approximate the MPA if carbonates are removed.

In the case that samples contain nonsulfidic acidity, TPA includes both sulfide and free acidities, and can be considered a practical estimate of MPA.

The method of O'Shay et al. (1990) may be used to remove excess H_2O_2 : add exactly 1 mL of 0.0157 M Cu^{2+} solution (prepared from $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) to a cool sample. A violent reaction is expected in control samples, so a cold-water or ice bath should be used to subdue the reaction, or incrementally add Cu^{2+} solution.

ORGANIC MATTER

Selective removal of organic matter from soil or sediment samples serves several purposes: disaggregating for isolation of particle-size fractions; more definitive interpretation of XRD, thermal, IR, CEC and specific surface analyses of minerals; and extracting elements (i.e., mostly trace metals) associated with the organic matrix (Jackson, 1979; Pickering, 1981; Theng et al., 1999; Mikutta et al., 2005). The most common oxidizers used for organic matter removal are NaOCl and H_2O_2 . Other oxidizers, such as KMnO_4 , $\text{Na}_2\text{S}_2\text{O}_8$ and $\text{K}_2\text{Cr}_2\text{O}_7$, are mainly used for quantitative analysis of organic C. Oxidation of organic C with NaOCl or H_2O_2 is not a controlled stoichiometric reaction, and the methods introduced are, therefore, qualitative. For soil dispersion or mineralogical analysis, excess reagents are usually added to ensure maximum removal of organic matter (Soukup et al. (2008, this volume), whereas for metal extraction, a reagent/sample ratio is often defined, but the residual organic matter content is not of concern (see Tables 3–4 and 3–5 later in this chapter).

There has been a debate about which reagent is more effective, although neither NaOCl nor H_2O_2 can destroy organic matter completely, and residual organic C usually occurs in the range of 0.1 to 1% for non-peat samples. Most experimental results, however, conclude that the two oxidizers are effective, and the conflicting results may come from the variations in experimental conditions used and soil characteristics. For example, NaOCl performed better than H_2O_2 when diluted H_2O_2 (3%) was used, but equally well when more concentrated H_2O_2 was used (Lavkulich and Wiens, 1970). The shaking time and reaction temperature could also have caused the differences observed between the two reagents. The major objection to the use of H_2O_2 is the undesirable effects caused by the treatment, which include formation of calcium oxalate precipitates interfering with XRD, IR and thermal analysis, exfoliation of clay minerals (particularly micas), dissolution of manganese oxides, increased acidity in nonbuffered extractions destroying some mineral components, and impurities in the reagent (Martin, 1954; Farmer and Mitchell, 1963; Jones and Beavers, 1963; Douglas and Fiessinger, 1971). Also, the reaction is very violent and can be a laboratory hazard. Anderson (1963) showed that repeated treatment with 5 to 6% NaOCl could achieve the results of H_2O_2 for removing organic matter, but had none of the above problems associated with H_2O_2 . However, the high pH (9.5) at which the oxidizer is most effective has the potential to effect dissolution of noncrystalline materials and produces permanganate in solution, and clays can become dispersed at high pH (Anderson and O'Connor, 1972; Zelazny and Qureshi, 1972). Because of these potential problems, Shuman (1985) lowered solution pH to 8.5 for metal extraction. One of the authors of this chapter (L. Zelazny) recommends removal of organic matter with H_2O_2 if the original sample is acidic and with NaOCl if the sample is alkaline.

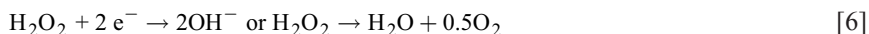
Hydrogen Peroxide

Principles

The reactions between organic C and H_2O_2 may be depicted as follows (though C^0 is not true in organic compounds):



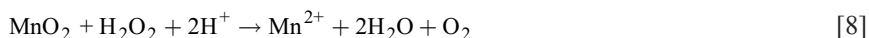
In a normal oxidation reaction, O_2 or OH^- are expected to be released with a rise in pH:



But, in organic matter oxidation, excess acid can be produced and pH can decrease:



Manganese oxides (MnO_2 or Mn_2O_3) are also dissolved through oxidation–reduction reactions, and the overall reaction would be similar to:



Mn^{IV} may be first oxidized to Mn^{VII} , which is a stronger oxidant than H_2O_2 , and then reduced to Mn^{2+} (Neaman et al., 2003).

Equipment

- Centrifuge and 100-mL centrifuge tubes
- Water bath
- Vortex stirrer, and rubber-tipped glass rod

Reagents

- **Hydrogen peroxide (H_2O_2), 30%, reagent grade, pH 2.** Adjust to pH 2 with HNO_3 .
- **Nitric acid (HNO_3), 0.02 M.** Transfer 1.25 mL of concentrated HNO_3 to 800 mL of distilled-deionized water, mix well, and dilute to 1 L with water.
- **Acidified ammonium acetate ($\text{CH}_3\text{COONH}_4$), 3.2 M in 20% (v/v) HNO_3 .** Dissolve 246.6 g of $\text{CH}_3\text{COONH}_4$ in 600 mL of distilled-deionized water in a 1-L beaker. Transfer 200 mL of concentrated HNO_3 to the beaker and mix the content well. Transfer the contents after cooling at room temperature to a 1-L volumetric flask and dilute to 1 L volume with water.

Procedure (Tessier et al., 1979)

1. Weigh 1 g of air-dried, <2-mm soil (or extracted sample) into a 100-mL centrifuge tube, add 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 adjusted to pH 2. Heat the mixture to 85°C in a water bath for 2 h with occasional agitation.
2. Add a second 3-mL aliquot of 30% H_2O_2 at pH 2 and keep the sample at 85°C for 3 h with intermittent agitation
3. After cooling, add 5 mL of acidified 3.2 M ammonium acetate, bring the sample volume to 20 mL with water and agitate it continuously for 30 min.

4. The extracts are collected for metal analysis by centrifugation at 1000 g for 15 min. or by filtration with Whatman no. 42 filter paper.

Comments

Addition of ammonium acetate is designed to prevent adsorption of extracted metals onto soil mineral components; 1 M ammonium acetate at pH 2 and 0.1 M $\text{Mg}(\text{NO}_3)_2$ are also used for the same purpose. The oxidation reaction for metal extraction is often performed at pH 2 and 85°C. Extraction normally lasts from 1 to several hours, and repeating the treatment is common. The solution/solid ratio ranges from 5 to 10 in general. For mineralogical pretreatment, a buffer pH of 5.0 is often recommended to minimize mineral dissolution.

Sodium Hypochlorite

Principles

The oxidation of organic C with OCl^- may be described as:



The redox potential of OCl^-/Cl^- system is +0.95 V at pH 9.5 and increases with decreasing pH. The residual C after NaOCl treatment and extracted Fe were shown to be lowest at pH 9.5 (Lavkulich and Wiens, 1970). Shuman (1983) lowered the NaOCl solution pH to 8.5 to reduce the pH effects on extractable metals.

Equipment

- Centrifuge and 100-mL centrifuge tubes
- Water bath

Reagents

- **Sodium hypochlorite (NaOCl), 5%, pH 8.5.** Sodium hypochlorite solution (5%), reagent grade, adjusted to pH 8.5 with 6 M HCl. The pH adjustment should be done just before the treatment.

Procedure (Anderson, 1963; Shuman, 1985)

1. Weigh 5 g of air-dried, <2-mm soil sample into a 100-mL centrifuge tube. Add 10 mL of 5% NaOCl solution and heat in a boiling bath for 30 min.
2. The mixture is centrifuged and the supernatant saved for metal analysis if metal extraction is intended.
3. Repeat the treatment three times for soils with <3% organic matter and up to five times for higher organic matter soils.

Comments

At the end of the treatment, samples are Na-saturated and can be dispersed in water without further treatments. However, additional washing with 2% Na_2CO_3 – NaHCO_3 solution was found necessary for fine tropical soils (Omuetti, 1980).

Caution: Lowering the pH of sodium hypochlorite solution with sulfuric acid should be avoided because a toxic chlorine gas could be produced.

It is recommended to use this method with neutral and high pH soils because of the possible dissolution of noncrystalline materials in acidic soils. As a pretreatment of mineralogical analysis, solution pH should be adjusted to 9.5, and commercial bleach can be used.

Shuman (1985) recommended two 30-min extractions for metal extraction.

EXTRACTABLE ALUMINUM, IRON, MAGANESE, AND SILICON

Pedogenesis results in the accumulation of soil colloids with compositions that are enriched in hydrous oxides of Al, Fe, and Mn relative to Si. In soils and sediments, these pedogenic oxides and hydroxides frequently occur as noncrystalline surface coatings (Follett et al., 1965; Jones and Uehara, 1973), hydrous metal oxide coatings on mineral surfaces (Davidtz and Sumner, 1965; Roth et al., 1968, 1969; Greenland et al., 1968; Jackson and Frolking, 1982), discrete crystalline minerals such as goethite and hematite that may have varying amounts of Al substitution (Callière et al., 1960; Norrish and Taylor, 1961; Schwertmann et al., 1977; Jackson and Frolking, 1982), and concretions or nodules (Winters, 1938; Drosdoff and Nikiforoff, 1940). For soils developed from volcanic ash and weathered pumice, dominant mineral components are noncrystalline and poorly crystalline aluminosilicates; organically complexed Al and Fe; and noncrystalline Al, Fe, and Si oxides and hydroxides (Wada, 1989). Knowledge of the nature and distribution of pedogenic oxides and hydroxides in soil profiles aids in understanding the pedological processes of soil formation (Oades, 1963) and in soil classification (McKeague and Day, 1966; Soil Survey Staff, 1975).

Free oxides and hydroxides (i.e., oxides, oxyhydroxides and hydrated oxides) include crystalline, poorly crystalline (short-range order), and noncrystalline (amorphous) compounds of Si, Al, Fe, Ti and Mn. The term *free* refers to compounds having mainly a single species of coordination cation, such as oxides of Si, Al, or Fe. Hydrous oxides containing two or more coordinating cation species are termed *combined oxides*, and examples are aluminosilicates or ferri-aluminosilicates (Jackson et al., 1986). The term *free iron oxides* used in the literature, however, is more operational and represents the iron extracted by dithionite. The term *noncrystalline* has been used to include both amorphous and short-range order materials in the literature (Jackson et al., 1986), and this usage is closely related to a group of noncrystalline and poorly crystalline aluminosilicates that are gel-like compounds containing mainly Al, Fe, Mn, Si, O, OH, and H₂O, but usually also containing more or less Mg, occasionally phosphate, and minor amounts of other ions. Some compounds in this group, which are associated mainly with weathered volcanic ash and have the morphology of hollow, irregular, spherical particles with diameters of 3.5 to 5 nm, and with a molar Si/Al ratio typically of 1:2 to 1:1, have been designated allophane (Wada, 1989). Another mineral in this group is called imogolite, which appears as threads consisting of well-defined fine tubes with inner and outer diameters of 1.0 and 2.0 nm, respectively (Brindley and Pedro, 1972). Allophanes are further divided into Al-rich and Si-rich groups based on the Si/Al ratio (Parfitt, 1990). In this article *short-range order* and *poorly crystalline* will be used equivalently and include allophanes and imogolite as well as ferrihydrite. The terms *amorphous* and *noncrystalline* are used to describe the materials that can be quantified by chemical selective dissolution but not isolated as separate phases, including aluminosilicates with Si/Al ratios <1:2 and >1:1, oxides/hydrous oxides, and interlayer and surface coatings of vermiculites, smectites, and other minerals. However, it should be pointed out that there is virtually a continuum from perfect crystallinity (long-range order) to disorder constrained only by alternating linkage of cations and anions as radial distribution functions (short-range order) (Jackson et al., 1986). Therefore, the boundary between the min-

eral phases with varying crystallinity is diffuse, which dictates the semiquantitative nature of selective dissolution.

Selective chemical dissolution for soil metal oxides and aluminosilicates has not been comprehensively altered since the earlier chapter coauthored by Jackson, Lim, and Zelazny (Jackson et al., 1986), except a few random and brief treatments on iron oxides (Borggaard, 1988; Loveland, 1988; Loeppert and Inskeep, 1996), aluminum oxides (Hsu, 1989; Bertsch and Bloom, 1996), hydroxy interlayer materials (Barnhisel and Bertsch, 1989), and aluminosilicates (Farmer and Russell, 1990; Dahlgren, 1994). The methods of acidified oxalate under darkness (AOD) and citrate-bicarbonate-dithionite (CBD) are still in use without any significant modification and are reported here with updated references and minor revisions. Two new classes of extractions, hydroxylamine hydrochloride and Tiron, that have shown promise for selectively removing soil non- and poorly crystalline materials are introduced.

Pyrophosphate

Principles

Pyrophosphate extraction has been a classical method used to isolate humic acids from soil and sediments (Swift, 1996). Aluminum and Fe associated with organics can also be removed by this extraction (Alexandrova et al., 1964). McKeague (1967) reported that 0.1 M Na-pyrophosphate did not significantly dissolve inorganic amorphous or crystalline Fe and Al substances, suggesting that the extracted Fe and Al might be organically bound. Higashi (1983) reported that Al was quantitatively recovered from synthetic Al-organic complexes by pyrophosphate extraction. Numerous experimental results have shown a good correlation between extracted Fe and Al and the amounts of extracted organic matter, particularly for soil samples with high organic matter or from spodic horizons (McKeague, 1967; McKeague et al., 1971; McKeague and Schuppli, 1982; Jersak and McColl, 1989). These findings lead to the belief that pyrophosphate extraction of soils at pH 10 can give a measure of organically bound Fe and Al, and thus it is suitable for investigating the coupling relationship between organic matter and Fe and Al in soils (Jarvis, 1986; Jersak and McColl, 1989; Jersak et al., 1992). A "pyrophosphate index" has been developed to help identify Podzolic (Spodic) horizons (McKeague et al., 1971; Agriculture Canada Expert Committee on Soil Survey, 1987). Another important application of this method, in conjunction with acidified oxalate, is to estimate the amounts of allophane and imogolite in soils (Parfitt and Henmi, 1982; Farmer et al., 1983; Parfitt, 1990; Dahlgren, 1994).

However, it has been shown that the pyrophosphate-extractable Fe and Al are poor indicators of organically bound pools that are of interest to plant nutrition (Bertsch and Bloom, 1996; Loeppert and Inskeep, 1996). This may simply be because pyrophosphate in general removes inorganic sources of Fe and Al, possibly in colloidal forms of noncrystalline and/or poorly crystalline Fe and Al oxides and hydroxides that may be coated with organic matter (McKeague, 1967; McKeague and Schuppli, 1982; Jarvis, 1986; Kaiser and Zech, 1996). Kaiser and Zech (1996) showed a significant release of Al from amorphous $\text{Al}(\text{OH})_3$ and gibbsite by pyrophosphate. It appears that pyrophosphate extracts Fe and Al from soil by both complexation with Fe^{3+} and Al^{3+} and also solubilization or peptization of organic-metal complexes through bond disruption of the organic matter matrix at high pH resulting in more negative charges in the system. The peptization mechanism may even be more significant than ligand complexation (Borggaard, 1988). This may explain the presence of suspended particles in extracts, which may have caused poor reproducibility leading to various experimental efforts to improve this extraction (McKeague,

1967; McKeague and Schuppli, 1982; Kassim et al., 1984; Loveland and Digby, 1984; Borggaard, 1988; Jersak and McColl, 1989). These experimental measures include higher centrifugation speeds, use of flocculants and Millipore filtration (Loveland, 1988). Another variation associated with this method is to use K-pyrophosphate instead of Na-pyrophosphate (Bascomb, 1968). There is insufficient information for evaluating the superiority of one salt over another except the Na salt would promote more dispersion of organic-associated colloidal phases (Bertsch and Bloom, 1996). In summary, pyrophosphate extracts organically bound Fe and Al, but not all pyrophosphate-extractable Fe and Al are from organic complexes (Campbell and Schwertmann, 1985; Parfitt and Childs, 1988; Kaiser and Zech, 1996), and the proportion of inorganic forms of Fe and Al extracted by pyrophosphate is larger for soils with low organic matter content (Jarvis, 1986). The amounts of Fe and Al extracted vary with experimental conditions, including centrifugation, flocculation, or filtration of extracts. Thus, Loveland's comments (1988) should be heeded: "it is vital to state exactly how the pyrophosphate determination has been made, and equally important to compare with care data obtained by different methods."

Equipment

- Centrifuge and 50-mL centrifuge tube or 250-mL centrifuge bottle

Reagents

- **Sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$), 0.1 M.** Dissolve 44.6 g of $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$ in about 800 mL of deionized water, adjust to pH 10 with NaOH, and dilute to 1 L. Minimize contact with CO_2 during storage.
- **Superfloc (American Cyanamid Co., Wayne, NJ).**

Procedure (McKeague, 1967; Ross and Wang, 1993)

1. Weigh 300 mg (0.01 mg) of air-dried, ground soil ($<149 \mu\text{m}$) into 50-mL centrifuge tube (if soil samples of <0.5 - or <2.0 -mm particle size are used, 2 g of soil is weighed into a 250-mL plastic bottle).
2. Add 30 mL of 0.1 M sodium pyrophosphate, and shake 16 h at room temperature (add 200 mL of the pyrophosphate solution to the 250-mL bottle containing 2-g sample, extracting at a solution/soil ratio = 100).
3. Centrifuge at $20,000 \times g$ for 10 min, or alternatively, add 0.5 mL of 0.1% Superfloc solution and centrifuge at $510 \times g$ for 10 min. Take an aliquot of the supernatant for metal analysis.
4. Keep the samples refrigerated and analyze within a few days. Extracted Al and Fe can be analyzed by inductively coupled plasma or atomic absorption spectroscopy using an air-acetylene gas mixture for Fe and nitrous oxide-acetylene for Al. The standards, samples, and blanks should have the same matrix composition (i.e., 0.1 M pyrophosphate or its dilutions). Refer to Sparks (1996, Chapters 4, 5, 18, and 23) for the analytical details.
5. If Fe and Al in pyrophosphate extracts are determined by colorimetry, pretreatments to remove organic matter are required. Digest 5 to 10 mL of the above pyrophosphate extract with 1 mL of concentrated HNO_3 and two drops of 9 M H_2SO_4 to dryness on a hotplate. Repeat the digestion with HNO_3 if the residue is still brown in color. Dissolve the residue in dilute HCl and heat the mixture in a water bath if the residue is not completely dissolved.

Calculation of Results

$$\text{Al (mg g}^{-1}\text{)} = \frac{\text{concentration in extract (}\mu\text{g mL}^{-1}\text{)} \times \text{extractant volume (mL)} \times \text{dilution factor}}{\text{soil weight on oven-dry basis (mg)}}$$

The calculations are similar for Fe and Mn contents.

Comments

Concentrations of metals in pyrophosphate extracts vary with centrifugation time and forces used; it is important to keep these experimental conditions consistent throughout an experiment.

If a flocculating agent is not used, filtration of supernatants with a 0.1- μm pore size membrane may remove suspended colloids. The 0.45- μm membranes may be too coarse for this purpose (Bertsch and Bloom, 1996).

Ammonium Oxalate

Principles

Acid ammonium oxalate allowed to react in darkness (AOD) for 2 h, modified from the original method of Tamm (1922), has been shown to be a selective reagent for dissolution of noncrystalline and poorly crystalline aluminosilicates, oxides, and hydroxides of Al, Fe, Mn, and Ti (Schwertmann, 1959, 1964, 1973; McKeague and Day, 1966; McKeague et al., 1971; Higashi and Ikeda, 1974; Fey and LeRoux, 1977; Fitzpatrick et al., 1978; Hodges and Zelazny, 1980). Complete removal of allophane and imogolite from a variety of soils was verified by IR spectroscopy (Parfitt and Henmi, 1982; Russell et al., 1981). The AOD extracts all ferrihydrite but minor amounts of lepidocrocite (Schwertmann et al., 1982; Campbell and Schwertmann, 1985). A prolonged AOD treatment, however, extracted lepidocrocite of large particle sizes (Wang et al., 1993). The AOD treatment has been reported to dissolve very little <200- μm hematite and goethite and only minor amounts (9%) of <200- μm magnetite (Baril and Bitton, 1969; McKeague et al., 1971). The severity of this treatment, however, increases with a decrease in particle size (McKeague et al., 1971). Chao and Zhou (1983) reported similar findings on goethite and hematite but a higher dissolution of magnetite (about 20%). Therefore, the presence of magnetite in samples may confound the interpretation of the AOD-Fe data as representative of short-range-order forms of Fe (Gamble and Daniels, 1972; Rhoton et al., 1981). Noncrystalline silica is not dissolved by this method (Wada, 1989), and extracted Si is due to the congruent dissolution of aluminosilicates; therefore, this method can be used to estimate allophane content (Dahlgren, 1994). Because of the strong complexing ability of oxalate at pH 3, organically bound Fe and Al were also extracted by the AOD treatment (Schwertmann, 1973; Cornell and Schwertmann, 1996).

McKeague and Day (1966) found that some interlayer Al was removed from artificially prepared Al-chloritized bentonite during a 40-h AOD treatment, but also noted that interlayer materials of natural Al-chloritized clays were only slightly affected by this treatment. Some highly weathered soils of a humid tropical region released a fraction of 1% each of Fe_2O_3 , Al_2O_3 , and SiO_2 with the AOD method (Fey and LeRoux, 1977; Makumbi and Jackson, 1977). The AOD treatment did not appear to be selective for interlayered Al (Iyengar et al., 1981; Matsue and Wada, 1988).

Mendelovici and Sagarzazu (1991) investigated the effects of AOD treatment on a group of Mn minerals and found that ground hausmannite, rhodochrosite, and mangan-

osite were completely dissolved, whereas pyrolusite was more resistant to the treatment. Oxalate formed a precipitate ($\text{MnC}_2\text{O}_4 \cdot 3\text{H}_2\text{O}$) with dissolved Mn.

There are conflicting reports on the susceptibility of clay minerals to AOD treatment. McKeague and Day (1966) reported that the AOD treatment has little effect on kaolinite, montmorillonite, and illite. Hodges and Zelazny (1980) reported similar results for gibbsite, kaolinite, and montmorillonite. Iyengar et al. (1981) showed that an AOD treatment revealed no XRD-detectable alteration to mineral phases present in the clay fraction of soils dominated by kaolinite or hydroxy-interlayered vermiculite. However, it seems that trioctahedral minerals are more susceptible to AOD treatment. Arshad et al. (1972) found that AOD treatment caused considerable decomposition of finely ground trioctahedral minerals (biotite and chlorite), as measured by solubilization of Fe, Al, and Mg, but with lesser amounts of Si. Pawluk (1972) observed that prolonged extraction with AOD extraction resulted in slight dissolution of hydrous mica and trioctahedral chlorite. These results suggest that the attacks on clay minerals may be related to particle size and reaction time. Hodges and Zelazny (1980) found that $<0.5\%$ of a trioctahedral vermiculite dissolved during a 2-h AOD treatment.

Protonation and complexation are undoubtedly two mechanisms responsible for the dissolution of Fe and Al in acid-oxalate solution under darkness (Hearing and Stumm, 1990; Cornell and Schwertmann, 1996). In theory, all Fe oxides could be dissolved if sufficient contact time is allowed (Borggaard, 1988). Since dissolution is a time-dependent process, extraction time for AOD is determined by a significant reduction in the dissolution rate that results from either a complete dissolution of a phase (e.g., allophane and imogolite) or the hindered reaction due to large particles and aggregates or change in crystallinity. A 2-h reaction time is generally used (Loveland, 1988), but a 4-h reaction is recommended for allophane and imogolite estimation (Parfitt, 1989; Dahlgren, 1994). The major variation with this method is the solution/soil ratio that ranges from 1:40 to 1:250 (Loveland, 1988), and the ratio is often adjusted according to the contents of extractable species in samples. Either Na-oxalate or NH_4 -oxalate may be used (Parfitt, 1989), but NH_4 -oxalate is easy to prepare because of its high solubility (Dahlgren, 1994).

Two other modifications of AOD are oxalate under UV light (AO-UV) and AOD-Fe(II) (DeEndredy, 1963; Chao and Theobald, 1976; Iyengar et al., 1981; Cornell and Schindler, 1987; Blesa et al., 1987; van Oorschot and Dekkers, 2001). Both methods are designed to extract crystalline Fe and Mn oxides and hydroxides. However, the AO-UV extraction is not consistent, and AOD-Fe(II) requires more calibration with pure minerals and more tests on a variety of natural samples.

Equipment

- **Analytical balance: 0.01-mg readability.** A wire hook attached to the frame of the balance pan or a light weight donut is used to hold centrifuge tubes in a vertical position, to reduce weighing errors resulting from variable orientation of tubes on the balance pan.
- **Vacuum desiccator with P_2O_5 as desiccant**
- **Oven.** Set to $110 \pm 2^\circ\text{C}$.
- **100-mL polypropylene centrifuge tubes.** Able to withstand heating to 110°C , carefully cleaned, completely dried and labeled.
- **Aluminum foil**

Reagents

- **Ammonium oxalate** $[(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}]$, **0.2 M at pH 3.0**. Dissolve 28.4 g of reagent-grade $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ in 900 mL of distilled-deionized water, adjust to pH 3.0 using NH_4OH or HCl , and dilute to a volume of 1 L. Alternatively, mix 700 mL of 0.2 M ammonium oxalate and 535 mL of 0.2 M oxalic acid (25.2 g in 1 L water), and adjust to pH 3.0 with either the oxalate solution or the dilute oxalic acid.
- **Ammonium carbonate** $[(\text{NH}_4)_2\text{CO}_3]$, **0.5 M**. Dissolve 47.0 g of reagent-grade $(\text{NH}_4)_2\text{CO}_3$ in 1 L of distilled-deionized water.
- **Phosphorus pentoxide** (P_2O_5). Reagent-grade powder.

Procedure (Schwertmann, 1964; Fey and LeRoux, 1977; Hodges and Zelazny, 1980)

1. The sample should be ground to $<149 \mu\text{m}$ (100-mesh sieve). If cation saturation is performed, NH_4^+ is preferred since the saturation would provide the closest weight comparison. The sample should have prior treatments to remove soluble salts, carbonates, and organic matter. The sample should be dried in a vacuum desiccator over P_2O_5 , with an aliquot dried at 110°C to determine sample moisture content; or the entire sample may be oven dried at 110°C , depending on the drying characteristics of the sample.
2. Weigh about 250 mg of pretreated sample into a preweighed and predried 100-mL polypropylene centrifuge tube (weight recorded to 0.01 mg). Care should be exercised in reducing weighing errors by only handling the tubes with forceps, drying all tubes in a 110°C oven, cooling in a vacuum desiccator containing P_2O_5 , and duplicating the time of weighing and exact position of the centrifuge tubes in a vertical position at the center of the balance pan. A set of blank tubes should be carried through each procedure to account for any weight loss by the centrifuge tubes on drying.
3. Add 50 mL of 0.2 M ammonium oxalate solution at pH 3.0 to the weighed centrifuge tube containing the sample, stopper, immediately wrap in aluminum foil to eliminate light, and shake for 2 h (4 h for samples containing allophane and imogolite).
4. Centrifuge the sample and decant the supernatant solution from the sample into a container for analyzing Al, Fe, Mn, and Si.
5. Wash the residue in the tube three times with 0.5 M $(\text{NH}_4)_2\text{CO}_3$ solution and once with the equal volume of distilled-deionized water to remove remaining dissolution treatment chemicals.
6. Dry the residue in the tube overnight in an oven at 110°C to volatilize excess $(\text{NH}_4)_2\text{CO}_3$ as NH_3 , CO_2 and H_2O . Inspection of the oven-dried samples should reveal no visible salt residues.
7. The tube should be allowed to cool in a vacuum desiccator over P_2O_5 and weighed for the final time to determine the amount of material dissolved by the AOD treatment.
8. Analyze Al, Fe, Mn, and Si in AOD extracts by atomic absorption or inductively coupled plasma spectroscopy. An air-acetylene gas mixture should be used for Fe and Mn analysis by atomic absorption, and nitrous oxide-acetylene for Al and Si. The standards, samples and blanks should have the same matrix composition (i.e., the extractant or its dilutions). For the instrumentation theories and analytical applications of atomic absorption and inductively coupled plasma spectroscopy, refer to Sparks (1996).

Iron in AOD extracts may be determined by orthophenanthroline or KSCN method, and Al by the colorimetric Aluminon method after destroying oxalate by digesting the oxalate extract with concentrated HNO_3 (Weaver et al., 1968; Jackson, 1979; Sparks, 1996). Alternatively, determine Fe and Al by the ferron method after digesting the oxalate extract with $\text{H}_2\text{SO}_4\text{--H}_2\text{O}_2$ (Tokashiki and Wada, 1972). More information on Fe determination was given by Loveland (1988). Silicon can be determined by the molybdosilicate-blue colorimetric method using excess molybdate to remove the interference from Mo-oxalate complexation (Fey and LeRoux, 1976).

Calculation of Results

$$\% \text{ noncrystalline material} = \frac{(\text{sample weight before AOD} - \text{sample weight after AOD}) \times 100}{\text{sample weight before AOD}}$$

$$\text{Fe, Al, Mn, or Si (mg g}^{-1}\text{)} = \frac{\text{concentration } (\mu\text{g mL}^{-1}) \times \text{extractant (mL)} \times \text{dilution factor}}{\text{sample weight before AOD (mg)}}$$

$$\% \text{ Fe, Al, Mn, or Si} = \text{Fe, Al, Mn, or Si content (mg g}^{-1}\text{)}/10$$

$$\% \text{ Fe}_2\text{O}_3 = \% \text{ Fe} \times f; f = 1.4297 \text{ for Fe, } 1.8894 \text{ for Al, and } 2.1393 \text{ for Si}$$

$$\% \text{ water of noncrystalline material} = \frac{(\% \text{ noncrystalline material} - \text{sum of \% oxides}) \times 100}{\% \text{ noncrystalline material}}$$

The empirical values of water contents added to the sum of % oxides (Fe_2O_3 , Al_2O_3 , and SiO_2) can also be found from Alexiades and Jackson (1966) and Jackson (1979):

$$\text{SiO}_2/\text{Al}_2\text{O}_3 \text{ molar ratio} = \frac{\% \text{ SiO}_2}{\% \text{ Al}_2\text{O}_3} \times 1.6969$$

$$\begin{aligned} \% \text{ water of oxides} &= 21\%, \text{ if } \text{SiO}_2/\text{Al}_2\text{O}_3 = 0.8 \text{ to } 1.4 \\ &15\%, \text{ if } \text{SiO}_2/\text{Al}_2\text{O}_3 = 1.5 \text{ to } 2.8 \\ &10\%, \text{ if } \text{SiO}_2/\text{Al}_2\text{O}_3 = 2.9 \text{ to } 4.5 \end{aligned}$$

$$\% \text{ allophane/imogolite} = \% \text{ Si}_{\text{AOD}} \times f$$

$$\begin{array}{rcl} (\text{Al}_{\text{AOD}} - \text{Al}_{\text{pyr}})/\text{Si}_{\text{AOD}} &= & 1.0 \quad 1.5 \quad 2.0 \quad 2.5 \quad 3.0 \quad 3.5 \\ f &= & 5 \quad 6 \quad 7 \quad 10 \quad 12 \quad 16 \end{array}$$

where Al_{pyr} = pyrophosphate-extracted Al (Parfitt, 1990).

Comments

Inherent sources of error in this technique include weighing errors, presence of salts due to poor rinsing, and sample loss during decantation.

Greater sources of error result from not excluding light. Addition of light, especially ultraviolet, to the ammonium oxalate treatment removes both crystalline and noncrystalline oxides from soils (DeEndredy, 1963; Schwertmann, 1964). The photolytic ammonium oxalate treatment largely dissolves hematite, goethite, and magnetite and does degrade nontronite and hydroxy-interlayered vermiculite from soils (LeRiche and Weir, 1963; Chao and Theobald, 1976; Iyengar et al., 1981; Cornell and Schindler, 1987).

Assuming that the noncrystalline content of a sample is primarily present in the clay-sized fraction is another important source of error. Hodges and Zelazny (1980) reported that the noncrystalline material determined in the clay fractions underestimated the noncrystalline material content for selected whole soils from 0 to 34%. Much of this difference is undoubtedly due to incomplete dispersion of coatings of noncrystalline materials from sand and silt particles because most noncrystalline soil materials exist as coatings that bind aggregates of minerals together (Follett et al., 1965; Jones and Uehara, 1973).

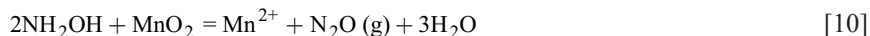
If the calculated water content of the noncrystalline material is much greater than the values published by Alexiades and Jackson (1966), it may indicate sources of error, especially the presence of undetermined metal oxides and/or hydroxides. The AOD extracts should be examined for Mn, Ti, or any other metals suspected of contributing to the loss of sample weight.

For less sensitive work or pretreatments for particle-size and mineralogical analyses, steps such as predrying, weighing, and ammonium-carbonate washing can be omitted from the procedure. Air-dried, ground, whole soil samples (sample moisture contents are determined separately) can be used without any pretreatments except for carbonates in calcareous soils (Ross and Wang, 1993; Loeppert and Inskeep, 1996). Iron, Al, Mn, and Si in supernatants are analyzed accordingly. For calcareous soils, samples should be treated with pH 5.0 acetate buffer to remove carbonates before an AOD extraction (Soukup et al., 2008, this volume).

Hydroxylamine Hydrochloride

Principles

Hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$, or HH) was used by Mann and Quastel (1946) as a reducing agent for estimating manganese dioxide by gasometry, and later by Dion et al. (1947) for determining "easily reducible" manganese in soils:



Chao and Zhou (1983) showed that this agent could also reduce ferric iron in oxides. Currently there are three methods utilizing this agent for the selective dissolution of Mn and Fe oxides and hydroxides in soils and sediments: (i) $\text{HH} + \text{HOAc}$ (Chester and Hughes, 1967), (ii) HH at pH 2 (Chao, 1972), and (iii) $\text{HH} + \text{HCl}$ (Chao and Zhou, 1983; Ross et al., 1985).

Chester and Hughes (1967) found that 1 M HH combined with 25% acetic acid dissolved 96% Mn and 90% Mn-associated Fe from ferromanganese nodules isolated from pelagic sediments, and 78% Mn and only 4% Fe in sediment samples. Therefore, this extraction method was considered selective for high Mn oxides and Mn-associated Fe in

soil and sediment samples, although a complete removal of Mn oxide phases is not usually achieved (Trolard et al., 1995). Tessier et al. (1979) incorporated this method into their sequential extraction protocol, which has been widely used by soil scientists and geochemists, to estimate the amounts of trace metals associated with Mn and Fe oxide phases. However, it should be pointed out that the removal of Fe oxides and hydroxides by the HH + HOAc method is not complete, and this method is not selective toward Fe oxide phases (Chao and Zhou, 1983; McAlister and Smith, 1999).

On the basis of the kinetic differentiation of dissolution between Mn and Fe oxide phases, Chao (1972) optimized the experimental conditions (reagent concentration, pH, temperature, and shaking time) to selectively dissolve Mn oxide phases with respect to Fe oxide phases. A 0.1 M HH at pH 2 (0.01 M HNO_3) removed 85% of Mn oxides and approximately 5% of Fe oxides from sediments with 30 min extraction, but about 50% of Mn oxides and <1% of Fe oxides from highly weathered soils. The selectivity of this method for soil Mn was confirmed by Shuman (1982), who reported 12 to 80% of soil total Mn was recovered by this method, which was as effective as any reducing agents used. Dion et al. (1947) reported that pyrolusite and a synthetic amorphous hydrous oxide (having a mean Mn valence of 2.73) were almost completely dissolved in HH solution buffered at pH 4.0 (1 M NH_4OAc), but only 15 to 25% of manganite and hausmannite dissolved. Tokashiki et al. (1986) showed that unacidified 0.1 M HH at room temperature destroyed birnessite and left lithiophorite and goethite unchanged, but lithiophorite could then be removed by repeated extractions at 60°C (Tokashiki et al., 1986, 2003) or by acidified HH (Golden et al., 1993). It seems that higher oxides of Mn and tubular and simple layer structured species are more readily dissolved in HH solution than low oxides and Al-interlayered Mn minerals (Neaman et al., 2003). Neaman et al. (2003) pointed out that the incomplete extraction of Mn oxides by Chao's method (Chao, 1972) may be due to insufficient extractant added and found that complete dissolution of Mn oxides could be achieved either by increasing HH concentrations or prolonging extraction time. However, the effort to achieve a complete dissolution of Mn oxides by HH from natural samples decreased its selectivity, increasing the extraction of other mineral components, particularly poorly crystalline fractions (e.g., ferrihydrite).

Chao and Zhou (1983) further developed from the work of Chao and Theobald (1976) another HH extraction version (0.25 M HH + 0.25 M HCl) for selectively removing amorphous and poorly crystalline Fe oxides and hydroxides. The amount of extracted Fe was similar to that by the Tamm (AOD) method at 50°C with a 1:250 solid/solution ratio for 30 min, with <1% crystalline minerals removed. Ross et al. (1985) modified this method for amorphous and poorly crystalline Fe, Al, and Si oxides by shaking samples at room temperature overnight and found excellent correlations for extracted Fe and Al between the modified HH method (Chao and Zhou, 1983) and the original HH method (Chao and Theobald, 1976) as well as AOD. Some variations in HH-extracted Fe compared with AOD Fe values were explained by the presence of AOD-extractable magnetite and other sample properties (Ross et al., 1985; Wang et al., 1987). Wang et al. (1987) suggested that HH Si was not a good estimate of AOD Si, but Lee et al. (1989) did not agree based on their data from 36 New Zealand soils (28 volcanic in nature). This method presumably performs better than AOD for amorphous Fe on magnetite-containing soils since HH dissolves much less Fe than AOD from magnetite (Chao and Zhou, 1983). However, little correlation was found between HH Fe minus AOD Fe and the abundance of magnetite in sand fractions (Lee et al., 1989). Ross et al. (1985) did not find any significant dissolution of chlorite in 0.25 M HH + 0.25 M HCl, as compared with that in AOD. The overnight HH extraction at room temperature only requires a regular shaker and no specialized heating bath, and

HH as a matrix causes much less interference than oxalate in analyzing Fe and Mn by atomic absorption.

Equipment

- Centrifuge and 50-mL polypropylene centrifuge tubes
- Reciprocal shaker

Reagents

- **Hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$), 0.1 M + 0.01 M HNO_3 (pH 2).** Dissolve 6.95 g of $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 800 mL of 0.01 M HNO_3 and bring to 1 L with 0.01 M HNO_3 .
- **Hydroxylamine hydrochloride, 0.25 M + 0.25 M HCl.** Dissolve 17.37 g of $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 800 mL of 0.25 M HCl and bring to 1 L with 0.25 M HCl.

Procedure—Reducible Mn (Chao, 1972)

1. Weigh 100 to 500 mg (to 0.1 mg) of an air-dried, finely ground soil or sediment sample (<0.5 mm or smaller) into a 50-mL centrifuge tube, and add 25 mL of 0.1 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ at pH 2.
2. Shake the contents for 30 min before centrifugation to separate the extracts (or filtration using Whatman no. 42 filter paper if the content is shaken in a flask).
3. Analyze Mn in the extracts by atomic absorption or inductively coupled plasma spectroscopy.

Procedure—Fe, Al, Mn, and Si in Non- and Poorly Crystalline Forms (Chao and Zhou, 1983; Ross et al., 1985)

1. Weigh 100 mg (to 0.1 mg) of a finely ground soil (<149 μm , passing a 100-mesh sieve) into a 50-mL centrifuge tube. Add 25 mL of the extractant (0.25 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ + 0.25 M HCl) and stopper the tube tightly.
2. Shake overnight (16 h).
3. Centrifuge at $510 \times g$ for 20 min, and keep the supernatant for Fe, Al, Mn, and Si analyses. All these elements can be analyzed by atomic absorption or inductively coupled plasma spectroscopy. Use air-acetylene gas mixture for Fe and Mn and nitrous oxide-acetylene for Al and Si for atomic absorption analysis. The standards, samples, and blanks should have the same matrix composition. For the instrumentation theories and analytical applications refer to Sparks (1996).

The Si in acidified HH extracts can also be determined by using the molybdate method (Wang and Schuppli, 1986).

Calculation of Results

$$\text{Fe, Al, Mn, or Si (mg g}^{-1}\text{)} = \frac{\text{concentration } (\mu\text{g mL}^{-1}) \times \text{extractant (mL)} \times \text{dilution factor}}{\text{sample weight on oven-dry basis (mg)}}$$

Comments

The HH solution at pH 2 (Chao, 1972) is selective for Mn over Fe in natural samples, but cannot selectively remove all Mn oxides. Its selectivity is more conclusive for amorphous and poorly crystalline materials than for crystalline oxides, although birnessite and

lithiophorite are soluble in the HH solution. This should be considered when the method is applied to extract other elements (e.g., metals) associated with Mn oxides in soils and sediments. The selectivity of this method is based on the dissolution kinetics of Fe and Mn oxides and hydroxides in the presence of HH under specified experimental conditions (HH concentration, acidity, shaking time, and sample/extractant ratio), which should not be altered if extraction of Mn over Fe is intended. Neaman et al. (2003) proposed a modified version of this method by shaking samples in 0.1 M HH without acid addition (pH 3.6) at a solution/solid ratio of 2000 for 2 h, and they reportedly extracted 85 to 100% of the Mn from natural samples with minimum amounts of extracted free-oxides of Fe and Al. The modification, however, needs to be tested on a wider range of samples for selectivity and the degree of dissolution.

Manganese oxides can also be selectively removed with hydrogen peroxide (Taylor and McKenzie, 1966). In fact, the use of hydrogen peroxide as a pretreatment to remove Mn oxides is recommended for clay mineralogical analysis (Jackson, 1979). For a complete dissolution and better selectivity, Neaman et al. (2003) suggested a 30-min extraction using 30% H_2O_2 in 0.5 M HNO_3 at a solution/solid ratio of 1000. The suitability of this extraction also needs more study.

To selectively remove birnessite from lithiophorite in soil samples, 0.1 M HH may be used without acidification at a solution/solid ratio of 200 at room temperature and with a 10-min extraction time, and the latter dissolves in the same solution but at 60°C during a 30-min reaction (Tokashiki et al., 1986, 2003). These treatments reportedly do not alter goethite, but a pretreatment with 5 M NaOH to concentrate Mn oxide minerals by removing kaolinite and gibbsite is required; furthermore, the extraction efficiency decreases in the presence of clay minerals, especially 2:1 minerals.

The HH (0.25 M) in 0.25 M HCl (Chao and Zhou, 1983) and the modified version (Ross et al., 1985) are equally effective for extracting amorphous Al, Fe, and Si, but the modified version of Ross et al. (1985) is experimentally more convenient. A change in solution/solid ratio from 250:1 to 400:1 may have some effects on the amount of elements extracted. More comparisons with AOD should be considered on a wider range of soils. It should be noted that the selectivity studies for this extraction have focused mainly on amorphous Al, Fe, and Si.

Tiron and Other Alkaline Extractants (Na_2CO_3 and NaOH)

Principles

Tiron (1,2-dihydroxybenzene-3,5-disulfonic acid, $\log K = 7.66$ and 12.6) was first introduced by Biermans and Baert (1977) as a complexing agent to extract amorphous Al, Fe, and Si oxides at pH 10.5. Further development and calibration of this method, however, is attributed to Kodama and his colleagues (Wang et al., 1981, 1986; Kodama and Jaakkimainen, 1981; Kodama and Hayashi, 1985; Kodama and Wang, 1989; Kodama et al., 1989; Kodama and Ross, 1991). The extraction is performed at 80°C for 1 h (Biermans and Baert, 1977; Hayashi and Yamada, 1990). The alkaline Tiron solution was found to be equally effective as AOD for extracting soil Al and Fe, but more effective than AOD for soil Si removal (Kodama and Wang, 1989). Using reference minerals, Kodama and Ross (1991) found that alkaline Tiron solution, like AOD, dissolved allophanes and imogolite completely, but extracted some Al from gibbsite, which is resistant to AOD, confirming the results of Biermans and Baert (1977). The Tiron solution dissolved little magnetite compared with a substantial dissolution by AOD, but both AOD and Tiron extracted Fe from siderite (about 50% total Fe). No significant dissolution was found for goethite, he-

matite, maghemite, and lepidocrocite, but some for akaganeite (Kodama and Ross, 1991). Kaolinite, halloysite, sepiolite, montmorillonite, illite, and chlorite are not attacked (Biermans and Baert, 1977; Hayashi and Yamada, 1990). Silicon removed by the alkaline Tiron solution was due to the dissolution of opaline silica, and this was confirmed on both pure mineral samples and soil samples from Spodosol and Spodosol-like soils by differential X-ray diffraction (Kodama and Wang, 1989; Kodama and Ross, 1991). The difference between Tiron Si and AOD Si may be used to measure the amount of opaline silica.

It seems that the Tiron method is equivalent to AOD and HH methods for extracting amorphous and poorly crystalline Al and Fe from soil, but the Tiron method is not suitable for estimating allophane and imogolite contents for soils having opaline silica. This method may also be helpful for the identification of soil magnetite. The method may be used as a pretreatment “clean-up” for X-ray diffraction and thermal analyses since better patterns can be obtained for crystalline phases, particularly nonphyllosilicate minerals, due to removing amorphous and poorly crystalline materials of Al, Fe and Si by this method (Wang et al., 1981; Kodama and Wang, 1989; Kodama and Ross, 1991). A prolonged extraction (>1 h) is not recommended because of the decrease in extracted Si, and an error in extractable Al and Fe for smectite-containing soils, which may be as great as 15% due to the interlayer adsorption of Al and Fe cations (Hayashi and Yamada, 1990).

Dilute Na_2CO_3 solutions (2–5%) and a boiling solution of 0.5 M NaOH have also been used as alkaline extractants for amorphous and poorly crystalline oxides and hydroxides of Al and Fe and aluminosilicates (allophanes, imogolite, and possibly halloysite and kaolinite) (Jackson et al., 1986). Neither method is effective for Fe. Because of alkaline conditions, free Fe oxides should be removed before the extraction to avoid the potential blocking effects of Fe oxides on dissolution. Studies show that Na_2CO_3 solutions are too mild to completely remove all amorphous and poorly crystalline Al from soil samples (Wang et al., 1981; Kodama and Ross, 1991), whereas NaOH has always been suspected of attacking crystalline layer silicates (Biermans and Baert, 1977; Kodama and Jaakkimainen, 1981; Bhattacharyya and Ghosh, 1986; Kodama and Ross, 1991). However, for soils with high amounts of amorphous and poorly crystalline materials, such as soils developed from volcanic ash, 0.5M NaOH boiling solution is as effective as AOD for allophanes, imogolite, and other amorphous fractions (Farmer et al., 1977). In view of the extractability and selectivity of all reviewed methods, these two methods do not seem to have advantages over others. Readers are referred to Jackson et al. (1986) for more details.

Equipment

- Water bath
- Centrifuge and 50-mL plastic centrifuge tubes
- Plastic containers and sample vials

Reagents

- **Sodium carbonate (Na_2CO_3), 0.5 M.** Dissolve 53 g of Na_2CO_3 in 800 mL of distilled-deionized water in a 1-L plastic volumetric flask or beaker, and bring to volume.
- **Sodium hydroxide (NaOH), 4 M.** Dissolve 160.2 g of NaOH in 800 mL of distilled-deionized water in a 1-L plastic volumetric flask or beaker, and bring to volume.
- **Tiron (disodium salt of 4,5-dihydroxy-1,3-benzene-disulfonic acid, $\text{C}_6\text{H}_4\text{Na}_2\text{O}_8\text{S}_2$), 0.1 M.** Dissolve 31.42 g in 800 mL of distilled-deionized water in a plastic beaker. The resulting solution is acidic (pH $\approx$ 5.5) with a light brownish yellow color. Add 100-mL of 0.5 M sodium carbonate to the beaker. After mixing, the Tiron solution is

greenish, and the pH is 10.2. Titrate the Tiron solution with 4 M NaOH until the pH reaches 10.5 (about 23.5 mL of sodium hydroxide solution). The total Tiron solution is brought to 1 L. After standing for about 0.5 h, the solution changes its color from greenish to brownish. Readjust the pH to 10.5 if it drops after storage. Store the Tiron solution in a plastic container under refrigeration.

Procedure (Biermans and Baert, 1977; Kodama and Ross, 1991)

1. Weigh 25 mg (to 0.1 mg) of finely ground materials (passing a 50- μm opening sieve, or soil silt and clay fractions) into a 50-mL plastic centrifuge tube. Add 30 mL of Tiron solution and weigh (sample + solution + tube). Larger samples may be used at the same solution/solid ratio.
2. The tube is covered loosely with a polypropylene cap, and placed in a water bath at 80°C for 1 h. Stir the contents occasionally by shaking manually.
3. At the end of extraction, cool the tube in a cold-water bath, dry the outside of the tube, and weigh it without the cap. Bring the weight back to the original amount by adding distilled-deionized water.
4. Mix the contents well and centrifuge at 17,600 g for 10 min. The supernatant is recovered in plastic vials for analyzing Al, Fe, and Si. Analyze Al, Fe, and Si in solution by atomic absorption or inductively coupled plasma spectroscopy. For atomic absorption analysis, use air-acetylene gas mixture for Fe and nitrous oxide-acetylene for Al and Si. The standards, samples, and blanks should have the same matrix composition. For the instrumentation theories and analytical applications of atomic absorption or inductively coupled plasma spectroscopy, refer to Sparks (1996).

To analyze Fe, Al, and Si colorimetrically by the methods mentioned in the “Sodium Hypochlorite” section, residual Tiron in solutions should be destroyed by treating with 30% H_2O_2 in a water bath until the color disappears (Wang and Schuppli, 1986; Kodama and Ross, 1991).

Calculation of Results

$$\text{Fe, Al, Mn, or Si (mg g}^{-1}\text{)} = \frac{\text{concentration in extracts (}\mu\text{g mL}^{-1}\text{)} \times \text{extractant (mL)} \times \text{dilution factor}}{\text{sample weight on oven-dry basis (mg)}}$$

Comments

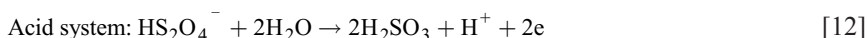
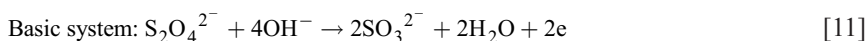
For a clean-up pretreatment before X-ray or thermal analysis, a single treatment with Tiron as described above appears to be sufficient. The dissolution of opaline silica makes it unsuitable for estimation of allophane or imogolite contents for soils containing significant amounts of opaline silica.

Citrate-Bicarbonate-Dithionite

Principles

Galabutskaya and Govorova (1934) used sodium dithionite (hydrosulfite) solution at $\sim 40^\circ\text{C}$ to remove free Fe oxides from kaolins. Since then, the dithionite method has evolved until the establishment of the current citrate-bicarbonate-dithionite (CBD) procedure of Mehra and Jackson (1960). The historic development of the dithionite method was reviewed by Jackson et al. (1986). With the CBD method, dithionite extraction is

performed at 75 to 80°C in the presence of citrate as a chelating agent to remove Fe, and the pH is buffered at 7.3 by 1 M bicarbonate (Mehra and Jackson, 1960; Jackson, 1979). Citrate, which contains a maximum of three donors to coordinate to a given metal center, commonly forms 1:1 complexes with Fe or Al, a (5+6)-membered joint chelate system involving a terminal –COOH, and the –COOH and –OH on the central carbon atom, and also bis and trinuclear species (Chaberek and Martell, 1959; Strouse et al., 1977; Rubini et al., 2002). These complexes are more stable than those formed from tartrate or oxalate anions. The strong chelating action of citrate prevents the precipitation of FeS at the near-neutral pH of extraction. At pH 7.3, a weak alkaline condition, the oxidation potential of the system is increased, compared with an acid system, because of the supply of four OH[−] groups used in the oxidation of each dithionite molecule:



The basic system has a theoretical redox potential of −1.12 V, while that of the acid system is −0.08 V (Jackson, 1979, p. 45). The redox potential at 7.3 is −0.7 V (Mehra and Jackson, 1960). Apparently earlier applications at lower pH did not produce effective removal of free oxides from soils high in Fe oxides because of the low oxidation potential of acid systems (Deb, 1950; MacKenzie, 1954). Another factor to be considered for the system pH is the solubility of Fe and Al oxides, which decreases as the third power of OH[−] concentration. The extraction efficiency of the dithionite method therefore is maximized at pH 7.3 after considering all factors. At pH 7.3, the dithionite extraction has the benefits of nonprecipitation of sulfur, rapid reaction rate, and little removal of Fe from silicates or effects on mineral cation exchange capacity.

The objectives of the CBD method are primarily the determination of free Fe oxides (goethite, hematite, and maghemite) and the removal of amorphous coatings and crystals of free Fe oxides acting as cementing agents for subsequent physical and chemical analysis of soils, sediments, and clay minerals. In addition to free oxides, Al-substituted crystalline hematite and goethite are dissolved by the dithionite treatment (Norrish and Taylor, 1961; Fey and LeRoux, 1976, 1977). Reduction of combined Fe³⁺ to Fe²⁺ (after oxidation with H₂O₂) can be effected in micaceous vermiculite (Roth et al., 1968, 1969; Veith and Jackson, 1974). Appreciable dissolution of structural Fe, change in oxidation state of Fe³⁺ to Fe²⁺, and change in Fe coordination environment in nontronite can be brought about by the CBD treatment (Dudas and Harward, 1971; Stucki et al., 1976; Russell et al., 1979). Magnetite and ilmenite, however, are not extracted by CBD. The effectiveness of CBD in Fe reduction is affected considerably by the particle size of crystalline Fe oxide minerals such as goethite and hematite (Borggaard, 1988; Kodama and Ross, 1991). Kodama and Ross (1991) reported that a single CBD treatment was not sufficient to dissolve clay-size goethite, that silt-size hematite required more than 10 repeated extractions, and that only about 60% of coarser silt-size hematite was dissolved after 19 extractions.

Dixon and Jackson (1962) reported that a considerable amount of Al was extracted from hydroxy aluminum interlayers of vermiculitic chlorite, but gibbsite was hardly attacked. Ghabru et al. (1990) removed Fe from the interlayer of soil vermiculites by repeated CBD and citrate-dithionite extractions. The infrared spectra of CBD-soluble fractions of volcanic ash soils suggest dissolution of allophane-like materials with a low SiO₂/Al₂O₃

molar ratio ranging from 0.2 to 0.4 and additional alumina-rich components with a ratio <0.2 (Wada and Greenland, 1970; Tokashiki and Wada, 1972; Wada and Tokashiki, 1972). The silica associated with Fe and Al oxides was also brought into solution by CBD (Beckwith and Reeve, 1964; Weaver et al., 1968).

Golden et al. (1993) confirmed with X-ray diffraction analysis that CBD treatment removed most of the Fe and Mn oxide minerals in an Oxisol from Hawaii, and only a trace of lithiophorite was left behind in the silt and sand fractions after two CBD treatments. Tokashiki et al. (1986) reported that removal of lithiophorite in soil Mn nodules required several CBD treatments, although the first extraction yielded most of the Mn. Their results also indicated a preferential reduction of Mn over Fe by CBD. Free MnO_2 , however, can be made soluble by H_2O_2 pretreatment before CBD (Jackson, 1979).

The ratio of AOD Fe to CBD Fe has been used as a relative measure of the degree of aging or crystallinity of free Fe oxides (Schwertmann, 1964) and as a supplementary criterion for deciding the age sequence of associated but noncontiguous Quaternary deposits (Alexander, 1974). In addition, the removal of amorphous and crystalline pedogenic oxides and hydroxides aids in the dispersion, purification, and concentration of silicate fractions. The CBD treatment is one of the standard pretreatments for soil particle-size and mineralogical analysis (Jackson, 1979; Kunze and Dixon, 1986).

Holmgren (1967) argued that the bicarbonate buffer is unnecessary as long as the dithionite/citrate mass ratio (both are as sodium salts) is small. Holmgren (1967) developed a routine procedure of shaking 2 g of sodium dithionite and 20 g of sodium citrate overnight with a sample containing up to 25% Fe. This citrate-dithionite combination has also been used to extract interlayer materials (Barnhisel and Bertsch, 1989; Ghabru et al., 1990). Trolard et al. (1995) employed CBD at room temperature to study the kinetic release of trace metals associated with Fe oxides from laterites. Acebal et al. (2000) replaced citrate with EDTA and found that the new combination was more effective for Fe extraction than CBD.

Equipment

- Water bath
- Centrifuge and 100-mL centrifuge tubes

Reagents

- **Sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), 0.3 M.** Dissolve 88 g of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ in 800 mL of distilled-deionized water and bring to 1 L.
- **Sodium bicarbonate (NaHCO_3), 1 M.** Dissolve 84 g of reagent-grade NaHCO_3 in 800 mL of distilled-deionized water and bring to 1 L.
- **Citrate-bicarbonate buffer.** Reagents 1 and 2 are combined at a volume ratio of 8:1 into one buffer solution. The pH is buffered at 7.3.
- **Sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$).** Use freshly opened, purest grade.
- **Sodium chloride (NaCl), saturated solution.** Shake 80 g of reagent-grade NaCl in 200 mL of distilled-deionized water.
- **Acetone.** Reagent grade.

Procedure (Mehra and Jackson, 1960; Jackson et al., 1986)

1. Weigh 5 g (to 0.001 g) of soil or 1 g (to 0.0001 g) of clay containing about 0.5 g of extractable free Fe_2O_3 or less into a 100-mL centrifuge tube.

2. To the tube, add 45 mL of citrate-bicarbonate buffer solution (alternatively, 40 mL of 0.3 M sodium citrate and 5 mL of 1 M NaHCO_3). Mix the contents and place the tube in a water bath at 75 to 80°C.
3. Add about 1 g of $\text{Na}_2\text{S}_2\text{O}_4$ powder with a calibrated spoon. Immediately stir thoroughly for one min using a glass stirring rod, and then intermittently for 5 min. Add a second dose (1 g) of $\text{Na}_2\text{S}_2\text{O}_4$ and continue occasional stirring for another 10 min.
4. After the 15-min extraction period, add 10 mL of saturated NaCl solution to promote flocculation, and centrifuge. If the suspension fails to flocculate with the NaCl, add 10 mL of acetone (particularly needed for volcanic ash soils), mix the contents, warm in a water bath, and centrifuge for 5 min at 1000 g. Decant the clear supernatant into a 500-mL volumetric flask.
5. Repeat the extraction for samples in which a brown, yellow, or red coloration persists and for those containing more than 5% Fe_2O_3 . Combine the extracts into the volumetric flask.
6. Wash the sample with sodium citrate solution or citrate-bicarbonate buffer solution. Two or more washings are needed for samples with more than 1 g of residues. Combine the washings with the previous extracts. Bring to volume with water.
7. Analyze Fe, Al, Mn, and Si in extracts by atomic absorption or inductively coupled plasma spectroscopy. An air-acetylene gas mixture should be used for Fe and Mn analysis by atomic absorption, and nitrous oxide-acetylene for Al and Si. The standards, samples and blanks should have the same matrix composition (i.e., the extractant or its dilutions). For the instrumentation theories and analytical applications of atomic absorption and inductively coupled plasma spectroscopy, refer to Sparks (1996).

When analyzing the Fe, Al, Mn, and Si in CBD extracts by colorimetric methods, follow the hints given above for AOD extraction. Adding an excess of molybdate in Al or Si determination may remove the effects of citrate (Weaver et al., 1968).

The sample residue after the CBD extraction can be preserved for further analyses (e.g., X-ray diffraction, thermal analysis, infrared analysis, total elemental analysis and others).

Calculation of Results

$$\text{Fe, Al, Mn, or Si (mg g}^{-1}\text{)} = \frac{\text{concentration } (\mu\text{g mL}^{-1}) \times \text{extractant (mL)} \times \text{dilution factor}}{\text{sample weight on oven-dry basis (mg)}}$$

$$\% \text{ Fe, Al, Mn, or Si} = \text{Fe, Al, Mn, or Si content (mg g}^{-1}\text{)} / 10$$

$$\% \text{ Fe}_2\text{O}_3 = \% \text{ Fe} \times f; f = 1.4297 \text{ for Fe, } 1.8894 \text{ for Al, and } 2.1393 \text{ for Si}$$

Or,

$$\% \text{ free oxides} = \frac{(\text{sample weight before CBD} - \text{sample weight after CBD}) \times 100}{\text{sample weight before CBD}}$$

Comments

Avoid CaCl_2 as a flocculent agent, because CaCO_3 could form. Avoid adding HCl, because HCl promotes sulfur precipitation.

The CBD-extracted Fe is often reported as oxide (Fe_2O_3), particularly in old literatures,

but the expression is not related to the molecular formula of Fe minerals in the sample.

A maximum of about 0.5 g of Fe_2O_3 can be dissolved in 40 mL of citrate solution. Sample size can be adjusted in accordance with the expected extractable Fe content, and may vary from 10 g for most temperate soils to 0.2 g (ground) for samples high in crystalline oxides such as hematite, goethite, or limonite.

Heating above 80°C is avoided because FeS forms at higher temperatures. If FeS is formed, the extraction is better repeated on a different sample, although a washing procedure can be followed (Jackson et al., 1986).

A pure white color of the residue after CBD treatment should not be expected, as many soils and colloids contain greenish, bluish, or cream-colored clays because of the presence of structural iron. Black minerals, such as ilmenite or magnetite, which are not dissolved, may also be present and leave a black color.

For routine analysis of a large set of samples, the dithionite-citrate method of Holmgren (1967) can be followed (Ross and Wang, 1993; Loeppert and Inskeep, 1996).

Ti(III) has been used as a reductant in combination with organic ligands (citrate or EDTA) to extract Fe and Mn oxides (Hudson and Morel, 1989; Ryan and Gschwend, 1991; Heron et al., 1994). Ryan and Gschwend (1991) reported that a solution of Ti(III)-citrate-EDTA ternary complex buffered at pH 7.0 with bicarbonate is equally effective as CBD for amorphous ferric oxides and slightly better than CBD for goethite, but less effective for hematite. There is an indication that CBD might extract structural Fe from Fe-containing layer silicates. Shuman (1982) proposed an ascorbic acid-oxalate combination to extract micronutrients associated with Fe and Mn oxides in soils, and found that this extractant (for 30 min at 97°C and 1:50 soil/solution ratio) extracted as much Fe as CBD and more Al than CBD from three soils. Ascorbic acid is known to affect the reductive dissolution of Fe oxides (Zinder et al., 1986; Dos Santos Afonso et al., 1990; Suter et al., 1991). Reyes and Torrent (1997) shook soil samples in 50 mL of 0.2 M sodium citrate-0.05 M sodium ascorbate solution at pH 6.0 for 16 h and found that the extraction is equally effective as the AOD method for amorphous and poorly crystalline Fe oxides and hydroxides, and dissolves only trace amounts of allophanes, imogolite, and magnetite. Therefore, the method seems more selective than the AOD method toward Fe. Chiarizia and Horwitz (1991) proposed a new mixture of complexing and reducing agents (1-hydroxyethane-1,1-diphosphonic acid and sodium formaldehydesulfoxylate) for rapid dissolution of Fe oxides at room temperature.

Summary Comments

Table 3–1 summarizes the extraction methods described in the preceding sections and the mineral species or phases targeted by a particular method. The combination of two or more extractions (i.e., sequential selective dissolution) is often applied to the same sample to estimate a particular mineral phase containing one or several minerals.

Assigning water content to oxides of Si, Al, and Fe derived from selective dissolution is problematic since noncrystalline materials have variable chemical composition (van Olphen, 1971) and thus may be expected to have variable water content. Constant water content has often been assumed for the noncrystalline components, resulting in assignment of these values without regard for sample composition (Alexiades and Jackson, 1966; de Villiers, 1971; Jackson, 1979). However, $\text{SiO}_2/\text{Al}_2\text{O}_3$ molar ratios have also been used to assign water contents within specified ranges (Jackson, 1979).

Fey and LeRoux (1976) assigned water content by comparing the $\text{SiO}_2/\text{Al}_2\text{O}_3$ molar ratio of samples with those of noncrystalline, synthetic aluminosilicate gels of known

water content. This method assumes that synthetic materials are adequate models for predicting the characteristics of natural materials. A more satisfactory method would be to measure the weight loss of a given sample after selective dissolution. Hodges and Zelazny (1980) made a comparison of measured weight loss to chemical determination with water content assigned on the basis of the $\text{SiO}_2/\text{Al}_2\text{O}_3$ molar ratio, according to the procedure of Jackson (1979) on AOD-treated soils. As would be expected from heterogeneous composition, the values were remarkably similar but not in complete agreement. It therefore seems possible to quantify the noncrystalline material content of soils without time-consuming chemical determinations or assuming sample homogeneity and water content. However, it is likely that methods used in conjunction with chemical analysis will result in more accurate characterization.

REMOVAL OF KAOLINITE AND GIBBSITE

Principles

Norrish and Taylor (1961) used boiling NaOH to dissolve clay minerals for their study of Al substitution in goethite, and the Al dissolved during the alkaline treatment was thought to be from Al-substituted goethite. The method takes advantage of the high solubility under alkaline condition of Al and Al-containing minerals such as kaolinite and gibbsite. This method was later used to concentrate Fe and Mn minerals in soil samples by selectively removing kaolinite and gibbsite (Tokashiki et al., 1986, 2003). The experimental procedure involves shaking soil sample in 5 M NaOH in a heating environment, followed by acid washing to remove residual NaOH (Norrish and Taylor, 1961; Tokashiki et al., 1986). However, this extraction has never been quantitatively examined against reference minerals and natural standard samples. Therefore, at best it should be considered a qualitative method for Al-containing minerals and a pretreatment for non-Al-containing minerals (e.g., Fe and Mn oxides).

Equipment

- Centrifuge and 100-mL centrifuge tubes
- Water bath

Reagents

- **Sodium hydroxide (NaOH), 5 M.** Dissolve 200 g of NaOH (reagent grade) in 800 mL of distilled-deionized water in a beaker. Transfer the content into a 1-L volumetric flask and bring to volume with distilled-deionized water.
- **Hydrochloric acid (HCl), 0.5 M.** Transfer 41.7 mL of concentrated HCl into 800 mL of distilled-deionized water in a beaker. Transfer the content into a 1-L volumetric flask and bring to volume with distilled-deionized water.

Procedure

1. Weigh 1 g of air-dried, ground (<2 mm or clay) sample into a 10-mL centrifuge tube. Add 100 mL of 5 M NaOH and place the sample tube in a water bath at 90 to 95°C for 1 h.
2. Cool the sample at room temperature and centrifuge at 1000 g for 10 min.
3. Wash with 10 mL of 0.5 M HCl and water to remove residual NaOH. The supernatants are discarded.

Table 3–1. Removal of Al, Fe, Mn, and Si by various treatments from soils and soil constituents (expanded from Table 6–4 in Jackson et al., 1986).†

Soil component	0.1 M Pyrophosphate	HH pH 2.0	HH + 0.25 M HCl	0.1 M Tiron pH 10.5	AOD pH 3.0	CBD	0.5 M NaOH
Organically bound							
Aluminum	+++	n	+++	+++	+++	+++	+++
Iron	+++	n	+++	+++	+++	+++	+++
Hydrous oxides							
Amorphous Al	+	n	+++	+++	+++	++	+++
Amorphous Fe	+	n	+++	+++	+++	+++	0
Amorphous Mn	n	+++	n	n	+++	+++	0
Opaline Si	n	n	+	++	0	0	+++
Ferrihydrite	+	+	+++	+++	+++	+++	0
Lepidocrocite	n	0	+	0	+	+++	0
Goethite	0	0	0	0	0	+++	0
Hematite	0	0	0	0	0	++	0
Maghemite	0	0	++	0	++	++	0
Magnetite	0	0	0	+	++	0	0
Birnessite	0	+++	n	n	+++	+++	0
Pyrolusite	0	+++	n	n	++	+++	0
lithiophorite	0	+	n	n	n	+++	0
Manganite	0	++	n	n	n	++	0
Hausmannite	0	++	n	n	+++	++	0
Gibbsite	0	0	n	++	+	0	++
Hydroxy- interlayers	0	0	n	+	++	++	n
Aluminosilicates							
Amorphous	+	n	+++	+++	+++	++	+++
Allophane	+	n	+++	+++	+++	++	+++
Imogolite	+	n	+++	+++	+++	++	+++
Halloysite	0	0	0	0	0	0	+
Kaolinite	0	0	0	0	0	0	+
2:1 layer minerals	0	0	0	0	‡	0	+

† +++ = excellent (>80%), ++ = good (10–80%), + = poor (<10%), 0 = not extractable, n = no information or not applicable.

‡ Trioctahedral minerals (biotite and chlorite) are more susceptible to AOD treatment.

FRACTIONATION OF PHOSPHORUS AND TRACE METALS BY SELECTIVE SEQUENTIAL EXTRACTION

Sequential fractionation is an extraction protocol consisting of a series of extraction procedures, which are successively applied to the same sample, resulting in an array of fractions containing the elements of interest. Selective chemical dissolution methods have more often been employed in sequential fractionation of agriculturally and environmentally significant elements in soils and sediments (e.g., P, micronutrients, and trace metals, which are intimately associated with various phases through adsorption, precipitation, or crystallization) than in mineralogical analysis (Chang and Jackson, 1957; Tessier et al., 1979). In this section, we tabulate the most widely used fractionation protocols for P and trace metals in soils, sediments, and environmental samples and provide some justifications for each extractant and scheme based on the SCD principles discussed in previous sections. Hopefully, our inputs are useful to the readers for data interpretation and future methodology improvements. In the following, commonly used functional terms (e.g.,

available, resistant) for elemental fractions are avoided because these functional names are arbitrarily given by individual researchers and are sometimes misleading.

Table 3–2 gives the chemical extractants that are used in P fractionation, the P forms intended to become soluble by each extractant, and their associated reaction mechanisms. Table 3–3 lists the six most widely used P fractionation schemes of more than 20 appearing in the literature. Each of the six protocols has merits and weaknesses, performing better in certain situations than others. The Hedley fractionation scheme (Hedley et al., 1982) probably is most tested in the last two decades for both soils and sediments, and the procedure reputes itself with a better biological relevance (Cross and Schlesinger, 1995). This fractionation scheme, however, is not specific to Fe-mineral occluded P, and thus is weak in studying P geochemical transformations. Almost every fractionation scheme includes an acid extraction, which is designated for Ca-P (carbonates- and apatite-associated P), but its interpretation becomes obscure in the case of acidic soils, though this fraction may be small for these soils. There is a trend of replacing CBD with other extractants, because of the difficulty encountered in P analysis and multiple phase-extraction by CBD reagents. However, there are no other methods currently available that can perform better than CBD in removing Fe oxides. It appears that P fractionation procedures can also be applied for arsenic (McLaren et al., 1998; Lomi et al., 2000).

Selected extractants and fractionation schemes for metal micronutrients and trace metals in soils and sediments are given in Tables 3–4 and 3–5. More thorough compilations can be found in the review articles by Kersten and Förstner (1995) and Filgueiras et al. (2002). There are possibilities for misuse of SCD methods in practice, such as the inappropriate use of hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$ or HH) for reducible mineral phases. This method extracts Mn oxides, amorphous Fe and Al compounds, and poorly crystalline aluminosilicates to crystalline Fe oxides depending on extraction time, concentration, and acidity (see “Hydroxylamine Hydrochloride” above). Any modification regarding these experimental conditions must be recalibrated with pure reference materials and natural samples. Another case is that pyrophosphate and oxidation procedures (H_2O_2 or NaOCl) for organic forms of metals are not synonymous; the former extracts the more “soluble” part of organic matter (on average 50% organic C; i.e., humus), while the latter oxidizes functional groups and is intended to remove organic matter totally, although this is never achieved. The difference between the two methods is primarily the humin fraction of organic matter, and whether humin is important for metal retention is unknown. Pyrophosphate was originally developed for extracting humus-bound Al and Fe, and extracting these two elements from other sources is negligible, but this may not be true for trace metals. Sodium acetate buffered at pH 5 is likely not sufficient to neutralize all carbonates for certain calcareous samples. The acidity/carbonate molar ratio required for a complete dissolution would be greater than the theoretical value of 2 because the reaction at this pH is kinetically controlled by removal of reaction products. A simple calculation before extraction with the sample’s carbonate content available would help determine the fate of this extraction. The last fractionation scheme for trace metals in soils given in Table 3–5 is compiled from several schemes developed by groups of soil scientists over 30 yr. Although the original versions have variations among them, their common features make it possible to compile them into one scheme with minimal adjustment. In practice, the fractionation steps included depend on the needs of the research problem and the dominance of mineral phases in the samples. In this scheme, the AOD method is replaced with the HH method described in this chapter to avoid the matrix effects of oxalate on metal analysis.

Although sequential fractionation provides meaningful information on nutrient cycling, mobility of metals, and transport of elements in nature ecosystems, nearly all schemes suf-

Table 3–2. Extractants used for P sequential fractionation in soils and sediments.

Extractants†	P forms intended to solubilize	Extraction mechanisms
Resin strips in water	soluble, exchangeable	exchange with HCO_3^-
1 M NH_4Cl , pH 7	soluble, loosely bound	dissolution and/or mass action displacement by Cl^-
1 M MgCl_2 , pH 8	soluble, exchangeable, loosely bound	formation of MgPO_4^- complexes and or mass action displacement by Cl^-
0.5 M NaHCO_3 , pH 8.5	HCO_3^- exchangeable organic and inorganic	HCO_3^- suppressing P-fixing cations Ca, Fe and Al in soil solution; hydrolysis of humic substances, exchange with HCO_3^-
0.5 M NH_4F , pH 8.2	Al-bound, Al-containing P minerals	formation of F^- complexes with Al from exchangeable sites, and as polymer or amorphous and crystalline compounds.
0.1 M or 1 M NaOH,	Al- and Fe-bound, adsorbed or Al-occluded	desorption from various adsorption sites; and hydrolysis of humic substances; dissolution of kaolinite and gibbsite at high NaOH concentrations
CBD	Fe^{3+} -bound, Fe oxides-occluded	reduction of Fe^{3+} ; unable to dissolve Fe-oxides in large particle sizes
0.3 M citrate/0.11 M NaHCO_3 (CB)	carbonates-bound, exchangeable	formation of Ca^{2+} complexes with citrate and anion exchange by citrate and HCO_3^-
0.11 M $\text{Na}_2\text{S}_2\text{O}_4$ /0.11 M NaHCO_3 (BD)	Fe^{3+} -bound, Fe oxides-occluded	reduction of Fe^{3+} , incomplete dissolution of Fe oxides, but no Ca-bound P removed by citrate; less efficient than CBD
1 M NaOAc/HOAc, pH 4	CaCO_3 -bound, carbonate fluorapatite, biogenic hydroxyapatite	acid dissolution and complexing Ca^{2+} by acetate
0.25–0.5 M H_2SO_4 or 0.5–1 M HCl	Ca-bound or carbonates-associated	acid dissolution of carbonates and apatites including detrital apatites
Conc. HCl, 80°C	stable inorganic and organic	mineral dissolution under harsh acidic conditions.
Ashing at 550°C/1 M HCl	organic	dry oxidation, acid dissolution

Table 3–3. Selected phosphorus fractionation schemes for soil and sediment samples.

Fractionation schemes	Remarks
Acid and neutral soils (Chang and Jacon, 1957; Kuo, 1996)	
a. 1 M NH ₄ Cl, pH 7, 50:1, 0.5 h	The scheme distinguishes Al-P from Fe-P; however, the exact forms of Al-P are not clear ranging from Al ³⁺ -bridged adsorbed P, P adsorbed on amorphous and crystalline Al compounds, Al-mineral occluded P, Al phosphates. The last acid extraction doesn't have a sound explanation for the forms of P extracted.
b. 0.5 M NH ₄ F, pH 8.2, 50:1, 1 h	
c. CBD, 45:1, 85°C, 15 min	
d. 0.25 M H ₂ SO ₄ or 0.5 M HCl, 50:1, 1 h	
Neutral and calcareous soils (Williams et al., 1971; Olsen and Summer, 1982)	
a. 0.1 M NaOH, 50:1, 17 h	Dilute NaOH removes sorbed Fe-P, Al-P, and organic P, which should be analyzed, and it also eliminates the complications associated with the earlier NH ₄ F extraction. But the soluble and loosely bound P fractions are not considered as a separate fraction in this procedure. Citrate in CB and CBD steps also extract substantial amounts of Ca-P so that the Ca-P by the HCl extraction needs to be redefined.
b. CB, 45:1, 15 min	
c. CBD, 45:1, 85°C, 15 min	
d. 1 M HCl, 50:1, 1 h	
Neutral and calcareous soils (Hedley et al., 1982; Tiessen and Moir, 1993)	
a. Resin strips in water, 60:1, 16 h	The resin-P and bicarbonate-P are most thoroughly calibrated among all P fractions with plant growth. Both organic and inorganic P are accounted for in the fractionation. Most of P fractions obtained bear biological significance, although definitions are operational. However, the hot HCl extraction and 1 M HCl in the case of acid soil don't have a sound CSD basis, and the scheme is not designed to selectively extract Fe- and Al-occluded P. Therefore, it has little or no relevance to P geochemical transformation.
b. 0.5 M NaHCO ₃ , pH 8.5, 60:1, 16 h	
c. 0.1 M NaOH, 60:1, 16 h	
d. 1 M HCl, 60:1, 16 h	
e. 12 M HCl, 20:1, 80°C, 10 min.	
f. 18 M H ₂ SO ₄ /H ₂ O ₂ , 360°C	
Calcareous sediments (Ruttenberg, 1992)	
a. 1 M MgCl ₂ , pH 8, 100:1, 2 h	It separates easily dissolved carbonate- and apatite-P from detrital apatite P, but organic P in the first four steps is not accounted for or partly cannot be analyzed (e.g., in CBD). Both the salt solution and CBD extract certain amounts of Ca-P, and it is not quantitative; therefore, underestimation of Ca-P in subsequent steps is likely. P re-sorption onto carbonates during the first two steps is possible.
b. CBD, pH 7.6, 90:1, 25°C, 8 h	
c. 1 M NaOAc/HOAc, pH 4, 100:1, 6 h	
d. 1 M HCl, 100:1, 16 h	
e. Ash at 550°C/1 M HCl	
Sediments (Kurmies, 1972; Hieltjes and Lijklema, 1980; Rydin and Welch, 1998)	
a. 1 M NH ₄ Cl, pH 7, 50:1, 2 × 2 h	In the BD step, omission of citrate and a lower experimental temperature has excluded apatite-P from the extraction and minimized the hydrolysis of phosphate from cells. However, the BD-P doesn't represent all Fe-P due to incomplete dissolution of Fe oxides, although it may correlate better with bioavailable P.
b. BD, 50:1, 40°C, 1 h	
c. 0.1 M NaOH, 50:1, 16 h	
d. 0.5 M HCl, 50:1, 16 h	
Sediments (Williams et al., 1980; Psenner et al., 1984; Psenner and Pucsko, 1988)	
a. H ₂ O, 50:1, 10 min	1 M NaOH extracts more P bound to Al and humus than 0.1M NaOH, but doesn't seem to edge the later as a bar for P bioavailability. The acid step may give a better estimate for Ca-P in this scheme than in others since carbonates-P and apatite-P are largely intact up to this step. The nature of hot-NaOH P is obscure, except more organic P is extracted. The BD-P is a better biological index than as a meaningful geochemical fraction.
b. BD, 50:1, 40°C, 0.5 h	
c. 1 M NaOH, 50:1, 16 h	
d. 0.5 HCl, 50:1, 16 h	
e. 1 M NaOH, 50:1, 85°C, 24 h	

Table 3—4. Chemical extractants and reaction mechanisms for various forms of metals in soils, sediments, and environmental samples.

Metal forms/associated components	Extractants	Extraction mechanisms and remarks
Water soluble	H ₂ O	Water soluble salts
Exchangeable	Na ⁺ , K ⁺ , NH ₄ ⁺ , Ca ²⁺ , Ba ²⁺ , or Mg ²⁺ salts of NO ₃ ⁻ , Cl ⁻ , or OAc ⁻	Cation exchange reaction; complexing with Cl ⁻ and C ₂ H ₃ OO ⁻ from specific adsorption sites; 1 M MgCl ₂ or NH ₄ OAc at pH 7 being most widely used.
Carbonates-bound or specific adsorbed	1 M NaOAc, pH 5 or 0.11–0.44 M HOAc	1 M NaOAc at pH 5 used for neutralizing carbonates, but also exchanging metals from specific adsorption sites (especially for carbonates-free samples), possible incomplete removal of carbonates; alternatively use 0.11–0.44 M HOAc; without prior-steps both extractions include soluble, adsorbed and carbonates-bound metals.
Mn oxides-bound	0.1 M NH ₄ OH-HCl, pH 2 (0.01 M HNO ₃)	Selectively dissolving Mn minerals if the method in “Hydroxylamine Hydrochloride” section is used (Chao, 1972), but possibly incomplete removal; for complete removal, 0.1 M NH ₄ OH-HCl at 2000:1 for 2 h suggested. It should not be confused with the method for reducible Mn (Chao and Zhou, 1983).
Humus-bound	0.1 M Na ₄ P ₂ O ₇ , K ₄ P ₂ O ₇ , or NaOH	The extractants were developed for humus-bound Al and Fe, also capable of extracting other forms by complexing; metals associated with humin remaining intact; better used when distinguishing humus-metals from sulfide-bound form is needed; humus present in extracts.
Sulfide-bound and Organic mater-associated	30% H ₂ O ₂ , 30% H ₂ O ₂ /1 M NH ₄ OAc, or 0.7 M NaOCl	By removal of organic matter or sulfide into sulfate by oxidation; not distinguishable between the two forms; use of NH ₄ OAc for preventing resorption; Mn oxides also removed by H ₂ O ₂ and better used after removal of Mn oxides.
Amorphous Fe and Al oxides, poorly crystalline Al silicates (specific adsorbed or physically occluded)	AOD	By completely removing the amorphous phases of Al and Fe oxides and hydroxides, poorly crystalline Al silicates, and part of Mn oxides (AOD); equivalent methods shown in sections “Procedure—Reducible Mn (Chao, 1972)” and “Procedure—Fe, Al, Mn, and Si in Non- and Poorly Crystalline Forms (Chao and Zhou, 1983; Ross et al., 1985)”; the Tiron method should follow an alkaline extraction removing humus.
Free Fe oxides-bound	CBD, 0.2 M (NH ₄) ₂ C ₂ O ₄ /0.2 M H ₂ C ₂ O ₄ /0.1 M ascorbic acid, or 1 M NH ₄ OH-HCl/25% HOAc	By removing free Fe oxides by reduction of Fe ³⁺ , no methods tested as reliable as CBD for this purpose; particle size is a significant factor. Ascorbic-acid method has less trouble with reagent purity, but requires more tests.
Residues	HNO ₃ -HCl-HF (micro-wave), or equivalent acid digestions	The nature of metals in this fraction is unknown.

Table 3–5. Representative and widely used sequential extraction schemes for metals in soil, sediment and environmental samples.

Fractionation schemes†	Remarks
Tessier (Tessier et al., 1979)	
a. 1 M MgCl ₂ , pH 7, 8:1, 1 h	Likely incomplete removal of carbonates (>3–5%); no equivalency between NH ₄ OH-HCl-HOAc and CBD, and the former only selective for Mn oxides and unknown amounts of Fe oxides; not know if Fe and Al oxides and poorly crystalline Al silicates removed; no differentiation between amorphous and crystalline phases for metal oxides; use of NH ₄ OAc for preventing resorption of extracted metal cations; uncertainty on the acid-attack on remaining mineral phases in the oxidation step. The scheme has been most widely applied and/or tested against.
b. 1 M NaOAc, pH 5, 8:1, 5 h	
c. CBD or 0.04 M NH ₄ OH-HCl-25% HOAc, 20:1, 96°C, 6h	
d. 3:5 v/v of 0.02 M HNO ₃ /30% H ₂ O ₂ , pH 2, 8:1, 85°C, 2 h; 3.2 M NH ₄ OAc-20% HNO ₃ , 20:1, 0.5 h	
e. 2:10 v/v of HClO ₄ /HF, 12:1	
SM&T or BCR† (Salomons and Förstner, 1984; Ure et al., 1993; Rauret et al., 1999)	
a. 0.11 M HOAc, 40:1, 16 h	HOAc-extractable including water-soluble, exchangeable, carbonates-bound and specific adsorbed metals, but quantitative basis only for carbonates-bound form; samples with <20% CaCO ₃ -equivalent only; 0.1M NH ₄ OH-HCl at pH 2 being selective for Mn oxides on synthetic samples but incomplete on nature samples; the optimized version of 0.5 M NH ₄ OH-HCl at pH 1.5 for better reproducibility but its selectivity not examined, and amorphous Fe and Al oxides (e.g., ferrihydrite) likely dissolved in the 16-h extraction. The scheme is used as a standard protocol in some European countries.
b. 0.1 M NH ₄ OH-HCl, pH 2, 40:1, 16 h	
c. 2 × (30% H ₂ O ₂ , 10:1, 85°C, 1 h); 1 M NH ₄ OAc, pH 2, 50:1, 16 h	
d. HNO ₃ -HClO ₄ -HF	
Shuman for soil micronutrients (Shuman, 1985)	
a. 1 M Mg(NO ₃) ₂ , pH 7, 10:1, 2 h	Water-soluble and exchangeable are in one fraction; not applicable for calcareous soils because there is no acid-dissolution step, or if used for calcareous samples, complications encountered in the following reducible fraction; the effects of high pH at Step b on metal speciation and subsequent extractability not known; no measures taken for preventing re-sorption; a questionable extraction for crystalline iron oxides by using AOD + 0.1 M ascorbic acid; the residues after step e subject to acid digestion for total metal contents or particle-size fractionation as described in the original paper.
b. 0.7 M NaOCl, pH 8.5, 10:1, 100°C, 0.5 h	
c. 0.1 M NH ₄ OH-HCl, pH 2, 20:1, 0.5 h	
d. AOD	
e. AOD + 0.1 M ascorbic acid, 50:1, 100°C	
Metals in soils (MacLaren and Crawford, 1973; Miller et al., 1986; Krishnamurti et al., 1995)	
a. H ₂ O, 40:1, 16 h	Water soluble and exchangeable as separate fractions; an overnight shaking may be used as a convenience for 0.1 M Mg(NO ₃) ₂ extraction; acid soluble with 0.11 M HOAc or increase solution/soil ratio; 0.1 M Mg(NO ₃) ₂ may be added for preventing resorption in Step c; HH extraction at a higher solution/soil ratio for complete removal of Mn minerals (see “Hydroxylamine Hydrochloride” section); humus-bound metals are comparable with pyrophosphate Al and Fe in “Pyrophosphate” section; the humin- and/or sulfide-bound metals by peroxide oxidation can be replaced by Shuman’s 5% NaOCl (pH 8.5) at 40:1 and 100°C for 0.5 h; the HH methods for non- and poorly crystalline metal oxides and silicates-bound metals uses a lower solution/solid ratio than described in the “Hydroxylamine Hydrochloride” section; reagent purity may impart problems for free Fe-oxides-bound metals by CBD.
b. 1 M Mg(NO ₃) ₂ , pH 7, 40:1, 2 h	
c. 0.11 M HOAc, 40:1, 16 h or 0.4 M HOAc, 40:1, 16 h	
d. 0.1 M NH ₄ OH-HCl, pH 2, 50:1, 0.5 h	
e. 0.1 M K ₄ P ₂ O ₇ , 100:1, 16 h	
f. 30% H ₂ O ₂ -0.1 M Mg(NO ₃) ₂ , pH, 5:1, 85°C, repeat	
g. 0.25 M NH ₄ OH-HCl-0.25 M HCl, 100:1, 16 h	
h. CBD	
i. HNO ₃ -HCl-HF (microwave)	

† SM&T (Standards, Measurements, and Testing program); BCR (Community Bureau of Reference).

fer some problems one way or another. Removing an entire mineral phase with a SCD method to release associated elements cannot reveal the physical and chemical status of extracted elements in the removed mineral phase. The limitations, such as incomplete or multiple-component extraction, of a SCD method are also present (Martin et al., 1987). In organizing a scheme, researchers are often required to change some parameters of the SCD methods for experimental convenience. For example, a single solution/sample ratio for the entire scheme and overnight (or 16 h) shaking are often found in use. Some of these modifications may alter the selectivity or efficiency of the original methods. Furthermore, SCD methods are individually tested for their efficiency and selectivity. When used in sequential fractionation, the extraction sequence itself becomes a factor determining the extraction efficiency (Miller et al., 1986). There are additional challenges in metal fractionation by SCD methods, including impurity of reagents and matrix effects caused by extractants in metal analysis. For example, dithionite and NaOCl were found not suitable for Zn extraction because of reagent contamination (Tessier et al., 1979; Filgueiras et al., 2002).

It should be pointed out that although a fractionation scheme can be applied to a group of elements, which have similar chemistry behaviors, it should not be used to extract multiple elements differing intrinsically in chemistry in the matrix from which the elements are extracted. Selenium in natural environments, for example, is present with multiple oxidation states (valences), and its fractionation is unique, different from those listed in Table 3–5 (Wright et al., 2003).

REFERENCES

- Acebal, S.G., A. Mijovilovich, E.H. Rueda, M.E. Aguirre, and C. Saragovi. 2000. Iron-oxide mineralogy of a mollisol from Argentina: A study by selective-dissolution techniques, X-ray diffraction, and Mössbauer spectroscopy. *Clays Clay Miner.* 48:322–330.
- Agriculture Canada Expert Committee Soil Survey. 1987. The Canadian system of soil classification. 2nd ed. Agric. Can. Publ. 1646. National Research Council of Canada, Ottawa.
- Alexander, E.B. 1974. Extractable iron in relation to soil age on terraces along the Truckee river, Nevada. *Soil Sci. Soc. Am. Proc.* 38:121–124.
- Alexiades, C.A., and M.L. Jackson. 1966. Quantitative clay mineralogical analysis of soils and sediments. *Clays Clay Miner.* 14:35–52.
- Alexandrova, L.N., O.V. Yurlova, and L.V. Lobitskaya. 1964. The distribution and composition of the humus substances and their organo-mineral derivatives in the granulometric fractions in some types of soils. *Trans. 8th Int. Cong. Soil Sci.* 3:109–116.
- Allison, L.E., and C.D. Moodie. 1965. Carbonate. p. 1379–1396. *In* C.A. Black (ed.) *Methods of soil analysis*. Part 2. Agron. Monogr. 9. ASA, Madison, WI.
- Amonette, J.E., and L.W. Zelazny (ed.) 1994. Quantitative methods in soil mineralogy. Misc. Publ. SSSA, Madison, WI.
- Anderson, J.U. 1963. An improved pretreatment for mineralogical analysis of samples containing organic matter. *Clays Clay Miner.* 10:380–388.
- Anderson, J.U., and G.A. O'Connor. 1972. Production of permanganate ion by sodium hypochlorite treatment to remove soil organic matter. *Soil Sci. Soc. Am. Proc.* 36:973–975.
- Arshad, M.A., R.J. St. Arnaud, and P.M. Huang. 1972. Dissolution of trioctahedral layer silicates by ammonium oxalate, sodium dithionite-citrate-bicarbonate, and potassium pyrophosphate. *Can. J. Soil Sci.* 52:19–26.
- Ashworth, J. 1997. Improvements to two routine methods for calcium carbonate determination in soils. *Commun. Soil Sci. Plant Anal.* 28:841–848.
- Baril, R., and G. Bitton. 1969. Teneurs élevées de fer libre et identification taxonomique de certains sols du Québec contenant de la magnetite. *Can. J. Soil Sci.* 49:1–9.
- Barnhisel, R.I., and P.M. Bertsch. 1989. Chlorites and hydroxy-interlayered vermiculite and smectite. p. 729–788. *In* J.B. Dixon and S.B. Weed (ed.) *Minerals in soil environments*. SSSA Book Ser. 1. 2nd ed. SSSA, Madison, WI.
- Barnhisel, R.I., and J. Harrison. 1976. Estimating lime requirements by a modified hydrogen peroxide potential acidity method. *Soil Testing Lab, Kentucky Agric. Ext. Stn., Lexington*.
- Bascomb, C.L. 1968. Distribution of pyrophosphate extractable iron and organic carbon. *J. Soil Sci.* 19:251–268.

- Beckwith, R.S., and R. Reeve. 1964. Studies on soluble silica in soils. II. The release of monosilicic acid from soils. *Aust. J. Soil Res.* 2:33–45.
- Berigari, M.S., and F.M.S. Al-Any. 1994. Gypsum determination in soils by conversion to water-soluble sodium sulfate. *Soil Sci. Soc. Am. J.* 58:1624–1627.
- Bertsch, P.M., and P.R. Bloom. 1996. Aluminum. p. 517–550. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- Bhattacharyya, T., and S.K. Ghosh. 1986. Comparison of some extractants for quantitative estimation of allophane content in soils. *J. Indian Soc. Soil Sci.* 34:387–392.
- Biermans, V., and L. Baert. 1977. Selective extraction of the amorphous Al, Fe and Si oxides using an alkaline Tiron solution. *Clay Miner.* 12:127–134.
- Blesa, M.A., H.A. Marinovich, E.C. Baumgartner, and A.J.G. Maroto. 1987. Mechanism of dissolution of magnetite by oxalic acid-ferrous ion solutions. *Inorg. Chem.* 26:3713–3717.
- Bloom, P.R., K. Meter, and J.R. Crum. 1985. Titration method for determination of clay-sized carbonates. *Soil Sci. Soc. Am. J.* 49:1070–1073.
- Borggaard, O.K. 1988. Phase identification by selective dissolution techniques. p. 83–98. *In* J.W. Stucki et al. (ed.) *Iron in soils and clay minerals*. NATO ASI Series. Series C, Mathematical and Physical Sciences; Vol. 217. D. Reidel Publ., Dordrecht, The Netherlands.
- Brindley, G.W., and G. Pedro. 1972. Report of the AIPEA nomenclature committee. *Assoc. Int. Etud. Argiles (AIPEA) Newsl.* 7:8–13.
- Callière, S., L. Gatineau, and St. Hénin. 1960. Preparation à base température d'hématite alumineuse. *C. R. Acad. Sci. Séance* 1960:3677–3679.
- Campbell, A.S., and U. Schwertmann. 1985. Evaluation of selective dissolution extractants in soil chemistry and mineralogy by differential X-ray diffraction. *Clay Miner.* 20:515–519.
- Carnicelli, S., A. Mirabella, G. Cecchini, and G. Sanesi. 1997. Weathering of chlorite to a low-charge expandable mineral in a Spodosol on the Apennine mountains, Italy. *Clays Clay Miner.* 45:28–41.
- Chaberek, S., and A.E. Martell. 1959. *Organic sequestering agents*. John Wiley & Sons, New York.
- Chang, S.C., and M.L. Jackson. 1957. Fractionation of soil phosphorus. *Soil Sci.* 84:133–144.
- Chao, T.T. 1972. Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride. *Soil Sci. Soc. Am. Proc.* 36:764–768.
- Chao, T.T., and P.K. Theobald, Jr. 1976. The significance of secondary iron and manganese oxides in geochemical exploration. *Econ. Geol.* 71:1560–1569.
- Chao, T.T., and L. Zhou. 1983. Extraction techniques for selective dissolution of amorphous iron oxides from soils and sediments. *Soil Sci. Soc. Am. J.* 47:225–232.
- Chester, R., and M.J. Hughes. 1967. A chemical technique for the separation of ferro-manganese minerals, carbonate minerals and adsorbed trace elements from pelagic sediments. *Chem. Geol.* 2:249–262.
- Chiarizia, R., and E.P. Horwitz. 1991. New formulations for iron oxides dissolution. *Hydrometallurgy* 27:339–360.
- Chou, L., R. Garrels, and R. Wollast. 1989. Comparative study of the kinetics and mechanisms of dissolution of carbonate minerals. *Chem. Geol.* 78:269–282.
- Cornell, R.M., and P.W. Schindler. 1987. Photochemical dissolution of goethite in acid/oxalate solution. *Clays Clay Miner.* 35:347–352.
- Cornell, R.M., and U. Schwertmann. 1996. *The iron oxides*. VCH Publishers, Weinheim, Germany.
- Cross, A.F., and W.H. Schlesinger. 1995. A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197–214.
- Dahlgren, R.A. 1994. Quantification of allophane and imogolite. p. 430–451. *In* J.E. Amonette and L.W. Zelazny (ed.) *Quantitative methods in soil mineralogy*. Soil Sci. Soc. Am., Madison, WI.
- Davidtz, J.C., and M.E. Sumner. 1965. Blocked charges on clay minerals in subtropical soils. *J. Soil Sci.* 16:270–274.
- Deb, B.C. 1950. The estimations of free iron oxide in soils and clays and their removal. *J. Soil Sci.* 1:212–220.
- DeEndredy, A.S. 1963. Estimation of free iron oxides in soils and clays by a photolytic method. *Clay Miner. Bull.* 9:209–217.
- de Villiers, J.M. 1971. The problem of quantitative determination of allophane in soil. *Soil Sci.* 112:2–7.
- Dion, H.G., P.J.G. Mann, and S.G. Heintze. 1947. The 'easily reducible' manganese of soils. *J. Agric. Sci.* 37:17–22.
- Dixon, J.B., and M.L. Jackson. 1962. Properties of intergradient chlorite-expandable layer silicates in soils. *Soil Sci. Soc. Am. Proc.* 26:358–362.
- Doner, H.E., and W.C. Lynn. 1989. Carbonate, halide, sulfate, and sulfide minerals. p. 279–330. *In*

- J.B. Dixon and S.B. Weed (ed.) Minerals in soil environments. SSSA Book Ser. 1. 2nd ed. SSSA and ASA, Madison, WI.
- Dos Santos Afonso, M., P.J. Morando, M.A. Blesa, S. Banwart, and W. Stumm. 1990. The reductive dissolution of iron oxides by ascorbate. *J. Colloid Interface Sci.* 138:74–82.
- Douglas, L.A., and F. Fiessinger. 1971. Degradation of clay minerals by H_2O_2 treatments to oxidize organic matter. *Clays Clay Miner.* 19:67–68.
- Dreimanis, A. 1962. Quantitative gasometric determination of calcite and dolomite by using Chittick apparatus. *J. Sediment. Petrol.* 32:520–529.
- Drosdoff, M., and C.C. Nikiforoff. 1940. Iron-manganese concretions in Dayton soils. *Soil Sci.* 49:333–345.
- Dudas, M.J., and M.E. Harward. 1971. Effect of dissolution treatment on standard and soil clays. *Soil Sci. Soc. Am. Proc.* 35:134–140.
- El Mahi, Y.E., I.S. Ibrahim, H.M. Abdel Magid, and A.M.A. Eltilib. 1987. A simple method for the estimation of calcium and magnesium carbonates in soils. *Soil Sci. Soc. Am. J.* 51:1152–1155.
- Evangelou, V.P., L.D. Whittig, and K.K. Tanji. 1984. An automated manometric method for quantitative determination of calcite and dolomite. *Soil Sci. Soc. Am. J.* 48:1236–1239.
- Fanning, D.S., and S.N. Burch. 2000. Coastal acid sulfate soils. p. 921–937. *In* R.I. Barnhisel et al. (ed.) Reclamation of drastically disturbed lands. Agron. Mongr. 41. ASA, CSSSA, and SSSA, Madison, WI.
- Farmer, V.C., and B.D. Mitchell. 1963. Occurrence of oxalates in soil clays following hydrogen peroxide treatment. *Soil Sci.* 96:221–229.
- Farmer, V.C., and J.D. Russell. 1990. Structures and genesis of allophanes and imogolite and their distribution in non-volcanic soils. p. 165–178. *In* M.F. de Boedt et al. (ed.) Soil colloids and their associations in aggregates. NATO ASI series. Series B, Physics; Vol. 215. Plenum Press, New York.
- Farmer, V.C., J.D. Russell, and B.F.L. Smith. 1983. Extraction of inorganic forms of translocated Al, Fe, and Si from a podzol Bs horizon. *J. Soil Sci.* 34:571–576.
- Farmer, V.C., B.F.L. Smith, and J.M. Tait. 1977. Alteration of allophane and imogolite by alkaline digestion. *Clay Miner.* 12:195–198.
- Fey, M.V., and J. LeRoux. 1976. Quantitative determinations of allophane in soil clays. p. 451–463. *In* Proc. Int. Clay Conf. 1975 (Mexico City). Vol. 5. Applied Publishing, Ltd., Wilmette, IL.
- Fey, M.V., and J. LeRoux. 1977. Properties and quantitative estimation of poorly crystalline components in sesquioxidic soil clays. *Clays Clay Miner.* 25:285–294.
- Filgueiras, A.V., I. Lavilla, and C. Bendicho. 2002. Chemical sequential extraction for metal partitioning in environmental solid samples. *J. Environ. Monit.* 4:823–857.
- Finkelman, R.B., and D.E. Giffin. 1986. Hydrogen peroxide oxidation: An improved method for rapidly assessing acid-generation potential of sediments and sedimentary rocks. *Reclam. Reveg. Res.* 5:521–534.
- Fitzpatrick, R.W., J. LeRoux, and U. Schwertmann. 1978. Amorphous and crystalline titanium and iron-titanium oxides in synthetic preparations, at near ambient conditions, and in soil clays. *Clays Clay Miner.* 26:189–201.
- Follett, E.A.C., W.J. McHardy, B.D. Mitchell, and B.F.L. Smith. 1965. Chemical dissolution techniques in the study of soil clays. I. & II. *Clay Miner.* 6:23–44.
- Galabetskaya, E., and R. Govorova. 1934. Bleaching of kaolin. *Min. Suir'e.* 9:27.
- Gamble, E.E., and R.B. Daniels. 1972. Iron and silica in water, acid ammonium oxalate, and dithionite extracts of some North Carolina coastal plain soils. *Soil Sci. Soc. Am. Proc.* 36:939–943.
- Ghabru, S.K., A.R. Mermut, and R.J. St. Arnaud. 1990. Isolation and characterization of an iron-rich chlorite-like mineral from soil clays. *Soil Sci. Soc. Am. J.* 54:281–287.
- Goh, T.B., R.J. St. Arnaud, and A.R. Mermut. 1993. Carbonates. p. 177–185. *In* M.R. Carter (ed.) Manual on soil sampling and methods of analysis. Can. Soc. Soil Sci., Ottawa, Canada.
- Golden, D.C., J.B. Dixon, and Y. Kanehiro. 1993. The manganese oxide mineral, lithiophorite, in an Oxisol from Hawaii. *Aust. J. Soil Res.* 31:51–66.
- Greenland, D.J., J.M. Oades, and T.W. Sherwin. 1968. Electron microscope observations of iron oxides in some red soils. *J. Soil Sci.* 19:123–126.
- Grube, W.E., R.M. Smith, R.N. Singh, and A.A. Sobek. 1973. Characterization of coal overburden materials and mine soils in advance of surface mining. p. 134–151. *In* Proc. Res. Appl. Technol. Symp. Mined-Land Reclamat., Pittsburgh, PA. 7–8 Mar. 1973. NCA/BCR, Pittsburgh, PA.
- Han, F.X., and A. Banin. 1995. Selective sequential dissolution techniques for trace metals in arid-zone soils: The carbonate dissolution step. *Commun. Soil Sci. Plant Anal.* 26:553–576.
- Hayashi, H., and M. Yamada. 1990. Kinetics of dissolution of noncrystalline oxides and crystalline clay minerals in a basic Tiron solution. *Clays Clay Miner.* 38:308–314.

- Hearing, J.G., and W. Stumm. 1990. Oxidative and reductive dissolution of minerals. p. 427–465. *In* M.F. Hochella, Jr. and A.F. White (ed.) Mineral–water interface geochemistry. Reviews in Mineralogy Vol. 23. Mineral. Soc. Am. Washington, DC.
- Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* 46:970–976.
- Heron, G., C. Crouzet, A.C.M. Bourg, and T.H. Christensen. 1994. Speciation of Fe(II) and Fe(III) in contaminated aquifer sediments using chemical extraction techniques. *Environ. Sci. Technol.* 28:1698–1705.
- Hietjes, A.H.M., and L. Lijklema. 1980. Fractionation of inorganic phosphates in calcareous sediments. *J. Environ. Qual.* 9:4:5–407.
- Higashi, T. 1983. Characterization of Al/Fe–humus complexes in dystrandepts through comparison with synthetic forms. *Geoderma* 31:277–288.
- Higashi, T., and H. Ikeda. 1974. Dissolution of allophane by acid oxalate solution. *Clay Sci.* 4:205–211.
- Hodges, S.C., and L.W. Zelazny. 1980. Determination of noncrystalline soil components by weight difference after selective dissolution. *Clays Clay Miner.* 28:35–42.
- Holmgren, G.G.S. 1967. A rapid citrate-dithionite extractable iron procedure. *Soil Sci. Soc. Am. Proc.* 31:210–211.
- Holtzclaw, K.M., G. Sposito, and J.D. Rhoades. 1986. Improved selective dissolution method for determining calcite and dolomite in soils. *Soil Sci.* 142:63–68.
- Hsu, P.H. 1989. Aluminum oxides and oxyhydroxides. p. 331–378. *In* J.B. Dixon and S.B. Weed (ed.) Minerals in soil environments. 2nd ed. SSSA, Madison, WI.
- Hudson, R.J.M., and F.M.M. Morel. 1989. Distinguishing between extra- and intracellular Fe in marine phytoplankton. *Limnol. Oceanogr.* 34:1113–1120.
- Iyengar, S.S., L.W. Zelazny, and D.C. Martens. 1981. Effect of photolytic oxalate treatment on soil hydroxy-interlayered vermiculites. *Clays Clay Miner.* 29:429–434.
- Jackson, M.L. 1979. Soil chemical analysis. Advanced course. 2nd ed. published by the author, Madison, WI.
- Jackson, M.L., and T.A. Froking. 1982. Mechanism of terra rossa red coloration. *Clay Res.* 1:1–5.
- Jackson, M.L., C.H. Lim, and L.W. Zelazny. 1986. Oxides, hydroxides, and aluminosilicates. p. 101–150. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA. Madison, WI.
- Jarvis, S.C. 1986. Forms of aluminum in some acid permanent grassland soils. *J. Soil Sci.* 37:211–222.
- Jennings, S.R., D.J. Dollhopf, and W.P. Inskeep. 2000. Acid production from sulfide minerals using hydrogen peroxide weathering. *Appl. Geochem.* 15:235–243.
- Jersak, J.M., and J.G. McColl. 1989. Aluminum release from solid-phase components of forest soil leached with citric acid. *Soil Sci. Soc. Am. J.* 53:550–555.
- Jersak, J.M., J.G. McColl, and J.F. Hetzel. 1992. Changes in extractability of iron, aluminum and silicon and dispersibility by storage of California forest soils. *Commun. Soil Sci. Plant Anal.* 23:993–1018.
- Jones, R.C., and G. Uehara. 1973. Amorphous coating on mineral surfaces. *Soil Sci. Soc. Am. Proc.* 37:792–798.
- Jones, R.L., and A.H. Beavers. 1963. Silt-size whewellite and weddellite by soil peroxidation. *Soil Sci. Soc. Am. Proc.* 27:356–357.
- Kaiser, K., and W. Zech. 1996. Defects in estimation of aluminum in humus complexes of podzolic soils by pyrophosphate extraction. *Soil Sci.* 161:452–458.
- Kassim, J.K., S.N. Gafoor, and W.A. Adams. 1984. Ferrihydrite in pyrophosphate extracts of podzol B horizons. *Clay Miner.* 19:99–106.
- Keller, L.P., G.J. McCarthy, and J.L. Richardson. 1986a. Laboratory modeling of Northern Great Plains salt efflorescence mineralogy. *Soil Sci. Soc. Am. J.* 50:1363–1367.
- Keller, L.P., G.J. McCarthy, and J.L. Richardson. 1986b. Mineralogy and stability of soil evaporates in North Dakota. *Soil Sci. Soc. Am. J.* 50:1069–1071.
- Kersten, M., and U. Förstner. 1995. Speciation of trace metals in sediments and combustion waste. p. 234–275. *In* A.M. Ure and C.M. Davidson (ed.) Chemical speciation in the environment. Blackie Academic & Professional, Glasgow, UK.
- Knabe, W. 1964. Methods and results of strip-mine reclamation in Germany. *Ohio J. Sci.* 64:75–105.
- Kodama, H., and H. Hayashi. 1985. Comparative study on chemical dissolution techniques—Application to identification and quantitative determination of noncrystalline silicates. *Clay Sci.* 25:139–147.

- Kodama, H., and M. Jaakkimainen. 1981. A comparative study of selective chemical dissolution methods for separating non-crystalline components produced by grinding of silicates. p. 399–410. *In* H. Van Olphen and F. Veniale (ed.) *Inter. Clay Conf.*, Bologna, Italy. Elsevier Science, New York.
- Kodama, H., L.S. Kotlyar, and J.A. Ripmeester. 1989. Quantification of crystalline and noncrystalline material in ground kaolinite by x-ray powder diffraction, infrared, solid-state nuclear magnetic resonance, and chemical-dissolution analyses. *Clays Clay Miner.* 37:364–370.
- Kodama, H., and G.J. Ross. 1991. Tiron dissolution method used to remove and characterize inorganic components in soils. *Soil Sci. Soc. Am. J.* 55:1180–1187.
- Kodama, H., and C. Wang. 1989. Distribution and characterization of noncrystalline inorganic components in Spodosols and Spodosol-like soils. *Soil Sci. Soc. Am. J.* 53:526–534.
- Krishnamurti, G.S.R., P.M. Huang, K.C.J. Van Rees, L.M. Kozak, and H.P. Rostad. 1995. Speciation of particulate-bound cadmium of soils and its bioavailability. *Analyst* 120:659–665.
- Kunze, G.W., and J.B. Dixon. 1986. Pretreatment for mineralogical analysis. p. 91–100. *In* A. Klute (ed.) *Methods of soil analysis. Part 1.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Kuo, S. 1996. Phosphorus. p. 869–920. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part. 3. Chemical methods.* SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- Kurmies, B. 1972. Zur fractionierung der bodenphosphate. *Phosphorsäure* 29:118–151.
- Lavkulich, L.M., and J.H. Wiens. 1970. Comparison of organic matter destruction by hydrogen peroxide and sodium hypochlorite and its effects on selected mineral constituents. *Soil Sci. Soc. Am. Proc.* 34:755–758.
- Lee, R., M.D. Taylor, B.K. Daly, and J. Reynolds. 1989. The extraction of Al, Fe and Si from a range of New Zealand soils by hydroxylamine and ammonium oxalate solutions. *Aust. J. Soil Res.* 27:377–388.
- LeRiche, H.H., and A.H. Weir. 1963. A method for studying trace elements in soil fractions. *J. Soil Sci.* 14:225–235.
- Lin, C., M.D. Melville, I. White, and Y.P. Hsu. 1996. Comparison of three methods of estimation of the reduced-S content in estuarine sediments. *Sci. Total Environ.* 187:1–9.
- Loeppert, R.H., C.T. Hallmark, and M.M. Koshy. 1984. Routine procedure for rapid determination of soil carbonates. *Soil Sci. Soc. Am. J.* 48:1030–1033.
- Loeppert, R.L., and W.P. Inskeep. 1996. Iron. p. 639–664. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part. 3. Chemical methods.* SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- Loeppert, R.H., and D.L. Suarez. 1996. Carbonate and gypsum. p. 437–474. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods.* SSSA Book Ser. 5. ASA and SSSA, Madison, WI.
- Lomi, E., R.S. Sletten, and W.W. Wenzel. 2000. Sequentially extracted arsenic from different size fractions of contaminated soils. *Water Air Soil Pollut.* 124:319–332.
- Loveland, P.J. 1988. The assay for iron in soils and clay minerals. p. 99–140. *In* J.W. Stucki et al. (ed.) *Iron in soils and clay minerals.* NATO ASI series. Series C, Mathematical and physical sciences; Vol. 217. D. Reidel Publ., Dordrecht, The Netherlands.
- Loveland, P.J., and P. Digby. 1984. The extraction of Fe and Al by 0.1 M pyrophosphate solutions: A comparison of some techniques. *J. Soil Sci.* 35:243–250.
- Lund, K., H.S. Fogler, and C.C. McCune. 1973. Acidization—I. The dissolution of dolomite in hydrochloric acid. *Chem. Eng. Sci.* 28:691–700.
- Lund, K., H.S. Fogler, C.C. McCune, and J.W. Ault. 1975. Acidization—II. The dissolution of calcite in hydrochloric acid. *Chem. Eng. Sci.* 30:825–835.
- MacKenzie, R.C. 1954. Free iron-oxide removal from soils. *J. Soil Sci.* 5:167–172.
- MacLaren, R.G., and D.V. Crawford. 1973. Studies on soil copper. I. The fractionation of copper in soils. *J. Soil Sci.* 24:172–181.
- Makumbi, M.N., and M.L. Jackson. 1977. Weathering of Karroo argillite under equatorial conditions. *Geoderma* 19:181–197.
- Mann, P.J.G., and J.H. Quastel. 1946. Manganese metabolism in soils. *Nature* 158:154–156.
- Martin, J.M., P. Nirel, and A.J. Thomas. 1987. Sequential extraction techniques: Promises and problems. *Mar. Chem.* 22:313–341.
- Martin, R.T. 1954. Calcium oxalate formation in soil from hydrogen peroxide treatment. *Soil Sci.* 77:143–145.
- Martin, S.E., and R. Reeve. 1955. A rapid manometric method for determining soil carbonate. *Soil Sci.* 79:187–197.
- Matsue, N., and K. Wada. 1988. Interlayer materials of partially interlayered vermiculites in Dystrochrepts derived from Tertiary sediments. *J. Soil Sci.* 39:155–162.

- McAlister, J.J., and B.J. Smith. 1999. Selectivity of ammonium acetate, hydroxylamine hydrochloride, and oxalate/ascorbic acid solutions for the speciation of Fe, Mn, Zn, Cu, Ni, and Al in Early Tertiary paleosols. *Microchem. J.* 63:415–426.
- McKeague, J.A. 1967. An evaluation of 0.1 M pyrophosphate and pyrophosphate-dithionite in comparison with oxalate as extractants of the accumulation products in Podzols and some other soils. *Can. J. Soil Sci.* 47:95–99.
- McKeague, J.A., J.E. Brydon, and N.M. Miles. 1971. Differentiation of forms of extractable iron and aluminum in soils. *Soil Sci. Soc. Am. Proc.* 35:33–38.
- McKeague, J.A., and J.H. Day. 1966. Dithionite- and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. *Can. J. Soil Sci.* 46:13–22.
- McKeague, J.A., and P.A. Schuppli. 1982. Changes in concentration of iron and aluminum in pyrophosphate extracts of soil and composition of sediment resulting from ultracentrifugation in relation to spodic horizon criteria. *Soil Sci.* 134:265–270.
- McLaren, R.G., R. Naidu, J. Smith, and K.G. Tiller. 1998. Fractionation and distribution of arsenic in soils contaminated by cattle dip. *J. Environ. Qual.* 27:348–354.
- Mehra, O.P., and M.L. Jackson. 1960. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. *Clays Clay Miner.* 7:317–327.
- Mendelovici, E., and A. Sagarzazu. 1991. Characteristics of products from the acid ammonium oxalate treatment of manganese minerals. *Clays Clay Miner.* 39:264–269.
- Mikutta, R., M. Kleber, K. Kaiser, and R. Jahn. 2005. Review: Organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate. *Soil Sci. Soc. Am. J.* 69:120–135.
- Miller, W.P., D.C. Martens, and L.W. Zelazny. 1986. Effects of sequence in extraction of trace metals from soils. *Soil Sci. Soc. Am. J.* 50:598–601.
- Misopolinos, N.D., and J.M. Kalovoulos. 1984. Determination of CEC and exchangeable Ca and Mg in non-saline calcareous soils. *J. Soil Sci.* 35:93–98.
- Morse, J.W., and R.S. Arvidson. 2002. The dissolution kinetics of major sedimentary carbonate minerals. *Earth Sci. Rev.* 58:51–84.
- Neaman, A., B. Waller, F. Mouélé, F. Trolard, and G. Bourrié. 2003. Improved methods for selective dissolution of manganese oxides from soils and rocks. *Eur. J. Soil Sci.* 54:1–8.
- Nelson, R.E. 1982. Carbonate and gypsum. p. 181–197. *In* A.L. Page et al. (ed.) *Methods of soil analysis. Part 2.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Nierode, D.E., and B.B. Williams. 1971. Characteristics of acid reaction in limestone formations. *Soc. Pet. Eng. J.* 11:406–417.
- Nommik, H. 1974. Examination of procedures for determining carbonates in soils and related materials. *Commun. Soil Sci. Plant Anal.* 5:319–330.
- Norrish, K., and R.M. Taylor. 1961. The isomorphous replacement of iron by aluminum in soil goethites. *J. Soil Sci.* 12:294–306.
- Oades, J.M. 1963. The nature and distribution of iron compounds in soils. *Soils Fert.* 26:69–80.
- Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. p. 403–430. *In* A.L. Page et al. (ed.) *Methods of soil analysis. Part 2.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Omuetti, J.A.I. 1980. Sodium hypochlorite treatment for organic matter destruction in tropical soils of Nigeria. *Soil Sci. Soc. Am. J.* 44:878–880.
- Orndorff, Z. 2001. Evaluation of sulfidic materials in Virginia highway corridors. Ph.D. diss. ed-10042001–123731. Dep. Crop and Soil Environ. Sci., Virginia Polytechnic Institute and State University, Blacksburg.
- O'Shay, T., L.R. Hossner, and J.B. Dixon. 1990. A modified hydrogen peroxide oxidation method for determination of potential acidity in pyritic overburden. *J. Environ. Qual.* 19:778–782.
- Paktunc, A.D. 1999. Mineralogical constraints on the determination of neutralization potential and prediction of acid mine drainage. *Environ. Geol.* 39:103–112.
- Parfitt, R.L. 1989. Optimum conditions for extraction of Al, Fe, and Si from soils with acid oxalate. *Commun. Soil Sci. Plant Anal.* 20:801–816.
- Parfitt, R.L. 1990. Allophane in New Zealand—A review. *Aust. J. Soil Res.* 28:343–360.
- Parfitt, R.L., and C.W. Childs. 1988. Estimation of forms of Fe and Al: A review and analysis of contrasting soils by dissolution and Mössbauer methods. *Aust. J. Soil Res.* 26:121–144.
- Parfitt, R.L., and T. Henmi. 1982. Comparison of an oxalate-extraction method and an infrared spectroscopic method for determining allophane in soil clays. *Soil Sci. Plant Nutr.* 28:183–190.
- Pawluk, S. 1972. Measurement of crystalline and amorphous iron removal in soils. *Can. J. Soil Sci.* 52:119–123.
- Petersen, G.W., and G. Chesters. 1966. Quantitative determination of calcite and dolomite in pure carbonates and limestones. *J. Soil Sci.* 17:317–327.

- Petersen, G.W., G. Chesters, and G.B. Lee. 1966. Quantitative determination of calcite and dolomite in soils. *J. Soil Sci.* 17:328–338.
- Pickering, W.P. 1981. Selective chemical extraction of soil components and bound metal species. *Crit. Rev. Anal. Chem.* 12:223–266.
- Plass, W.T. 2000. History of surface mining reclamation and associated legislation. p. 1–20. *In* R.I. Barnhisel et al. (ed.) Reclamation of drastically disturbed lands. Agron. Monogr. 41. ASA, CSSSA, and SSSA, Madison, WI.
- Plummer, L.N., and E. Busenberg. 1982. The solubilities of calcite, aragonite and vaterite in CO_2 – H_2O solutions between 0 and 90°C, and an evaluation of the aqueous model for the system CaCO_3 – CO_2 – H_2O . *Geochim. Cosmochim. Acta* 46:1011–1040.
- Plummer, L.N., D.L. Parkhurst, and T.M.L. Wigley. 1979. Critical review of the kinetics of calcite dissolution and precipitation. p. 537–573. *In* E. Jenne (ed.) Chemical modeling—Speciation, sorption and solubility, and kinetics in aqueous systems. Am. Chem. Soc., Washington, DC.
- Porta, J. 1998. Methodologies for the analysis and characterization of gypsum in soils: A review. *Geoderma* 87:31–46.
- Psenner, R., and R. Pucsko. 1988. Phosphorus fractionation: Advantages and limits of the method for the study of sediments P origins and interactions. *Adv. Limnol.* 30:43–59.
- Psenner, R., R. Pucsko, and M. Aager. 1984. Die fraktionierung organischer und anorganischer phosphorverbindungen von sedimenten. *Arch. Hydrobiol. Suppl.* 70:111–155.
- Raad, A.A. 1978. Carbonates. p. 86–98. *In* J.A. McKeague (ed.) Manual on soil sampling and methods of analysis. Can. Soc. Soil Sci., Ottawa, Canada.
- Rauret, G., J.F. López-Sánchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, and Ph. Quevauviller. 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.* 1:57–61.
- Reyes, I., and J. Torrent. 1997. Citrate-ascorbate as a highly selective extractant for poorly crystalline iron oxides. *Soil Sci. Soc. Am. J.* 61:1647–1654.
- Rhoades, J.D. 1996. Salinity: Electrical conductivity and total dissolved solids. p. 417–435. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 5. Chemical methods. Part 3. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- Rhoton, F.E., J.M. Bigham, L.D. Norton, and N.E. Smek. 1981. Contribution of magnetite to oxalate-soluble iron in soils and sediments from the Maumee River basin of Ohio. *Soil Sci. Soc. Am. J.* 45:645–649.
- Rivers, E.D., C.T. Hallmark, L.T. West, and L.R. Drees. 1982. A technique for rapid removal of gypsum from soil samples. *Soil Sci. Soc. Am. J.* 46:1338–1340.
- Ross, G.J., and C. Wang. 1993. Extractable Al, Fe, Mn, and Si. p. 239–246. *In* M.R. Carter (ed.) Manual on soil sampling and methods of analysis. Can. Soc. Soil Sci., Ottawa, Canada.
- Ross, G.J., C. Wang, and P.A. Schuppli. 1985. Hydroxylamine and ammonium oxalate solutions as extractants for iron and aluminum from soils. *Soil Sci. Soc. Am. J.* 49:783–785.
- Roth, C.B., M.L. Jackson, E.G. Lotse, and J.K. Syers. 1968. Ferrous-ferric ratio and CEC changes on deferration of weathered micaceous vermiculite. *Isr. J. Chem.* 6:261–273.
- Roth, C.B., M.L. Jackson, and J.K. Syers. 1969. Deferration effect on structural ferrous-ferric iron ratio and CEC of micaceous vermiculites and soils. *Clays Clay Miner.* 17:253–264.
- Rubini, P., A. Lakatos, D. Champmartin, and T. Kiss. 2002. Speciation and structural aspects of interactions of Al(III) with small biomolecules. *Coord. Chem. Rev.* 228:137–152.
- Russell, J.D., B.A. Goodman, and A.R. Fraser. 1979. Infrared and Mössbauer studies of reduced nontronites. *Clays Clay Miner.* 27:63–71.
- Russell, M., R.L. Parfitt, and G.G.C. Claridge. 1981. Estimation of the amounts of allophane and other materials in the clay fraction of an Egmont loam profile and other volcanic ash soils, New Zealand. *Aust. J. Soil Res.* 19:185–195.
- Ruttenberg, K.C. 1992. Development of a sequential extraction method for different forms of phosphorus in marine sediments. *Limnol. Oceanogr.* 37:1460–1482.
- Ryan, J.N., and P.M. Gschwend. 1991. Extraction of iron oxides from sediments using reductive dissolution by titanium (III). *Clays Clay Miner.* 39:509–518.
- Rydin, E., and E.B. Welch. 1998. Aluminum dose required to inactivate phosphate in lake sediments. *Water Res.* 32:2969–2976.
- Salomons, W., and U. Förstner. 1984. Metals in the hydrocycle. Springer-Verlag, Berlin.
- Schwertmann, U. 1959. Die fraktionierte Extraktion der freien Eisenoxide in Boden, ihre mineralogischen Formen und ihre Entstehungsweisen. *Z. Pflanzenernähr. Dueng. Bodenk.* 84:194–204.
- Schwertmann, U. 1964. Differenzierung der Eisenoxide des Bodens durch photochemische Extraktion mit saurer Ammoniumoxalate-Lösung. *Z. Pflanzenernähr. Dueng. Bodenk.* 105:194–202.
- Schwertmann, U. 1973. Use of oxalate for Fe extraction from soils. *Can. J. Soil Sci.* 53:244–246.

- Schwertmann, U., R.W. Fitzpatrick, and J. LeRoux. 1977. Al substitution and differential disorder in soil hematites. *Clays Clay Miner.* 25:373–374.
- Schwertmann, U., D.G. Schulze, and E. Murad. 1982. Identification of ferrihydrite in soils by dissolution kinetics, differential x-ray diffraction and Mössbauer spectroscopy. *Soil Sci. Soc. Am. J.* 46:869–875.
- Sherman, L.A., and P. Barak. 2000. Solubility and dissolution kinetics of dolomite in $\text{Ca-Mg-HCO}_3/\text{CO}_2$ solutions at 25°C and 0.1 M Pa carbon dioxide. *Soil Sci. Soc. Am. J.* 64:1959–1968.
- Shuman, L.M. 1982. Separating soil iron- and manganese-oxide fractions for microelement analysis. *Soil Sci. Soc. Am. J.* 46:1099–1102.
- Shuman, L.M. 1983. Sodium hypochlorite methods for extracting microelements associated with soil organic matter. *Soil Sci. Soc. Am. J.* 47:656–660.
- Shuman, L.M. 1985. Fractionation method for soil microelements. *Soil Sci.* 140:11–22.
- Sjöberg, E.L., and D.T. Rickard. 1984. Temperature dependence of calcite dissolution kinetics between 1 and 62°C at pH 2.7 to 8.4 in aqueous solutions. *Geochim. Cosmochim. Acta* 48:485–493.
- Skarie, R.L., J.L. Arndt, and J.L. Richardson. 1987. Sulfate and gypsum determination in saline soils. *Soil Sci. Soc. Am. J.* 51:901–905.
- Skinner, S.I.M., R.L. Halstead, and J.E. Brydon. 1959. Quantitative manometric determination of calcite and dolomite in soils and limestones. *Can. J. Soil Sci.* 39:197–204.
- Sobek, A.A., W.A. Schuller, J.R. Freeman, and R.M. Smith. 1978. Field and laboratory methods applicable to overburden and minespoils. EPA Publ. 600/2-78-054. U.S. Gov. Print. Office, Washington, DC.
- Sobek, A.A., J.G. Skousen, and S.E. Fisher, Jr. 2000. Chemical and physical properties of overburdens and minespoils. p. 77–104. *In* R.I. Barnhisel et al. (ed.) Reclamation of drastically disturbed lands. Agron. Monogr. 41. ASA, CSSSA, and SSSA, Madison, WI.
- Soil Survey Staff. 1975. Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys. U.S. Gov. Printing Office, Washington, DC.
- Soukup, D.A., B.J. Buck, and W. Harris. 2008. Preparing soils for mineralogical analyses. p. 13–32. *In* A.L. Ulery and L.R. Drees (ed.) Methods of soil analysis. Part 5. Mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Sparks, D.L. (ed.) 1996. Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- St. Arnaud, R.J., and A.J. Herbillon. 1973. Occurrence and genesis of secondary magnesium-bearing calcites in soils. *Geoderma* 9:279–298.
- Stern, R., N. Alperovitch, and G.J. Levy. 1989. Rapid removal of gypsum by resin prior to particle-size-distribution analysis in soils. *Soil Sci.* 148:448–451.
- Strouse, J., S.W. Layten, and C.E. Strouse. 1977. Structural studies of transition metal complexes of trionized and tetraionized citrate. Models for the coordination of the citrate ion to transition metal ions in solution and at the active site of aconitase. *J. Am. Chem. Soc.* 99:562–572.
- Stucki, J.W., C.B. Roth, and W.E. Baitinger. 1976. Analysis of iron-bearing clay minerals by electron spectroscopy for chemical analysis (ESCA). *Clays Clay Miner.* 24:289–292.
- Stumm, W., and J.J. Morgan. 1981. Aquatic chemistry. John Wiley & Sons, New York.
- Suter, D., S. Banwart, and W. Stumm. 1991. Dissolution of hydrous (III) oxides by reductive mechanisms. *Langmuir* 7:809–813.
- Swift, R.S. 1996. Organic matter characterization. p. 1011–1069. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- Tamm, O. 1922. Eine methode zur bestimmung der anorganischen komponenten des gelkomplexes in boden. *Medd. Statens Skogförsöksanstalt* 19:385–404.
- Taylor, R.M., and R.M. McKenzie. 1966. The association of trace elements with manganese minerals in Australian soils. *Aust. J. Soil Res.* 4:29–39.
- Tessier, A., P.G.C. Campbell, and M. Bisson. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Can. Anal. Chem.* 51:844–851.
- Theng, B.K.G., G.G. Ristori, C.A. Santi, and H.J. Percival. 1999. An improved method for determining the specific surface areas of topsoils with varied organic matter content, texture and clay mineral composition. *Eur. J. Soil Sci.* 50:309–316.
- Tiessen, H., and J.O. Moir. 1993. Characterization of available P by sequential extraction. p. 75–86. *In* M.R. Carter (ed.) Manual on soil sampling and methods of analysis. Can. Soc. Soil Sci., Ottawa, Canada.
- Tokashiki, Y., J.B. Dixon, and D.C. Golden. 1986. Manganese oxide analysis in soils by combined x-ray diffraction and selective dissolution methods. *Soil Sci. Soc. Am. J.* 50:1079–1084.
- Tokashiki, Y., T. Hentona, M. Shimo, and L.P. Vidhana Arachchi. 2003. Improvement of the successive selective dissolution procedure for the separation of birnessite, lithiophorite, and goethite in

- soil manganese nodules. *Soil Sci. Soc. Am. J.* 67:837–843.
- Tokashiki, Y., and K. Wada. 1972. Determination of silicon, aluminum and iron dissolved by successive and selective dissolution treatments of volcanic ash soil clays. *Clay Sci.* 4:105–114.
- Trolard, F., G. Bourrie, E. Jeanroy, A.J. Herbillon, and H. Martin. 1995. Trace metals in natural iron oxides from laterites: A study using selective kinetic extraction. *Geochim. Cosmochim. Acta* 59:1285–1297.
- Ure, A.M., Ph. Quevauviller, H. Muntau, and B. Griepink. 1993. Improvements in the determination of extractable contents of trace metals in soil and sediment prior to certification. CEC/BCR Information Report, EUR 14763 EN, Brussels.
- USDA-SCS. 1984. Procedures for collecting soil samples and methods of analysis for soil survey. Soil Survey Invest. Rep. 1. (Rev.) U.S. Gov. Print. Office, Washington, DC.
- U.S. Salinity Laboratory Staff. 1954. Diagnosis and improvement of saline and alkali soils. USDA Handb. 60. USDA, Washington, DC.
- van Olphen, H. 1971. Amorphous clay materials. *Science* 171:91–92.
- van Oorschot, I.H.M., and M.J. Dekkers. 2001. Selective dissolution of magnetic iron oxides in the acid-ammonium oxalate/ferrous iron extraction method. I. Synthetic samples. *Geophys. J. Int.* 145:740–748.
- Veith, J.A., and M.L. Jackson. 1974. Iron oxidation and reduction effects on structural hydroxyl and layer charge in aqueous suspensions of micaceous vermiculites. *Clays Clay Miner.* 22:345–353.
- Wada, K. 1989. Allophane and imogolite. p. 1051–1087. *In* J.B. Dixon and S.B. Weed (ed.) *Minerals in soil environments*. 2nd ed. SSSA Book Ser. 1. SSSA, Madison, WI.
- Wada, S.I., and S. Furumura. 1994. Microdiffusion method for determination of small amount of carbonate in noncalcareous soils: Development and evaluation. *Soil Sci. Plant Nutr.* 41:141–145.
- Wada, K., and D.J. Greenland. 1970. Selective dissolution and differential infrared spectroscopy for characterization of ‘amorphous’ constituents in soil clays. *Clay Miner.* 8:241–254.
- Wada, K., and Y. Tokashiki. 1972. Selective dissolution and differential infrared spectroscopy in quantitative mineralogical analysis of volcanic-ash soil clays. *Geoderma* 7:199–213.
- Wang, C., H. Kodama, and N.M. Miles. 1981. Effect of various pretreatments on x-ray diffraction patterns of clay fractions of podzolic B horizons. *Can. J. Soil Sci.* 61:311–316.
- Wang, C., J.A. McKeague, and H. Kodama. 1986. Pedogenic imogolite and soil environments: Case study of Spodosols in Quebec, Canada. *Soil Sci. Soc. Am. J.* 50:711–718.
- Wang, C., and P.A. Schuppli. 1986. Determining ammonium oxalate-extractable Si in soils. *Can. J. Soil Sci.* 66:751–755.
- Wang, C., P.A. Schuppli, and G.J. Ross. 1987. A comparison of hydroxylamine and ammonium oxalate solutions as extractants for Al, Fe, and Si from spodosols and spodosol-like soils in Canada. *Geoderma* 40:345–355.
- Wang, H.D., G.N. White, F.T. Turner, and J.B. Dixon. 1993. Ferrihydrite, lepidocrocite, and goethite in coatings from east Texas vertic soils. *Soil Sci. Soc. Am. J.* 57:1381–1386.
- Weaver, R.M., J.K. Syers, and M.L. Jackson. 1968. Determination of silica in citrate-bicarbonate-dithionite extracts of soils. *Soil Sci. Soc. Am. Proc.* 32:497–501.
- Williams, D.E. 1948. A rapid manometric method for the determination of carbonate in soils. *Soil Sci. Soc. Am. Proc.* 13:127–129.
- Williams, J.D.H., T. Mayer, and J.O. Nriagu. 1980. Extractability of phosphorus from phosphate minerals common in soils and sediments. *Soil Sci. Soc. Am. J.* 44:462–464.
- Williams, J.D.H., J.K. Syers, R.F. Harris, and D.E. Armstrong. 1971. Fractionation of inorganic phosphate in calcareous lake sediments. *Soil Sci. Soc. Am. Proc.* 35:250–255.
- Wilson, M.J. 1987. X-ray powder diffraction methods. p. 26–98. *In* M.J. Wilson (ed.) *A handbook of determinative methods in clay mineralogy*. Chapman and Hall, New York.
- Winters, E. 1938. Ferromanganiferous concretions from some podzolic soils. *Soil Sci.* 46:33–40.
- Wright, M.T., D.R. Parker, and C. Amrhein. 2003. Critical evaluation of the ability of sequential extraction procedures to quantify discrete forms of selenium in sediments and soils. *Environ. Sci. Technol.* 37:4709–4716.
- Yoneda, S. 1961. Studies on polder soils in Japan. XVI. Rapid method for determining oxidizable sulfur and change of soil reaction of sea muds and polder soils. *Soil Sci. Rep. Fac. Agric. Okayama Univ.* 17:39–46.
- Zelazny, L.W., and J.N. Qureshi. 1972. Chemical pretreatment effects on clay separation and mineralogical analysis of selected Florida soils. *Proc. Soil Crop Sci. Soc. Fla.* 32:117–121.
- Zinder, B., G. Furrer, and W. Stumm. 1986. The coordination chemistry of weathering: II. Dissolution of Fe(III) oxides. *Geochim. Cosmochim. Acta* 50:1861–1869.
- Zipper, C.E. 2000. Coal mine reclamation, acid mine drainage, and the clean water act. p. 169–191. *In*

- R.I. Barnhisel et al. (ed.) Reclamation of drastically disturbed lands. Agron. Monogr. 41. ASA, CSSSA, and SSSA, Madison, WI.
- Zwarich, M.A., and J.G. Mills. 1971. Evaluation of three methods for the quantitative determination of calcite and dolomite in soils and geological sediments. *Can. J. Earth Sci.* 8:967–972.

X-ray Diffraction Techniques for Soil Mineral Identification

WILLIE HARRIS, University of Florida, Gainesville

G. NORMAN WHITE, Texas Commission on Environmental Quality, Austin

Soil mineralogy is determined routinely because of its strong influence on soil behavior, its use in soil classification, and its relevance to soil genetic processes. Soils commonly contain primary minerals, which are formed from magma and provide insight into characteristics such as parent material provenance, uniformity, and weathering rates. Soils also contain secondary minerals, which are formed from weathering processes, and may have crystallographic characteristics that strongly influence the physical and chemical properties of soils. For example, some phyllosilicates are termed *expansible* because they adsorb water and cations on interior surfaces within the crystal structure itself. Expansion of the interior surfaces, or interlayers, imparts high cation exchange capacity (CEC) relative to nonexpansible phyllosilicates and most other secondary minerals. X-ray diffraction (XRD) is the technique most heavily relied on in soil mineralogical analysis.

X-ray diffraction is a technique that provides detailed information about the atomic structure of crystalline substances. It is a powerful tool in the identification of minerals in rocks and soils. The bulk of the clay fraction of many soils is crystalline, but clay particles are too small for optical crystallographic methods to be applied. Therefore, XRD has long been a mainstay in the identification of clay-sized minerals in soils. However, its usefulness extends to coarser soil fractions as well. X-ray diffraction analysis can be conducted on single crystals or powders. This chapter will be devoted to X-ray powder diffraction (Reynolds, 1989a), since that is the technique most applicable to soil mineralogy.

The objective of this chapter is to provide a detailed procedural reference for soil mineralogical determination by XRD. This chapter will emphasize well-established techniques, as well as some of the more recent innovative procedures. Alternative approaches will be cited to the extent possible, along with their advantages and disadvantages. We are indebted to preceding works addressing XRD methodologies applicable to soils (Jackson, 1956; Brown and Brindley, 1980; Whittig and Allardice, 1986).

Some familiarity of principles underlying XRD analysis is advisable before undertaking the procedures and interpretations. Many sources are available that address these principles in detail (e.g., Klug and Alexander, 1974; Cullity, 1978). Other useful sources of information about XRD theory and interpretation applied specifically to powder methods include Bish and Post (1989), Zevin and Kimmel (1995), and Moore and Reynolds (1997). The following section provides a brief introduction to some fundamental aspects of XRD.

PRINCIPLES OF X-RAY DIFFRACTION

Generation of X-rays

The part of the electromagnetic spectrum ranging in wavelength from approximately 10^{-3} to 10 nm is considered X-radiation, or X-rays. X-rays are produced by the rapid deceleration of fast-moving electrons as they impinge on matter (Klug and Alexander, 1974). Production of X-rays for XRD analysis is accomplished using an X-ray tube consisting of a filament electron source and a metal target (Fig. 4-1). The tubes are evacuated to minimize absorption of electrons accelerated from the filament (cathode) to the target (anode). Activation of the tube entails passing a current through the filament to establish a current (e.g., 10–30 mA) under high voltage (e.g., 30–50 kV) between the filament and target. X-rays generated from the target during the rapid deceleration of electrons from the filament emerge from windows in the tube. The material comprising the window has a minimal tendency to absorb X-rays.

X-ray diffraction analysis uses monochromatic radiation. Intense X-radiation at a specific wavelength can be produced when electrons from a source (e.g., tube filament) dislodge inner shell electrons from the atoms of the metal target. Instantaneous replacement of the dislodged electron by an electron from a specific lower energy shell results in the quantum release of a packet of energy corresponding to a specific wavelength and termed *characteristic radiation* (Fig. 4-2). The characteristic radiation used in XRD, termed $K\alpha$, is produced by replacement of K shell electrons by L shell electrons. The $K\alpha$ wavelength (λ) is element specific and relates to the atomic number of the element selected for the target in the X-ray tube. Hence, tubes can be selected based on the wavelength that is most advantageous for the material being analyzed by XRD (see "Selection of X-ray Wavelength" below).

An X-ray tube also generates X-rays at other wavelengths in addition to the $K\alpha$ radiation of its metal target. These include (i) a continuous spectrum across a broad range of wavelengths and (ii) another significant monochromatic intensity maximum arising from K shell electrons being replaced by M shell electrons ($K\beta$) (Fig. 4-2). Modern XRD instruments (X-ray diffractometers) are equipped with filters and/or monochromators that reduce relative amounts of unwanted radiation.

X-ray Diffraction

X-ray diffraction occurs when X-rays are scattered by atoms arranged in an orderly array in crystals. The atoms serve as scattering centers (Moore and Reynolds, 1997), re-emitting X-rays at the same wavelength as the incident radiation in all directions (coherent scattering). The orderly arrangement of atoms results in the scattered X-rays within the crystal being (i) in phase in specific directions dictated by symmetry and atomic spacings and (ii) out of phase in all other directions (Fig. 4-3). The X-rays that are in phase constructively

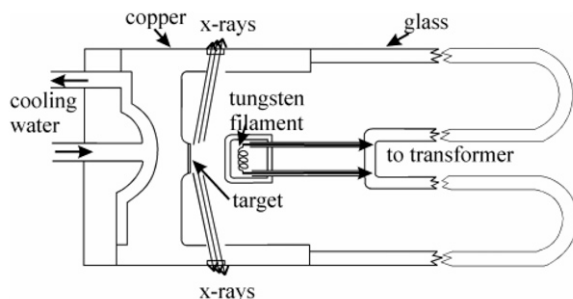


Fig. 4-1. Schematic cross section of a sealed-off filament X-ray tube (after Cullity, 1978). High voltage accelerates electrons from the filament to the metal target, where X-rays are generated from their rapid deceleration. The X-rays exiting one of the windows are collimated and focused in X-ray diffraction analysis.

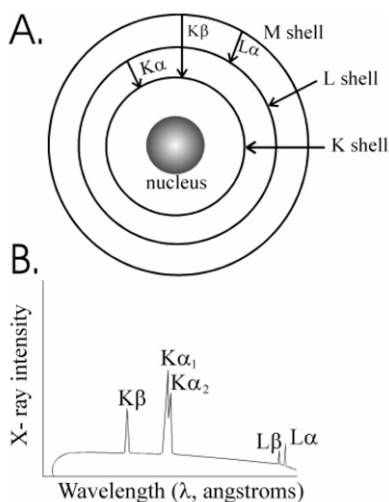


Fig. 4-2. (A) Schematic of an atom, depicting electron shells and the energy transitions for $K\alpha$, $K\beta$, and $L\alpha$ characteristic radiation. $K\alpha$ arises from the replacement of K-shell electrons by electrons from the L shell; $K\beta$, by replacement of K-shell electrons by M-shell electrons, and $L\alpha$, by replacement of L-shell electrons by M-shell electrons. (B) Generalized depiction of an X-ray spectrum, showing peaks in intensity at wavelengths (energy levels) corresponding to characteristic radiation. The highest-energy (shortest wavelength) characteristic radiation shown is $K\beta$. Peaks marked $K\alpha_1$ and $K\alpha_2$, which are seldom resolved in XRD data, arise from contribution of electrons from two sublevels in the L shell.

interfere and emerge as intense beams (diffracted beams) from the crystal, while those that are out of phase destructively interfere and hence have minimal emergence. This systematic combination of constructive and destructive interference arising from the periodicity of atoms in crystals is X-ray diffraction. Detailed information about the internal symmetry and arrangement of atoms in crystals can be gained from XRD. However, this chapter will emphasize only those aspects that are most pertinent to the identification of naturally occurring minerals in soils.

A simplifying way to intuitively comprehend the relatively complex phenomenon of XRD is to envision regularly spaced planes of atoms in mineral structures (Fig. 4-3). The distance between a given set of planes is termed *d-spacing*. The d-spacing, although on a scale of Angstroms, can be determined quite accurately using XRD. The principles underlying this determination are elegantly expressed by the Bragg equation:

$$n\lambda = 2d \sin \theta$$

[1]

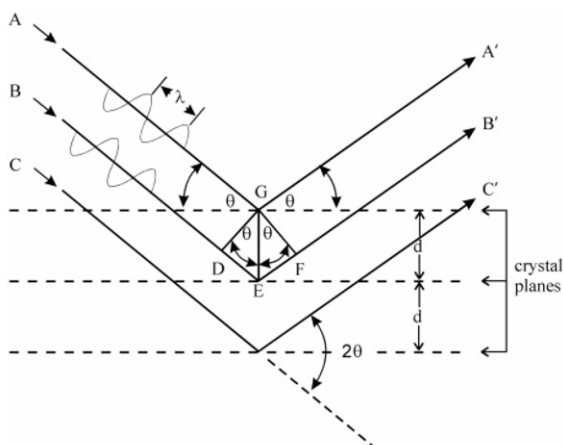


Fig. 4-3. Schematic representation of XRD by regularly spaced planes of atoms in a crystal. Theta (θ) is the angle that the beam makes with the atomic planes; 2θ is the angle that the diffracted beam deviates from the primary beam; d is the distance between equivalent atomic planes in the crystal (*d-spacing*); and λ is wavelength of the radiation. Note that when $DE + EF = n\lambda$, where n is an integer, the diffracted beams from each plane of atoms would be in phase, leading to constructive interference which accounts for XRD. In effect, when that condition is met, an XRD peak is observed. The Bragg equation can be used to calculate *d-spacing* from the 2θ angle at which the diffraction peak occurs.

where n is an integer, λ is wavelength of the radiation, d is d-spacing, and θ is the angle between the planes and the incident X-ray beam. This equation expresses the condition for diffraction, in effect, that for a given d-spacing and wavelength of radiation diffraction will occur at a unique angle between the beam and the set of planes in question.

The factor in the Bragg equation of interest to mineralogists is the d-spacing, which can be determined in XRD analysis by fixing λ and measuring the θ angle where a peak in X-ray intensity occurs. Such measurements can be made for a single crystal, for a mineral in powder form, or for a mixture of minerals in powder form. Information gained from diffraction angles and relative peak intensities for pure minerals can be used to establish structural details of those minerals. X-ray diffraction can also be used to identify the minerals present in a mixture, such as the soil clay fraction.

X-ray Powder Diffraction Analysis

Obtaining useful information from XRD requires the ability to control and/or measure angular relations between incident and diffracted radiation. Two types of instruments have been used to perform X-ray powder diffraction analysis: the XRD powder camera and the X-ray diffractometer. The powder camera approach entails recording diffraction maxima “cones” on cylindrically mounted photographic film surrounding the specimen. The diffractometer records the intensity of the diffracted beam electronically at precise angles as the specimen is scanned over an angular range (Fig. 4–4). Modern diffractometers have a number of advantages over the powder camera and are the more commonly used instruments in soil mineralogy. Therefore, the diffractometer approach will be emphasized in this chapter, but the d-spacing and intensity data obtained from either type of instrument are interpreted the same way.

SAMPLE PREPARATION FOR X-RAY DIFFRACTION ANALYSIS

Sample preparation is an important aspect of soil mineralogical analysis by XRD. Preparatory procedures must be judiciously selected according to the objectives of the analyst. Accurate assessment of soil mineralogy requires a thorough understanding of these procedures and their consequences for data interpretation.

The simplest preparation procedure is to grind the soil “as is” into a powder fine enough to mount in the focal plane of the diffractometer. However, the minerals occurring in soil clay fractions are often very different in nature from minerals that dominate the coarser fractions. Grinding the whole soil makes it difficult to detect and identify the clay-sized minerals due to dilution and other confounding effects of the coarser components. This is a disadvantage because the clay-sized minerals often have a disproportionate effect on chemical and physical properties of soils. Thus, if the objective is an effective characterization of all minerals, it is best to analyze individual particle size fractions separately.

Some preparatory procedures are necessary for definitive identification of expansible phyllosilicates. The actual spacing between “basal” (001) atomic planes in expansible phyllosilicates varies with temperature, relative humidity, and exchangeable cations in the interlayer region of these minerals. Therefore, comparison of the variable d-spacings (d_{001}) must be made under standardized conditions (e.g., temperature, cation saturation) to enable identification based on differential responses to these conditions.

The following sections provide explanations and detailed procedures pertaining to sample preparation.

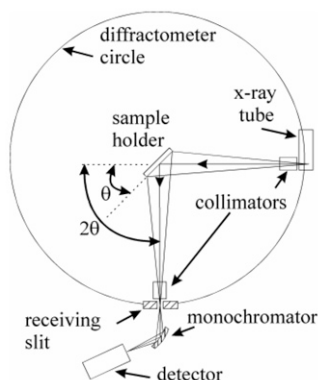


Fig. 4-4. Schematic representation of the components of an X-ray diffractometer.

Mounting Samples for X-ray Diffraction—Low Salt Samples

Principles

The way that a sample is mounted can have major effects on the resulting data (Harward and Theisen, 1962; Harward et al., 1962; Gibbs, 1965; Bish and Reynolds, 1989). It is important to be aware of these effects as well as their underlying causes. Some of the critical factors that should be accounted for are degree of preferred orientation of crystals, accurate positioning of the planar sample surface within the instrument, differential settling (for mounts prepared from particles in suspension), and infinite thickness (i.e., thick enough that no increase in diffraction intensity occurs with further thickening).

One fundamental consideration is that diffraction is only detected for the atomic crystal planes that are coplanar with the focal plane of the diffractometer (see “Nature of X-ray Diffraction Data”). This means that crystal planes would be evenly represented only if there is random orientation of crystals in the sample (Thompson et al., 1972). Random orientation occurs naturally for anhedral, equidimensional crystals. However, there is a tendency for crystals with a dominant crystal face (e.g., platy crystal habit) or elongated morphology to be oriented with plates or long axes parallel to the surface of the mount. The degree of this preferred orientation increases in proportion to the pressure applied in establishing the planar surface of the powder. Thus, the applied pressure must be minimal if a near-random orientation is sought. The distinction between random and preferred orientation is moot if all minerals in the mount are anhedral and equidimensional. See “Important Considerations in Mount Selection” below for a discussion of advantages of preferred vs. random orientation.

It is critical that the planar upper surface of the powder be precisely positioned in the focal plane of the instrument to obtain accurate d-spacing. An offset of that position is possible for cavity mounts, which require that the powder be packed into recessed cavities. For such mounts it is necessary that the powder be level with the upper surface of the mount.

Some mounting techniques entail the deposition of particles in suspension onto the surface of the mount (suspension mounts). That means that particles undergo settling until the liquid phase evaporates or is removed by suction. Differential settling based on differences in particle density, shape, etc. can occur during this time, resulting in a stratification which biases the resulting XRD data (Gibbs, 1965). In effect, the particles that settle slowest are concentrated at the top of the cake and hence are over-represented relative to their mass fraction. Differential sedimentation is most pronounced when (i) the liquid phase is left to evaporate (e.g., sedimentation on glass slide), and (ii) there is a wide range

in particle size and/or density. The effect can be reduced by rapidly removing the liquid phase using suction (e.g., through use of a filter of ceramic tile) or by reducing the range in particle sizes.

Infinite thickness of the sample is desirable for a number of reasons (Bish and Reynolds, 1989; Moore and Reynolds, 1997). For one thing, it enables greater reproducibility, because at infinite thickness, sample thickness no longer affects relative diffraction intensity. Also, infinite thickness ensures that the mounting medium itself will have no influence on the XRD results. The energy of the beam at infinite thickness is effectively consumed by diffraction and absorption phenomena (Moore and Reynolds, 1997). Failure to have infinite thickness results in greater attenuation of peaks at higher 2θ angles than at lower angles because the depth of beam penetration is less for the latter. For example, the depth of vertical penetration for a beam capable of 200- μm total penetration would be 3.5 μm at $2^\circ 2\theta$ and 100 μm at $60^\circ 2\theta$. Calculation of infinite thickness can be difficult because it is dependent on such factors as beam intensity, packing density, and X-ray absorption characteristics of the components in the mixture. However, it can be verified for the materials being analyzed by adding incremental amounts of sample material until high angle intensities show no further increase. Also, if the underlying mount generates a diffraction peak itself at a reasonably high angle, then the disappearance of that peak with incremental additions would verify infinite thickness.

Particle-Size Considerations

Sample particle size must be fine enough to (i) obtain adequate statistical representation of the components and their various diffracting crystal planes and (ii) avoid diffraction-related artifacts (Bish and Reynolds, 1989). This means that sand-sized particles require grinding, as by ball milling, mortar and pestle, or blender. Samples with platy or fibrous minerals are most effectively ground using bladed devices such as blenders. It is generally recommended that a cooling liquid, such as acetone or alcohol, be used in grinding to avoid sample degradation from heat. A summary of grinding methods was provided by Bish (1994). He recommended that particles be ground to 0.01 mm for quantitative assessments.

Important Considerations in Mount Selection

It is necessary to know whether preferred or random orientation best serves the objectives of the analysis. Mounting approaches can be selected to minimize or maximize preferred orientation. Random orientation is desirable if the objective is to obtain information about all crystal planes, while preferred orientation is advantageous if the primary objective is to detect and identify platy minerals such as phyllosilicates. In the latter case, however, the preferred orientation effect must be accounted for in the interpretation of the data.

A randomly oriented powder is the appropriate choice in cases where information about crystal planes oblique to the dominant cleavage face is required. Such cases include (i) XRD characterization of a mineral, (ii) verifying the identity of a mineral using powder diffraction reference data, and (iii) discriminating between related mineral species that produce similar XRD patterns in oriented mounts, a common purpose in soil mineralogical analysis. For example, the *b* crystallographic axis dimension, which can be used to differentiate dioctahedral from trioctahedral phyllosilicates, can only be measured if a sufficient number of crystals are oriented with the dominant cleavage face oblique or perpendicular to the focal plane of the goniometer.

Intuitively, random orientation may appear to be a pure and unbiased approach to sample mounting. However, it is often advantageous to preferentially orient certain crystal faces, as in the case of identifying expansible phyllosilicates. Furthermore, random orientation is difficult (if not impossible) to achieve for samples containing platy or elongated crystals. The analyst faces the dilemma of creating a planar surface for a powder while not applying enough force to orient plates, laths, or tubes parallel to the plane. Even the slightest pressure can induce preferred orientation of some samples. This sensitivity to pressure as applied to the surface of the powder makes it difficult to attain acceptable precision. In effect, relative X-ray intensities may vary significantly between different mounts of the same sample. It is generally much more difficult to achieve good precision with random orientation than with preferred orientation for samples with platy minerals because these minerals naturally orient parallel to the packing plane.

Suspension mounts (see below) prepared using a porous mounting medium (e.g., unglazed ceramic tile, filter) enable convenient cation saturation treatments and provide preferred orientation, both of which are advantageous for identification of expansible phyllosilicates. Suspension mounts should generally be avoided for silt-sized material (including ground sand) due to differential settling of material during the drying period. However, some silt and sand fractions may contain expansible phyllosilicates whose identification would be made easier by using a suspension mount. A separate suspension mount could be made, in addition to a dry mount for the purpose of identifying the phyllosilicates.

Achieving rapid dewatering under suction (to minimize differential settling) is difficult for some samples (e.g., fine clay may clog pores). Effective orientation can be accomplished without differential sedimentation by preparing the mount from a paste.

Mount Storage

Identification of expansible phyllosilicates requires that conditions of relative humidity (RH) be controlled, as will be elaborated on later. Therefore, it is necessary to store the samples under conditions that maintain the targeted humidity, such as in a desiccator, until they are scanned on the X-ray diffractometer. (See “Relative Humidity Control” under “Mineral Identification from XRD Data”).

Dry Mounting Techniques

Random Powder Mount. The dry powder material is mounted in such a way as to minimize the force required to form a planar surface of the powder that is to be positioned in the diffractometer. A common technique is to loosely drop the material into the cavity of the mount “from the side,” that is, such that the plane to be mounted is in a vertical position as the mount is prepared (Fig. 4–5). This is accomplished by machining the sample holder to have a shallow rectangular cavity with one open side as positioned horizontally. A glass slide with an etched (frosted) surface toward the powder is used (avoiding a smooth contact surface, which might induce orientation) to retain the powder in the mount as it is being loaded edge-wise and vertically. Binder clips can be used to hold the glass slide in place and to provide a “stand” to hold the mount in a vertical position. Once the cavity is filled with the powder, the mount is positioned horizontally and the glass slide removed by lifting it straight up to avoid inducing preferred orientation. Another method for attaining random orientation involves the use of an air brush to spray dry samples (Hughes and Bohor, 1970; Hillier, 1999). This method, which produces a powder of spherical aggregates, reportedly minimizes preferred orientation and yields XRD data that are highly reproducible. However, the heat required by the method can dehydrate expansible phyllosilicates and thereby hinder their identification.

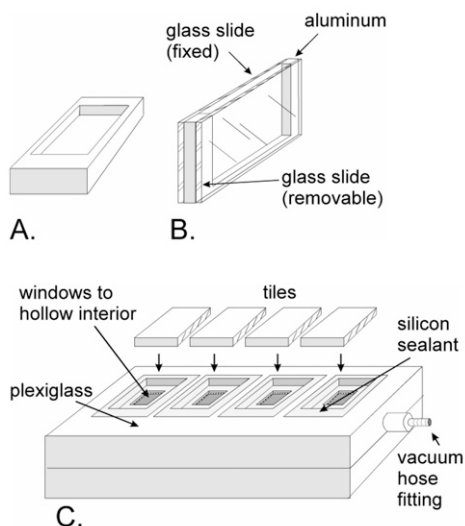


Fig. 4-5. Drawings of mounts and mounting paraphernalia used in X-ray diffraction analysis. (A) Cavity mount—powder is packed into cavity and leveled parallel to the top of the mount surface. (B) Mount used for side-packing powder to minimize preferred orientation; removable glass slide is braced against the aluminum frame, held in place by a binder clip. Powder is dropped into the cavity from the side while the mount is positioned vertically. Binder clips can serve as the dual purpose of holding the removable glass slide in place while providing a stand to hold the mount in a vertical position. When the powder has been loaded into the mount cavity, the slide is placed horizontally with the removable glass slide to the top. Then the slide is removed by lifting it vertically. Commonly, a frosted glass slide is used to further minimize preferred orientation. (C) Example of a setup for applying suction to porous ceramic tiles to prepare oriented mounts of clay. Tiles fit tightly into the silicon-lined cavities, forming a seal when suction is applied. A water trap (e.g., stoppered flask) is installed on the vacuum line between the plexiglass manifold and the vacuum pump to protect the pump. Other approaches have been used to accomplish the same objective (Rich, 1969).

Oriented Powder Mount. This mounting technique can be used with any cavity mount. The sample is packed into the cavity of the mount using a flat surface, such as a spatula blade or glass slide, with uniform and sufficient force to promote the preferred orientation of platy and elongated crystals. Reasonably good precision can be attained if care is taken in applying uniform, firm pressure in packing the mount with each preparation.

Wet Mounting Techniques (For <2- μ m Material)

Oriented Suspension Mounts. The term *suspension mount* is used here to denote mounts that are prepared by depositing the material to be analyzed onto a planar surface as a viscous suspension. The suspension is then dewatered either by evaporation or applied suction. Suspension mounts achieve effective adhesion of the sample to a surface, obviating the packing of the sample into a sample holder cavity. They generally produce a very flat surface for XRD, which is desirable in maintaining optical precision within the goniometer. They also achieve a high degree of preferred orientation if platy or elongated crystals are present, which is an advantage for phyllosilicate differentiation. Suspension mounts on porous material have the additional advantage that cation saturation and glycerol solvation (described in subsequent sections) can be accomplished directly on the ceramic tile while still under suction. Some samples tend to crack and peel away from the surface during drying. This is often a result of a difference in the mineralogy of the material between the finer and coarser grains and may require further particle size fractionation. Peeling may also be prevented if a thinner deposit is applied or if the sample is not air-dried (e.g., maintained moist in a desiccator). Several thin deposits using dilute suspensions can be progressively made to produce sufficient sample thickness.

Glass Slide Mount. A simple way to prepare a suspension mount is to pipette the suspension onto a glass slide in a quantity sufficient to cover the slide and be held by

surface tension, then allow it to dry. Cation saturations should be made before the material is pipetted onto the slide. Drying can be accelerated (to lessen differential sedimentation) by placing the slide in an oven at slightly elevated temperature (e.g., 40°C), but oven drying can exacerbate peeling. Care must be taken that the suspension be free of any salt that may have been previously added to the suspension (e.g., in inducing flocculation to reduce water volume). The optimal concentration of the suspension is a balance between having sufficient material on the slide for effective XRD assessment and not having so much as to result in the development of cracks upon drying. A first approximation of “sufficient material” is about 30 mg cm⁻².

Ceramic Tile Mount. Suspension mounts can also be prepared by depositing the suspension onto a porous (unglazed) ceramic tile and applying suction to accelerate dewatering and hence lessen the extent of differential sedimentation. Occasionally, clay saturated with monovalent cations disperses sufficiently that some of the material passes through the tile pores, but this is not usually a serious problem. Dewatering by suction permits the addition and rinsing of salt solutions to the sample in the process of cation saturation. Also, heat treatments (explained in a subsequent section) can be performed while the sample is on the tile. Different laboratories have devised various means to apply suction to ceramic tiles (e.g., Rich, 1969). The common denominators are a rectangular slot that forms an elastic seal around the edge of the tile, a means of bracing the tile in a level position, and a means of applying suction to the underside of the tile (Fig. 4–5). An effective sealing material is commercial silicon sealant, which dries to a soft but durable and elastic solid. The tile is pressed into a tightly fitting rectangular cavity formed by the sealant. The top of the tile is positioned to extend above the sealant, such that suspensions can be retained on the tile by surface tension. The ceramic tile XRD mount is prepared as follows, assuming the area of the tile to be about 10 cm² (deviations should be adjusted proportionately for the mass of material specified):

1. Place the tile in the suction apparatus and apply suction.
2. Wet the slide with a few milliliters of deionized water and allow it to pull through.
3. Pipette about 8 to 10 mL of suspension at a particulate solids concentration of approximately 30 mg mL⁻¹ onto the tile, being careful that it stays on the tile via surface tension.
4. Continue suction until the excess water is removed, leaving the material to be analyzed plated on the tile.
5. Rinse the material by washing several times (totaling about 25 mL) with deionized water to ensure that all salts are removed, or selected salt solutions can be applied if controlled cation saturation is pursued. If salt solutions are used to saturate the exchange complex with a cation, wash the slide (25 mL) to remove the excess salt.
6. Tiles can be stored in a desiccator when preparation is complete, which permits control of relative humidity and prevents dust from contaminating the surface.

0.45- μ m Membrane Filter Mount. This mounting approach (Drever, 1973; McAlister and Smith, 1995) can be used as an alternative to the ceramic tile approach and has the same advantages that come with dewatering by suction. Also, filters and filter suction devices are easily purchased from vendors of scientific supplies, in contrast to unglazed ceramic tiles and the customized suction devices required for their use. The use of filters involves transferring the material from the filter to a rigid support such as a glass slide, which requires practice and skill. Be careful to watch for fine clay material being pulled

through the pores of the filter. The filter mount is prepared as follows, assuming the area of the filter surface to be about 10 cm^2 (deviations should be adjusted proportionately for the mass of material specified):

1. Place filter on its suction manifold mount, and apply suction.
2. Apply a few milliliters of deionized water to wet the filter and allow the water to be pulled through the filter.
3. Pipette about 8 to 10 mL of suspension at a particulate solids concentration of approximately 30 mg mL^{-1} onto the filter and allow it to drain under suction.
4. Rinse the material with about 25 mL of deionized water to ensure that all salts are removed, or selected salt solutions can be applied and rinsed if controlled cation saturation is pursued.
5. Remove filter carefully with tweezers and place on a tissue or other absorbing surface, sample side up.
6. Allow the filter to dry for 5 to 10 min.
7. The sample must now be transferred from the filter to a glass slide. Pick up the filter by the edge with tweezers, and carefully place it, centered on top of the glass slide, sample side down. Alternatively, the glass slide can be positioned on top of the filter cake and a tissue can be used to turn over the slide and filter.
8. Gently rub the bottom of the filter to ensure good contact of the sample with the glass slide.
9. Using a glass rod, crease the filter along the edges of the glass slide by pressing down at an angle and sliding the rod along both edge contacts between filter and slide.
10. Gently peel the filter off the slide longitudinally. This usually results in the sample adhering to the slide.
11. Slides can be stored in a desiccator when preparation is complete, which permits control of relative humidity and prevents dust from contaminating the surface.

Oriented Paste (Smear) Mount. This mounting approach entails spreading the sample as a paste onto a flat surface (Thiesen and Harward, 1962; Gibbs, 1965). A “smear” mount eliminates the risk of differential sedimentation, but lacks some of the advantages of suspension mounts. For example, cation saturation cannot be performed directly on the smear mount. A smear mount can be prepared as follows:

1. Work the sample into the consistency of a paste (putty-like), moist enough to have plasticity but dry enough not to be excessively sticky.
2. Place an aliquot of the paste on the edge of a glass slide.
3. Using a spatula, spread the paste all the way across the slide in one motion. The paste should be spread such that the surface is smooth and flat, which sometimes requires practice.

Mounting High-Soluble Salt Samples

Samples in arid regions can contain appreciable concentrations of soluble salts. These salts are important minerals and should be preserved in the process of mineralogical analysis. Preservation of salts precludes all washing procedures and requires dry mounting approaches, such as random or oriented powder mounts as described above, for XRD analysis. Soils with high salt concentrations can be analyzed on a whole-soil basis or after me-

chanical sieving to obtain a finer particle size fraction (e.g., $<50\ \mu\text{m}$). Some grinding may be necessary, particularly if the whole soil is analyzed, to avoid diffraction-related artifacts (Bish and Reynolds, 1989). It can be advantageous for some purposes to physically separate zones of high salt concentration when they are evident and to mount them separately after grinding (Buck and Van Hoesen, 2002).

Cation Saturation

Rationale

Soils commonly contain expansible phyllosilicates, which have the unusual trait that their d-spacing for (001) crystal planes varies with the cation population on their exchange complex (Barshad, 1950; Mielenz et al., 1955). The variable spacing for a given expansible phyllosilicate arises from changes in the balance of expansive and contractive forces within the hydrated interlayer region of the mineral (Walker, 1957; Grim, 1968). This balance hinges on the hydration energy and hydrated radii of cations vs. the mutual attraction of the adjacent layers of the mineral for the cations. Thus, the resultant d-spacing is affected by both cation species and charge properties of the mineral, including charge density and origin of layer charge. In effect, mineral identification requires control of the saturating cation so that mineral characteristics can be inferred from d-spacings.

Control of cation saturation is accomplished by creating a monoionic exchange complex. The most common cations used diagnostically in soil mineralogical analysis are Mg and K. The contrasting hydration characteristics of these two cations induce differential effects on interlayer spacing, and hence, d-spacing. Magnesium has sufficient hydration energy to maintain a relatively uniform and well-ordered distribution of water molecules in the interlayer, producing a d-spacing of approximately 14 Å in common expansible phyllosilicates. Potassium, on the other hand, due to its size, interaction with adjacent layers, and low hydration energy, tends to promote dehydration and collapse of interlayers. The differential effects of Mg and K on d-spacing, in conjunction with glycerol solvation can be used diagnostically in distinguishing and identifying specific phyllosilicates. Details regarding the diagnostic procedures will be provided in the section addressing mineral identification.

Reagents

- 1 M MgCl_2
- 1 M KCl
- Deionized water

Materials

- 0.45- μm membrane filters or unglazed ceramic tiles
- Suction filtering apparatus

Procedure

1. Prepare two 0.45- μm membrane-filter mounts or ceramic-tile mounts per sample, as described under “Oriented Suspension Mounts.” The cation designation “Mg” or “K” should be included in the mount labels. Use a heat-resistant labeling marker if heat treatments are to be conducted.
2. Apply 25 mL of the 1 M MgCl_2 or KCl to the “Mg” and “K” mounts, respectively, or about 25 mL per 300 mg of clay on the slide. This can be applied all at once if the filter apparatus accommodates the whole volume, or in smaller (e.g., 5 mL) aliquots as may be necessary in the case of ceramic tiles.
3. Apply 25 mL of deionized water as a rinse.

Comments

Previous publications addressing soil mineralogical methods (e.g., Jackson, 1956; Whittig and Allardice, 1986) have presented procedures for cation saturation that entail numerous washing and rinsing steps using a centrifuge. The final product of these procedures is a salt-free suspension of homoionic clay. That procedure may be expedient if the glass slide mount is used. The procedure presented here is simpler and faster, since the washing and rinsing steps are accomplished directly on the mount while it is under suction. An additional advantage is that the clay can be stored in a higher ionic strength medium, which reduces hydrolysis and microbial growth.

Homoionic clay for dry mounting purposes, if needed, can be obtained as follows:

1. Remove the clay from the filter or ceramic tile while still moist by gently scrapping with a rubber policeman. (this may require several filters, depending on the amount of clay needed).
2. Transfer it to the surface of a glass slide and allow it to air-dry.
3. Gently crush and store as a powder until ready for use.

Glycerol Solvation

Rationale

Cation saturation alone is not usually sufficient for confident distinction between common expansible phyllosilicates. Saturation with Mg sometimes produces similar d-spacings (about 14 Å) for these minerals. Glycerol [$C_3H_5(OH)_3$] is used diagnostically in conjunction with Mg saturation because of its tendency to solvate (due to its polar nature) the interlayer regions in bilayer configuration for smectites and as monolayers for vermiculites (Walker, 1950; Brindley, 1966). The resistance of vermiculites to solvation by glycerol arises from its higher charge density and hence cation occupancy. The layers of vermiculite are mutually attracted to cations, and this force of attraction is sufficient to deter glycerol incursion. The lower layer charge of smectites allow the solvated interlayers to expand to a d-spacing of about 18 Å, thereby distinguishing these minerals from vermiculites.

A number of polar organic intercalates other than glycerol can form interlayer complexes with smectite (MacEwan and Wilson, 1980; Whittig and Allardice, 1986), including alcohols, ethers, amines, and polyamines. However, glycerol has several advantages for phyllosilicate identification, including good peak separation between solvated and non-solvated phyllosilicates, less variability of the d-spacing as a function of layer charge, and high stability of the complex (Whittig and Allardice, 1986). Therefore glycerol is specified below for routine analyses. However, ethylene-glycol is also used extensively for solvation, and is more prone than glycerol to form a bilayer in smectite with high charge of tetrahedral origin (beidellite).

Reagents

- 30% Glycerol [$C_3H_5(OH)_3$]/water mixture
- Deionized water

Materials

- 0.45- μ m membrane filter or unglazed ceramic tiles
- Suction filtering apparatus

Procedure for Ceramic Tile Mounts

1. Apply a sufficient quantity of 30% glycerol to cover the Mg-saturated clay plated on

- top of the tile by surface tension (see “Oriented Suspension Mounts” and “Cation Saturation”), and allow excess to pull through.
2. Remove tile from suction apparatus and allow to air dry for a few hours, being careful to protect it from dust.
 3. Conduct X-ray scan, or store in glycerol desiccator until scanning is performed.

Procedure for 0.45- μm Membrane Filter Mounts

The membrane filter mount differs from the ceramic tile in that for the former the clay ends up on a glass slide rather than on the original medium (i.e., filter). The glycerol application is best made after the transfer from the filter to the slide has been performed. This can be done by spraying a 10 to 20% glycerol–water solution directly on the Mg-saturated clay plated on the slide using a very fine misting device, covering the surface completely but lightly. It is best to then allow the slides to equilibrate in a glycerol desiccator for at least 24 h.

Comments

Previous publications addressing soil mineralogical methods (e.g., Jackson, 1956; Whittig and Allardice, 1986) have presented procedures of glycerol solvation that entail numerous washing and rinsing steps using a centrifuge. There is some uncertainty in these procedures as to an adequate but not excessive amount of glycerol to be applied. One way to avoid this uncertainty is simply to apply the glycerol directly to the mounted Mg-saturated clay using either 0.45- μm membrane filter mounts or ceramic tile mounts while they are moist. Adding to air-dried sample surface may result in incomplete penetration into the sample.

X-RAY EXAMINATION OF SAMPLES

X-ray Diffractometers

Presumably most readers will have access to and some familiarity with an X-ray diffractometer. Alignment and optimization involve instrument-specific procedures and will not be discussed here, but a brief summary of some basic aspects of diffractometers most pertinent to soil mineralogical analysis will be presented. There are a number of sources where more detailed information about diffractometers can be found (e.g., Klug and Alexander, 1974; Cullity, 1978; Moore and Reynolds, 1997).

An X-ray diffractometer is a sophisticated system designed to generate X-rays, optically focus them onto a sample, scan with precise angular control, and monitor the relative intensity of the diffracted beam as a function of 2θ (the angle is conventionally expressed as 2θ rather than θ , since 2θ is the actual angular deviation from the incident beam). The main components are a generator to produce high voltage, a tube to produce X-rays, a beam collimator, a sample mounting stage, a detector, a monochromator, and a goniometer (Fig. 4–4). The goniometer controls the angular relationship between the incident beam, sample surface, and detector. For routine analysis, the goniometer maintains an equal angle between the focal plane and both the incident beam and the path to the detector that receives the diffracted beam. This means that diffraction from crystal atomic planes that are coplanar with the focal plane will be registered as a peak in intensity by the detector at the 2θ angle that “solves” the Bragg equation for the d-spacing of that particular set of atomic planes.

The sample stage is positioned in the instrument such that the sample is exposed to the incident radiation within a focal plane in the center of what is termed the *goniometer circle*,

while the radiation source and detector are positioned on the circle itself. The goniometer controls the angle that the incident radiation makes with the focal plane, as well as the relative position of the detector. Effective control of the X-ray beam requires some “optical” collimation and focusing. The beam is collimated as it emerges from the X-ray tube, such that only X-rays parallel to the goniometer circle are incident to the sample. There is focusing of the beam, in that it diverges slightly from the collimator to the sample, and converges to the detector (Fig. 4–4).

Monochromatic radiation is necessary for XRD analysis using a powder diffractometer. The X-ray tube emits intense characteristic ($K\alpha$) radiation from its metal target, but some white- and $K\beta$ radiation is emitted as well. Modern X-ray diffractometers are equipped with devices in the beam path that serve to reduce intensities along the spectrum other than the wavelength of choice. One such device is a metal filter, with a precipitous X-ray absorption maximum (absorption “edge”) that results in selective absorption of $K\beta$ radiation generated from the target of the X-ray tube. The metal used for the filter must be selected based on the $K\beta$ wavelength of the target metal. A more effective device for the purpose of achieving a high degree of wavelength purity is the crystal monochromator. This is a crystal positioned in the beam path between the sample and diffracted beam detector such that it diffracts only $K\alpha$ radiation into the detector.

Modern diffractometers are computer controlled and collect data digitally. They have a stepping motor, which enables the operator to select the step width (fraction of a degree 2θ at which counts are collected) and dwell time (the time interval for each step). Peak/background ratios can be increased by using longer dwell times. Digital data have a number of advantages over analog strip chart data recordings, standard for older diffractometers. Data collected digitally can be conveniently manipulated graphically and mathematically. The area under diffraction peaks, an index of relative intensity, can be rapidly calculated using computer programs or spreadsheets. Also, the diffraction data can be modeled, as will be discussed in a subsequent section.

Choice of X-ray Wavelength

Shorter-wavelength (higher energy) radiation has the advantages of lower attenuation in air and less absorption by the sample. Longer-wavelength radiation, however, produces better resolution of peak maxima (a larger 2θ interval between adjacent peaks, as can be verified by applying the Bragg equation), which is particularly advantageous for the large d-spacings of expansible phyllosilicates. The most commonly used radiation for soil mineralogical analysis is $\text{CuK}\alpha$ ($\lambda = 1.54 \text{ \AA}$) because it is a reasonably good tradeoff with respect to energy and peak separation. $\text{MoK}\alpha$ ($\lambda = 0.71 \text{ \AA}$), $\text{FeK}\alpha$ ($\lambda = 1.94 \text{ \AA}$), and $\text{CrK}\alpha$ ($\lambda = 2.28 \text{ \AA}$) are higher- and lower-energy alternatives.

Safety: An Important Consideration

X-ray diffraction analysis requires high voltage to generate and detect potentially harmful high-energy radiation. Hence, not only is there the hazard of X-ray exposure, but of contact with high voltage as well. Careless, ill-informed operation of XRD equipment can result in serious injury or death. It is imperative that all persons who are permitted access to the equipment be thoroughly trained in safe operating procedures and aware of the potential risks. Fortunately, modern X-ray diffractometers have a number of safety features that minimize the risk of exposure to X-rays. These include lead-lined cabinets or other enclosures and safety switches that close the shutter to the X-ray tube when the X-ray chamber is accessed. It is best, however, not to become complacent about safety even when using modern “fail safe” equipment. For example, it is a good idea to routinely check

the beam path using an appropriate X-ray detection instrument even when the shutter light indicates that it is closed.

Conducting Scans

The X-ray tube is energized by establishing a voltage potential and current flow between the filament and the target (commonly Cu). The magnitude of the current and voltage is established by optimization procedures for the diffractometer. The sample mount is braced into the focal plane of the goniometer by means of a mechanical device provided for the sample stage. It is important to verify at this time that the sample surface is not protruding above or recessed below the focal plane. Displacement from the plane will result in inaccurate d-spacing determinations. Routine operation for diffraction analysis involves a scan from lower to higher 2θ angle. The selection of scanning range is based on expectations regarding likely components. The starting 2θ angle is commonly set at 2° , which is sufficiently low to detect peaks of large d-spacings characteristic of expansible phyllosilicates. It is generally a good idea to have at least one scan from 2 to 60° to cover the total range where peaks of common soil minerals are likely to occur. Additional scans for different saturation-, solvation-, or heat treatments need only cover the range necessary to get the needed discriminatory information.

Nature of X-ray Diffraction Data

The results of XRD using a powder diffractometer are generally plotted as 2θ angle on the x axis vs. X-ray “intensity” as measured by the detector on the y axis (Fig. 4–6). The intensity is usually expressed as counts per second, although it is most accurately a relative measurement affected by various conditions, including the current and voltage at which the X-ray tube is operating and the counter efficiency. Relative intensity is measured as relative areas under diffraction peaks. The relative intensity of a diffraction peak produced by a given set of atomic planes in a crystal, assuming all atomic planes are equally represented (random orientation), is dictated by the composition and arrangement of atoms in the unit cell (i.e., the smallest atomic unit displaying the symmetry of the crystal). In effect, elements of the crystal differ in the way that they scatter and absorb X-rays, and their position in the unit cell affects the degree to which X-rays emerge in phase for a given direction. Thus, relative intensity does not provide a simple 1:1 index of the mass fraction of minerals in a mixture; calibrating relative intensities to mass fractions is one of numerous challenges to quantification by XRD, as will be discussed below.

It is important to understand that a diffractometer only monitors diffraction for crystal planes that are coplanar with the focal plane. This does not mean that diffraction isn’t occurring for crystal planes that are otherwise oriented, but simply that the detector is positioned uniquely (angle of incidence = angle of diffraction) to “solve” the Bragg angle for the focal plane. It follows that the greater the number of crystals of a given mineral with a particular crystal face oriented in the focal plane, the greater the diffraction intensity will be for that face. Hence, preferred orientation of a given crystal plane (i.e., nonrandom orientation) is a major factor to be accounted for in the interpretation of X-ray powder diffraction data. Mounting procedures that enhance or minimize preferred orientation are described in the section entitled “Mounting Samples for Diffraction.”

MINERAL IDENTIFICATION FROM X-RAY DIFFRACTION DATA

Mineral identification is based on d-spacings and relative peak intensities. All minerals generate multiple diffraction peaks. Identification is much simpler if only one mineral

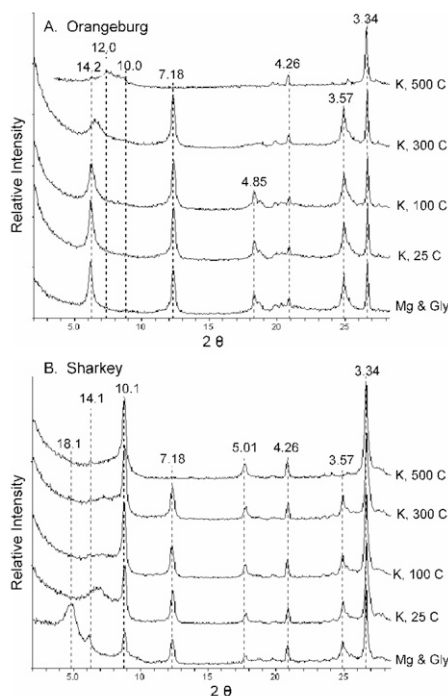


Fig. 4-6. Sequences of X-ray diffraction patterns for soil clays (2.0–0.2 μm) scanned after specified cation-saturation (Mg and K), glycerol (Gly) solvation, and heat treatments. Peaks are labeled by d-spacings (Å). (A) The clay from the Orangeburg series (Ap horizon, Georgia) shows a 14-Å peak that is minimally affected by cation saturation and shifts and broadens with heat treatment to a peak at 12 Å. This behavior typifies hydroxy-interlayered vermiculite as it occurs in the coastal plain of the southeastern USA. There is a small peak that persists at 14 Å at 500°C, which is due to a small amount of chlorite. Also present are kaolinite (7.18 and 3.57 Å), gibbsite (4.85 Å), and quartz (4.26 and 3.34 Å). Note that peaks for gibbsite and kaolinite disappear at 300 and 500°C, respectively, due to dehydroxylation. (B) Clay from the Sharkey soil (Ap, Louisiana) shows peaks at 18 and 14 Å that have mainly shifted to 10 Å at 300°C. Note the increase in intensity of the 10-, 5- (second-order), and 3.34-Å (third-order) peaks with heat. This behavior suggests that both smectite and vermiculite are present. A small peak (not labeled) intermediate between 14 and 10 Å may be attributable to some resistance to collapse of these expandable phyllosilicates. The small 14-Å peak is attributable to chlorite. Also present are mica (illite) (10, 5, and 3.34 Å), kaolinite (7.18 and 3.57 Å), and quartz (4.26 and 3.34 Å). Note that at 25°C the 10-Å peak is completely attributable to mica, but is enhanced by the collapse of expandable phyllosilicates with increasing temperature. Also, quartz, mica, smectite, and vermiculite all contribute to the 3.34-Å peak at 500°C, whereas only mica and quartz contribute to it at 25°C. (These soils were analyzed as part of USDA Regional Project S-207. Special acknowledgment is due Dr. Ben Hayek at Auburn University, who conducted the XRD analyses.)

is present in the sample, but even then it is not necessarily a matter of certainty and may require corroborating data (e.g., elemental or thermal analysis). Mixtures of minerals can produce complex XRD patterns that present a challenge in mineral identification. However, several factors mitigate the complexity somewhat for soils. Most soils contain only a few minerals, and these minerals tend to segregate into particle size fractions, which are normally analyzed separately to further reduce complexity. Also, the minerals that occur frequently in soils constitute only a small fraction of the >40,000 that have been identified. Generally, the analyst quickly acquires a familiarity with minerals likely to be found in soils from specific environments and parent materials.

Relative Humidity Control

In our discussion of XRD mounts, we pointed out that it is important to store samples under specific RH before analysis because expandable phyllosilicate d-spacings are dependent on RH. Also, some minerals (e.g., halloysite) are prone to irreversible dehydration and d-spacing change upon air-drying; hence, it is advisable to maintain the soil (and clay fraction obtained from it) in a field-moist state if such minerals are expected. The mineral

identification approaches given below specify treatments that influence RH. Thus, it is necessary to ensure that the appropriate conditions apply. This can be accomplished by using desiccators with atmospheres controlled with various substances. For example, a desiccant such as P_2O_5 or anhydrous $CaSO_4$ can be used for low humidity, water for high humidity, and a saturated solution of $Mg(NO_3)_2$ (RH $\approx$ 54%) for a stable intermediate RH.

General Method

1. A reference of mineral powder diffraction data is needed for identification of unknown minerals. Table 4–1 contains limited diffraction data for some minerals that commonly occur in soils. However, it is useful to have a comprehensive reference such as the Mineral Powder Diffraction Files compiled by the Joint Committee on Powder Diffraction Standards (JCPDS) and published by the International Centre for Diffraction Data.
2. Determine relative peak areas (i.e., relative integrated intensities) and calculate the d-spacings from the 2 θ peak maxima by means of the Bragg equation. Most diffraction systems are equipped with software that automatically calculates peak areas and d-spacings, but the latter can also be performed using a spreadsheet.
3. Start by identifying the peaks for the minerals that you know to be present. For example, many soils contain quartz. Soils described as calcareous probably contain calcite, but may also contain dolomite. Mark these peaks, but remember that these peaks may be shared with other minerals in the sample.
4. Begin with the d-spacing for the highest intensity peak not already identified. Refer to Table 4–1 in this chapter or other reference source to determine prospective minerals with a maximum intensity peak matching that d-spacing. Then check to see if other high-relative-intensity peaks for the prospective mineral are also present. If so, then tentatively mark these peaks with a designation for that mineral.
5. Find the most intense peak not explained by the first mineral tentatively identified. Then follow the same procedure as described in Step 3 to tentatively identify a second mineral, then a third, etc., until all peaks are accounted for. Often two or more minerals can have common or proximal peaks, and this must be taken into account in assessing relative peak intensities. The intensities are additive, such that common peaks would be more intense than if only one mineral were generating them. Relative peak intensities reported for reference diffraction files are a guide as to what to expect, but will not necessarily correspond perfectly to your results even if there is only one mineral contributing to the peak. Many factors can influence relative intensity, with a particularly important one being tendency for preferred orientation. Different specimens of the same mineral can differ in crystal habit and hence in the tendency to orient preferentially.
6. Heat treatments can be used to support the identification of common soil minerals that undergo thermal decomposition or crystallographic shifts in response to dehydration. Normally, routine mineralogical analysis of the soil clay fraction includes heat treatments (e.g., 25, 110, 300, and 550°C) to establish the diagnostic criteria for phyllosilicate identification. However, the identification nonphyllosilicate minerals, such as gibbsite, goethite, and gypsum, can also be corroborated when their peaks disappear at temperatures corresponding to their thermal decomposition (Table 4–2, Fig. 4–6).
7. Phyllosilicates cannot usually be identified without interpretations from special diagnostic treatments (cation saturations, glycerol solvation, heat treatments). These interpretations are described in the next section.

Table 4–1. Major d-spacings for some minerals that occur in soils, listed for each mineral in the commonly observed order of decreasing XRD peak intensity. Spacings given for expandable phyllosilicates are approximate values for Mg saturation at room temperature; d-spacings for these minerals can vary with changes in relative humidity even for homoionic conditions. This is a partial listing of soil minerals and d-spacings; it is recommended that analysts consult a comprehensive reference such as the Mineral Powder Diffraction Files compiled by the Joint Committee on Powder Diffraction Standards (JCPDS) and published by the International Centre for Diffraction Data.

Mineral groups	Minerals	Major d-spacings Å
Amphiboles	hornblende	8.52, 3.16, 2.73
	riebeckite	8.40, 3.12, 2.73
	tremolite	8.38, 3.12, 2.71
Carbonates	aragonite	3.40, 1.98, 3.27
	calcite	3.03, 1.87, 3.85
	dolomite	2.88, 2.19, 1.80
Chlorites	chamosite	7.05, 3.53, 2.52, 14.1
	clinochlore	3.54, 7.07, 4.72, 14.1
Expandable phyllosilicates	montmorillonite	18.0, 9.0, 4.49
	vermiculite	14.4, 7.18, 4.79, 3.60
Feldspars	albite	3.19, 4.03, 3.21
	anorthite	3.20, 3.18, 4.04
	microcline	3.24, 3.29, 4.22
	orthoclase	3.31, 3.77, 4.22
Kaolins	halloysite	10.7–10.0 (hydrated), 7.6, 4.4, 3.4
	kaolinite	7.17, 3.58,
Micas	biotite	10.1, 3.37, 2.06
	muscovite	10.0, 3.33, 5.0
Oxides, hydroxides	anatase	3.51, 1.89, 2.38
	gibbsite	4.85, 4.37, 2.39
	goethite	4.18, 2.45, 2.70
	hematite	2.69, 2.59, 1.69
	ilmenite	2.74, 2.52, 1.72
	quartz	3.34, 4.26, 1.82
	rutile	3.26, 1.69, 2.49
	fluorapatite	2.80, 2.70, 2.77
Phosphates	strengite	4.38, 5.50, 3.11
	variscite	4.29, 5.39, 4.83
	wavellite	8.67, 8.42, 3.22
	augite	2.99, 3.23, 2.95
Pyroxenes	enstatite	3.18, 2.88, 2.54
	antigorite	7.29, 2.53, 3.61
Serpentines	chrysotile	7.31, 3.65, 4.57
	epsomite	4.21, 5.35, 2.68
Sulfates	gypsum	7.56, 3.06, 4.27
	jarosite	3.08, 3.11, 5.09
Sulfides	marcasite	2.69, 3.43, 1.75
	pyrite	1.63, 2.71, 2.43
Zeolites and related minerals	analcime	3.43, 5.60, 2.93
	clinoptilolite	3.97, 8.99, 3.91
	heulandite	3.92, 2.96, 8.85
	palygorskite	10.4, 4.47, 4.26
	sepiolite	12.1, 2.56, 4.31

Table 4–2. Selected diagnostic d-spacings (Å) of common soil minerals at specified conditions of cation saturation, glycerol solvation, and heat treatment. See also Table 4–1 for additional XRD reference data for soil minerals.

Mineral	Diagnostic d-spacings†					
	Mg	Mg + glycerol	K, 25°C	K, 110°C	K, 300°C	K, 550°C
	Å					
Smectites‡	14–15	15–18	10–14	10–12	10	10
Hydroxy-interlayered smectite	14–15	17–18	11–14	12–14	11–12	10–11
Vermiculites	14	14	10–12	10–11	10	10
Hydroxy-interlayered vermiculite	14	14	14	13–14	11–13	10–12
Chlorites	14, 7	14, 7	14, 7	14, 7	14, 7	14, 7
Diocahedral mica	10	10	10	10	10	10
Triocahedral mica	10.3	10.3	10.3	10.3	10.3	10.3
Halloysite	10	10	10	7.2	7.2	no peak
Kaolinite	7.2	7.2	7.2	7.2	7.2	no peak
Gibbsite	4.86	4.86	4.86	4.86	no peak	no peak
Goethite	4.18	4.18	4.18	4.18	no peak	no peak
Gypsum	7.56	7.56	7.56	7.56	3.50	3.50

† Values given in whole numbers should be interpreted flexibly; for example, a certain vermiculite specimen might have a 14.2-Å d-spacing.

‡ Smectites (e.g., montmorillonite, beidellite, and montmorillonite) require further analyses for species-level distinction. See “Methods for Discriminating Among Phyllosilicates” section.

8. Soils high in soluble salts pose a challenge for XRD mineral identification because they may contain several salt minerals with overlapping peaks, resulting in overprinting of important accessory mineral peaks by peaks of more abundant minerals. Complementary approaches such as electron microscopy in conjunction with elemental microanalysis may prove necessary to obtain a thorough mineralogical assessment of soils high in salts.

Methods for Discriminating Among Phyllosilicates

Phyllosilicates minerals are distinguished from one another mainly by the XRD peaks generated from atomic planes parallel to their dominant cleavage face (001). Multiple peaks for a given mineral may be generated from these planes over a typical 2θ scan because constructive interference can occur when the path difference is an integral number of wavelengths (consider the n of the Bragg equation). These peaks are commonly referred to as “first-order” or d001, “second-order” or d002, etc. They are also sometimes termed *basal reflections*. They appear evenly spaced on an XRD pattern, which is a “tip off” for the experienced eye that phyllosilicates are present. Intensities (peak areas) of different order peaks for a mineral commonly vary due to specific structural and compositional effects of the unit cell on the diffracted beam. The d-spacing of higher-order peaks calculate to be equal to the d001 spacing divided by the order (i.e., $d_{002} = d_{001}/2$). It is important to recognize and account for these peaks, so they can be allocated to the mineral and not be mistaken for other minerals. Also, sometimes they occur at the same position as major peaks for other common soil minerals, and their effect must be accounted for in explaining the relative intensities of peaks. For example, the third-order peak for micas (d003; or for two-layer polytypes, d006) (3.33 Å) is not easily resolvable from the most intense peak for quartz (3.34 Å).

Expansible phyllosilicates have variable d00L spacings that relate to cation saturation and relative humidity, while nonexpansible species are insensitive to these variables. Therefore, nonexpansible species can be distinguished from expansible species by their fixed d-spacings in XRD patterns that have undergone different treatments, and from each other (in most cases) by d-spacings and relative intensities of different ordered peaks. Distinctions between expansible phyllosilicates require the application of diagnostic criteria based on response to cation saturations, glycerol solvation, and heat treatments. Different species of expansible phyllosilicates respond differently to these diagnostic conditions. Based on these general characteristics of phyllosilicates, a system for distinguishing phyllosilicate species is given below.

Begin by conducting XRD scans for Mg-saturated, glycerol-solvated samples from 2 to 60° 2 θ ; and for K-saturated samples, from 2 to 32° 2 θ at room temperature and after heating for 4 h at 110, 300, and 550°C. The longer scan for the Mg-glycerol treatment is for the purpose of detecting some soil nonphyllosilicate minerals whose largest peaks occur above 32°. Alternatively, the long scan can be run for the K-saturated sample.

Mark all peaks by the d-spacing value and refer to Table 4–1. The table can serve as a quick reference and general guide except for cases where there is significant interstratification. Any mineral for which all the conditions along its row are met is likely to be present, although there are exceptions that can't all be captured in a simple table. The steps below provide some elaboration and qualifications.

1. Does the Mg-glycerol XRD pattern show a peak at approximately 18 Å? If so, does this peak shift to a lower d-spacing with K saturation, and collapse to 10 Å by 300°C? If both of these conditions are met then a smectite mineral is likely present. Low-charge smectites with interlayer charge arising mainly from the octahedral sheet (e.g., montmorillonite, hectorite) generally have d-spacings 18 Å. Higher-charge smectites, particularly those with significant layer charge arising from the tetrahedral sheet (e.g., beidellite, saponite, nontronite) may show less expansion (17–18 Å) and a tendency to collapse more readily (Malik and Douglas, 1987). However, distinction between smectite species generally requires special procedures. A simple distinction reported by Borchardt (1989) for octahedrally vs. tetrahedrally charged smectites is that the former tend to expand to 18 Å when exposed to glycerol vapor only, while the latter require exposure to liquid glycerol. Other approaches involving Li saturation and heat treatment exploit the tendency for Li to enter vacant sites in the octahedral layer of montmorillonite, thereby neutralizing layer charge and eliminating interlayer expansion (Greene-Kelly, 1952, 1953; Lim and Jackson, 1986). Random interstratification of smectite with other phyllosilicates can also produce d-spacings intermediate between 14 and 18 Å. Generally, interstratification should be suspected if the peak is very broad. Further explanation of interstratification will be given below.
2. A broad peak in the range of 14 to 15 Å that undergoes only partial collapse (i.e., does not collapse to a d-spacing of 10 Å at 300°C) is generally interpreted to be hydroxy-interlayered smectite (i.e., it contains appreciable nonexchangeable hydroxy-Al polymers) (Barnhisel and Bertsch, 1989).
3. Does the Mg-glycerol XRD pattern show a peak at approximately 14 Å? If so, does this peak shift to a lower d-spacing with K saturation, and collapse to 10 by 300°C? This behavior is indicative of vermiculite.
4. A 14-Å mineral is generally interpreted to be hydroxy-interlayered vermiculite if it shows little change with K saturation at room temperature and collapses to a d-spacing >10 Å at 300°C (Barnhisel and Bertsch, 1989) (Fig. 4–6).
5. Does the Mg-glycerol XRD pattern show a peak at approximately 14 Å that does

not change with K saturation or heat treatment at least up to 300°C and usually up to 550°C? If so, this mineral is most likely a chlorite. In some cases, chlorite can be present at too low a concentration to be observed at treatments before the 550°C heat treatment but is observed in this treatment because of the appearance due to intensification of the peak near but usually slightly lower than 14 Å.

6. Does the Mg-glycerol XRD pattern show a peak at approximately 10 Å that shifts to approximately 7.2 after heating to 110°C and disappears at 550°C? If so, this mineral is halloysite. Alternatively, if the kaolinite peak is very broad, is there a peak that rises sharply near 4.2 Å and decreases at a much slower rate over the next 3 to 4°? This may be the sign of dehydrated halloysite.
7. Does the Mg-glycerol XRD pattern show a peak at approximately 10 Å that does not change with any other heat or saturation treatment? If so, the mineral is most likely mica.
8. Does the Mg-glycerol XRD pattern show a peak at approximately 7.2 Å that disappears with 550°C heat treatment? If so, the mineral is most likely kaolinite.

Interstratification (Mixed Layering) in Phyllosilicates

Phyllosilicate crystals sometimes occur as randomly or regularly alternating layers of two or more phyllosilicates (MacEwan and Ruiz-Amil, 1975; Reynolds, 1980), rather than as crystallographically discrete phases. This condition is referred to as interstratification or mixed layering. It results in a series of XRD peaks that differ from those that would have appeared if the interstratified minerals had occurred as independent phases. Some samples contain the same two minerals in both interstratified form and discrete phases. As a further complication, mixtures of elementary clay particles can apparently produce the same effect as interstratification on XRD patterns (Nadeau et al., 1984).

Interstratification is one of the major challenges to XRD mineral identification and quantification. It is essential to be able to recognize and interpret the effects of interstratification. Computer models that aid in detecting and quantifying the extent of interstratification are described below in "Computer Diffraction Modeling."

The simplest form of interstratification to interpret from XRD data is regular interstratification, which in the ideal case is a perfect alternating pattern of layers of the type (MSMSMS, etc.). Regular interstratification is common in soils where primary phyllosilicates (derived from crystalline rocks) such as mica or chlorite are being altered to secondary expandable phyllosilicates. For example, the weathering of chlorite from mafic metamorphic rocks can involve a stage of regularly interstratified chlorite-vermiculite (Johnson, 1964). Two primary phyllosilicates (e.g., chlorite and mica) can also occur as a regularly interstratified phase. Regular interstratification results in a d_{001} that is the sum of the d_{001} spacings for the two phases, since the two phases together rather than individually establish the repeating periodic pattern of the unit cell. Thus, the lowest angle peak (d_{001}) for a regularly interstratified chlorite-mica would be 14 + 10, or 24 Å. Remember, though, that the intensity of peaks can vary according to structural and composition variables, and the 24-Å peak is sometimes weak and hard to see on what is commonly a steep baseline at low angles. Often the second-order peak is more intense.

Response to saturation and heat treatments for regularly interstratified phyllosilicates must be interpreted in light of both interstratified layer types. For example, regularly interstratified mica-vermiculite would have a second-order peak with Mg saturation at 25°C at about 12 Å. This peak would collapse to 10 Å with K saturation after heating to 110°C. Regularly interstratified mica-chlorite, on the other hand, would have a second-order 12-

Å peak that would not change with heat treatment due to the nonexpansible nature of the interstratified phases.

Randomly interstratified minerals are affected by heat treatments and cation saturations in much the same manner as the minerals that form the interstratification, but the locations of the peak are more complex. For example, if the d-spacing for a peak from an interstratified mineral is higher in the Mg glycerol pattern when compared with the Mg-saturated XRD pattern, then the interstratification includes smectite. If there is no difference between these two patterns, but there is a change when the mineral is K-saturated and heated to 110°C, then the mineral contains vermiculite. These effects are especially important when there are low amounts of one mineral interstratified with another. For example, if 10% smectite is interstratified in 90% kaolinite or chlorite, the Mg-saturated XRD pattern will appear to contain only those minerals because the smectite d001 is the same as that of chlorite and the d002 is the same as the d001 of kaolinite. When the sample is glycerol solvated, however, the sample peaks will broaden due to the deviation of the d00L of the smectite from that of the other minerals, allowing the detection of the smectite interstratification.

Interpretation of randomly interstratified phases is challenging because the diffraction effects result from packets of varying amounts of pure endmember minerals. The location of peaks is the result of a weighting of the effects of diffraction for each mineral. Where the peaks for both minerals are close, a sharp peak results. Where the peaks for the minerals greatly differ, no peak or a broad peak may be formed (for a detailed explanation see the method of Mering as described in Sawhney, 1989). Another indication of random interstratification is irrational higher order peaks (basal reflections).

Crystallite Size

Often the size of diffracting crystallites is important in describing the activity of a mineral in soils, because surface area increases with decreasing particle size. The Scherrer equation (Klug and Alexander, 1974) gives a first approximation of the value for this factor:

$$L = (\lambda K) / (\beta \cos \theta) \quad [2]$$

where L is the mean crystallographic dimension in the same units as the wavelength λ , K is a constant near unity, β is the width of a peak at half height in radians of 2π corrected for instrumental factors, and θ is the location of the peak. The Scherrer equation may underestimate particle thickness, however, because it does not account for effects of atomic substitutions. Another caveat is that it is actually crystal domain thickness rather than direct particle thickness that is calculated; it is common for soil mineral particles to contain numerous (smaller) crystal domains.

QUANTIFICATION: LIMITATIONS AND APPROACHES

Limitations

We begin with limitations to stress the fact that in most cases soil mineral quantification is “semiquantitative” at best. Accurate mineral quantification by XRD can only be achieved under ideal conditions that include the following:

- Mineral phases are discrete and well crystalline, which rarely applies for soils.
- The degree of preferred orientation for each phase can be controlled or accounted for.
- Mounting artifacts like differential sedimentation are avoided.
- Relatively few phases are present.

Deviation from these ideal conditions can result in considerable interlaboratory disparity in mineral proportions reported for the same samples (Ottner et al., 2000).

Quantification requires accurate calibration of the relative integrated XRD intensities with relative weight fractions of phases present. A major impediment to attaining quantitative results for soils is the compositional and structural heterogeneity of soil minerals, which results in variability in peak intensity even within a given mineral species. Solid solution series such as with feldspars also add complexity to XRD interpretations, as compounded by specimen to specimen variability in such critical properties as crystal habit and tendency for preferred orientation. Phyllosilicate quantification is further complicated by varying degrees of interstratification and interlayer occupancy with nonexchangeable metal polymers. Hydroxy-interlayered minerals, which are common secondary phyllosilicates in highly weathered soils, do not occur in pure deposits and hence have not been well characterized for their chemical and crystallographic properties. Furthermore, the nature and abundance of interlayers as well as characteristics of the 2:1 components probably display appreciable geographic variability.

Chapters are already available that address mineral quantification techniques using XRD in relative detail (e.g., Brindley, 1980; Reynolds, 1989b; Snyder and Bish, 1989; Bish, 1994; Hughes et al., 1994). Also, some approaches to mineral quantification use other methods (e.g., chemical composition, cation exchange capacity, thermal analysis) to complement XRD (e.g., Jackson, 1969; Karathanasis and Hajek, 1982). Such sources should be consulted if quantification is the major goal of the analyst. We will cover XRD quantification in only an introductory way in this section, addressing basic principles and summarizing two commonly used techniques. A subsequent section, "Computer Diffraction Modeling," addresses modeling applications to aid in quantifying phyllosilicate mixtures for oriented samples.

Theoretical Considerations

The integrated intensity (I) of a given XRD peak is controlled by a variety of instrumental, mounting, and sample-related factors, including weight fraction of the phase generating the peak. Absolute measurements of I are impractical and unnecessary for the purposes of quantification, thanks to the following relationship,

$$I_a/I_b = K_{ab} W_a/W_b \quad [3]$$

where I_a and I_b are relative intensities of selected peaks for minerals "a" and "b," respectively, and W_a and W_b are weight fractions of minerals a and b relative to each other in the sample. Noncrystallographic variables cancel out in the derivation of this equation. The calibration constant, K_{ab} , a key to mineral quantification by XRD, is also termed the *mineral intensity factor* (MIF) (Reynolds, 1989b). The K_{ab} (or MIF) can be determined empirically by mixing standards of two minerals at a known weight fraction and conducting an XRD scan, in which case it can be calculated as the only unknown in the above equation. However, there is no guarantee that the standard will accurately match the species as it occurs in the sample with respect to diffraction characteristics. Alternatively, it can be calculated from the scattering factor as determined using XRD modeling computer programs (Reynolds, 1989b). The latter approach is faster and eliminates the necessity of having multiple standards on hand. Also, it can enable an accounting for compositional (e.g., Fe vs. Mg) variations by iteratively specifying the elemental proportions until relative intensities of specific basal reflections (for phyllosilicates) of the modeled pattern match the actual pattern.

Equation [3] is the basis for several approaches to mineral quantification by XRD. Two approaches, the intensity ratio method and the internal standard method, are explained in the next sections. Another approach involving full-pattern fitting (the Rietveld method; Rietveld, 1969), originally developed for crystal structure refinement, has been adapted for quantitative XRD analysis (Bish and Howard, 1988; Smith, 1989; Bish, 1994). Full-pattern fitting for mineral quantification is most advantageous for mixtures of well-ordered minerals, but it has been applied successfully to soils and geologic materials (e.g., Jackman et al., 1997; Aylmore and Walker, 1998; Gualtieri, 2000; Jones et al., 2000; Plançon and Drits, 2000; Altomare et al., 2001; Srodon et al., 2001). The full-pattern approach involves computer modeling, which is addressed in a later section of this chapter.

Intensity Ratio Method

An important caveat is that this method and the one that follows (internal standard method) are based on theoretically sound principles, but they are unlikely to yield absolute quantitative results for soil minerals due to the factors summarized under “Limitations,” above. Nevertheless, these approaches do provide objective means of comparing relative amounts of minerals within limited contexts (e.g., changes with depth, landscape). Usually it would be best to report data as an index (e.g., relative peak area index) rather than as percentage.

Equation [3] can be rearranged as follows:

$$W_a = (1/K_{ab})(I_a/I_b)W_b \quad [4]$$

Of course, $1/K_{ab}$ is still a constant, which we could label K_{ba} . Hence,

$$W_a = K_{ba}(I_a/I_b)W_b \quad [5]$$

We can represent a mixture of several minerals in terms of the weight fractions of the minerals, assuming all components are crystalline and detectable, and therefore the sum of weight fractions is one:

$$W_a + W_b + W_c = 1 \quad [6]$$

We can determine the calibration constants K_{ca} and K_{cb} , say, by mixing known weights of minerals a and c and minerals b and c and determining the values of I_a/I_c and I_b/I_c (alternatively, the constants could be calculated from computer XRD modeling software). In effect, we would determine the calibration constant for all minerals in the mixture in relation to one selected mineral in the mixture, in this case mineral c:

$$K_{ca}(I_a/I_c)W_c + K_{cb}(I_b/I_c)W_c + W_c = 1 \quad [7]$$

We can then solve for W_c , the only unknown variable in the equation. Knowing W_c allows us to calculate W_a and W_b from the expressions representing them in the equation.

The intensity ratio method has a theoretical basis, is objective, and can be done with high precision. However, it fails to provide a check for accuracy since it “forces” the calculated weight fractions to total one. This is a disadvantage, because accuracy is not constrained to follow precision, and hinges on the degree to which the standard minerals match their counterparts in the sample with respect to diffraction intensities (Bish, 1994). The internal standard method, explained next, does not require the constraint that the calculated weight fractions total one.

Internal Standard Method

Equation [5] is also the basis for the internal standard method. However, Eq. [6] becomes:

$$W_a + W_b + W_c = ? \quad [8]$$

Also, all the expressions representing weight fractions on the left side of Eq. [4] can be calculated directly. This is because the mineral c would be added in known amounts (W_c) to the sample, hence the term *internal standard*. Favorable characteristics of an internal standard mineral are that (i) it has intense diffraction peaks, (ii) it doesn't occur in the sample, (iii) its strongest peak does not overlap with peaks of the minerals in the sample, and (iv) it occurs in the same 2θ range as the peaks of the minerals being assessed in the sample. Corundum is a mineral that generally meets these characteristics and is a frequently used internal standard. Pyrophyllite is sometimes used as an internal standard for samples containing appreciable phyllosilicate components because its orientation properties and peak position are similar to other phyllosilicates.

COMPUTER DIFFRACTION MODELING

There are many programs that simulate XRD data. Some require detailed knowledge of the crystal structure, including atom locations, symmetry, unit cell size, and types and amounts of substitution in the structure. These programs can give valuable information for interpreting diffraction patterns, but often require information that is not available to the researcher. (Also, most methods are designed to simulate random powder diffraction patterns rather than oriented patterns.) The principle reason for modeling XRD patterns is that it allows you to interpret at least qualitatively differences that are occurring between patterns. For example, models of a set of XRD patterns for a series of depths in a profile may allow you to explain the types of changes in the mineralogy that accompany weathering, such as increases in a mineral relative to others or decreases in mineral crystallinity.

Any program expected to simulate observed XRD patterns with any accuracy requires the correction for diffractometer parameters, including the radiation source, slit sizes, and the presence of a theta compensating slit (device that maintains constant area of incident beam exposure on sample). Proper use requires that these be preset to values that are used by the diffractometer producing the observed patterns so that the calculated patterns are consistent with those observed. Failure to set these parameters correctly can produce a spurious match with the observed patterns.

In general, there are two types of computer programs available for use in soil mineralogy: those that yield specific data about individual minerals in samples and those that can be used to model entire diffraction patterns to quantify the minerals in the sample.

Mineral Characterization Programs

Several programs have been written to help in the characterization of individual minerals in clay fractions. The most well known program is NEWMOD (Reynolds, 1985), and less well known but potentially useful are the MUDMASTER and GALOPER packages (Eberl et al. (1996, 2000).

When a sample is examined by XRD, the resulting peaks are averages resulting from billions of individual crystals of different sizes and with different amounts of strain. The MUDMASTER and GALOPER packages are written to aid in the characterization of crystallite size distributions and strain on the XRD peaks in the sample. Both programs assume

that the mineral examined is not interstratified. MUDMASTER (Eberl et al., 1996) uses profile analysis of peaks to determine the crystallite size in the crystal direction corresponding to the peak. Proper use of the program requires high-quality XRD data collected at relatively close steps ($0.02^\circ 2\theta$) to calculate the crystallite size distribution. GALOPER (Eberl et al., 2000) is a companion program to MUDMASTER that calculates crystal size distributions (CSDs) that result from crystal growth in open and closed systems using several growth mechanisms. The program also calculates the effects that two types of weathering reaction should have on crystal size. When used together, the programs have potential for interpreting the origin of a mineral in a soil.

NEWMOD (Reynolds, 1985) is probably the most useful computer program available for interpreting oriented XRD patterns for clay samples. The program calculates one-dimensional (oriented) XRD patterns for pure phyllosilicates and for interstratifications of any two phyllosilicates. Once these minerals are individually simulated, the simulated patterns may be combined to simulate a mixture of minerals. It is not possible to model XRD patterns for samples with nonphyllosilicates or the palygorskite-sepiolite group using this program. NEWMOD allows for the calculation of effects of isomorphous substitution, ordering of interstratification, and particle size on the minerals present in a sample. The results are singularly useful for the estimation of isomorphous substitution within those phyllosilicates with a high degree of substitution. A small amount of Fe substitution, for example, in most 2:1 phyllosilicates may result in orders of magnitude changes in total XRD intensity. This substitution can be estimated with NEWMOD using the changes in the relative intensities of the 00L peaks observed in the XRD pattern. NEWMOD is probably the best available program for the interpretation of patterns resulting from interstratifications of one mineral with another. Calculation of particle size on the diffraction peaks of phyllosilicates using peak broadening and shifts is also possible, and the program allows custom size distributions such as those determined from MUDMASTER and GALOPER. Interpretation of differences in hydration in smectite XRD patterns is also possible using the program. A subprogram within NEWMOD can also be used for mineral quantification using simulated patterns if the only nonphyllosilicate present is quartz (which although not included in the program is used as an intensity standard and can be added as a data file).

Mineral Quantification Programs

Mineral quantification programs can vary from relatively simple to extremely detailed and complex. In general, the programs use methods described earlier in our discussion of quantification. The more easily applied programs, such as FULLPAT (Chipera and Bish, 2003) and ROCKJOCK (Eberl, 2003), use reference patterns. More rigorous Rietveld programs require more detailed information about the minerals in the sample.

The most common method, used in the programs FULLPAT (Chipera and Bish, 2003) and ROCKJOCK (Eberl, 2003), involve the use of patterns of reference standards, preferably run on the same XRD unit under the same conditions as the sample. Both methods involve the use of internal standards both in the reference standards and in the samples. Both programs are also written to work as Microsoft Excel spreadsheets. The mathematics of the quantification is that covered in the "Internal Standard Method" section above. The use of the internal standards has the advantage that it does not require the total to equal 100%. The disadvantage is that the reference standards need to have similar characteristics as the minerals in study samples. Application of these methods requires a consistent internal standard. Therefore, anyone using this method is advised to obtain a large supply of the mineral used as the internal standard to prevent problems resulting from small differences between batches.

Another approach involving full-pattern fitting is the Rietveld method (Rietveld, 1969), which was originally developed for crystal structure refinement and was adapted for quantitative XRD analysis (Bish, 1994). This approach uses no reference patterns but calculates the pattern based on the crystal structures of the minerals present in the samples. This method requires much more knowledge about the minerals present in the sample and at least some knowledge of crystallography. The use of the method has been simplified somewhat by use of modern graphical user interface, such as the EXPGUI package for the General Structure Analysis System (GSAS) program (Toby, 2001), but it is still beyond the capabilities of all but dedicated mineralogists. The programs work well for quantification of well-ordered minerals, but can be used with soils and geologic materials. The biggest limitation is that a known crystal structure or a close approximation to the structure is required. On the other hand, the programs can be used to refine a crystal structure if you can come up with an approximation, even if it is in a mixture.

Computer Modeling of Oriented X-ray Diffraction Patterns

The first step in proper use of any XRD simulation program is to identify the minerals in the sample examined. This requires interpretation of the observed patterns. It is impossible to model observed data without knowing what minerals are present in the sample. Examine all patterns carefully. Know how standard minerals react when treated in the same manner as the samples. Do not forget to look at all the fractions even if there is especial emphasis on one fraction because a mineral may be more easily identified in another fraction (see Towe, 1974, for an example of the importance of this practice).

Although any of the programs listed earlier could be used to model oriented XRD patterns, only NEWMOD is designed specifically for that purpose. The programs that use reference patterns could be utilized if the reference patterns were also from oriented samples. Rietveld programs would not be useful for quantification of patterns resulting from oriented samples. Modeling patterns with randomly interstratified phyllosilicates will not be possible without NEWMOD.

In any method, the steps outlined above in the "Mineral Identification from X-ray Diffraction Data" section must be made to prepare for the analysis. When using NEWMOD, the next steps would be:

1. Look for shifts in peak locations and widths between patterns with different cation saturations and heat treatments. Only peaks from expansible minerals should change due to cation saturation and low temperature (<200°C) heat treatments. If peaks identified for nonexpansible minerals are different between patterns, the minerals may be incorrectly identified, or if the changes are very small, there may be a small amount of interstratification with an expansible phyllosilicate.
2. If peaks change in width or position between patterns, determine the amount of change. If the changes differ significantly from standard minerals, this may be the result of interstratification. The amount of change is important in determining the proportion of expansible phyllosilicates in an interstratified phyllosilicate.
3. Once the minerals and any irregularities resulting from interstratification are identified, then the individual minerals in the sample are simulated. When simulating XRD patterns for individual minerals to match observed data, the effects of isomorphous substitution and the range of diffracting domain thicknesses need to be modeled. Some data on the isomorphous substitution can be obtained by looking at the relative heights of the 00L ("basal reflections" of phyllosilicates) whose relative intensities are orientation independent with respect to each other (i.e., they are equally affected by degree of orientation). Many studies have been made to obtain data for

this use (e.g., Brown and Brindley, 1980; Moore and Reynolds, 1997), but these values can only be used as a first approximation because they were not corrected for the instrumental parameters of the instrument used to obtain the observed data. To obtain the best possible results, it is helpful if there is data from other methods. Infrared and other spectroscopic techniques may give indications of the octahedral cations of the mineral, as may total elemental analysis. There are also a few chemical tests, such as the Greene-Kelly test (Greene-Kelly, 1952, 1953), that provide information on the octahedral sheet of the mineral. The more data integrated into the interpretation, the more likely the results will be accurate. The effects of substitution on peak intensity cannot be overemphasized. Substitution of Fe for Al in a structure may result in a 10-fold increase in peak intensity.

4. Particle size and defects affect peak size, shape, and location when the thickness of the diffracting domains falls below a certain value. In most cases, there is not a single value for this correction but a range of values. The high end of the range affects the sharpness of the peaks, the low end of the range is expressed by the broadness of the peaks at their base, and the intermediate values affect the overall shape of the peak. Once an approximate value is obtained, choose values for the thickness parameters that include this value and revise the data on the basis of these results until satisfied with the match to the data.
5. After each of the individual minerals has been modeled, combine the patterns by adding them together in proportion to their concentration in the sample. Use whatever data are available describing the minerals (e.g., thermal, CEC, total K, FTIR) to assist in the estimations and model the remainder.

DIFFERENTIAL X-RAY DIFFRACTION

Principles and Applications

Modern XRD systems, which provide data in a digital format, allow the user to identify minerals by producing a differential XRD pattern (DXRD). Subtracting one XRD pattern from another produces a DXRD pattern. In most cases, one pattern is scaled such that when the two patterns are subtracted, one or more peaks from a mineral disappear from the resulting pattern.

For instance, knowledge on the changes of the feldspar species in a weathering sequence in which quartz decreases with depth may be desired, but the strong quartz peaks overwhelm the XRD pattern. Assuming that different feldspar species in the sample are not being selectively weathered, a DXRD pattern can be produced by scaling the pattern with higher quartz peaks such that the quartz peaks are the same height as in the other pattern, then subtracting the scaled pattern from the other pattern. The resulting pattern would not have the quartz peaks interfering with the feldspar peaks making it easier to identify the feldspars. It is also possible to determine whether there is selective dissolution of some feldspar species, as some feldspars would have negative peaks and some would have positive peaks.

Another situation in which DXRD patterns are useful is in the identification of oxide minerals in a mixed sample where XRD peaks of other minerals overlap those of the oxide peaks. Many oxide minerals have importance even when present in low concentrations. It may not be possible to positively identify the mineral because of low concentration, overlapping peaks, or poor diffraction efficiency. If the oxide mineral or the mineral with overlapping peaks can be removed by chemical or physical means, then the diffraction pattern of the sample after the removal of the mineral can be compared to the pattern before the removal to identify the minerals present in the sample. Subtracting the diffraction pattern of the sample before treatment from the pattern after treatment can identify the mineral

that is removed.

To perform a pattern subtraction with some form of digital data, scale one of the patterns to the other by multiplying the counts by a factor that would result in zero intensity for the peaks of a mineral in the sample. Ideally, this factor would be constant for all peaks in a sample resulting from a mineral. This is rarely the case. A very small lateral mismatch of 2θ values between the two patterns will result in peaks with positive and negative tails. For instance, a quartz pattern is shifted by $\pm 0.05^\circ 2\theta$ and subtracted from itself, and DXRD peaks are obtained in the $26.6^\circ 2\theta$ region (Fig. 4–7). The net area for the peak is by definition zero counts, but there are positive and negative regions of the peak resulting from the peak shifts.

Orientation changes in one or more of the minerals between two patterns used to produce a DXRD pattern may confound results. This is because some peaks on the difference pattern will be negative and others will be positive even if the minerals producing the peaks were not affected by the treatment. Even more of a problem is that some peaks of a mineral may change in intensity relative to other peaks from the same mineral as a result of orientation changes.

It is helpful if the mineral is present in a relatively high concentration or gives strong XRD peaks and if peaks don't overlap with other minerals. Manganese oxides, for instance, are typically disordered with broad peaks and have peak overlaps with phyllosilicates. Identification of these minerals in concentrations of less than about 5 to 10%, except in ideal situations, is not feasible.

Most pattern subtraction methods involve direct subtraction of the data on a step interval basis. In these methods, patterns may be shifted along the 2θ axis by steps that are multiples of the steps used in the step scans. A better approach is to use a spline-fitting program such as described by Schulze (1986). A spline-fitting program fits the curves to a spline function (a cubic spline function in the case of Schulze, 1986) before performing the pattern subtraction. Spline programs are much superior to the pattern subtraction routines that come with most XRD units. Such programs allow for the subtracting of the patterns after moving one relative to the other by 2θ values different from the data increments.

Another technique that might help obtain even better DXRD data is that of Wang et al. (1993). This technique involves selective dissolution of a relatively thin layer of the clay fraction sedimented onto a quartz slide. The difference pattern is produced by subtracting the pattern after dissolution from the pattern before dissolution. Selective dissolution of the material without removal from the slide minimizes the variability resulting from orientation changes and results in better DXRD data.

When making a DXRD pattern, the easiest way to get started is to choose a mineral that is unaffected by the treatment (e.g., quartz) and adjusting the scaling factors (con-

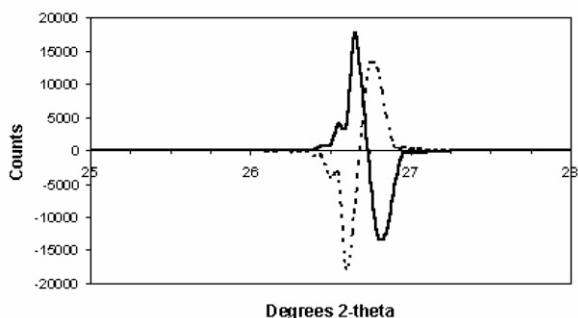


Fig. 4–7. Example of the results obtained using differential X-ray diffraction in which a quartz pattern is subtracted from itself after being shifted along the 2θ axis. The solid line represents a shift of $+0.05^\circ 2\theta$, and the dashed line represents a shift of $-0.05^\circ 2\theta$.

trolled using the XRD computer software options) between the two patterns until the XRD peaks for the mineral are gone. In DXRD patterns obtained from randomly oriented samples, the use of a factor sufficient to remove the XRD peaks for one mineral often results in some other mineral, which was not affected directly by the treatment, having positive or negative XRD peaks due to changes in orientation.

Differential X-ray Diffraction by Selective Dissolution on a Slide

Selective dissolution on a slide was introduced in Wang et al. (1993). The technique can be used with any selective dissolution treatment. For example, ammonium oxalate in the dark can be used to remove poorly crystalline Fe oxides, or hydroxylamine hydrochloride can be used to remove Mn oxides. Unlike cases when quantification is desired, dissolution of all of a mineral is not required to identify the mineral by DXRD. In many cases, dissolving too much of a sample results in a disruption of the fabric of the material on the slide, negating the advantages of the selective dissolution on the slide technique. For that reason, a weaker solution and shorter reaction time is desirable to decrease the amount of dissolution and better preserve the fabric of the material on the slide. In a situation analogous to the carpenter's axiom, "measure twice, cut once," it is always possible to perform another dissolution treatment if the resulting DXRD pattern shows insufficient change. After the subsequent treatment, it is possible to subtract the XRD pattern from the XRD pattern for untreated material or from the pattern performed after the first treatment. There may be important differences between the material removed initially and that removed later (see, e.g., Wang et al., 1993).

Required materials include a nonreflecting quartz slide, beakers, a mister, and the solution used for the dissolution. The quartz slide cut to result in no XRD lines (readily available commercially) has much lower background interference than a glass slide. The flat background allows for the more definitive identification of poorly crystalline minerals such as ferrihydrite.

The optimal sample for this technique should be a clay fraction. Coarser particles may not adhere well to the slide. The sample should also be NH_4^+ or K^+ saturated for the selective dissolution technique. This is especially important for samples containing smectite or vermiculite, which are sensitive to cation saturation. Use of NH_4^+ or K^+ saturated samples will prevent problems due to the shifting of phyllosilicate peaks.

Mounting Technique for Differential X-ray Diffraction

Materials

- Low-background quartz crystal mount
- Disposable pipettes

Procedure

1. Saturate the clay with K^+ or NH_4^+ .
2. Wash by adding distilled water, shaking, centrifuging and pouring off the supernatant. Washing the sample removes excess salts that, once dried, may exhibit peaks on the XRD pattern.
3. Dry the sample. This will cause expandable minerals to collapse and become insensitive to cations. The lack of sensitivity of these peaks to the saturating cation will greatly aid in the ability to obtain good pattern subtractions near the expandable mineral XRD peaks.
4. Disperse the clay fraction in a small quantity of distilled water as follows:

- (i) Add about 2 to 3 mL of water with about 125 mg of clay in a small diameter test or centrifuge tube.
 - (ii) Use the same disposable pipette that will be used to apply the sample to the slide. Suck up some of the solution with the pipette and discharge it with force at the clay and solution in the tube. Repeat this process until the sample appears dispersed and completely suspended.
5. When the clay is dispersed, suck up some of the dispersed clay suspension and deposit it onto the nonreflecting quartz slide.
 6. Allow the clay to dry naturally at room temperature. Addition of heat increases the chance of the sample peeling from the slide. If the sample peels from the slide as it dries, dilute the dispersed solution and try again. If the sample is too thin, add more material. It is preferable to have a thin layer completely covering the area of the slide undergoing diffraction than a thicker, less uniform coating. The sample material is applied thinly to allow the selective dissolution chemicals to react through the material more evenly, with less disruption of sample fabric. The best DXRD patterns are obtained when the fabric is unaffected. The material is sedimented to increase adhesion to the slide. The strong adhesion of the clay to the slide results in (hopefully) less disturbance during DXRD treatment and hence less difference in orientation of the undissolved material between the before and after slides.

Obtaining an X-ray Diffraction Pattern for the Slide Before Treatment

Use a much longer dwell time per 2θ step than normally used (8–10 times as long) to reduce interferences from background noise and to increase the counting statistics. This is especially important if the material to be removed does not diffract strongly, is present only in minute amounts, or has peaks that overlap strongly with the peaks of other minerals. A better DXRD pattern will be obtained for the weak peaks of a mineral if the peaks are easily resolvable from the background noise. A longer count time per step reduces the variability in the background. As the pattern may require a long period of XRD time, the scan can be run overnight. Experience has shown that the use of shorter 2θ steps does not necessarily result in better diffraction data if the material to be identified has broad peaks relative to the step size. The less variable background from longer step times is more important than an increased number of data points.

Conducting Differential X-ray Diffraction Treatment

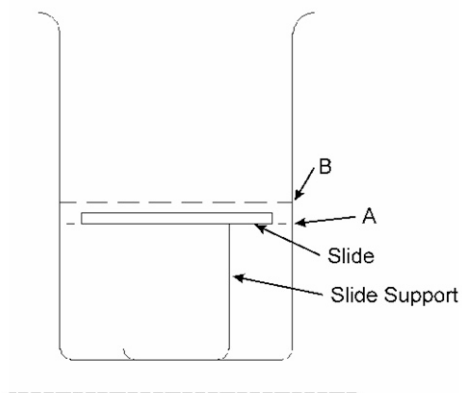
Materials

- Low-background quartz crystal mount
- Large and small beakers
- Disposable pipettes

Procedure

1. Place a small beaker, with a diameter smaller than the length of the slide, into a second beaker just larger enough to allow the slide to be placed face up on the first beaker (Fig. 4–8). The smaller beaker is added to elevate the slide above bottom of the second beaker and can be replaced by any level object that is unreactive in the treatment being applied. The purpose of this beaker is to allow for a relatively large volume of reacting liquid, thereby resulting in a large solution/solid ratio while allowing the slide to be barely suspended within the liquid.
2. Add the reacting liquid to the beakers until the level of the liquid is approximately level with the top of the inner beaker.

Fig. 4-8. Schematic of apparatus used for *in situ* selective dissolution on a quartz slide.



3. Mist the sample and slide with distilled water or reacting liquid. This step helps to prevent the material from lifting off the slide by reducing the wetting angle. It also lessens potential orientation changes on the slide.
4. Place the slide face up on the inner beaker. The level of reacting liquid at this point should be just lower than the face of the slide. If the solution is deeper, there is a risk of having the sedimented layer lift off the slide as a unit as it is immersed.
5. Use a disposable pipette to add more of the reacting liquid to the outer beaker until the solution flows across the slide. Add the liquid slowly, allowing it to flow down the inner side of the beaker to reduce turbulence. Add the solution especially cautiously when the liquid is beginning to flow across the top of the slide.
6. When the treatment time has elapsed, reduce the level of the liquid in the beaker until it is below that of the slide using the same pipette used to add the liquid being careful to prevent turbulence.
7. Remove the slide from the beaker. Replace the reacting solution in the beakers with distilled water and repeat Steps 5 and 6 to wash salts off the slide.
8. Allow the slide to dry and obtain an XRD pattern of the residue as performed in Step 4. Use a DXRD program to obtain a difference pattern.

REFERENCES

- Altomare, A., M.C. Burla, C. Giacovazzo, A. Guagliardi, A.G.G. Moliterni, G. Polidori, and R. Rizzi. 2001. Quanto: A Rietveld program for quantitative phase analysis of polycrystalline mixtures. *J. Appl. Crystallogr.* 34:392-397.
- Aylmore, M.G., and G.S. Walker. 1998. The quantification of lateritic bauxite minerals using x-ray powder diffraction by the Rietveld method. *Powder Diffr.* 13:136-143.
- Barshad, I. 1950. The effect of interlayer cations on the expansion of the mica-type crystal lattice. *Am. Mineral.* 35:225-238.
- Barnhisel, R.I., and P.M. Bertsch. 1989. Chlorites and hydroxy-interlayered vermiculite and smectite. p. 729-788. *In* J.B. Dixon and S.B. Weed (ed.) *Minerals in soil environments*. 2nd ed. SSSA Book Ser. 1. SSSA, Madison, WI.
- Bish, D.L., and S.A. Howard. 1988. Quantitative phase analysis using the Reitveld method. *J. Appl. Crystallogr.* 21:86-91.
- Bish, D.L., and J.E. Post (ed.) 1989. *Modern powder diffraction*. Mineralogical Society of America, Washington, DC.
- Bish, D.L. 1994. Quantitative x-ray analysis of soils. p. 267-296. *In* J.E. Amonette and L.W. Zelazny

- (ed.) Quantitative methods in soil mineralogy. SSSA, Madison, WI.
- Bish, D.L., and R.C. Reynolds, Jr. 1989. Sample preparation for x-ray diffraction. p. 73–100. *In* D.L. Bish and J.E. Post (ed.) Modern powder diffraction. Mineralogical Society of America, Washington, DC.
- Borchardt, G. 1989. Smectites. p. 675–727. *In* J.B. Dixon and S.B. Weed (ed.) Minerals in soil environments. 2nd ed. SSSA, Madison, WI.
- Brindley, G.W. 1966. Ethylene glycol and glycerol complexes of smectites and vermiculites. *Clay Miner.* 6:237–260.
- Brindley, G.W. 1980. Quantitative x-ray mineral analysis of clays. p. 411–438. *In* G.W. Brindley and G. Brown (ed.) Crystal structures of clay minerals and their X-ray identification. *Miner. Soc. Monogr.* 5. Mineralogical Society, London.
- Brown, G., and G.W. Brindley. 1980. X-ray diffraction procedures for clay mineral identification. p. 305–359. *In* G.W. Brindley and G. Brown (ed.) Crystal structures of clay minerals and their X-ray identification. *Mineral. Soc. Monogr.* 5. Mineralogical Society, London.
- Buck, B.J., and J. Van Hoesen. 2002. Snowball morphology and SES analysis of pedogenic gypsum, southern New Mexico, USA. *J. Arid Environ.* 51:469–487.
- Chipera, S.J., and D.L. Bish. 2003. FULLPAT: A full pattern quantitative analysis program for x-ray powder diffraction using measured and calculated patterns. *J. Appl. Cryst.* 35:744–749.
- Cullity, B.D. 1978. Elements of x-ray identification. Addison-Wesley Publ., Reading, MA.
- Drever, J.I. 1973. The preparation of oriented clay mineral specimens for x-ray analysis by a filter membrane filter technique. *Am. Mineral.* 58:553–554.
- Eberl, D.D. 2003. User's guide to ROCKJOCK—A program for determining quantitative mineralogy from powder x-ray diffraction data. USGS Open-File Rep. 03-78. USGS, Denver, CO.
- Eberl, D.D., V.A. Drits, J. Srodon, and R. Nüesch. 1996. MudMaster: A computer program for calculating crystallite size distributions and strain from the shapes of X-ray diffraction peaks. USGS Open-File Rep. 96-171. USGS, Denver, CO.
- Eberl, D.D., V.A. Drits, and J. Srodon. 2000. User's guide to GALOPER—A program for simulating the shapes of crystal size distributions—and associated programs. USGS Open-File Rep. OF 00-505. USGS, Denver, CO.
- Gibbs, R.J. 1965. Error due to segregation in quantitative clay mineral x-ray diffraction mounting techniques. *Am. Mineral.* 50:741–751.
- Greene-Kelly, R. 1952. Irreversible dehydration in montmorillonite. *Clay Miner. Bull.* 1:221–227.
- Greene-Kelly, R. 1953. Irreversible dehydration in montmorillonite. Part II. *Clay Miner. Bull.* 2:52–56.
- Grim, R.E. 1968. *Clay mineralogy*. 2nd. ed. McGraw-Hill, New York.
- Gualtieri, A.F. 2000. Accuracy of XRPD QPA using the combined Rietveld-RIR method. *J. Appl. Crystallogr.* 33:267–278.
- Harward, M.E., and A.A. Theisen. 1962. Problems in clay mineral identification by x-ray diffraction. *Soil Sci. Soc. Am. Proc.* 26:335–341.
- Harward, M.E., A.A. Theisen, and D.D. Evans. 1962. Effect of iron removal and dispersion methods on clay mineral identification by x-ray diffraction. *Soil Sci. Soc. Am. Proc.* 26:535–541.
- Hillier, S. 1999. Use of an air brush to spray dry samples for x-ray powder diffraction. *Clay Miner.* 34:127–135.
- Hughes, R., and B. Bohor. 1970. Random clay powders prepared by spray drying. *Am. Mineral.* 55:1780–1786.
- Hughes, R.E., D.M. Moore, and H.D. Glass. 1994. Qualitative and quantitative analysis of clay minerals in soils. p. 330–359. *In* J.E. Amonette and L.W. Zelazny (ed.) Quantitative methods in soil mineralogy. SSSA, Madison, WI.
- Jackman, J.M., R.C. Jones, R.S. Yost, and C.J. Babcock. 1997. Rietveld estimates of mineral percentages to predict phosphate sorption by selected Hawaiian soils. *Soil Sci. Soc. Am. J.* 61:618–625.
- Jackson, M.L. 1956. *Soil chemical analysis—Advanced course*. Publ. by author, Madison, WI.
- Jackson, M.L. 1969. *Soil chemical analysis—Advanced course*. Revised ed. Publ. by author, Madison, WI.
- Johnson, L.J. 1964. Occurrence of regularly interstratified chlorite-vermiculite as a weathering product of chlorite in a soil. *Am. Mineral.* 49:556–572.
- Jones, R.C., C.J. Babcock, and W.B. Knowlton. 2000. Estimation of the total amorphous content of Hawaiian soils by the Rietveld method. *Soil Sci. Soc. Am. J.* 64:1100–1108.
- Karathanasis, A.D., and B.F. Hajek. 1982. Revised method for rapid quantitative determination of minerals in soil clays. *Soil Sci. Soc. Am. J.* 46:419–425.
- Klug, H.P., and L.E. Alexander. 1974. X-ray diffraction procedures for polycrystalline and amorphous

- materials. 2nd. ed. John Wiley & Sons, New York.
- Lim, C.H., and M.L. Jackson. 1986. Expandable phyllosilicate reactions with lithium on heating. *Clays Clay Miner.* 34:346–352.
- Malik, P.B., and L.A. Douglas. 1987. Identification of expanding layer silicates: Layer charge vs. expansion properties. p. 277–283. *In* L.G. Schultz et al. (ed.) *Proc. Int. Clay Conf.* Denver. 28 July–2 Aug. 1985. Clay Minerals Soc., Bloomington, IN.
- MacEwan, D.M.C., and A.A. Ruiz-Amil. 1975. Interstratified clay minerals. p. 265–334. *In* J.E. Giesking (ed.) *Soil components*. Vol. 2. Springer-Verlag, New York.
- MacEwan, D.M.C., and M.J. Wilson. 1980. Interlayer and intercalation complexes of clay minerals. p. 197–248. *In* G.W. Brindley and G. Brown (ed.) *Crystal structures of clay minerals and their X-ray identification*. Mineral Soc. Monogr. 5. Mineralogical Society, London.
- McAlister, J.J., and B.J. Smith. 1995. A rapid preparation technique for x-ray diffraction analysis of clay minerals in weathered rock materials. *Microchem. J.* 52:53–61.
- Mielenz, R.C., N.C. Schieltz, and G.E. King. 1955. Effects of exchangeable cation on x-ray diffraction patterns and thermal behavior of montmorillonite clay. *Clays Clay Miner.* 3:146–173.
- Moore, D.M., and R.C. Reynolds, Jr. 1997. *X-ray diffraction and the identification and analysis of clay minerals*. 2nd ed. Oxford Univ. Press, New York.
- Nadeau, P.H., J.M. Tait, W.J. McHardy, and M.J. Wilson. 1984. Interstratified X-ray diffraction characteristics of physical mixtures of elementary clay particles. *Clay Miner.* 19:67–76.
- Ottner, F., Gier, S., Kuderna, M., Schwaighofer. 2000. Results of inter-laboratory comparisons of methods for quantitative clay analysis. *Appl. Clay Sci.* 17:223–243.
- Plançon, A., and V.A. Drits. 2000. Phase analysis of clays using an expert system and calculation programs for x-ray diffraction by two- and three-component mixed-layer minerals. *Clays Clay Miner.* 48:57–62.
- Reynolds, R.C., Jr. 1980. Interstratified clay minerals. p. 249–303. *In* G.W. Brindley and G. Brown (ed.) *Crystal structure of clay minerals and their x-ray identification*. Mineral Soc. Monogr. 5. Mineralogical Society, London.
- Reynolds, R.C., Jr. 1985. NEWMOD: A computer program for the calculation of one-dimensional diffraction patterns of mixed-layer clays. R.C. Reynolds, Hanover, NH.
- Reynolds, R.C., Jr. 1989a. Principles of powder diffraction. p. 1–18. *In* D.L. Bish and J.E. Post (ed.) *Modern powder diffraction*. Mineralogical Society of America, Washington, DC.
- Reynolds, R.C., Jr. 1989b. Principles and techniques of quantitative analysis of clay minerals by x-ray powder diffraction. p. 4–37. *In* D.R. Pevear and F.D. Mumpton (ed.) *Quantitative mineral analysis of clays*. Clay Minerals Society, Evergreen, CO.
- Rietveld, H.M. 1969. A profile refinement method for nuclear and magnetic structures. *J. Appl. Cryst.* 2:65.
- Rich, C.I. 1969. Suction apparatus for mounting clay specimens on ceramic tiles for x-ray diffraction. *Soil Sci. Soc. Am. Proc.* 33:815–816.
- Sawhney, B.L. 1989. Interstratification in layer silicates. p. 789–828. *In* J.B. Dixon and S.B. Weed (ed.) *Minerals in soil environments*. 2nd ed. SSSA, Madison, WI.
- Schulze, D.G. 1986. Correction of mismatches in 2 scales during differential x-ray diffraction. *Clays Clay Miner.* 34:681–685.
- Smith, D.K. 1989. Computer analysis of diffraction data. p. 183–216. *In* D.L. Bish and J.E. Post (ed.) *Modern powder diffraction*. Mineralogical Society of America, Washington, DC.
- Snyder, R.L., and D.L. Bish. 1989. Quantitative analysis. p. 101–145. *In* D.L. Bish and J.E. Post (ed.) *Modern powder diffraction*. Mineralogical Society of America, Washington, DC.
- Srodon, J., V.A. Drits, D.K. McCarty, J.C.C. Hsieh, and D.D. Eberl. 2001. Quantitative x-ray diffraction analysis of clay-bearing rocks from random preparations. *Clays Clay Miner.* 49:514–528.
- Theisen, A.A., and M.E. Harward. 1962. A paste method for preparation of slides for clay mineral identification by x-ray diffraction. *Soil Sci. Soc. Am. Proc.* 26:90–91.
- Thompson, A.P., D.M.L. Duthie, and M.J. Wilson. 1972. Randomly oriented powder for quantitative x-ray determination of clay minerals. *Clay Miner.* 9:345–348.
- Toby, B.H. 2001. EXPGUI, a graphical user interface for GSAS. *J. Appl. Cryst.* 34:210–213.
- Towe, K.M. 1974. Quantitative clay petrology; the trees but not the forest? *Clays Clay Miner.* 22:275–278.
- Walker, G.F. 1950. Vermiculite–organic complexes. *Nature* 166:695–697.
- Walker, G.F. 1957. On the differentiation of vermiculites and smectites in clays. *Clay Miner. Bull.* 3:154–163.
- Wang, H.D., G.N. White, F.T. Turner, and J.B. Dixon. 1993. Ferrihydrite, lepidocrocite, and goethite in coatings from East Texas Vertic soils. *Soil Sci. Soc. Am. J.* 57:1381–1386.
- Whittig, L.D., and W.R. Allardice. 1986. X-ray diffraction techniques. p. 331–362 *In* A. Klute (ed.)

Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
Zevin, L.S., and G. Kimmel. 1995. Quantitative X-ray diffractometry. Springer, New York.

Thermal Analysis of Soil Minerals

A. D. KARATHANASIS, University of Kentucky, Lexington, Kentucky

The International Confederation for Thermal Analysis and Calorimetry (ICTAC) defines thermal analysis (TA) as “a group of techniques in which a property of a sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed” (Brown, 2001). Simply put, TA is a group of techniques in which changes in physical properties of a substance are measured as a function of controlled temperature (Hatakeyama and Liu, 1998). The changes in properties of a sample could be associated with the state of the sample (temperature, mass, volume), processes (e.g., heat of transition), or sample properties (e.g., heat capacity, chemical composition, crystalline structure). These changes may occur at a constant or varying sample temperature and are usually associated with thermal events that either generate or consume heat. The controlled-temperature program may involve heating or cooling at a fixed or variable rate of temperature change or holding the temperature constant (isothermal), or any sequence of these. Changes occurring in the sample properties are detected and measured by sensors and transformed into electrical signals. Quantitative interpretations of these changes require calibration of the signal to the true value of the quantity sought. The ability of TA to provide qualitative and quantitative characterizations to a broad range of materials over a considerable temperature range has been pivotal in establishing it as a widely accepted analytical tool for a variety of research applications.

A large number of TA techniques are available, including thermogravimetry (TG), derivative thermogravimetry (DTG), differential thermal analysis (DTA), differential scanning calorimetry (DSC), thermomechanical analysis (TMA), thermodilatometry (TD), thermooptometry (TOA) and others (Brown, 1998; 2001; Wendlandt, 1986; Haines, 1995). In addition, simultaneous TA techniques (TG-DTG, TG-DSC, DTA-TMA), or TG techniques coupled with evolved gas analysis (TG-EGA), gas chromatography (TG-GC), mass spectroscopy (TG-MS) and others are employed to improve interpretations (Brown, 2001; Paulik, 1995; Charsley and Warrington, 1992; Wendlandt, 1986). A classification scheme of major types of thermoanalytical methods currently in use for various applications is presented in Table 5–1. The incorporation of microcomputer controllers and advanced analytical software in this instrumentation has greatly expanded the versatility and power of these methods. Computer-controlled instruments provide great flexibility and reproducibility in the experiments, allowing the use of multiple heating rates or isothermal data collection. Computerization has also streamlined calibration, data acquisition, storage and processing, graphics capabilities and advanced the analytical precision of the measurements. These technological advancements have expanded the range of TA applications to a large variety

Table 5–1. Classification scheme of thermoanalytical and calorimetric methods used in soil mineralogy (Brown, 2001).

TA method	Property under study
Thermogravimetry (TG)	mass
Differential Thermal Analysis (DTA)	temperature
Differential Scanning Calorimetry (DSC)	heat/enthalpy
Thermomechanical Analysis (TMA)	dimension/mechanical properties
Thermo-optometry (TO)	optical properties
Evolved Gas Analysis (EGA)	composition of evolved gases
Emanation Thermal Analysis (ETA)	radioactive gases
Thermoluminescence (TL)	intensity of radiation
Thermomagnetometry	magnetic properties
Thermoacoustimetry (TAA)	acoustic waves

of materials, including geological and soil samples, polymers, plastics, ceramics, composites, fibers, fossil fuels, and pharmaceuticals.

Many soil constituents undergo thermal reactions and transitions at specific temperature regions upon heating, which can serve as a diagnostic property for their identification and quantification. Some of these reactions, such as dehydration and evaporation of sorbed water, occur at low temperatures (20–300°C). Other reactions, like dehydroxylation (loss of crystal-lattice OH) or decarbonation (conversion of CO_3^{2-} to CO_2), usually occur at higher temperatures, generally above 250°C. Samples containing reduced organic or metallic compounds may undergo oxidation state changes at intermediate temperature ranges (250–500°C). Thermal reactions may be endothermic (heat gain) or exothermic (heat loss) and may be associated with phase or crystal structure changes. Advanced and more sophisticated TA equipment in the last decade has allowed not only a more precise identification and quantification of minerals in soil and geological samples, but through combined or coupled TA techniques a more detailed characterization of various mineral associations with industrial compounds or environmental contaminants.

TYPICAL THERMAL REACTIONS OF SOIL MINERALS

Water loss is the main transition that most soil minerals experience upon heating. Water can be present in different forms and held with different energies on soil minerals. Sorbed water is present as adsorbed films on external and/or internal surfaces and voids of mineral particles, as well as in the hydration cells of associated exchangeable cations. This type of water is held with relatively low energy and is lost readily upon heating in low temperature regions. Water loss upon low temperature heating is generally reversible without permanent consequences to most mineral structures, although there are exceptions. The overall loss of both sorbed and hydration water is mainly a function of the hydration state and energy of the saturating cation and the water vapor pressure or relative humidity of the surrounding environment. Therefore, quantitative approaches based on sorbed water losses should be used only when these conditions are strictly monitored. In contrast, OH-water is present in fixed positions within the crystal lattice, and its removal is accompanied by irreversible changes or complete destruction of the mineral structure. OH-water is held with greater energy and is generally driven off at higher temperatures than other forms of water. Because the energy (heat) required to expel OH-water from the crystal lattice varies in different minerals, the dehydroxylation reaction usually occurs at different temperature regions, thus enabling differentiation between minerals. Quantitative assessments of dehydroxylation reactions are based on comparisons with ideal or pure OH mineral compositions. Some TA methods (DTA, DSC) may also be useful in identifying

the types of crystal phase changes occurring upon heating, such as the α - β inversion of quartz (Earnest, 1984).

The application of TA methods for mineral identification, and especially quantification, is not without pitfalls and limitations. Extensive weathering in soil environments may cause slight changes in crystal-lattice OH-water and lead to erroneous quantification. Furthermore, some minerals have partially overlapping dehydroxylation or dehydration and dehydroxylation reactions, which may hinder quantitative assessment. In certain temperature ranges gross errors may also result from interferences due to the presence of organic matter and carbonates. For example, some types of organic matter may interfere with the dehydration and/or dehydroxylation region (200–500°C) of aluminosilicates, while carbonates may or may not be a problem, depending on sample composition. Therefore, sample pretreatment to remove extraneous constituents may be required. Reduced metallic ions or compounds, such as ferrous iron or sulfides, may be present in reduced soils and sediments, and may gain weight upon oxidation during the heating process, which could counterbalance weight losses from dehydroxylation reactions. The type of pretreatment required for a soil or clay sample for thermal analysis depends on the objective of the analysis and the nature of the sample. Qualitative information can be obtained by analyzing the whole soil sample without any pretreatment, particularly if it is low in organic matter content and monomineralic. However, detailed quantitative assessments usually require specific pretreatment phases, performed in multiple steps. The pretreatment methods are described in Chapter 2 (Soukup et al., 2008, this volume).

INSTRUMENTATION PRINCIPLES

Thermogravimetry

Thermogravimetry (TG) is the technique in which changes in the mass of a substance or a mineral are measured as a function of temperature as the sample is subjected to a controlled-temperature program. Therefore, TG applications are limited to reactions involving mass or weight changes. The record produced is the TG curve with temperature on the x axis and absolute (mg) or relative (%) mass changes based on the original sample mass on the y axis. Mass changes in the sample upon heating are measured with a thermobalance assembly, comprised of a suitable electronic microbalance, a furnace, a temperature programmer, and a computer controller that allows the sample to be simultaneously heated and weighed in a controlled manner (Fig. 5–1). The balance is maintained in a suitably en-

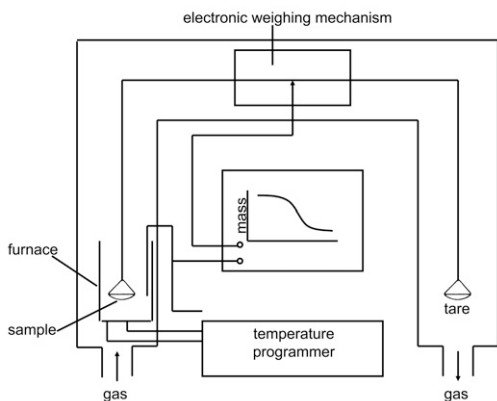


Fig. 5–1. Schematic representation of a thermogravimetry (TG) system (Brown, 1998; Karathanasis and Harris, 1994).

closed system so that the atmosphere surrounding the sample and the weighing mechanism are kept under controlled conditions. Several types of microbalance mechanisms are available, including the deflection type, which monitors mass changes via direct deflections of the balance beam about a fulcrum, or the null type, which measures, via an electromagnetic mechanism, the restoring force required to maintain a constant position of the sample. The null-type weighing mechanisms are favored in modern microbalances because they ensure that the sample remains in the same zone of the furnace irrespective of mass changes, thus minimizing heating variations. Typical thermobalance sensitivities and maximum loads are in the order of $1\mu\text{g}$ and 1 g, respectively. Electronic taring of the microbalance and gain (scale normalization and expansion) of the output in mass loss, derivative mass loss (DTG), or percentage of the original mass is accomplished through suitable software programs. The sample may be suspended from the balance beam or placed on a rigid vertical support above the balance beam for a top-loading option.

The furnace within which the sample is being heated is usually an electrical resistance heater, although infrared and microwave heaters have also been employed in some applications. The furnace is operated through a temperature-programming unit, which provides a uniform temperature zone around the sample and holder that does not affect the balance mechanism. Typical conduction-type furnaces able to reach 1200°C include Nichrome or Kanthal resistive heating elements. The furnace-lining consists of an inert material that does not undergo thermal reactions. A ceramic tube composed of fused alumina or silica refractories usually surrounds the sample. The furnace temperature is usually measured by a platinum-rhodium or a chromel-alumel thermocouple. The temperature signal from the furnace thermocouple is continuously compared with and adjusted to that of the programmer through heat flow sensors. Shorter response times required for balancing the temperature differences between the furnace and the controller (thermal lag time) mean that higher heating rates can be employed. The most common temperature-control program used in soil mineral studies is a linear heating or cooling ramp at 10 or $20^{\circ}\text{C min}^{-1}$. Slower heating rates allow better quantitative analysis. Proportional type furnace-temperature controllers use sophisticated feedback mechanisms to attain heating rate accuracies of $0.1^{\circ}\text{C min}^{-1}$. Isothermal programs are also used to measure mass changes as a function of time for kinetic studies. More complex temperature programs may contain combinations of linear and isothermal segments, as well as variable heating rates, to maintain a constant rate of mass changes for improved resolution (Ford and Bertsch, 1999; Xi et al., 2004).

The sample temperature is usually monitored by a platinum-rhodium thermocouple, which can measure temperatures up to 1700°C , that is located near (but not in contact with) the sample. Since separate sensors are used to control the furnace temperature and to measure the sample temperature, offset between the two temperatures is accommodated through calibration procedures. It is very important that calibration be performed under similar conditions to those used in actual experiments to improve reproducibility. Two of the most suitable materials for temperature calibration are nickel metal ($T_c = 354^{\circ}\text{C}$) or perkalloy ($T_c = 596^{\circ}\text{C}$), which are ferromagnetic at low temperature, but which lose their ferromagnetism at a well-defined Curie point (C_p). Therefore, a magnetic material being heated under a magnetic field (provided by a magnet placed under the sample holder) will lose its magnetism (undergo mass loss) at its specified Curie temperature point. The mid temperature of the magnetic transition or the peak of the DTG curve is used as the fixed point for calibration.

The sample is commonly purged with an inert gas (usually N_2) to suppress oxidation reactions and minimize interferences from gases evolved during sample reactions. The flow rate of the purging gas is controlled by flow meters or mass-flow controlling devices

so that sample-mass changes from thermomolecular gas fluxes can be avoided. Purge gas flow rates that are too high may disturb the balance mechanism, while low flow rates may not efficiently remove gaseous products. The typical flow rate range is between 10 and 60 $\text{cm}^3 \text{min}^{-1}$. Using small-mass sample holders and samples minimizes buoyancy effects on the sample from the flow of gaseous molecules. When the evolved gases are of interest, their composition can be monitored spectroscopically by coupled TG-EGA methods (Morgan et al., 1988). Experiments conducted without a purge gas usually result in higher dehydration and dehydroxylation temperatures.

The nature, precision, and accuracy of TG results may be influenced by a number of instrument and sample characteristics. Important instrument factors include furnace heating rate, furnace atmosphere, geometry of the sample holder and furnace, and the sensitivity of the recording mechanism. Generally, faster heating rates result in higher temperatures for the onset of the reaction, the completion of the reaction, and the difference between the onset and completion of reaction temperatures, even though the mass losses associated with these transitions should remain unchanged. Employing small-mass samples allows the use of faster heating rates without jeopardizing reaction resolution, but lower heating rates in dehydration reaction regions are generally preferred. A flowing atmosphere of an inert gas is usually preferred within the furnace to decrease condensation of reaction products on the thermobalance, and flush out gaseous products, thus decreasing secondary reactions. The choice of purge gas is determined by its reactivity with the sample, as well as cost, availability, purity, density, and thermal conductivity. Sample holders range from flat pans to small crucibles made of platinum, alumina, glass, and other metals and metallic alloys, depending on the heating range employed. Sample and furnace geometry and heat capacity may affect the rate of heat transfer to and from the sample, and therefore, the sensitivity of the measurement. Sensitivities of $<1 \mu\text{g}$ and low mass noise TG curves can be attained by modern thermobalances with $<10\text{-mm}$ inside diameter tubes under atmospheric conditions with samples $<20 \text{ mg}$.

The most important sample characteristics influencing the nature and quality of TG data are: sample mass, particle size distribution, crystallinity, degree of packing, heat of reaction, and thermal conductivity (Wendlandt, 1986). Modern TG instruments typically accommodate very small sizes (10–100 mg), which minimize thermal gradients within the mass of the sample, reduce deviations in transition temperatures, and provide a more efficient diffusion of gaseous products. Increasing sample mass and degree of packing may increase transition temperatures and lower the resolution of overlapping transitions. A more detailed account of sample characteristics affecting TA analysis is provided under the “Methodology” section.

Differential Thermal Analysis

Differential thermal analysis (DTA) is a technique in which the difference in temperature (ΔT) between the sample and a reference material is monitored and recorded while both are subjected to the same heating program. In “classical” DTA instruments, the sample and reference material are placed in symmetrical cavities (mounts) of a single block or a chamber, which serves as a heat sink, and are heated in a furnace under controlled temperature and atmosphere conditions. Sample holders of low thermal conductivity used between the block and the sample ensure an adequate ΔT signal, which is monitored through a pair of differentially connected thermocouples in contact with the sample and reference during each thermal event (Fig. 5–2a). In the “calorimetric” DTA, the sample and reference are placed in similar sample holders, located on individual ther-

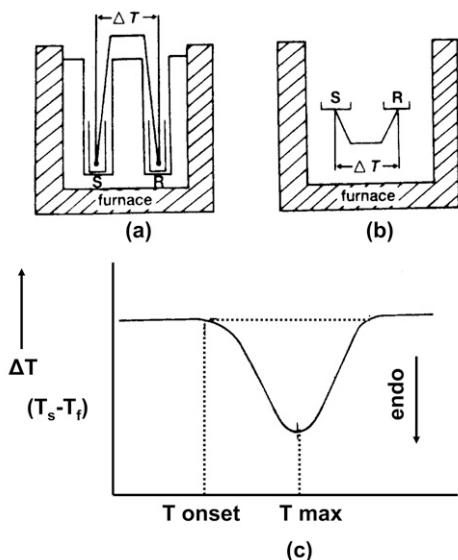


Fig. 5-2. Schematic representation of a differential thermal analysis (DTA) system with temperature sensors (a) inside or (b) outside the sample holders and (c) signal output; S, sample; R, reference; ΔT , difference in temperature between sample and reference material; T_{onset} , initial reaction temperature; T_{max} , peak reaction temperature; T_r , temperature increase with time; endo, endothermic reaction (Brown, 1998).

mally conducting bases with attached thermocouple junctions (Fig. 5-2b). The advantage of this configuration is that the output signal is less dependent on the thermal properties of the sample since the thermocouples are not touching the sample or reference material, but the response to temperature differences is somewhat slower. The DTA curve is usually a plot of the difference in temperature (ΔT) as the ordinate against the temperature (T) or time as the x axis (Fig. 5-2c).

The furnaces used in DTA are generally electrical resistance heaters for low and intermediate temperature ranges (0–900°C) or electrically conducting graphite systems for high temperatures (up to 1500°C). The temperature of the furnace is programmed through a computer controller and measured by sensors similar to those used to monitor the sample temperature. Modern instruments offer heating rates between 0.1 and 100°C min⁻¹ and very sophisticated programmed temperature regimes, including variable ramp and isothermal settings. The use of inert gases, such as nitrogen, argon, or helium, and reactive gases, like air or oxygen, is possible with most DTA instruments. Flow rates are usually in the region of 10 to 100 cm³ min⁻¹. Typical temperature sensors and junctions in various configurations used in DTA are similar to those used for TG, with the most common being chromel-alumel (0–1000°C) and platinum-rhodium (0–1500°C) thermocouples and platinum resistance sensors. Sample holders are made of aluminum (0–600°C), graphite (0–900°C), and platinum or alumina ceramic (0–1500°C), and can be open, lidded, or sealed (with a pinhole to remove gaseous products).

The reference material should not undergo thermal events beyond the operational temperature range, should not react with the sample holder or thermocouple, and should have similar thermal conductivity and heat capacity as the sample. For mineralogical analyses, alumina, Al₂O₃, carborundum, and SiC have been extensively used as reference DTA materials. When an endothermic event occurs in the sample, its temperature (T_s) lags behind that of the reference material (T_r), which keeps pace with the heating program, and a plot of the temperature difference (ΔT) against T will produce a peak pointing downward (Fig. 5-2). During exothermic transitions (such as oxidations), the ΔT response is in the opposite direction. The baseline from which negative (endotherms) or positive (exotherms)

deflections occur is ideally a horizontal line, which corresponds to $\Delta T = 0$. A complete description of each thermal event includes the initial displacement from the baseline (onset), the maximum deviation (peak), and the return to the baseline at the end of the transition. The area under the peak is related to the enthalpy change (ΔH) associated with the thermal event. Generally, phase transitions, dehydration, dehydroxylation, and some decomposition reactions produce endothermic peaks, while oxidation, recrystallization, and certain decomposition reactions generate exothermic peaks. Standard reference materials are used to calibrate the DTA instrument with respect to transition temperature and heat of reaction (ΔH) for specific temperature regions. Typical calibration materials (and their transition temperatures) include indium (156.6°C), tin (231.9°C), zinc (419.5°C), and aluminum (660.3°C), with ΔH values of 28.7, 60.5, 108.6, and 401.3 J g⁻¹, respectively.

Most DTA applications involve heating a sample in a furnace at a constant rate. The ΔT signal obtained is related to the thermal resistance (R) of furnace and samples, the heating rate (dT/dt) employed, and the difference in heat capacity between the sample and reference materials ($C_s - C_r$) by the following equation (Brown, 1998):

$$\Delta T = R(dT/dt)(C_s - C_r) \quad [1]$$

However, the applicability of this expression is partially limited by the difficulty in measuring actual R values and the fluctuation in the heating rate between the sample and reference during thermal events. These fluctuations can be minimized by diluting the sample with the thermally inert reference, but at the expense of reduced sensitivity (Mackenzie and Calliere, 1975). An estimate of ΔH (enthalpy change) associated with a thermal event can also be obtained from the following theoretical expression (Kerr and Kulp, 1948):

$$(\Delta H)m = gk \int_{t_1}^{t_2} \Delta T dt \quad [2]$$

where m is sample mass, g is a geometry constant related to the shape of sample housing, k is the thermal conductivity of the sample, and t is time. Some limitations associated with the application of this equation include the failure to account for: (i) differential heating rates between the sample and reference during thermal events, (ii) changes in the specific heat of the sample as it undergoes transitions, and (iii) temperature gradients occurring within the mass of the sample. In spite of these limitations, subsequent refinements of this expression in modern instrumentation convey adequately the proportionality between ΔH and the area under the peak for most DTA applications (Wendlandt, 1986; Turi, 1996).

Factors influencing DTA curves are similar but even more numerous than those mentioned for TG analysis. Instrumental parameters affecting the nature and precision of DTA data include heating rate, furnace atmosphere, sample-holder material and geometry, and thermocouple type, junction, and position. Generally, increasing the heating rate will increase reaction initial temperature (T_i), reaction peak temperature ($\Delta T_{\max}$), reaction final temperature (T_f), peak amplitude, and the area under the peak (ΔH). A higher heating rate will also decrease the resolution of two partially overlapping peaks. In contrast, very low heating rates may significantly lower peak areas and intensities, thus hindering instrument sensitivity and detectability.

The evolution or sorption of gaseous products during thermal events also affects the temperature and shape of the DTA peaks. Under the dynamic flow conditions of an inert gas, increases in flow rate usually cause decreases in peak temperatures as a result of respective reductions in the partial pressure of the gaseous products above the sample dur-

ing the transition. Considerable differences may also be observed in DTA curves of samples containing oxidizable components run under ambient vs. inert purged gas conditions, where endothermic and exothermic peaks may be shifted, or partially counterbalanced, thus inhibiting the resolution of thermal events.

Different sample-holder materials and geometry have variable heat transfer and diffusivity rates, which may cause significant changes and distortions to the shape of DTA peaks. These distortions may be minimized by using small-mass samples and small-radius sample holders. Similarly, peak resolution may be improved by using sample holders made of low thermal-conductivity ceramic materials for endothermic reactions, and high thermal-conductivity metallic materials for exothermic reactions.

Different thermocouple types and sizes may also produce temperature shifts and variable shapes in the DTA peaks because of their differential electrical resistance and thermal conductivity. Generally, the thicker the thermocouple, the more pronounced the peaks obtained. Finally, positioning the thermocouple away from the sample and not maintaining a symmetrical arrangement in reference to the sample and the sample-holder walls may affect transition temperature, peak shape and size, and lead to baseline distortions. Experiments comparing furnace temperature vs. sample temperature suggested that the latter provided a more accurate measure of the actual transition temperature, with $\Delta T_{\max}$ being a better indicator than T_i and T_f .

Important sample characteristics influencing the quality of DTA measurements include sample mass, sample particle-size, packing, and diluent effects (Anderson et al., 1977) and are discussed further in the "Methodology" section.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a technique that measures the difference in heat flow (energy) as a function of temperature or time between a sample and a reference material as they are being subjected to a controlled temperature program. Therefore, DSC instruments can provide precise calorimetric determinations of heats of reaction or enthalpy changes (ΔH) associated with various thermal events. Although DTA can be used for higher temperature ranges than DSC, the results of the latter are usually more quantitatively reliable. There are two major types of DSC instruments, based on different operation principles: (i) heat-flux DSC, which is closely related to classical DTA, and (ii) power-compensated DSC (Fig. 5–3).

In heat-flux DSC instruments, the sample and the reference material are heated from the same source, and the temperature difference (ΔT) signal is measured and converted to a power difference via a calorimetric sensitivity function. Unlike DTA, the thermocouple junctions in heat-flux DSC are not in direct contact with the sample and reference material. Instead they are attached to individual thermally conducting bases upon which the sample and reference material are positioned, usually in flat pans. This configuration minimizes the dependence of the output signal on the thermal properties of the sample and allows for a better control of instrumentation effects through suitable calibrations.

In power-compensated DSC instruments, the sample and reference are heated by separate heaters to maintain the temperature difference ($\Delta T = T_s - T_r$) close to zero throughout the controlled temperature program. The signal recorded is the difference in electrical power needed to maintain equal temperatures and is expressed as heat flow (q) according to the equation:

$$\Delta(dq/dt) = (dT/dt)(C_s - C_r) \quad [3]$$

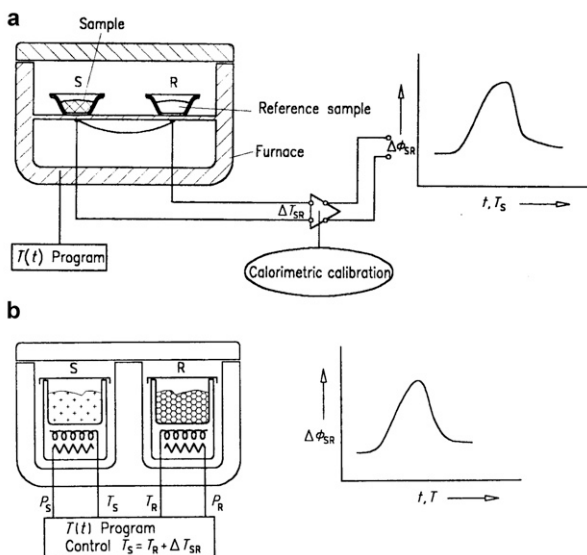


Fig. 5-3. Schematic representation of (a) disc-type and (b) power compensating differential scanning calorimetry (DSC) systems with signal outputs; S, sample; R, reference; ΔT_{SR} , temperature difference between sample and reference; $\Delta \phi_{SR}$, heat flow difference between sample and reference (Brown, 1998).

Since $\Delta T \approx 0$, then $R \approx 0$. Therefore, there is no thermal resistance, and no temperature correction for the cell constant is required. Thermal events occurring in the sample appear as deviations from the baseline, in either the endothermic or exothermic direction, depending on whether more or less energy needs to be supplied to the sample relative to the reference material. In DSC, endothermic responses are represented as positive (above the baseline) displacements, corresponding to increased heat transfers to the sample compared with the reference material. Note that this convention is exactly the *opposite* of that used in DTA, where endothermic responses are negative (below the baseline) displacements representing negative temperature differences between the sample and the reference.

The operating temperature range of DSC instruments is generally lower (750°C max.) than that of DTA instruments. It is important that the temperature control program and temperature range employed are compatible with the melting point of the pan material to avoid damage to the sample-holder assembly and the sensor junctions. For heating ranges <600°C, the samples are loaded into aluminum pans, which may be open, lidded, or crimped, depending on the type of samples used and the reactions to be monitored. Aluminum melts at 660°C and usually accommodates complete transitions for the dehydroxylation of kaolinite. Copper, gold, and graphite pans are available for extended temperature ranges. The reference material used in most DSC applications is an empty pan similar to that used for the sample. The heating chamber with the sample-holder assembly is usually purged with an inert gas to flush gaseous products and prevent corrosion of the chamber and thermal sensors from reactive substances. However, specially designed DSC instruments allow experiments to be conducted under vacuum, oxygen, or other atmospheres, as well as under high atmospheric pressure (7 MPa) conditions. High-pressure DSC systems have been used to explain hydrothermal stability of phyllosilicate minerals, such as smectites and kaolinites (van Groos and Guggenheim, 1990). Thermocouples similar to those used in DTA are used as the sample and reference sensors in heat-flux DSC instruments, but platinum resistors are preferred in power-compensated DSC. Unlike the ceramic-lined electrical heaters with electronic control used in DTA, many DSC instruments employ a resistance-heated furnace enclosure of silver. The high thermal conductiv-

ity of silver ensures a uniform temperature throughout the heating chamber (cell). The need for a suitable fast-pace microprocessor is paramount in DSC, since the ΔT signal must be converted to a power difference signal through calorimetric sensitivity function before heat flow (q) data can be recorded. Therefore, instrument calorimetric sensitivity is improved with small-mass samples (10–100 mg), which keep temperature gradients to a minimum. Heating rates, calibration, and instrumental and sample effects on DSC data are similar to those discussed for DTA (Haines et al., 1998).

Thermomechanical and Thermo-optical Analysis

Thermomechanical analysis (TMA) involves a set of methods in which the deformation of a sample is monitored as a function of temperature or time as it is being subjected to a controlled temperature program. If the method measures expansion or contraction changes under negligible loads as a function of temperature, the term *thermodilatometry* (TD) is used. For analyses involving tension, compression, flexure, or penetration measurements under variable loads, the term *thermomechanical analysis* (TMA) is more appropriate. Finally, the term *dynamic thermomechanical analysis* (DTMA) is used for dynamic modulus experiments involving frequency and temperature dependence of solids (relaxation studies). While TMA analysis has a wide range of applications in ceramics, metallurgy, and polymeric materials, TD is the only thermal method that has been used to study length or volume changes in soil materials (Hajek, 1979; Dyer, 1981; Karathanasis and Hajek, 1985). The variation in length (ΔL) or volume (ΔV) experienced by a material as a function of temperature is commonly expressed by the equations:

$$\Delta L = L_f - L_i = \alpha L_i (\Delta T) \quad [4a]$$

or

$$\Delta V = V_f - V_i = \alpha V_i (\Delta T) \quad [4b]$$

where L_f , L_i , V_f , and V_i are final and initial lengths and volumes, respectively; $\Delta T = T_f - T_i$; and α is the linear coefficient of thermal expansion. In the case of volume changes, α is assumed to be equal in all three dimensions, which may not be true for all materials. In the absence of a phase transition in the temperature range investigated, L or V changes are recorded as a function of temperature, and assuming that α is constant, the result will be a straight line with a slope of α . A differential expression of Eq. [4] with respect to time yields:

$$dL/dt = \alpha L_i (dT/dt) \quad [5]$$

where dT/dt is the heating rate, which is usually constant. Therefore, under a constant heating rate, the signal recorded is directly proportional to the coefficient of thermal expansion α . With appropriate calibration, α can be directly obtained from the curve.

In the most common TD instruments, changes in length (ΔL) or volume (ΔV) as a function of temperature or time are detected by an electrical transducer through an output voltage proportional to the displacement (Fig. 5–4a). The sample is placed on the platform of a quartz sample-tube and is attached to a quartz probe connected to the armature of the transducer. The probe assembly includes a weight tray for optional loading applications, which is supported by a plastic float rigidly fixed to a shaft and immersed in a high-density fluid (Wendlandt, 1986). Different types of sample-probes are available, depending on the mode of the experiment (e.g., expansion, compression, penetration) (Fig. 5–4b). Typical furnaces used in TD instruments are similar to those used in DSC, with a heating

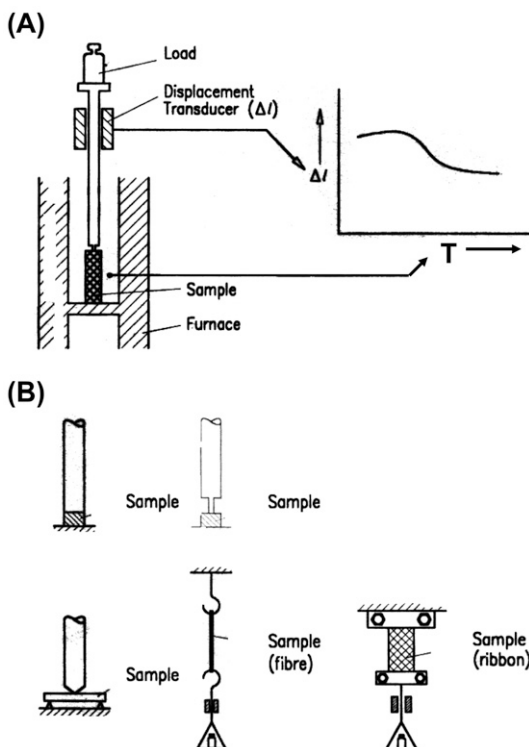


Fig. 5-4. Schematic representation of (a) a thermomechanical analysis (TMA) system (b) with various probes used for analysis of the mechanical behavior of the sample (Δl = length change) (Brown, 1998).

range up to 700°C. Experiments can be conducted under ambient or purged gas conditions. Instrument sensitivity can be improved to $<0.01 \mu\text{m}$ through the use of amplifiers and even to $<0.01 \text{ nm}$ in laser dilatometers or optoelectronic transducers (Brown, 2001). Instrument calibration is conducted with a solid of known expansion coefficient (aluminum metal) under conditions similar to those used for the experiment (Wetton, 1998; Brown, 2001).

Thermoptometry (thermo-optical analysis, TO) or thermomicroscopy is a technique in which a sample is heated by a temperature-controlled program while morphological and structural changes as a function of temperature are observed through a microscope. Such changes are usually the result of physical or chemical alterations. The main components include a specially designed thermomicroscope with hot or cold stage capability, a sample holder, a light source, optical recording and documentation devices, and an image and data analysis processor. The use of special objective lenses with optimized numerical aperture, physically separated from the hot sample and the incorporation of optical quality windows in the stage shell, allow for satisfactory resolutions at magnifications of up to 600 $\times$. Hot-stage microscopes are equipped with electrical furnaces for low (25–350°C) or high (25–2700°C) temperature ranges. The low-temperature range is used mainly for organic compounds, while the high-temperature range is employed for studying physical property changes, reaction kinetics, and phase transformations of metals, ceramics, and minerals (Wiedemann and Felder-Casagrande, 1998). Heater-atmosphere reactions are minimized with the use of vacuum, while image quality is enhanced by high-intensity lamps and filters. Sapphire glass pans appear to be the most suitable sample holders for this application, especially in combined TO-DSC instruments. The sample temperature is monitored with a platinum sensor placed close to the sample. In TO-DSC systems a sample and a refer-

ence pan are used, and the temperature difference is measured with a gold or nickel thermopile. Heat protection filters and a blower are used to shield and cool down the optical system (Brown, 2001).

Combined Thermal Analysis Techniques

Combined TA techniques may be simultaneous, concurrent, or parallel. Simultaneous techniques involve measurements of two or more properties of the same portion of a sample during a single temperature program (van Humbeeck, 1998). Each of the properties may be monitored continuously or in a repetitive sequence to allow data processing from both measurements. Parallel techniques monitor different portions of a sample using different instruments interfaced with each other, while concurrent techniques conduct measurements on different portions of a sample placed in different containers, but in the same furnace under a common temperature program.

The advantages provided by the combined techniques include (i) savings in time and amount of sample, (ii) more accurate correlation between thermal events, (iii) a greater amount of information usually obtained compared with individual techniques, and (iv) a lower cost for the combined equipment compared with individual instruments. Some disadvantages involve (i) potential reductions in sensitivity because of compromises in instrumental design, (ii) operation of more complicated instruments because different sensors and detectors need to be combined, and (iii) incompatibility of certain instruments. Complementary techniques may include a wide range of physical, chemical, mechanical, and morphological property measurements, as well as many forms of spectroscopic analyses (Brown, 2001). Some of the most common combinations of classical TA techniques with potential applications in soil mineralogy include TG-DTG, TG-DTA, TG-DSC, DSC-XRD, DSC-TO, and coupled TA with evolved gas analysis (EGA) techniques, such as TG-MS, TG-FTIR, and TG-GC (Paulik, 1995; Mullens, 1998; van Humbeeck, 1998).

TG-DTG, TG-DTA, and TG-DSC Techniques

The most common single-sample combination TA technique is the derivatograph, developed by Paulik and Paulik (1978), which allows simultaneous measurements of TG, DTG, and DTA (Fig. 5–5). Initial versions of this instrument did not have reference sample weighing capability. Modern designs are based on a thermobalance modified to weigh and record the temperature of both sample and reference. Typical designs contain the sensor elements in the lever arm of the TG balance at the position of the sample and reference. In heat-flux DSC-TG combinations, the sample and reference materials are contained in crucibles placed on a plate with temperature sensors connected to the thermobalance and detector systems. In power-compensated DSC-TG combinations, there is no mechanical contact of the TG sample pans with the detectors of the DSC tubes, which in conjunction with the symmetrical twin-furnace design enhances weighing accuracy. Other instruments have a dual-beam horizontal design in which ceramic beams support the DSC sample and reference holders, while dual meter movements are used to monitor weight changes in the sample and reference materials. An important advantage of TG-DSC or TG-DTA combinations is the possibility of temperature calibration by using only the DSC or DTA sensor, because endothermic or exothermic transitions at invariant points can be much more accurately detected than weight changes. Another advantage is the capability of directly relating endo- or exothermic heat fluxes to actual mass changes of the reacted sample fraction. Since both measurements are made on a single sample, a high accuracy of heat exchange per unit mass can be obtained for chemical reactions where the energetic effect has caused the mass change. Suitable software allows simultaneous plotting of all three signals (TG,

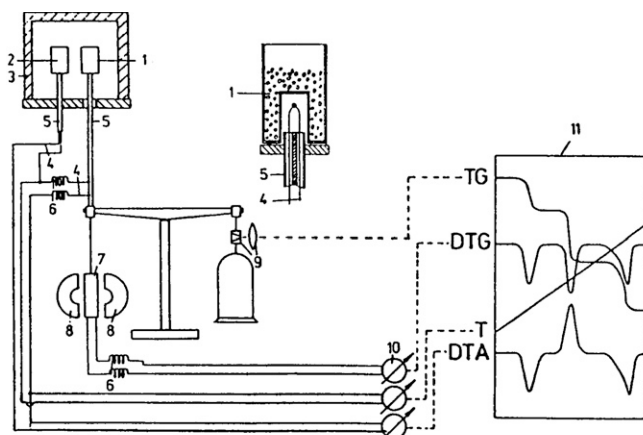


Fig. 5-5. A derivatograph system setup for simultaneous thermal gravimetry (TG), differential thermogravimetry (DTG) and differential thermal analysis (DTA) (Paulik, 1995) (1 = sample, 2 = reference, 3 = furnace, 4 = thermocouples, 5 = corundum tubes, 6 = flexible junction, 7 = coil, 8 = magnet, 9 = optical system, 10 = galvanometers, 11 = recorder).

DTG, DTA, or DSC), thus making interpretations easier and more accurate (Paulik et al., 1982; Paulik, 1995; Haines, 1995; van Humbeeck, 1998).

DSC-XRD and DSC-TO Techniques

While DSC-XRD combinations have been used mainly to monitor microstructural changes of organic compounds as a function of temperature, they offer considerable promise for soil mineral applications. A broad range of X-ray scattering angles have been used including synchrotron radiation ($0.005^\circ < 2\theta < 0.05^\circ$), small angle X-ray scattering ($0.25^\circ < 2\theta < 10^\circ$), wide angle X-ray diffraction ($5^\circ < 2\theta < 70^\circ$), and energy dispersive X-ray analysis (EDAX). Within these ranges, structural features ranging in size from 0.1 to 500 nm can be studied. X-ray powder diffraction patterns are recorded while the sample is heated in a low or high temperature furnace by a temperature-controlled program. This technique can provide useful information for a variety of applications, including phase transitions, temperatures and degree of crystallization, and oxidation reactions, but requires a high flux of radiation and a fast detector. Sample holders are made from aluminum, graphite, or boron nitride, which have X-ray transparent windows and low diffuse scattering with few, or well-defined Bragg reflections. Care must be taken to avoid interferences of the high flux X-ray beam with controlled-temperature effects through radiation-induced decomposition of the sample (Haines, 1995; Haines et al., 1998).

The combination of DSC with TO allows simultaneous visual examinations of the sample and quantitative measurements of DSC or DTA transitions occurring while the sample is heated, thus providing more meaningful results. The main difference in combined TO systems is the modification of the hot stage to allow simultaneous measurements of heat flow and optical observations of the events induced by the temperature change. Typical DSC-TO systems have a heat-flux DSC sensor made of thin-film thermopiles deposited on a special glass-disc, with the sample and reference being placed in sapphire crucibles. Other designs have used thermocouples as both specimen holder and heating source. Sample illumination is provided by transmitted light. Suitable microprocessors are able to control the temperature and light transmission and measure temperature and heat flow changes. On-line observations of morphological changes at resolutions down to 0.01 mm and corresponding thermal data can be monitored on a multiscreen monitor and processed with image-analysis software (Wiedemann and Felder-Casagrande, 1998).

TG-EGA Techniques

Combinations of TG with evolved gas analysis (EGA) equipment allow simultaneous monitoring of mass changes as a function of temperature and characterization of volatile products formed as a result of desorption or decomposition while the sample is heated under a controlled-temperature program (Morgan et al., 1988). The main EGA techniques coupled with TG include mass spectrometry (MS), fourier transform infrared spectrometry (FTIR), and gas chromatography (GC). The coupling is accomplished through an on-line serial connection, which provides continuous sampling (via interface) and/or intermittent sampling (via valve) capabilities, or an off-line connection with only intermittent sampling capability via condensing traps. The advantage of the continuous sampling is that it provides continuous measurements for the selected gas property, and hence, good correlation with the thermal analysis curves. However, this may require elaborate interfacing and more careful selection of the experimental conditions because of temperature and pressure gradients between the two techniques. Intermittent sampling does not require extensive modification of the detector of the coupling system, thus providing optimum sensitivity, but synchronization of thermal and gas evolution events and accurate compositions of evolved products may not be attainable (Haines, 1995; Hatakeyama and Liu, 1998; Brown, 2001).

The gas flow conditions in the combined instruments need to be carefully controlled through a suitable calibration so the data sets produced by the two techniques are adequately correlated. The purge gas from the TA unit becomes the carrier for sweeping evolved gases to one or more evolved gas detectors (EGD), which are located near the sample to decrease vapor condensation, secondary reaction, and lag time effects. The most commonly used detectors are based on thermal conductivity, gas density, ionization, or infrared radiation principles. The sensitivity of the EGD can be optimized by selecting a purge gas carrier with markedly different value for the measured property compared with that of the evolved gas. The thermal conductivity of H_2 and He gases is very high, while for Ar is very low, thus making them suitable carriers, but their influence on thermal effects needs to be determined to avoid artifacts. Flame ionization detectors are very sensitive for gases evolved during decomposition of organic compounds, but not for water vapor products.

In TG-MS combinations (Fig. 5-6) the gas detector is replaced with a mass spectrometer (Mullens, 1998). One difficulty in this combination is that the MS requires high vacuum, while the TG unit usually operates at atmospheric pressure ranges. Therefore, the coupling interface needs to accommodate the pressure differences between TG and MS during the transfer of an evolved gas sample from one system to another for analysis. This is usually achieved by bringing into the MS system only a small fraction of the gases leaving the TG unit via a heated inert ceramic capillary. Recent advances in the design of quadrupole mass spectrometers, which require simpler interfacing options have made the TG-MS a particularly appealing combination. However, problems may still arise from vapor condensation in the sampling system, and detection desensitization from high carrier gas/evolved gas concentration ratios. Quantitative calibrations of TG-MS systems are accomplished either by injecting a known concentration of a calibration gas into the carrier gas stream, or by measuring the decomposition products of a solid with a simple stoichiometric reaction in the TG system. Helium is the most commonly used carrier gas, although its high diffusivity may interfere with the shape of the MS signal in some quantitative applications. Similar interferences may be caused by fluctuations in the gas flow rate, gas transfer delay, and condensation of evolved gases. Evolved gases entering the MS ionization chamber are bombarded with electrons and undergo fragmentation into positive ions. These positive ions are separated by the quadrupole on the basis of their mass/charge ratio. Software produces scans of the intensity of the ion current signal at each mass/charge

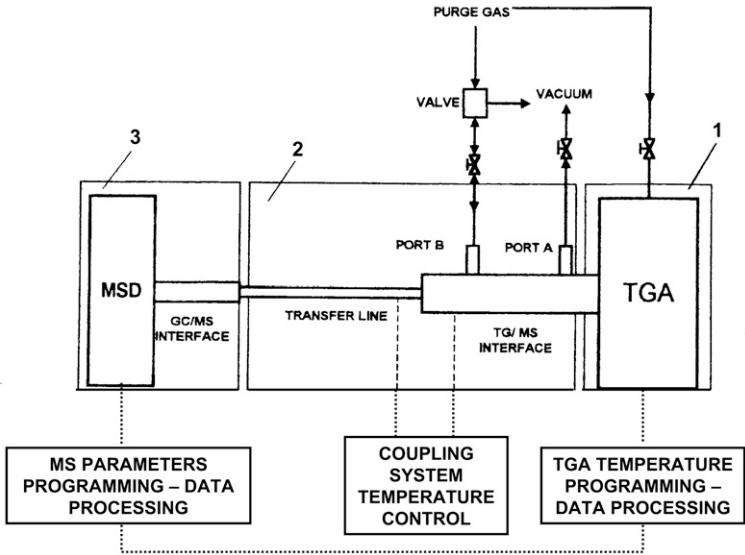


Fig. 5-6. A Thermogravimetry-mass spectrometry (TG-MS) system configuration (Statheropoulos et al., 1998) (1 = TG system, 2 = coupling system, 3 = mass spectrometer detector).

ratio as a function of temperature or time. The data may be recorded as complete mass spectra, selected mass numbers, or single mass numbers monitored continuously. The results are usually plotted as the integral of the signal over the entire mass range scanned or the intensity of a fixed mass number against temperature. Although the fragmentation of some parent product molecules occurring in MS systems with limited ionization potential may somewhat complicate gas identification, the technique is considered extremely sensitive even for molecules without dipole moment (Charsley and Warrington, 1992; Mullens, 1998).

The coupling between TG and FTIR offers the advantage of scanning the whole mid infrared (IR) spectrum (± 400 to ± 4000 cm^{-1}) several times per second, thus providing a fast analysis of gases released during a TG experiment. Other IR regions may also be used with proper detectors. The interfacing requires a transfer line, which is usually heated at least to 200°C to prevent condensation, and a heated gas cell of about 20-mL capacity with appropriate (e.g., KBr, ZnSe, CH) “windows.” A liquid nitrogen-cooled MCT (Hg-Cd-Te $600\text{--}4800$ cm^{-1}) detector is commonly used. Since purge gases normally used in TG experiments are transparent to the IR beam, the flow ensures fast transfer (a few seconds) of gas molecules from the TG to the FTIR system, thus minimizing the lag time between the release and detection. However, the sensitivity of the coupled system is somewhat compromised by the low heating rates and moderate gas flow rates required by the TG experiment, which may not be ideal for FTIR detection. In contrast to the TG-MS system, the whole gas flow stream should pass through the FTIR cell; hence, the transfer line should maintain the same flow rate and gas pressure set for the TG system. On-line combinations of TG-FTIR allow identification of all gas molecules or bonds with an oscillating dipole with a high degree of resolution through the entire range of gas-phase IR spectra. The results are recorded as FTIR spectra over the full range of the instrument or selected windows (specific bands) as a function of temperature. Full or partial IR spectra at selected sections of the TG

curve are evaluated to identify the gas species evolved at each stage (Kaiserberger, 1997; Mullens, 1998; Brown, 2001).

In TG-GC combinations the heating chamber is connected to a set of gas-sampling valves, which at programmed intervals withdraw gas samples evolved from various thermal events and pass them through single or multiple chromatographic columns before reaching the detectors. The type of adsorbed material used in the GC columns depends on the gaseous molecules to be analyzed. Because various gases have different retention times, an ample amount of time is required for the elution of all the species, thus limiting the system to intermittent sampling only. This is the main disadvantage of the TG-GC combination, since the time required for adequate separations may be several minutes. Therefore, only a few samplings may be possible during a thermal event, and some rapid reactions may be missed altogether. However, a suitable selection of column-packing materials and detectors and the use of multiple-loop gas sampling valves can overcome some of these limitations. Also, reducing the size of the heating chamber may decrease the time delay in gas transfer and provide results equivalent to those obtained from MS characterizations (Wendlandt, 1986; Kaiserberger, 1997).

Other Techniques

Some other less common thermal techniques not extensively utilized for mineralogical investigations—often also requiring more specialized instrumentation—include emanation thermal analysis (ETA), thermoluminescence (TL), thermomagnetometry, and thermoacoustimetry (TAA). In ETA, the release of trapped inert gas (usually radioactive, such as Ra or Th) from a sample is monitored, while the sample is heated in a specified atmosphere at a programmed rate. Mineralogical applications of ETA include studies of structural deformations and alterations of various salts, coals, carbonates, and other minerals, the dehydration and decomposition behavior of various salts and hydroxides, as well as solid–solid, solid–liquid, and solid–gas interactions on the surfaces of metal oxides (Brown, 1998). Thermoluminescence involves the measurement of light emitted as a function of annealing defects present in a sample, which is heated in a specified atmosphere at a programmed rate. The defects usually represent the previous thermal history or oxidative state of the sample. Therefore, this method has been used extensively for archaeological dating, geochemical investigations of limestone deposits and lava flows, and geological thermometry applications (Wendlandt, 1986; McKeever, 1988). Thermomagnetometry applications include the derivation of Curie points for magnetic minerals, such as hematite, maghemite, ferrihydrite, or ilmenite, from which information about their origin can be deduced. In thermoacoustimetry, the characteristics of imposed acoustic waves after passing through a sample are monitored as a function of temperature, as the sample is being heated in a specified atmosphere at a programmed rate. One geological application of thermoacoustimetry includes the distinction of different grades of oil shales based on changes in acoustic velocities as a function of temperature and organic C content (Brown, 1998).

METHODOLOGY

Sample Preparation

Type of Sample

Thermal analysis is suitable for any solid or liquid sample, but as a mineralogical method is mostly used for solid sample characterization. Whole soil or sediment samples can be analyzed, as well as various organic or mineral fractions separated by an array

of fractionation procedures typically used for mineralogical and chemical investigations (Karathanasis and Hajek, 1982a, 1982b; Wendlandt, 1986). The type of soil or sediment fraction to be used and the sample preparation (pretreatment) before analysis depend on the objectives of the investigation and the desired precision and detection limits. In most cases, the sample consists of fine particulate material, with a certain degree of size aggregation, following a light grinding in a mortar and dry sieving, preferably through a <104- μm (<140-mesh) sieve. The latter is important to maintain some standardization, which allows a better sample representation, more uniform packing, greater signal stability, and improved reliability in sample comparisons. Particle-size differences may affect variably the diffusion of gaseous products and alter the temperature of the thermal transition, and the shape of the curve, especially where surface reactions are involved. Large mineral grains may decrepitate, causing abrupt weight losses and spikes in TG curves. The smaller the particle size, the more uniform the thermal conductivity within the overall mass of the sample. Generally, the thermal reaction temperature decreases with a decrease in sample particle size and crystallinity (Smykatz-Kloss, 1974; Wendlandt, 1986; Earnest, 1988; Bish and Duffy, 1990). Diverse particle-size distribution may also produce variable heats of reaction and accentuate the discrepancy between sample and furnace temperature in sensitive isothermal kinetic measurements. Because of these effects, consistent sample preparation, involving uniform particle size and similar mass, is necessary for quantitative comparisons between samples.

Variations in the mineralogical and structural composition of the sample may also have significant effects on thermal reactions. Soil mineralogy examples of such compositional effects include: (i) the nature and hydration of the saturating cation in dehydration reactions of 2:1 and zeolite minerals, (ii) the composition of the octahedral sheets in dehydroxylation temperatures of 2:1 minerals, (iii) the vacancy type (cis- vs. trans-vacancy) in dioctahedral 2:1 layer silicates (Drits, 2003), and (iv) structural composition variations in the decarbonation of carbonate minerals (Smykatz-Kloss, 1974; Bish and Duffy, 1990).

The resolution of TA is also affected by the presence of impurities. Organic matter produces strong exothermic reactions (even under inert gas flow conditions), which may obscure characteristic endothermic transitions and weight losses of certain minerals. Therefore, for organically enriched samples (surface soils, spodic or histic materials) in which characterization of the organic fraction is not of interest, organic matter removal with H_2O_2 or other oxidizing agents is recommended. Such a treatment usually enhances the discrimination and intensity of the gibbsite, goethite, and kaolinite dehydroxylation transitions in the 250 to 550°C temperature region. Occasional problems arising from the formation of insoluble oxalates during the peroxidation process, which are thermally active, may be alleviated by ammonium presaturation and washing with hot water. Another concern in selecting either whole soil samples with relatively low clay content or the sand fractions for DTA or DSC analysis, is the dilution factor caused by an abundance of quartz, which usually obscures endothermic peaks of minerals present in small quantities. Whole soil samples and coarse sand fractions usually produce transitions of low intensity, while fine-sand and silt fractions are generally more suitable. In the latter, the amount of gibbsite, goethite, and kaolinite may be large enough to produce detectable thermal transitions by TG or DTA/DSC analysis.

Clay fractions are generally the most suitable for quantitative thermal analysis. Total clay fractions, as well as coarse- or fine-clay fractions can be used, depending on the scope of the study and the mineralogical composition of the sample. Thermal analysis of clay fractions is also more sensitive to cation saturation and hydration/solvation conditions of the sample (Fig. 5-7). The variable hydration properties of different cations markedly

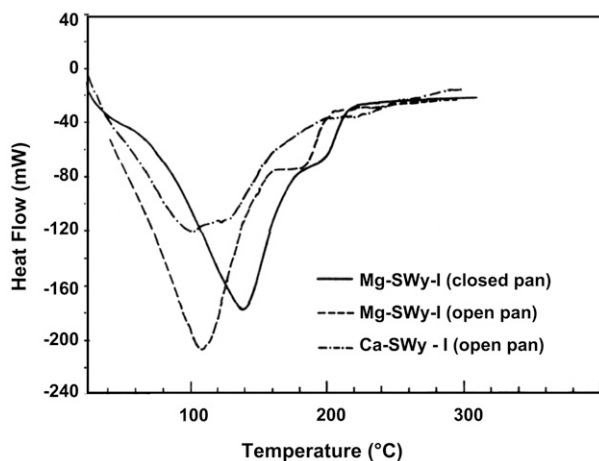


Fig. 5-7. Effect of saturating cation on the differential scanning calorimetry (DSC) dehydration curves of a smectite sample from Wyoming (SWy) (van Groos and Guggenheim, 1990).

affect dehydration transitions with respect to magnitude of weight loss (TG), and intensity and shape of endothermic peaks (DTA/DSC). These effects are more noticeable in samples with considerable amounts of smectite, vermiculite, and zeolite minerals (Dyer, 1981; El-Akkad et al., 1982; Karathanasis and Evangelou, 1986; Schilling, 1990; Bish and Duffy, 1990). Therefore, for comparative studies involving routine mineralogical characterizations, monoionic saturation with the same cation (Mg or Ca) is preferred. When TA analysis is combined with XRD, Mg is the most common saturating cation, although investigators have used a variety of saturating cations (El-Barawy et al., 1986; van Groos and Guggenheim, 1990).

Low-temperature transitions are also sensitive to hydration and moisture conditions of the sample. Fine clay fractions with large specific surface area may sorb substantial amounts of water, especially if they contain high proportions of smectite, vermiculite, poorly crystalline minerals, or organic matter. Such samples, and all clay fractions in general, should be equilibrated under a constant relative humidity environment to assure that thermal transitions, interpretations, and comparisons can be made on a similar relative basis. Equilibration is usually achieved by placing the samples over a saturated aqueous solution of salt that provides a stable relative humidity atmosphere overnight in a desiccator (Weast, 1980). A typical salt used to establish a relative humidity level of ~52% is saturated MgNO_3 (Karathanasis and Hajek, 1982b). Organic soil fractions, separated by a variety of extraction and purification procedures, can also be analyzed by TG or DTA/DSC methods (Tan, 1978; Schnitzer and Kodama, 1972). Humic and fulvic acids exhibit exothermic decomposition peaks (DTA) in the 400 and 500°C temperature region, respectively, but with considerable drift due to different cation saturation (Tan, 1978). With increased analytical care and instrument sensitivity, TG-DTG patterns could be used to infer the biochemical stability of various soil organic fractions. Finally, selective chemical dissolutions can also aid thermoanalytical interpretations of soil clays. A typical example involves the overlapping endothermic reactions of gibbsite and goethite at around 300°C, which can be resolved by the citrate-bicarbonate-dithionite (CBD) pretreatment that dissolves goethite (see the discussion of pretreatment in Chapter 2; Soukup et al., 2008, this volume). Similar overlapping of poorly crystalline or amorphous mineral phases with various aluminosilicates may be circumvented by alkali dissolutions (see the discussion of selective chemical dissolution in Chapter 3; Shang and Zelazny, 2008, this volume).

For thermomechanical analysis (TMA), the sample is usually a packed or pelletized powder of any soil fraction, a small undisturbed moist soil core or clod, or a thick soil paste (mud) placed into a mold of known volume dimensions (Karathanasis and Hajek, 1985). For combined DSC-XRD, DSC-TO, and TG/DSC-EGA techniques, hand-picked mineral grains or powdered or pelletized soil or sediment samples can be used, following some degree of purification or characterization (Wicks and Ramik, 1990) to facilitate the resolution of the gaseous products. Samples that cannot be manually purified can be centrifuged in heavy-liquid density columns or treated by magnetic separation techniques.

Regardless of the type of sample used, it is essential that a complete description of its source, pretreatments, and known physical and chemical characteristics is provided. If the sample is diluted with another inert or thermally active material, the composition of the mixture should also be given because the diluent can greatly alter the thermal conductivity of the sample.

Sample Size and Packing

Sample mass and packing density are important parameters in thermoanalytical studies. The greater sensitivity of modern instruments allows for the use of smaller samples (5–50 mg) with efficient detectability. The smaller mass maintains a uniform heat transfer throughout the sample and an effective diffusion of the gaseous products. However, in mixtures containing small amounts of certain minerals, larger sample sizes may be necessary, and more replicates need to be examined to assure reproducibility (Anderson et al., 1977). Similar problems may arise in some DTA cases, where the use of a diluent, such as Al_2O_3 , is necessary (Mackenzie, 1981). In very small samples (<2 mg) the signal produced may be too weak or too noisy to be resolved from the baseline. In modern instruments sample masses of 10 to 100 mg are adequate for producing high quality DTA curves, while minimizing thermal gradients and heat transfer interferences within the mass of the sample. Increasing the particle-size distribution of the sample above 1 μm generally increases the temperature and the heat of transition. Similar results are also obtained with increasing sample crystallinity, but the effects of sample grinding have been inconsistent (Wendlandt, 1986; Tan et al., 1986). This is probably due to the fact that the area under the peak is inversely proportional to the thermal conductivity of the sample, which is dependent not only on the particle-size distribution, but also the packing of the sample. Finally, the type, quantity, and degree of mixing of the diluent with the sample may cause shifts in the baseline and the temperature of transition, as well as changes in the shape and area of peaks. Therefore, it is critical that similar sample masses be used for comparative studies, particularly in quantitative thermal analysis.

One of the most operator-dependent variables in thermal analysis is the packing of the sample in the instrument sample holder. While in most cases thinly and uniformly spreading a loose powdered sample in the sample holder may be sufficient, some DTA or DSC applications require packing the sample to assure a good thermal contact between the sample and the holder. This can be achieved by hand tapping or judicious tamping of the sample with a tool matching the inside diameter of the holder or the sandwich-packing technique. In the latter, the sample is placed around the thermocouple as a sandwich between two layers of reference inert material (Mackenzie, 1981; Tan et al., 1986). Reproducibility in sample packing is important because differences in the bulk density of the sample may cause variable thermal conductivity and diffusivity rates. Loosely packed, coarse materials contain large pores that reduce the thermal conductivity of the sample, while finely ground and densely packed samples have higher thermal conductivity. Tight sample packing may

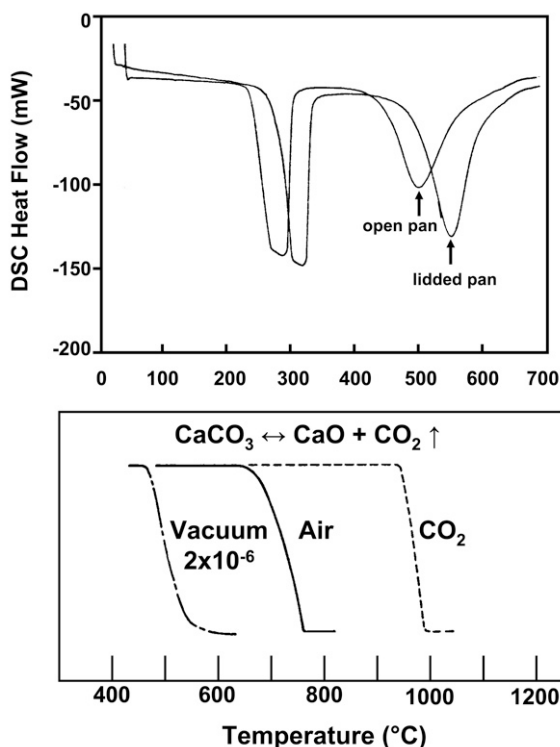


Fig. 5-8. The effect of (a) lidded vs. open pans on the differential scanning calorimetry (DSC) peaks of gibbsite and kaolinite and (b) furnace atmosphere on the thermogravimetry (TG) curve of CaCO_3 (Wendlandt, 1986).

impede the diffusion of volatiles through the mass of clay samples, causing broader DTA and DTG peaks shifted to higher temperatures (Schilling, 1990). The same effect is observed when using lidded sample pans as compared with open pans for DSC analysis (Fig. 5-8a). Inconsistent packing may produce considerable variation in mineral thermal behavior, particularly at low temperature ranges, where heat transfer is controlled by conduction. The effects are less apparent at high temperature regions where heat transfer occurs mainly through radiation. Packing consistency also applies to the reference materials used so that uniform bulk densities are achieved to minimize baseline drift.

Operating Conditions

Furnace Environment

The furnace atmosphere has a significant effect on the heat transfer rate through the sample, the type of thermal reactions taking place, and the gaseous products formed. Changing the furnace atmosphere may cause shifts to normal thermal transition temperatures, enhancement or elimination of certain reactions, and various interactions between the inert gas and the sample gaseous products that may obscure characteristic peaks (Wendlandt, 1986; Tan et al., 1986). Even if there is no interaction between the sample and the furnace atmosphere, the heat transfer by the inert gas will affect the thermal results. A number of different atmospheres may be employed separately or in combination to enhance thermo-analytical interpretations, depending on the scope of the experiment, ranging from high vacuum (10^{-4} Pa) to high pressure (70 bar) of inert, oxidizing, reducing or corrosive gases (Brown, 2001). At low pressures (10^{-2} to 270 Pa), thermomolecular flow

problems caused by temperature gradients along the sample holder may result in spurious mass changes. At high pressures and flow rates, buoyancy effects may require certain corrections. The advantage provided by instruments modified to accommodate high-pressure atmospheres is that they allow studies of mineral behavior and interactions under simulated hydrothermal conditions (van Groos and Guggenheim, 1990). At atmospheric pressure, the atmosphere can be static or dynamic (flowing). Under static conditions, gaseous products cannot be removed rapidly from the vicinity of the sample and the buildup of the gas partial pressure results in an increased reaction temperature. Under dynamic conditions, the evolved gases are swept away from the sample, thus avoiding a partial pressure buildup that would delay the thermal reaction. A flowing atmosphere also has the advantages of limiting the condensation of reaction products, decreasing secondary reactions, flushing out potentially corrosive products, and acting as a coolant of the sample chamber. The gases employed in TA experiments are classified as inert (N_2 , Ar, He, and CO_2), oxidizing (air, O_2), reducing (H_2 , CO), self-generated gases produced by the thermally reactive sample (CO_2 , water vapor), and corrosive (Cl_2 , F_2 , SO_2). The latter are very dangerous and are rarely used in mineralogical investigations.

The choice of purge gas is determined by the scope of the experiment, the reactivity with the sample, cost, availability, purity, density and thermal conductivity. Most mineralogical experiments are conducted under inert gas atmospheres (N_2) to suppress the oxidation of organic constituents present in soil and sediment samples. This allows a better resolution of minerals undergoing dehydration or dehydroxylation in the temperature region in which exothermic reactions from organic C oxidation may interfere with their identification. Inert atmospheres may also eliminate exothermic reactions associated with the oxidation of Fe-hydroxides or Fe-hydroxyoxide minerals following their dehydroxylation reaction, which may obscure weight or heat flux measurements. The use of CO_2 as an inert gas is not recommended because at high temperatures it may undergo dissociation to CO and O. However, its use is highly advantageous for studying the decomposition of carbonate mineral mixtures because of the greater resolution of the decarbonation reactions. Generally, the presence of product gas atmosphere in the furnace (flow atmosphere) or the slow or incomplete removal of the gaseous products (static atmosphere) results in shifts of the thermal reaction to higher temperatures and/or generation of double peaks (Fig. 5–8b). Examples include the decarbonation of carbonate minerals under CO_2 atmosphere and the dehydration of gypsum in water vapor atmosphere, respectively.

Recent innovations in the use of coupled TA and EGA instruments provide great advantages in the diagnosis of the evolved gases during each thermal reaction, the detection of the mineral phases present, and the type of the thermal transitions involved (Morgan et al., 1988; Wicks and Ramik, 1990). The development of complex TA techniques further allows multistage heating programming accompanied by furnace gas atmosphere changes during the course of individual runs. Examples of this application include proximate analysis of coal, oil shale, fly ash, different sorbents, and various waste materials (Charsley and Warrington, 1992). The flow rate of the purging gas also affects the thermal conductivity of the sample, the temperature of the thermal reactions, the nature of the baseline, and the size of the peaks. Average flow rates recommended for thermal studies of soil minerals are in the range of 50 to 100 mL min⁻¹. Flow rates that are too low may not flush the generated gaseous products effectively, while flow rates that are too high may cause significant turbulence and result in noisy signals. Atmosphere and flow rate control is achieved by a variety of devices, including a range of relatively inexpensive valves and flow meters to more costly mass flow controllers.

Heating Rate

Experimental results of thermal analysis are markedly affected by the heating rate employed because the transfer of heat from the furnace to different parts of the sample is not instantaneous, but depends on conduction, convection and radiation processes. Usually there is a thermal lag between the furnace and the sample, which increases with increasing heating rate. At low heating rates ($<5^{\circ}\text{C min}^{-1}$), the temperature difference inside and outside the sample mass is negligible, and near equilibrium conditions are attained. Under these conditions the thermal reaction is finalized in lower temperatures, and the resolution of overlapping thermal events is greater. At higher heating rates, the thermal reaction takes place at a faster speed, but due to a greater thermal lag within the sample, it takes longer to be completed, thus shifting the peaks to higher temperatures (Fig. 5–9). Under these conditions, the reaction is completed within a narrower temperature interval, causing the derivative curves to be sharper. However, when successive reactions are involved within a short temperature range, the high heating rate may not allow these reactions to be separated, while a slow heating rate will provide a better resolution of the overlapping peaks. Rates of 5 to $20^{\circ}\text{C min}^{-1}$ are commonplace in mineralogical investigations. Low rates are preferred for samples in which the resolution of overlapping transitions is important at the expense of extra time and for samples containing a large amount of water undergoing several intermediate dehydration stages (Wendlandt, 1986). In contrast, for routine thermal studies rates of 10 to $20^{\circ}\text{C min}^{-1}$ provide the advantage of speed of determination and increased DTA or DSC peak size, which is significant for the detection of minor components. Although heating rate changes have been reported to affect peak height, peak width, peak temperature, and peak area, this has seldom been a problem in mineralogical investigations as long as the same heating rate is used for comparative studies. Recent advances in TA instrumentation allow for the use of variable (controlled) heating rates during the same thermal run (Reading, 1998). The terms used for this mode of operation are *quasi-isothermal*, *stepwise isothermal*, or *dynamic rate thermal analysis*, in which the heating rate is programmed to change as a function of changes in the thermal behavior of the sample (i.e., mass loss, temperature change, heat transfer). For example, the sample is heated at a constant heating rate up to the beginning of a thermal transition, when the linear temperature rise cycle is interrupted and the temperature of the sample either remains constant (isothermal) or increases at a slower rate (dynamic) until the end of the transition, after which the programmed initial heating rate resumes. This technique provides more diagnostic inflections for intermediate mineral phases and better resolution of overlapping peaks

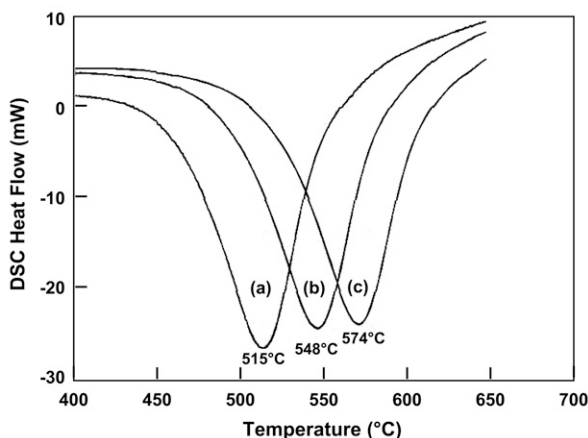


Fig. 5–9. The effect of heating rate at 2 min^{-1} (a), $5^{\circ}\text{C min}^{-1}$ (b), and $10^{\circ}\text{C min}^{-1}$ (c) on the differential scanning calorimetry (DSC) peak of kaolinite.

(Wendlandt, 1986; Charsley and Warrington, 1992; Paulik, 1995; Haines, 1995; Ford and Bertsch, 1999; Xi et al., 2004).

Data Processing

The manufacturers of modern TA equipment offer both the hardware and the software necessary to conduct most thermal analyses with a high degree of automation, and to calculate the proper thermal parameters from the collected data. However, the hardware and software provided are specific to the manufacturer's equipment, and it is nearly impossible to alter them to suit all the operator's requirements. The development of most software programs used in TA has been based on principles and algorithms applied in gas chromatography. In DTA and DSC the data processing includes the establishment and distinction of the baseline signal, the detection of peak onsets and maxima, the resolution of overlapping peaks, and the determination of peak areas by numerical integration. In TG the required routines include smoothing and numerical differentiation, onset and return weight loss calculation, buoyancy corrections, and possibly polynomial regression. Other more specialized procedures, such as kinetic analysis of certain thermal transitions, require specific software packages provided by the manufacturer or could be performed by importing data files into spreadsheets and analyzing them by general software programs.

The sequence of operations in running a thermal experiment includes

1. Selection of a heating program, including the temperature range, the heating rate, and the furnace atmosphere
2. Loading the sample and starting the run
3. Capturing and storing the data
4. Displaying the data being acquired for preliminary examination
5. Manipulating the collected data
6. Baseline correction
7. Smoothing and scaling data
8. Processing the data
9. Numerical differentiation
10. Peak integration
11. Other calculations

The first four steps are hardware specific, while data manipulation/scaling and baseline correction can be easily accomplished following the manufacturer's software guidelines. The availability of powerful spreadsheet packages has virtually removed the necessity of using the dedicated software provided by the manufacturer for smoothing and processing thermal data. Various algorithms incorporated in spreadsheet or chromatographic software programs can efficiently smooth, integrate, or differentiate the data to meet the operator's specifications. This is a particularly useful option in cases where deconvolution of overlapping peaks is necessary for diagnostic mineralogical determinations because the currently available manufacturers' software is completely inadequate for such applications.

QUALITATIVE INTERPRETATIONS

Qualitative mineralogical interpretations from TA curves are based on fingerprint comparisons with standard or well-known minerals. Although the thermal characteristics of a particular mineral, especially those found in soil mixtures, may vary somewhat as a function of origin, crystallinity, structural and chemical composition, and pretreatment, its

Table 5–2. Thermogravimetric (TG) weight loss regions used for quantitative estimation of selected soil minerals (Karathanasis and Harris, 1994).

Mineral	Temperature region °C	Type of reaction	Weight loss† g kg ⁻¹	Reference
Oxides/hydroxides				
Gibbsite	250–350	-OH → H ₂ O	312	Karathanasis and Hajek (1982a)
Goethite	300–400	-OH → H ₂ O	101–112	Todor (1976)
Carbonates/sulfates/sulfides/phosphates				
Calcite	750–900	-CO ₃ → CO ₂	440	Todor (1976)
Dolomite	500–950	-CO ₃ → CO ₂	476	Todor (1976)
Magnesite	500–750	-CO ₃ → CO ₂	524	Todor (1976)
Siderite	450–600	-CO ₃ → CO ₂	379	Todor (1976)
Na ₂ CO ₃	500–1000	-CO ₃ → CO ₂	415	Asomoza et al. (1978)
Gypsum	100–350	H-OH → H ₂ O	209	Todor (1976)
Alunite	450–560	-OH → H ₂ O	131	Arazi and Krenkel (1970)
Jarosite	250–460	-OH → H ₂ O	108	Paulik, 1995
Pyrite	400–550	-S → SO ₂	Variable	Paulik et al. (1982)
Strengite	200–400	-OH → H ₂ O	190	Nathan et al. (1988)
1:1 Layer silicates				
Kaolinite	400–600	-OH → H ₂ O	140	Jackson (1975)
Halloysite	400–600	-OH → H ₂ O	122	Jackson (1975)
Endellite	400–600	-OH → H ₂ O	122	Jackson (1975)
Antigorite	600–900	-OH → H ₂ O	129	Todor (1976)
2:1 Layer silicates				
Mg-smectite (52% RH)	25–250	H-OH → H ₂ O	228	Karathanasis and Hajek (1982a)
	600–900	-OH → H ₂ O	50	Jackson (1975)
Mg-vermiculite (52% RH)	220–250	H-OH → H ₂ O	24	Karathanasis and Hajek (1982a)
Hectorite	500–900	-OH → H ₂ O	193	Earnest (1988)
Mica	500–900	-OH → H ₂ O	50	Jackson (1975)
Talc	850–1050	-OH → H ₂ O	50	Jackson (1975)
2:2 Layer silicates				
Chlorite	540–800	-OH → H ₂ O	123	Jackson (1975)

† Pure mineral on an air-dry weight basis.

thermal behavior is usually expressed within relatively well-defined temperature limits. Particular emphasis should be placed in providing a complete description of the instrument, the method, the sample, and the conditions of each experiment so that valid comparisons can be made. Tables 5–2 and 5–3 list characteristic TG and DTA/DSC temperature regions commonly used for the identification and quantification of soil minerals.

Thermal Gravimetric Analysis

In TG curves, inflections associated with mass losses or gains within characteristic temperature regions are compared with standard or reference minerals for mineral identification (Table 5–2). It is often difficult to identify precisely the starting and ending inflection points for a specific reaction against a background of continuous mass loss, especially

Table 5-3. Differential thermal analysis (DTA) or DSC temperature regions and respective heats of reaction (ΔH) used for quantitative estimation of selected soil minerals (Karathanasis and Harris, 1994).

Mineral	Temperature region °C	Type of reaction	$\Delta H^\dagger$ J g ⁻¹	Reference
Oxides/hydroxides				
Gibbsite	250–350‡	-OH → H ₂ O	1075.3	Karathanasis and Hajek (1982a)
Goethite	300–400‡	-OH → H ₂ O	895	Smykatz-Kloss (1974)
Quartz	~573‡	$\alpha \rightarrow \beta$ inversion	5.4	Bartenfelder and Karathanasis (1989)
Carbonates/sulfates/sulfides/ phosphates				
Calcite	900–990	-CO ₃ → CO ₂	1787	Mackenzie (1970)
Magnesite	650–750	-CO ₃ → CO ₂	1345	Wendlandt (1986)
Dolomite	700–950	-CO ₃ → CO ₂	1565.5	Dubrawski and Warne (1988)
Gypsum	180–220	H-OH → H ₂ O	657.7	Wendlandt (1986)
Alunite	430–590	-OH → H ₂ O	878.6	Arazi and Krenkel (1970)
1:1 Layer silicates				
Kaolinite	500–600‡	-OH → H ₂ O	656.9	Karathanasis and Hajek (1982a)
Halloysite	500–600‡	-OH → H ₂ O	694.5	Barshad (1952)
2:1 Layer silicates				
Mg-smectite/vermiculite	50–250	H-OH → H ₂ O	592.5	Karathanasis and Hajek (1982a)
Mg-vermiculite	220–250	H-OH → H ₂ O	159.0	Barshad (1952)

† Pure mineral on an air-dry weight basis.

‡ Tan et al. (1986).

in the presence of overlapping reactions. The use of DTG or dynamic rate TG analysis may aid significantly in the resolution of these reactions and the identification of the respective minerals. The TG patterns are usually plotted as absolute mass loss or gain in milligrams or percentage mass loss or gain relative to the original weight of the sample as a function of temperature. A typical TG scan of a soil clay, equilibrated at 52 to 56% relative humidity (RH), between ambient and 1000°C at 20° min⁻¹ under N₂ atmosphere will usually produce reactions associated only with mass losses, since the presence of the inert gas will inhibit oxidation reactions (Fig. 5-10).

If the sample contains significant amounts (>20%) of smectite or allophane group minerals, an initial inflection associated with the dehydration of sorbed water will be observed commencing at the starting temperature of the thermal run and ending at about 250°C. The latter stages (150–250°C) of this dehydration reaction may include loss of water coordination-bonded with the saturating cation(s) or associated with the surfaces of smectites. The amount of water loss is dependent on the type of saturating cation.

Mass losses associated with sorbed water of mixed mineralogy samples dominated by other 2:1 phyllosilicates or kaolinite (1:1) group minerals are usually substantially lower than 5% in this temperature region. Significant overlap of mass loss events may occur in this temperature region with samples containing hydrated halloysite, allophane, gypsum,

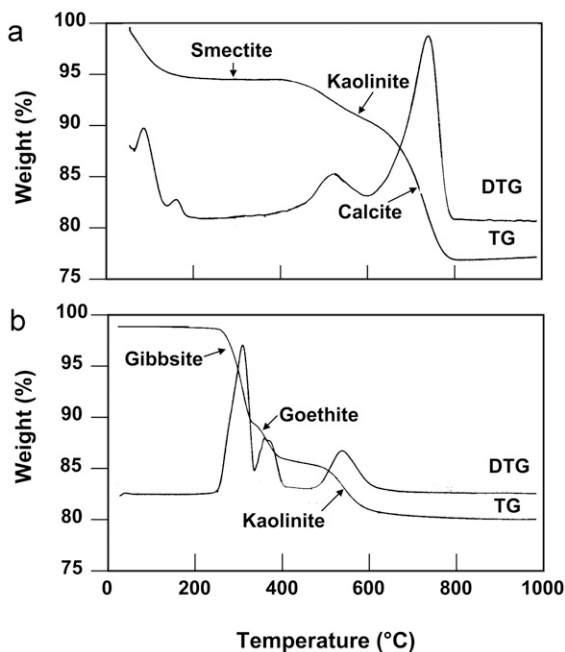


Fig. 5-10. Typical thermogravimetry/differential thermogravimetry (TG/DTG) curves of two soil clays containing: (a) smectite, kaolinite, and calcite, and (b) gibbsite, goethite, and kaolinite.

or gibbsite. Hydrated halloysite and allophane lose their sorbed water at 100 to 200°C, gypsum at 140 to 200°C, and gibbsite dehydroxylates at 220 to 300°C. Therefore, all of these reactions may theoretically overlap with the dehydration of smectite type minerals. Since the coexistence of smectite and gibbsite in natural soil samples is unusual, the only real concerns apply to samples with smectite, hydrated halloysite, allophane, and gypsum. In samples containing gypsum, running the sample before and after treating it with alcohol to dissolve gypsum may circumvent one of the problems. For differentiation of the allophane component a pretreatment with hot NaOH or $(\text{Na})_2\text{CO}_3$ solutions may be necessary. In the case of hydrated halloysite, its differentiation may rely on the temperature of its dehydroxylation reaction, which is similar to kaolinite at 450 to 600°C and some trioctahedral smectites.

In samples with Fe-hydroxides or Fe-oxyhydroxides (goethite), the next mass loss associated with their dehydroxylation reaction occurs in the region between 250 and 350°C, which usually overlaps with the dehydroxylation of gibbsite. If DTG peaks are not very helpful in resolving such interferences, a free iron oxide removal with sodium bicarbonate-citrate-dithionite will provide positive quantification of the mineral(s). In the region between 350 and 500°C the dehydroxylation of several Fe and Al hydroxysulfate and palygorskite or sepiolite minerals may interfere with the initial dehydroxylation stages of kaolinite. The coexistence of Al-hydroxy-interlayered minerals with kaolinite is typical in semitropical environments, and a gradual dehydroxylation of the interlayer phase may mask the initial inflection of the kaolinite dehydroxylation reaction.

The region of 450 to 600°C is typified by mass losses associated with the dehydroxylation of minerals in the kaolinite group. Although there are some subtle differences in the dehydroxylation patterns of kaolinite, dehydrated halloysite, and dickite, it is difficult to decipher the effect of crystallinity and particle size on this variability. Generally,

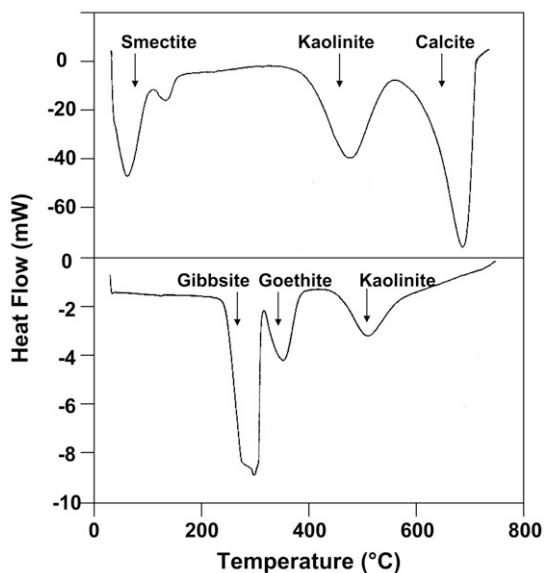


Fig. 5-11. Differential scanning calorimetry (DSC) curves of the two soil clays in Fig. 5-10.

the better the structural order and the greater the particle size, the higher the temperature of the completion of the dehydroxylation reaction. In samples containing Fe-rich chlorites and trioctahedral smectites (saponite, hectorite), the final inflection point of the kaolinite dehydroxylation may interfere with the early stages of their dehydroxylation reactions (450–600°C).

The majority of 2:1 (smectites, vermiculites, illites) and “2:1:1” (chlorites, Al-hydroxy-interlayered) minerals, as well as some serpentine type minerals, exhibit dehydroxylation within the 600 to 750°C temperature region. The initial and final dehydroxylation temperatures may vary slightly, depending on structural and compositional variation of the minerals (e.g., Fe-rich chlorites and trioctahedral smectites have lower dehydroxylation temperatures). Although most carbonates experience decomposition mass losses above 700°C, siderite and magnesite may undergo decarbonation at lower temperatures (600–700°C) and overlap with the dehydroxylation reactions of the above 2:1 and hydroxy-interlayered minerals. In these cases, an acid pretreatment may resolve the interference (Soukup et al., 2008, this volume). Finally, the temperature region between 700 and 950°C is characterized by mass losses associated with the decarbonation of other carbonate minerals. Dolomite usually decomposes at 700 to 800°C, calcite at 800 to 900°C, and aragonite at 850 to 950°C. The temperature of decarbonation is strongly dependent on cationic composition and structural order. Using a CO₂ furnace atmosphere will provide a better separation of the decarbonation reactions, while complementary XRD patterns may add greatly in the identification of the carbonate mineral species.

Differential Thermal Analysis/Differential Scanning Calorimetry Analysis

The advantage of DTA/DSC patterns is that not only do they exhibit endothermic or exothermic peaks associated with dehydration, dehydroxylation, decomposition, or oxidation reactions, they also show endothermic or exothermic peaks caused by mineral transformation reactions or phase transitions not necessarily associated with mass losses or gains, and therefore, not detected by TG/DTG analysis (Fig. 5-11). As a result, DTA/DSC

patterns can provide additional information about the identity of the minerals present, thus improving the diagnostic quality of the thermo-analytical data (Table 5–3). Differential thermal analysis patterns are probably more useful in this case because they can span a greater temperature range (up to 1200°C) compared with the 750°C of most DSC instruments. Nevertheless, DSC patterns will show a sharp endotherm at 573°C associated with the reversible $\alpha \rightarrow \beta$ inversion of quartz, and exothermic peaks between 450 and 550°C corresponding to transitions of γ - Al_2O_3 and γ - Fe_2O_3 to α - Al_2O_3 and α - Fe_2O_3 polymorphs, respectively. Similarly, DTA curves will show sharp exotherms associated with the recrystallization of dehydroxylated dioctahedral mineral phases, such as kaolinite, montmorillonite, or illite to spinel type minerals (MgAl_2O_4) at 900 to 1000°C, and to mullite ($\text{Al}_6\text{Si}_2\text{O}_{13}$) or corundum (α - Al_2O_3) at 1000–1200°C. Trioctahedral minerals, such as serpentine, talc, saponite, vermiculite, and chlorite would produce similar exotherms in the 700 to 1100°C region associated with the formation of forsterite (Mg_2SiO_4) or enstatite (MgSiO_3) mineral phases (Brindley and Lemaire, 1987).

Differential thermal analysis, and especially DSC patterns, because of their better precision in low temperature ranges (smaller mass, better equilibration environment), have also been useful in deciphering the effects of the saturating cation in smectites and vermiculites on their di- or trioctahedral nature, charge origin, and compositional variability. For example, some beidellites and vermiculites with high tetrahedral charge show a more pronounced two-stage loss of sorbed water (double endotherm at the 100–250°C region), associated with the better coordination and higher dehydration energy of some of the interlayer water molecules. In the kaolinite group of minerals, endotherm peak temperature and shape of the dehydroxylation reaction can also provide useful qualitative information. There is a general tendency for endotherm peak temperatures to decrease with decreasing structural order. Aside from the low-temperature (100–250°C) endotherm associated with the dehydration of interlamellar water in halloysite, dehydrated halloysite can usually be distinguished from better crystalline kaolinites by the lower temperature and greater asymmetry of its dehydroxylation peak caused by increased structural disorder. On the other hand, dickite produces dehydroxylation endotherms with a shoulder on the low temperature side, which reflects structural stacking differences from kaolinite. DTA/DSC peak shifting and deformation has also been used to detect Fe^{2+} for Mg^{2+} substitutions in dolomites, Fe^{3+} for Al^{3+} substitutions in goethites and smectites, and Mg^{2+} for Fe^{3+} substitutions in chlorites (Smykatz-Kloss, 1974). Although particle size, morphology, and crystal defects may cause some variability, careful evaluations of DTA/DSC peak shape and temperature trends may be of considerable value in distinguishing among polymorphs or even compositionally variable members of the same group of minerals, particularly if supported by other analyses.

QUANTITATIVE INTERPRETATIONS

Calculations from Thermogravimetry and Differential Scanning Calorimetry Patterns

To calculate the weight loss for a particular thermal reaction of a specific mineral the starting and ending temperature points of the transition must be defined on the TG curve. The use of DTG curves can greatly facilitate the identification of these points because the corresponding peaks are sharper than TG inflections, and their initial and final inflections usually correspond well with starting and ending reaction temperatures (Fig. 5–12).

Another approach, which is used alone or in conjunction with DTG curves, is the graphical extrapolated onset and return technique, in which the intercepts of a tangent

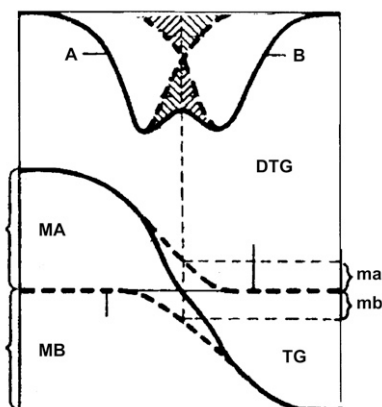


Fig. 5-12. The use of differential thermogravimetry (DTG) for quantitative analysis of minerals with overlapping reactions; MA and MB = mass losses for minerals A and B, respectively; ma and mb = overlapping mass losses for the two minerals corrected by DTG (Paulik, 1995).

drawn at the middle point of the reaction step ($T_{1/2}$) with the extrapolated initial and final baselines of the reaction are used to define the starting and ending temperatures (Fig. 5-13). The technique usually provides satisfactory quantitative results except for samples with considerable overlapping of thermal reactions. In such cases, if DTG curves do not provide the desired resolution, curve decomposition software can be used to improve discrimination and quantification options (Fig. 5-14). The latter option is particularly helpful in samples with overlapping peaks. However, such programs for TA are generally not provided by the manufacturers and are usually developed by the investigators to suit their needs.

Changing the conditions under which the analysis is conducted may be required to obtain better resolution. For example, in samples with multiple sequential weight losses in which the slope of the baseline at the end of the reaction does not flatten out (return to horizontal) before the start of the next reaction, employing a lower heating rate may improve resolution. Once the starting and ending points of the thermal reaction are defined, the corresponding percentage of weight loss is calculated from the TG curve and compared with either the theoretical weight loss of a pure mineral or the relevant weight loss of a reference mineral run under the same conditions. Figure 5-15a demonstrates the application of these techniques for the quantification of gibbsite and kaolinite in a soil sample from Georgia. The amount of weight loss calculated for the dehydroxylation of gibbsite in the 250 to

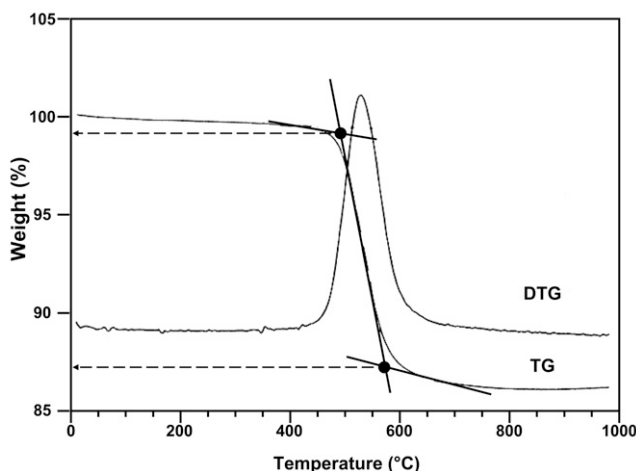


Fig. 5-13. The application of the graphical extrapolated onset and return technique for thermogravimetry (TG) quantifications.

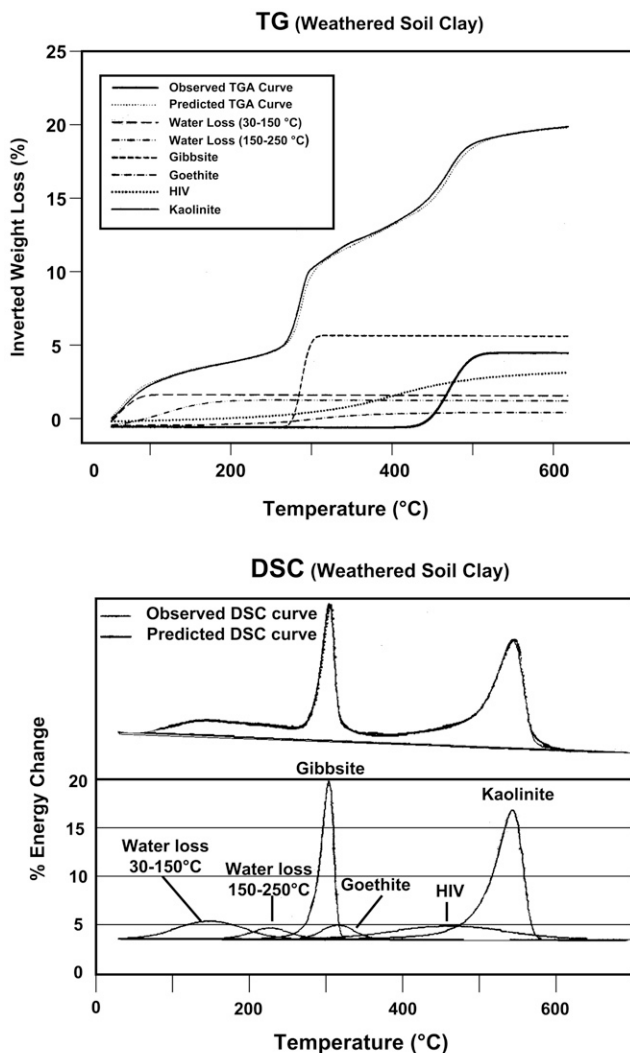


Fig. 5-14. A deconvolution software program used for thermogravimetry (TG) and differential scanning calorimetry (DSC) analysis of a soil clay (Beck, 2003).

300°C region was 3.2%, while the respective weight loss for the dehydroxylation of kaolinite (400–550°C) was 2.9%. Therefore, the quantity of each mineral in the sample can be estimated from the respective weight losses compared with the ideal hydroxyl water contents of reference gibbsite (31.2%) and kaolinite (14%) minerals, following the equations:

$$\text{Gibbsite} = (3.2/31.2) \times 100 = 10.2\% \quad [6]$$

$$\text{Kaolinite} = (2.9/14.0) \times 100 = 20.7\% \quad [7]$$

In modern TA equipment the calculation of the heat change, or enthalpy (ΔH), of a thermal reaction in a DSC curve or the area under the peak in a DTA curve is performed with an integration software program provided by the manufacturer. This expedites quantification, which in the past relied on manual measurements of peak size by planimetry or weighing. Still, defining the initial and final points of each thermal transition is a critical

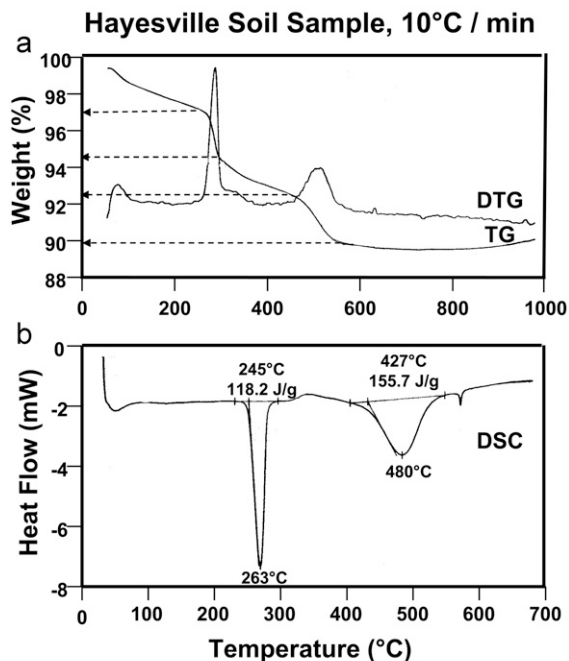


Fig. 5-15. Gibbsite and kaolinite quantification in a Hayesville soil sample from Georgia by (a) thermogravimetry (TG) and (b) differential scanning calorimetry (DSC) analysis.

step in the quantification process. With relatively symmetrical peaks, a simple extrapolation of the baseline connecting the initial and final inflection points (interpolated baseline) may provide adequate quantification. However, when a significant displacement of the baseline occurs between the initial and final inflection points, various graphical procedures have been used to compensate for the drift and produce reasonable quantitative estimates (Fig. 5-16). In modern DSC instrumentation several graphical baseline correction options are available and may be incorporated in the software package provided by the manufacturer. Once a satisfactory baseline has been defined, the area under the endotherm or

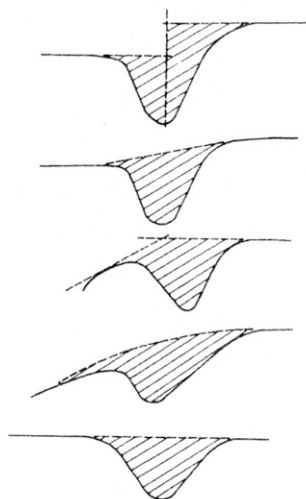


Fig. 5-16. Graphical procedures used for baseline drift compensation in quantitative differential scanning calorimetry (DSC) analysis (Brown, 2001).

exotherm is determined by software-driven numerical integration. The measured area is assumed to be proportional to the heat evolved or consumed during the reaction (enthalpy change, ΔH), and therefore, the quantity of the mineral undergoing the thermal reaction. The ΔH of the reaction is calculated from the following equation:

$$\Delta H = (k_T' A)/m \quad [8]$$

where k_T is the calibration coefficient for the temperature region of the reaction, A is peak area, and m is the mass of the sample. For power-compensated DSC instruments k_T is virtually independent of temperature, but for DTA and heat-flux DSC units a different k_T value should be determined from calibrations over the desired reactive temperature region. For most soil and geological materials tested by DSC, three calibrations at the low, medium, and high temperature reactive regions with indium ($T_f = 156.6^\circ\text{C}$, $\Delta H = 28.7 \text{ J g}^{-1}$), tin ($T_f = 231.9^\circ\text{C}$, $\Delta H = 60.5 \text{ J g}^{-1}$) or lead ($T_f = 327.5^\circ\text{C}$, $\Delta H = 23.0 \text{ J g}^{-1}$), and zinc ($T_f = 419.5^\circ\text{C}$, $\Delta H = 108.6 \text{ J g}^{-1}$) provide satisfactory results (Brown, 2001). Figure 5–15b demonstrates the application of these procedures for the quantification of gibbsite and kaolinite by DSC in the soil sample from Georgia. The enthalpy changes (ΔH) calculated from the DSC thermogram for the dehydroxylation of gibbsite and kaolinite were 118.2 and 155.7 J g^{-1} , respectively. Compared with the ΔH of reference pure gibbsite and kaolinite minerals of 1075.3 and 656.9 J g^{-1} , respectively (Karathanasis and Harris, 1994), the estimated quantities are

$$\text{Gibbsite} = (118.2/1,075.3) \times 100 = 11.0\% \quad [9]$$

$$\text{Kaolinite} = (155.7/656.9) \times 100 = 23.7\% \quad [10]$$

which are very close to the values estimated by TG.

Smectite and Vermiculite

Quantification of smectite- and vermiculite-type minerals in soil and geological materials by TG or DTA/DSC methods is based on thermal assessments of water molecules sorbed on their monocationic forms under controlled relative humidity conditions (Mackenzie and Calliere, 1975; Smalley and Xidakis, 1979; Karathanasis and Hajek, 1982a, 1982b; Karathanasis et al., 1986; Post et al., 1997). The applicability of the method is highly dependent on the type of saturating cation and the equilibrium environment (atmosphere), but its sensitivity and reproducibility have improved substantially with recent advances in DSC instrumentation. In most cases the samples are air-dry, powdered, Mg-saturated clay fractions used for XRD analysis, either before or after Fe-removal, which are equilibrated for 48 h in chambers with controlled RH at $52 \pm 2\%$. In cases where the ambient RH deviates significantly from that of the chamber, purging the furnace with N_2 gas passing through the saturated solution used to maintain the desired RH minimizes errors.

In TG thermograms the amount of smectite is estimated from weight losses in the 25 to 250°C temperature region compared with reference smectite minerals run under the same conditions (Table 5–2). In DSC/DTA curves the quantity of smectite is estimated by integrating the area under the endotherm (25–250°C) corresponding to the heat of the dehydration reaction and comparing it with that of reference smectite minerals analyzed under the same conditions (Table 5–3). In samples containing smectite and vermiculite the quantitative estimate reflects the sum of the two minerals because they exhibit similar water adsorption characteristics. Agreement between TG and DSC estimations of smec-

tite-vermiculite mixtures is reasonably good, unless the sample contains organic material, halloysite, allophane, gibbsite, gypsum, zeolite, or phosphate minerals. The variable amount of sorbed water associated with expanding 2:1 minerals may overestimate quantities of smectite and vermiculite without appropriate pretreatments. When the sample contains hydroxy-interlayered forms of smectite or vermiculite, their amounts are usually underestimated because a part of their interlayer space is not accessible to water sorption. The quantities of smectite plus vermiculite estimated by TG and DSC analysis in 49 Mg-saturated soil clays of the southern U.S. region compared quite favorably with the values obtained from surface area and cation-exchange capacity measurements (Karathanasis and Hajek, 1982b). Good agreement was also observed between DTA estimates of smectite in selected soil clays of Jamaica and Australia compared with other mineralogical methods (Davis and Holdridge, 1969; Smalley and Xidakis, 1979). In clays containing both smectite and halloysite, the relationship between water sorption at multilayer coverage and DTA peak area has been used to estimate the quantities of the two minerals, suggesting that the binding energy of water molecules varies with the degree of hydration (Mackenzie, 1970). The nature of the saturating cation also markedly affects the peak-area-water sorption relationship because of differences in cation hydration energies (Karathanasis and Evangelou, 1986). In samples containing both smectite and vermiculite, an estimate of the amount of vermiculite can be made from a characteristic weight loss (TG) or endotherm peak area (DSC/DTA) between 250 and 300°C, provided that gibbsite and goethite are absent. These inflections are associated with the dehydration of strongly coordinated water molecules around the saturating cations of vermiculite (Barshad, 1952; Boss, 1967; Karathanasis and Hajek, 1982a). Attempts to quantify smectite and vermiculite on the basis of weight losses or heat changes associated with their dehydroxylation reactions (550–700°C) have also been reasonably successful in samples lacking interfering minerals, such as illite, chlorite, and Fe-carbonate minerals (Mackenzie, 1970).

Gibbsite and Goethite

Gibbsite is one of the most successfully quantified minerals by TA methods, based on weight loss (TG) or endothermic peak size (DTA/DSC) associated with its dehydroxylation reaction in the 220 to 320°C temperature region. Quantitative estimates are made following respective comparisons with suitable reference gibbsite minerals tested under the same experimental conditions (Tables 5–2 and 5–3). Dehydroxylation reactions of poorly crystalline gibbsite usually occur between 220 and 250°C, while well-crystalline gibbsite loses its hydroxyls at temperatures >250°C. Therefore, it is advantageous to quantify gibbsite in CBD-pretreated clay samples to eliminate interferences with goethite or other hydroxide minerals having overlapping dehydroxylation reactions in this temperature region. Similarly, pretreatment with cold or hot Na_2CO_3 solutions may enhance the quantitative resolution of poorly crystalline gibbsite from well-crystalline gibbsite (Jorgensen et al., 1970) or smectite minerals (Tullock and Roth, 1975). Generally there is a good agreement between TG and DTA/DSC estimates of gibbsite when proper pretreatments eliminate interfering minerals (Karathanasis and Harris, 1994). Gibbsite quantification errors may also result from the presence of partially hydroxy-interlayered vermiculite or smectite phases, which undergo dehydroxylation in the 320 to 450°C temperature region. This is more of a problem, however, in the quantification of poorly crystalline kaolinite than well-crystallized gibbsite (Barnhisel and Bertsch, 1989). Perhaps the most common difficulty in untreated clay samples is the quantification of gibbsite in the presence of goethite. When a CBD pretreatment is not feasible, quantitative resolution can be enhanced by relying on DTG peaks or by using deconvolution software programs (Fig. 5–14).

Goethite quantification is based on measurements of TG weight losses or DTA/DSC endotherm peak area associated with its dehydroxylation in the 300 to 400°C region, following comparison with suitable reference minerals (Tables 5-2 and 5-3). This region generally coincides with the dehydroxylation of lepidocrocite and ferroxhyte, thus making these polymorphs practically indistinguishable. Some discrepancies in the value of theoretical weight loss and heat of reaction for the dehydroxylation of pure goethite are the result of impurities, variable crystallinity, or Al-substitutions in the goethite structure. Although CBD extractions are preferred for goethite quantification in soil clays, TA estimates are generally in good agreement, particularly when DTG curves are used to improve quantitative resolution (Karathanasis et al., 1983). Precise goethite quantification has also been accomplished by using DTA under variable atmosphere conditions (Lodding and Hammell, 1960). Under H₂ atmosphere goethite loses its hydroxyls below 300°C and recrystallizes to magnetite at 300 to 360°C. Replacing H₂ with N₂ at 400°C and then air, two exotherms are produced at 400 and 775°C, corresponding to the oxidation of magnetite to maghemite and the transformation of maghemite to hematite. Integration of the latter exotherm yields a quantitative estimate of the hematite formed and, therefore, the amount of goethite present in the sample. If the sample contains both gibbsite and goethite, the amount of gibbsite is estimated from the difference between the <300°C endotherm (gibbsite + goethite dehydroxylation) and the 775°C exotherm peak areas. Double or triple dehydroxylation peaks occasionally observed in DTA/DSC thermograms usually reflect various degrees of Al substitution or different types of hydroxyl groups, which may cause erroneous goethite quantifications (Mackenzie, 1981; Schwertmann, 1984). Goethite crystallinity may also be assessed from bulk dehydroxylation temperatures measured by high resolution thermogravimetry (Ford and Bertsch, 1999).

Kaolinite and Halloysite

Kaolinite is one of the most extensively studied and most successfully quantified soil minerals by thermal analysis. Quantitative estimates are based on assessments of weight losses (TG), heat (enthalpy) changes (DSC), or endothermic peak areas (DTA) associated with its dehydroxylation in the 400 to 600°C temperature region following comparisons with suitable reference minerals (van der Marel, 1956; Karathanasis and Hajek, 1982a) (Tables 5-2 and 5-3). In spite of the generally good agreement in the kaolinite estimates obtained between TA methods, considerable variability may be observed in samples containing Al-hydroxy-interlayered minerals, with TG values being usually higher than DSC estimates (Karathanasis and Harris, 1994). Greater discrepancies in kaolinite quantification have been observed between DTA and selective dissolution methods, particularly in comparisons of coarse- and fine-clay fractions due to the presence of poorly crystalline or expansible components (Dixon, 1966). One of the chief deterrents in kaolinite quantification by TA methods is the effect of particle size and degree of crystallinity on the inflection temperatures and the area and shape of the dehydroxylation endotherm. In DTA/DSC thermograms the width and area of the dehydroxylation endotherm (400–600°C) decreases as particle size and crystallinity decreases, thus producing distorted peaks and lower estimates for finer-grained and disordered kaolinite minerals (Anderson et al., 1977). In contrast, well-crystallized and coarse-grained kaolinites yield more symmetrical peaks, which facilitate the quantification process. The symmetry of the dehydroxylation endotherm, expressed in terms of the slope ratio between the lower- and the higher-temperature side tangents, has also been used to distinguish kaolinite from halloysite (Earnest, 1984). Halloysite and poorly crystalline kaolinite usually exhibit higher slope ratios than well-crystalline kaolinite of the same particle size.

Thus, the selection of kaolinite reference minerals with similar particle size and crystallinity is a critical step in the quantification process. In samples containing hydroxy-interlayered minerals, mica, smectite, vermiculite, or chlorite, which interfere with the shape of the kaolinite endotherm, drawing a symmetrical half-peak to that of the well-defined temperature side should minimize quantification errors. When both kaolinite and halloysite are present in the same sample, the sum of the two minerals is estimated from TG or DSC/DTA thermograms, while individual mineral estimates are based on XRD halloysite/kaolinite peak area ratios (0.72/1.04 nm) of formamide-intercalated samples (Churchman et al., 1984). In the absence of kaolinite, halloysite can be estimated from weight losses (TG) or enthalpy changes (DSC) associated with its dehydroxylation reaction, but OH-water losses and dehydroxylation enthalpies may be more variable than those of kaolinite (Minato, 1988). It is important that halloysite-containing samples must be processed and preserved appropriately (field moisture conditions) or the halloysite could dehydrate before analysis.

Carbonates

Carbonate mineral quantification by TG and DTA methods has been used more extensively in geological materials than in soil materials (Todor, 1976; Warne et al., 1981; Paulik et al., 1982; Sobecki and Karathanasis, 1987; Smykatz-Kloss, 2002). Carbonate quantification is based on weight losses (TG) or endotherm peak area changes (DTA) associated with their decomposition reaction in the 550 to 1000°C temperature region (Tables 5-2 and 5-3). Even multiple carbonate species can be quantified in this region utilizing the proper furnace atmosphere because of differential thermal stability of their associated cations ($\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Fe}^{2+}$). The best quantitative resolution of carbonate mineral mixtures is obtained under a purified CO_2 atmosphere, but particle size, heating rate, crystallinity, and impurities may shift thermal decomposition temperature regions considerably. Generally, the suggested temperature ranges for quantification are: 550–600°C for siderite, 600–700°C for magnesite, 700–800°C for the MgCO_3 phase of dolomite, and >900°C for the CaCO_3 phase of dolomite and calcite (Mackenzie, 1970; Todor, 1976; Karathanasis and Harris, 1994). In the presence of dolomite, magnesite can be resolved with relative ease, but dolomite–calcite, and dolomite–magnesite–calcite mixtures may require greater effort and skill, even with the benefit of DTG curves. Samples containing kaolinite–carbonate mixtures may require two thermal runs, one before and one after acid dissolution to resolve dehydroxylation–decarbonation overlapping. Comparisons of TG, XRD, and selective dissolution analysis estimates of total carbonate quantities are usually in good agreement, but dolomite and calcite quantities may differ significantly due to different crystallinity and Ca/Mg ratios (Sobecki and Karathanasis, 1987). The utilization of CO_2 atmosphere greatly improves DTA peak resolution and detection limit in calcite–dolomite soil mixtures, while suppressing oxidation effects of Fe and organic impurities on the decarbonation of siderite, ferroan dolomite, and ankerite minerals (Warne et al., 1981). High-temperature DSC (900–1000°C) instruments under variable atmospheres can enhance quantitative resolution and precision to the point that Fe substitution in ankerites and Mg substitution in calcites can be precisely estimated (Dubrawski and Warne, 1988).

Other Minerals

Quartz quantification by DTA or DSC is based on the intensity or the area of the ~573°C endotherm associated with the $\alpha \rightarrow \beta$ inversion of the mineral (Table 5-3). The small size of the peak and its susceptibility to geological origin and lattice defects and

substitutions make quantitative estimates somewhat suspect, but the sensitivity of modern DSC instruments has improved the precision of the method. In spite of the increased sensitivity and detection limit, DSC quantification of quartz in soils and materials of different geological origin may vary by as much as 400% compared with other methods (Mackenzie, 1970; Barwood and Hajek, 1979; Bartenfelder and Karathanasis, 1989). Therefore, it is important that any attempts for quartz quantification by DTA or DSC should include a calibration procedure with a reference quartz mineral with similar particle size, origin, and crystallinity. Microcrystalline and cryptocrystalline (short-range order) quartz samples generally produce lower intensity and smaller area endotherms, thus underestimating the amount of quartz. Extensive grinding of the sample before TA analysis should be avoided because it forms an amorphous layer on the surface of quartz grains, which affects peak size and intensity (Dubrawski, 1987). Interferences in quartz quantification from the kaolinite dehydroxylation endotherm may be overcome by rerunning the same sample after cooling or replotting the first run in cooling mode, because the $\alpha \rightarrow \beta$ transition is reversible.

Gypsum is the most common sulfate mineral found in soils and geological materials. Quantitative assessments by DTA/DSC methods are based on peak area or enthalpy changes associated with its dehydration endotherm (often a doublet) in the 140 to 200°C temperature region (Table 5–3). However, because several aluminosilicates (smectite, vermiculite, halloysite, allophane) exhibit dehydration endotherms in this region, the adequacy of gypsum quantification relies on the absence of interfering mineral phases. In such samples or monomineralic gypsum determinations, quantitative DTA/DSC estimates are generally excellent, with detection sensitivity <2% (Dunn et al., 1980; Karathanasis and Harris, 1994). Thermal analyses of other sulfate and sulfide minerals have been mainly qualitative or at best semiquantitative, with significant complementary contributions from XRD, optical microscopy, and chemical analytical techniques (Karathanasis and Harris, 1994).

A number of phosphate minerals undergo dehydration and dehydroxylation reactions upon heating, but their reactive temperature region usually overlaps with that of several aluminosilicate minerals found in soils and geological materials. Therefore, quantification approaches are suitable only on samples with minimal interferences from overlapping reactions. Phosphate minerals successfully quantified by DTA or DSC methods include, crandallite, wavellite, strengite, variscite, brushite, and cacoenite, following complementary analytical determinations by other methods (Karathanasis and Harris, 1994).

Kinetics

Thermogravimetry and DTA/DSC methods have been very helpful in deducing kinetic characteristics of various minerals, including rates, mechanisms, and activation energies associated with mineral dehydration, dehydroxylation, decomposition reactions or other mineral transitions occurring upon heating under isothermal or constant rate conditions. To obtain kinetic data under isothermal mode the sample is heated at different isothermal temperatures to the desired reaction temperature range and the extent of reaction completion (reacted fraction = α) at each isothermal temperature setting is expressed as a function of time (t). Plots of $d\alpha/dt$ against time are used to calculate different kinetic parameters expressing the reaction rates at different reaction stages and compared with theoretical kinetic functions (Wendland, 1986; Brown, 1998) to deduce the rate controlling processes (mechanisms). A formal kinetic equation used for the calculations is expressed as $-d\alpha/dt = k(1 - \alpha)^n$, with k the reaction rate constant and n the order of the reaction. The reaction rate constant (k) calculated from this equation is used to estimate the activation energy for the reaction through the Arrhenius equation $\ln k = \ln Z - (E_a/RT)$, where Z is the Arrhenius

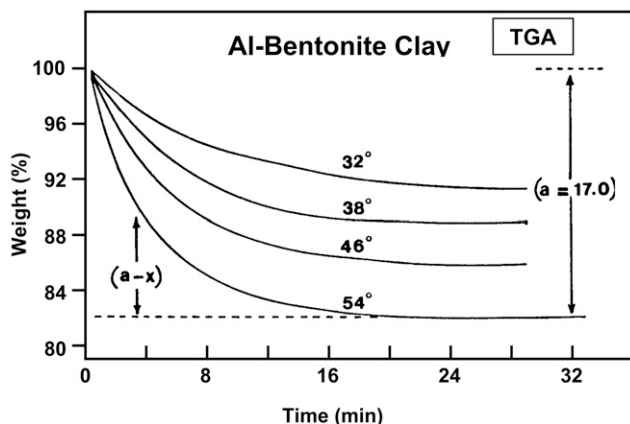


Fig. 5-17. Isothermal thermogravimetry (TG) mass losses ($a - x$) vs. time plots used to derive dehydration kinetics data for an Al-saturated bentonite sample (Karathanasis and Evangelou, 1987).

frequency factor (min^{-1}), E_a is activation energy (J mol^{-1}), R is the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), and T is absolute temperature (K). A variety of mathematical treatments of kinetic functions have been used in thermal experiments, including differential and integral formulations to deduce the proper reaction mechanisms (Sharp and Wentworth, 1969). In nonisothermal experiments, plots of $\ln(d\alpha/dt)_\alpha$ against T^{-1} for the different heating rates employed or $\ln(\beta)$ against $1/T_\alpha$ are the most typical. From these plots the activation energy for the reaction is calculated using the equation $\ln(d\alpha/dt)_\alpha = \ln(\beta) = -E_a/RT$, where β is heating rate and T_α is the temperature of reaction at the specific heating rate.

Examples of isothermal TG kinetic approaches in soil mineralogy include the determination of reaction rates, mechanisms and activation energies for the dehydration of Al- and Ca-saturated clays (Karathanasis and Evangelou, 1987) (Fig. 5-17), and the identification of Mg-enriched pedogenic carbonates in calcic horizons based on activation energy differences of decomposition reactions (Sobecki and Karathanasis, 1987). Nonisothermal TG kinetics have also been used to calculate activation energy changes associated with cationic composition of carbonate minerals (Sharp and Wentworth, 1969; Gallagher and Johnson, 1973). Nonisothermal DSC kinetic data were used by Bartenfelder and Karathanasis (1989) to determine activation energy differences of the $\alpha \rightarrow \beta$ inversion of soil quartz found in different loess deposits (Fig. 5-18).

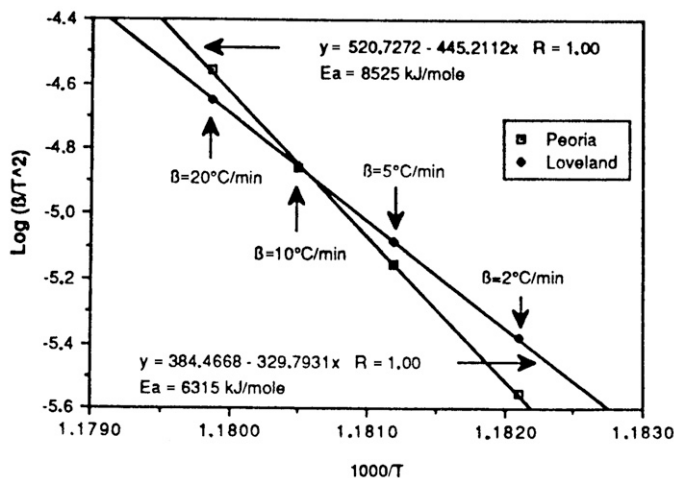


Fig. 5-18. Kinetics plots derived from differential scanning calorimetry (DSC) curves at four heating rates for the inversion of quartz present in two loessial deposits used to estimate its activation energy (Bartenfelder and Karathanasis, 1989).

The quality of the obtained experimental thermal kinetic data and their respective fit into theoretical kinetic expressions representing different mechanisms strongly depends on various experimental factors, such as the type of DTA/DSC/TG sample holder, atmosphere, particle size, specific heat, thermal conductivity, heating rate, and packing density. Better quality thermograms for kinetic experiments are usually obtained under isothermal conditions in a vacuum atmosphere, using uniform samples of small particle size placed in uncovered sample holders. Nonisothermal experiments, however, can provide reasonable kinetics data for certain experiments at a faster rate. The choice between isothermal or nonisothermal mode depends on the material, the reaction type, and the experimental conditions. Generally, TG curves produce less reliable kinetic data than DSC thermograms because they are more sensitive to experimental conditions (Wendlandt, 1986; Karathanasis and Harris, 1994).

Thermomechanical Analysis Applications

Thermomechanical analysis (TMA) is a very useful method for measuring volume or length changes of soil, clay, and ceramic materials. An atlas of TMA curves of clay minerals has been published by Schomburg and Storr (1984) with a comprehensive review of TMA techniques in mineralogy and ceramic industry. One application includes the measurement of the coefficient of linear extensibility (COLE), which is used as a criterion for differentiating vertic from typic subgroup in U.S. soil taxonomy (Hajek, 1979; Karathanasis and Hajek, 1985) (Fig. 5-19). The samples are usually moist soil peds at about field capacity or molded clay samples at the plastic point, which are heated to 250°C at a constant rate of 5 to 10°C, and ped length changes (ΔL , in mm) are recorded as a function of temperature. A derivative function of the ΔL signal can also provide soil length rate changes as a function of temperature. This is a rapid (20–30 min) and precise ($\pm 5\%$ for representative samples) technique for soil or clay shrink–swell potential estimation requiring

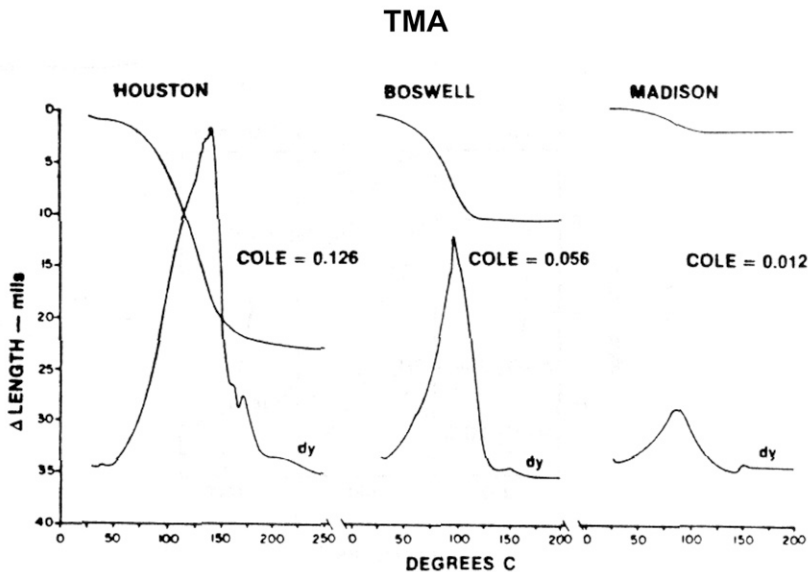


Fig. 5-19. Thermomechanical analysis (TMA) curves of three soil clays, showing length change and rate from which their coefficient of linear extensibility (COLE) value was estimated (Karathanasis and Hajek, 1985).

minimum sample preparation. Thermomechanical analysis has also been used to measure volume increases associated with the $\alpha \rightarrow \beta$ inversion of quartz in ceramic samples, which is proportional to the peak area under the derivative dL/dt curve (Yariv, 1989). Although some of the volume increase due to quartz may be compensated by the decreased volume of the heated sample, the procedure provides a rapid semiquantitative estimate of quartz in ceramic samples. Quartz samples containing fine-grain or disordered quartz may produce erroneous estimates because they exhibit an extended $\alpha \rightarrow \beta$ transition range. Therefore, separate calibration curves are required for different types of ceramic materials. Similar approaches have been used to measure volume changes associated with water or solute adsorption-desorption and ion migration between heteroenergetic sites in zeolite minerals (Dyer, 1981). Combined TMA-TG or TMA-DSC/DTA techniques have also been useful in detecting volume changes associated with dehydroxylation, decomposition, or recrystallization processes. For example, simultaneous TMA-TG-DTA analysis of kaolinitic clays has revealed that the recrystallization of metakaolinite to mullite at around 950°C is accompanied by a volume increase of approximately 2%, which may cause cracks in over-heated ceramic clays (Paulik and Paulik, 1978). Because of its sensitivity to orientation effects, TMA has also been employed in assessing the degree of orientation of clay strata (Mackenzie, 1981).

Thermal Analysis-Evolved Gas Analysis Applications

Coupled thermal analysis-evolved gas analysis (TG-EGA) and DTA/DSC-EGA techniques have allowed identification and quantification of volatile materials evolved during the process of heating soil, clay, or geological samples. The most common volatiles released from such samples are H_2O , CO_2 , SO_2 , and less frequently H_2 and CO , which can be detected with GC, FTIR, or MS detectors. Combined TA-EGA approaches have been used successfully not only to yield better quantitative data for certain constituents of various mineral mixtures and associations, but also to provide additional insight into their physicochemical interactions with potential environmental contaminants (Morgan, 1977; Yariv, 1989; Dunn, 1998). Besides enabling the detection of mineral and organic impurities within a sample, these approaches have shown much greater sensitivity than XRD for certain minerals (calcite, magnesite), reaching detection limits of parts per million accuracy (Yariv, 1989). TG-EGA and DTA-EGA combinations employing GC or IR detectors have been used extensively for quantification of carbonate minerals and other clay mixtures (Morgan et al., 1988; Kaiserberger, 1997), dehydroxylation of Fe- and Mn-hydroxyoxide minerals (Yariv, 1989), Al-pillared smectites (Acemana et al., 1999) and hectorites (Kloprogge et al., 2000), transformations of carbonate apatites (Tonsuaadu et al., 1995), oxidation of sulfide minerals (Dunn, 1997), pyrolysis of oil shales (Morgan, 1977), dehydration patterns of smectite and zeolite groups of minerals (Sadek and Mekhamer, 2000; Yariv, 1989), and adsorption of organic molecules by clay minerals (Yariv, 1996; Ovadyahu et al., 1996; Dunn, 1998; Xie and Pan, 2001).

In TA-MS mode, scanning could be continuous in the mass range of 1 to 200 m/e, or preset to 10 to 15 preprogrammed channels corresponding to anticipated masses of volatiles. Although magnetic-sector mass spectrometers exhibit better sensitivity and improved resolution, quadrupole mass spectrometers provide a good balance of fast response, reasonable analytical mass range, and good sensitivity at a reasonable cost. For mineral analysis, the typical mass numbers monitored include 2 (H_2^+), 18 (H_2O^+), 28 (N_2^+ , CO^+), 32 (O_2^+), 44 (CO_2^+), and 64 (SO_2^+), with the remaining channels left for optional settings. In new instruments the scanning mode settings and spectrometric analysis are software-driven (Wicks and Ramik, 1990). A variety of TA-MS setups have been used to increase

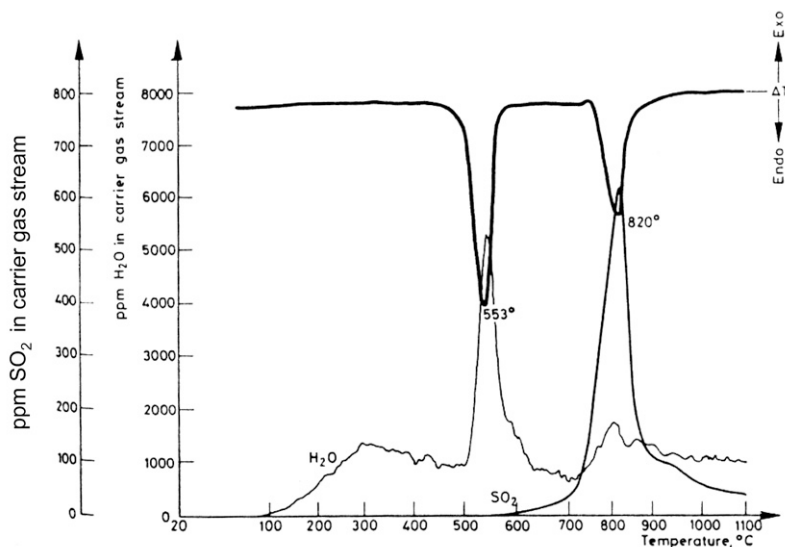


Fig. 5–20. Differential thermal analysis-evolved gas analysis (DTA-EGA) curves of an alunite sample, showing H₂O and SO₂ gas detection (Morgan et al., 1988).

the detection sensitivity of the evolved gases (Statheropoulos et al., 1998) in mineralogical investigations, including the determination of organic C, pyrite, and carbonate minerals in clay samples (Morgan et al., 1988); the compositional characterization of various clay mixtures, coal samples, and oil shales (Earnest, 1988; Charsley and Warrington, 1992; Carleer et al., 1998; Frost and Ding, 2003); the distinction between nontronite and palagonite in the Mars surface exploration project (Heidbrink et al., 1996); the fractional characterization of soil organic matter and hydrocarbon-sludge soil mixtures (Bracewell and Robertson, 1980; Remmler and Kopinke, 1995); the assessment of catalytic activity of modified clays (Charsley and Warrington, 1992); the sorption–desorption behavior of environmental contaminants on clay surfaces (Remmler and Kopinke, 1995); thermal decomposition of jarosites and alunites (Frost et al., 2005, 2006); and others (Fig. 5–20). More recently, a TA/GC–MS interface has been utilized, which provides an even better resolution of complex mixtures of gases evolved from various mineral fertilizers (Pelovski, 2000) and clay mixtures containing carbonates, phosphates, and organomineral fractions (Kettrup et al., 1997; Carleer et al., 1998; Koel et al., 1998).

MAJOR SUPPLIERS OF EQUIPMENT

The following is an incomplete alphabetical list of some major suppliers of TA equipment used for mineralogical investigations with their websites. Their listing is only informative and does not constitute an endorsement or recommendation by the author.

- Mettler-Toledo Instruments AG, Switzerland, www.mt.com
- Netzsch-Geratebau GmbH, Germany, www.netzsch.com
- PerkinElmer Corporation, USA, www.instruments.perkinelmer.com
- Shimadzu Corporation, Japan, www.shimadzu.com
- TA Instruments, USA, www.tainst.com
- Thermo Cahn, USA, www.cahn.com

REFERENCES

- Acemana, S., N. Lahav, and S. Yariv. 1999. A thermo-FTIR-spectroscopy analysis of Al-pillared smectites differing in source of charge, in KBr disks. *Thermochim. Acta* 340:349–366.
- Anderson, H., W. Besch, and D. Haberland. 1977. Investigation about methods of quantitative evaluation of DTA curves. *J. Therm. Anal.* 12:59–68.
- Arazi, S.C., and T.G. Krenkel. 1970. Dehydroxylation heat of alunite. *Am. Mineral.* 55:1329–1337.
- Asomoza, P.M., M.L. Razo, T.L. Chaidez, and S.R. Cassillas. 1978. Quantitative evaluation of Na_2CO_3 and NaCl content of the clays of the ex-lake of Texcoco (Valley of Mexico) by means of thermogravimetry. *J. Therm. Anal.* 13:327–339.
- Barnhisel, R.L., and P.M. Bertsch. 1989. Chlorites and hydroxyinterlayered vermiculite and smectite. p. 729–788. *In* J.B. Dixon and S.B. Weed (ed.) *Minerals in soil environments*. 2nd ed. SSSA Book Ser. 1. SSSA, Madison, WI.
- Barshad, I. 1952. Temperature and heat of reaction calibration of the DTA apparatus. *Am. Mineral.* 37:667–694.
- Bartenfelder, D.C., and A.D. Karathanasis. 1989. A differential scanning calorimetry evaluation of quartz status in geogenic and pedogenic environments. *Soil Sci. Soc. Am. J.* 53:961–967.
- Barwood, H.L., and B.F. Hajek. 1979. Differential thermal characteristics of soil and reference quartz. *Soil Sci. Soc. Am. J.* 43:626–627.
- Beck, J. 2003. Qualitative and quantitative analysis of highly weathered clays. Ph.D. diss. Auburn University, AL.
- Bish, D.L., and C.J. Duffy. 1990. Thermogravimetric analysis of minerals. p. 96–157. *In* J. W. Stucki et al. (ed.) *Thermal analysis in clay science*. Clay Minerals Society, Boulder, CO.
- Boss, B.D. 1967. DTA of biotitic vermiculite to determine vermiculite content. *Am. Mineral.* 52:293–298.
- Bracewell, J.M., and G.W. Robertson. 1980. Pyrolysis-mass spectrometry studies of humification in a peat and a peaty podzol. *J. Anal. Appl. Pyrolysis* 2:53–62.
- Brindley, G.W., and J. Lemaitre. 1987. Thermal, oxidation, and reduction reactions of clay minerals. p. 319–370. *In* A.C.D. Newman (ed.) *Chemistry of clays and clay minerals*. Monogr. 6. Mineralogical Society, London.
- Brown, M.E. 1998. *Handbook of thermal analysis and calorimetry*. Vol. 1. Principles and practice. Elsevier, Amsterdam.
- Brown, M.E. 2001. *Introduction to thermal analysis*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Carleer, R., G. Reggers, M. Ruysen, and J. Mullens. 1998. TG-MS analysis as a tool for the evaluation of clay mixtures. *Thermochim. Acta* 323:169–178.
- Charsley, E.L., and S.B. Warrington (ed.) 1992. *Thermal analysis: Techniques and applications*. Royal Society of Chemistry, Cambridge, UK.
- Churchman, G.J., J.S. Whitton, G.G.C. Claridge, and B.K.G. Theng. 1984. Intercalation method using formamide for differentiating halloysite from kaolinite. *Clays Clay Miner.* 32:241–248.
- Davis, C.E., and D.A. Holdridge. 1969. Quantitative estimation of clay minerals by DTA. *Clay Miner.* 8:193–200.
- Dixon, J.B. 1966. Quantitative analysis of kaolinite and gibbsite in soils by DTA and selective dissolution methods. *Clays Clay Miner.* 14:83–89.
- Drits, V.A. 2003. Structural and chemical heterogeneity of layer silicates and clay minerals. *Clay Miner.* 38:403–432.
- Dubrawski, J.V. 1987. The effect of particle size on the determination of quartz by DSC. *Thermochim. Acta* 120:257–260.
- Dubrawski, J.V., and S.S.J. Warne. 1988. Differential scanning calorimetry of the dolomite-ankerite mineral series in variable atmospheres. *Thermochim. Acta* 135:225–230.
- Dunn, J.G. 1997. The oxidation of sulfide minerals. *Thermochim. Acta* 300:127–139.
- Dunn, J.G. 1998. Applications of thermal methods of analysis to raw and processed minerals. *Thermochim. Acta* 324:59–66.
- Dunn, J.G., B.T. Sturman, and W. Van Bronswijk. 1980. The determination of gypsum and lime in small samples of set plaster by a DSC-computer method. *Thermochim. Acta* 37:337–341.
- Dyer, A. 1981. Thermomechanical analysis of zeolites. *Anal. Proc.* (October):447–450.
- Earnest, C.M. 1984. *Thermal analysis of clays, minerals, and coals*. Perkin-Elmer, Norwalk.
- Earnest, C.M. (ed.) 1988. *Compositional analysis by thermogravimetry*. ASTM, Philadelphia, PA.
- El-Akkad, T.M., N.S. Flex, N.M. Guindy, S.R. El-Massry, and S. Nashed. 1982. Thermal analyses of mono- and divalent montmorillonite cationic derivatives. *Thermochim. Acta* 59:9–17.

- El-Barawy, K.A., B.S. Girgis, and N.S. Felix. 1986. Thermal treatment of some pure smectites. *Thermochim. Acta* 98:181–189.
- Ford, R.G., and P.M. Bertsch. 1999. Distinguishing between surface and bulk dehydration-dehydroxylation reactions in synthetic goethites by high-resolution thermogravimetric analysis. *Clays Clay Miner.* 47:329–337.
- Frost, R.L., and Z. Ding. 2003. Controlled rate thermal analysis and differential scanning calorimetry of sepiolites and palygorskites. *Thermochim. Acta* 397:119–128.
- Frost, R.L., D.L. Wain, R.A. Wills, A. Musemeci, and W. Martens. 2006. A thermogravimetric study of the alunites of sodium, potassium, and ammonium. *Thermochim. Acta* 443:56–61.
- Frost, R.L., M.L. Weier, and W. Martens. 2005. Thermal decomposition of jarosites of potassium, sodium, and lead. *J. Therm. Anal. Calorim.* 82:115–118.
- Gallagher, P.K., and D.W. Johnson. 1973. The effects of sample size and heating rate on the kinetics of the thermal decomposition of CaCO_3 . *Thermochim. Acta* 6:67–83.
- Haines, P.J. 1995. Thermal methods of analysis: Principles, applications, and problems. Blackie Academic and Professional, London.
- Haines, P.J., M. Reading, and F.W. Wilburn. 1998. Differential thermal analysis and differential thermal calorimetry. p. 279–361. *In* M.E. Brown (ed.) *Handbook of thermal analysis and calorimetry*. Elsevier, New York.
- Hajek, B.F. 1979. COLE determination by thermomechanical analysis. *Soil Sci. Soc. Am. J.* 43:427–428.
- Hatakeyama, T., and Z. Liu. 1998. *Handbook of thermal analysis*. John Wiley and Sons, New York.
- Heidbrink, J.L., J. Li, W.P. Pan, J.L. Gooding, S. Aubuchon, J. Foreman, and C.J. Lundgren. 1996. Distinction of nontronite from palagonite by thermal analysis and evolved-gas analysis: Application to Mars surface exploration. *Thermochim. Acta* 284:241–251.
- Jackson, M.L. 1975. *Soil chemical analysis—advanced course*. 2nd ed. M.L. Jackson, Madison, WI.
- Jorgensen, S.S., A.C. Birnie, B.F.L. Smith, and B.D. Mitchell. 1970. Assessment of gibbsitic material in soil clays by DTA and alkali dissolution methods. *J. Therm. Anal.* 2:227–282.
- Kaiserberger, E. (ed.) 1997. *Coupling thermal analysis and gas analysis methods*. *Thermochim. Acta* 295:1–186.
- Karathanasis, A.D., F. Adams, and B.F. Hajek. 1983. Stability relationships in kaolinite, gibbsite, and Al-hydroxyinterlayered vermiculite soil systems. *Soil Sci. Soc. Am. J.* 47:1247–1251.
- Karathanasis, A.D., and V.P. Evangelou. 1986. Water sorption characteristics of Al- and Ca-saturated clays. *Soil Sci. Soc. Am. J.* 50:1063–1068.
- Karathanasis, A.D., and V.P. Evangelou. 1987. Low temperature dehydration kinetics of Al- and Ca-saturated clays. *Soil Sci. Soc. Am. J.* 51:1072–1078.
- Karathanasis, A.D., and B.F. Hajek. 1982a. Revised methods for rapid quantitative determination of minerals in soil clays. *Soil Sci. Soc. Am. J.* 46:419–425.
- Karathanasis, A.D., and B.F. Hajek. 1982b. Quantitative evaluation of water adsorption of soil clays. *Soil Sci. Soc. Am. J.* 46:1321–1325.
- Karathanasis, A.D., and B.F. Hajek. 1985. Shrink–swell potential of montmorillonitic soils in udic moisture regimes. *Soil Sci. Soc. Am. J.* 49:159–166.
- Karathanasis, A.D., and W.G. Harris. 1994. Quantitative thermal analysis of soil materials. p. 360–411. *In* J.E. Amonette and L.W. Zelazny (ed.) *Quantitative methods in soil mineralogy*. SSSA, Madison, WI.
- Karathanasis, A.D., G.W. Hurt, and B.F. Hajek. 1986. Properties and classification of montmorillonite-rich Hapludults in the Alabama Coastal Plain. *Soil Sci.* 142:1247–1251.
- Kerr, P.F., and J.L. Kulp. 1948. Multiple differential thermal analysis. *Am. Mineral.* 33:387–419.
- Kettrup, A., G. Matuschek, H. Utschick, C. Namendorf, and G. Brauer. 1997. A macro STA-system for environmental samples. *Thermochim. Acta* 295:119–131.
- Klopprogge, J.T., R.L. Frost, and L. Hickey. 2000. Infrared emission spectroscopic study of the dehydroxylation of some hectorites. *Thermochim. Acta* 345:145–156.
- Koel, M., M. Kudrjasova, K. Tonsuaadu, M. Peld, and M. Veiderma. 1998. Evolved gas analysis of apatite materials using thermochromatography. *Thermochim. Acta* 322:25–32.
- Lodding, W., and L. Hammell. 1960. Differential thermal analysis of hydroxides in reducing atmosphere. *Anal. Chem.* 32:657–662.
- Mackenzie, R.C. 1970. *Differential thermal analysis*. Academic Press, London.
- Mackenzie, R.C., and S. Calliere. 1975. The thermal characteristics of soil minerals and the use of these characteristics in the qualitative and quantitative determination of clay minerals in soils. p. 529–571. *In* J.E. Gieseking (ed.) *Soil components*. Vol. II. Elsevier, New York.
- Mackenzie, R.C. 1981. Thermoanalytical methods in clay studies. p. 5–29. *In* J.J. Fripiat (ed.) *Advanced techniques for clay mineral analysis*. Elsevier, Amsterdam, The Netherlands.
- McKeever, S.W.S. 1988. *Thermoluminescence of solids*. Cambridge Univ. Press, London.

- Minato, H. 1988. Dehydration energy of halloysite by means of DSC methods with the relationships of its mineralogy and modes of occurrence. *Thermochim. Acta* 135:279–283.
- Morgan, D.J. 1977. Simultaneous DTA-EGA of minerals and natural mineral mixtures. *J. Therm. Anal.* 12:245–263.
- Morgan, D.J., S.B. Warrington, and S.J. Warne. 1988. Earth sciences applications of evolved gas analysis: A review. *Thermochim. Acta* 135:259–266.
- Mullens, J. 1998. EGA-evolved gas analysis. p. 509–546. *In* M.E. Brown (ed.) *Handbook of thermal analysis and calorimetry*. Elsevier, New York.
- Nathan, Y., G. Panzer, and S. Gross. 1988. The thermal analysis of some phosphate minerals: Strengite, lipscombite, cyrilorite, and goyazite. *Thermochim. Acta* 135:259–266.
- Ovadياهو, D., S. Shoval, I. Lapides, and S. Yariv. 1996. Thermo-IR-spectroscopy study of the mechanochemical adsorption of 3,5-dichlorophenol by TOT swelling clay minerals. *Thermochim. Acta* 282:369–383.
- Paulik, F. 1995. *Special trends in thermal analysis*. Wiley, Chichester, UK.
- Paulik, F., and J. Paulik. 1978. Simultaneous techniques in thermal analysis. *Analyst* 103:417–437.
- Paulik, J., F. Paulik, and M. Arnold. 1982. Simultaneous TG, DTG, DTA, and EG techniques for the determination of carbonate, sulfate, pyrite, and organic material in minerals, soils, and rocks. *J. Therm. Anal.* 25:327–340.
- Pelovski, Y. 2000. Thermal analysis for control of the properties of mineral fertilizers. *J. Therm. Anal. Calorim.* 60:53–58.
- Post, J.L., B.L. Cupp, and F.T. Madsen. 1997. Beidellite and associated clays from the Delamar Mine and Florida Mountain area, Idaho. *Clays Clay Miner.* 45:240–250.
- Reading, M. 1998. Controlled rate thermal analysis and related techniques. p. 423–443. *In* M.E. Brown (ed.) *Handbook of thermal analysis and calorimetry*. Elsevier, New York.
- Remmler, M., and F.D. Kopinke. 1995. Thermoanalytical methods for characterizing hydrocarbon-sludge soil mixtures. *Thermochim. Acta* 263:101–122.
- Sadek, O.M., and W.K. Mekhamer. 2000. Ca-montmorillonite clay as thermal energy storage material. *Thermochim. Acta* 363:47–54.
- Schilling, M.R. 1990. Effects of sample size and packing in the thermogravimetric analysis of Ca-montmorillonite STx-1. *Clays Clay Miner.* 38:556–558.
- Schnitzer, M., and H. Kodama. 1972. Differential thermal analysis of metal-fulvic acid salts and complexes. *Geoderma* 7:93–103.
- Schomburg, J., and M. Storr. 1984. *Dilatometerkurvenatlas der tonmineralrohstoffe*, Akademie-Verlag, Berlin.
- Schwertmann, U. 1984. The double dehydroxylation peak of goethite. *Thermochim. Acta* 78:39–46.
- Shang, C., and L.W. Zelazny. 2008. Selective dissolution techniques for mineral analysis of soils and sediments. p. 33–80. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Sharp, J.H., and S.A. Wentworth. 1969. Kinetic analysis of thermogravimetric data. *Anal. Chem.* 41:2060–2062.
- Smalley, I.J., and G.S. Xidakis. 1979. Thermogravimetry of an expansive clay soil from Adelaide: Approximate mineralogical analysis using standard montmorillonite. *Clay Sci.* 5:189–193.
- Smykatz-Kloss, W. 1974. *Differential thermal analysis: Applications and results in mineralogy*. Springer-Verlag, New York.
- Smykatz-Kloss, W. 2002. Differential thermal analysis of Mg-bearing carbonates and sheet silicates. *J. Therm. Anal. Calorim.* 69:85–92.
- Sobecki, T.M., and A.D. Karathanasis. 1987. Quantification and compositional characterization of pedogenic calcite and dolomite in calcic horizons of selected Aquolls. *Soil Sci. Soc. Am. J.* 51:683–690.
- Soukup, D.A., B.J. Buck, and W. Harris. 2008. Preparing soils for mineralogical analyses. p. 13–32. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Statheropoulos, M., S. Kyriakou, and N. Tzamtzis. 1998. Performance evaluation of a TG/MS system. *Thermochim. Acta* 322:167–173.
- Tan, K.H. 1978. Formation of metal-humic acid complexes by titration and their characterization by differential thermal analysis and infrared spectroscopy. *Soil Biol. Biochem.* 10:123–129.
- Tan, K.H., B.F. Hajek, and I. Barshad. 1986. Thermal analysis techniques. p. 151–183. *In* A. Klute (ed.) *Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9*. ASA and SSSA, Madison, WI.
- Todor, D.N. 1976. *Thermal analysis of minerals*. Abacus Press, Kent, England.
- Tonsuaadu, K., M. Reid, T. Leskela, R. Mannonen, L. Niinisto, and M. Veiderma. 1995. A thermoanalytical study of synthetic carbonate-containing apatites. *Thermochim. Acta* 256:55–65.

- Tullock, R.J., and C.B. Roth. 1975. Stability of mixed iron and aluminum hydrous oxides on montmorillonite. *Clays Clay Miner.* 23:27–32.
- Turi, E.A. (ed.) 1996. *Thermal characterization of polymeric materials*. 2nd ed. Academic Press, New York.
- van der Marel, H.W. 1956. Quantitative differential thermal analysis of clays and other minerals. *Am. Mineral.* 41:222–244.
- van Groos, A.F.K., and S. Guggenheim. 1990. High pressure differential thermal analysis: Application to clay minerals. p. 50–94. *In* J.W. Stucki et al. (ed.) *Thermal analysis in clay science*. Clay Minerals Society, Boulder, CO.
- van Humbeeck, J. 1998. Simultaneous thermal analysis. p. 497–508. *In* M.E. Brown (ed.) *Handbook of thermal analysis and calorimetry*. Elsevier, New York.
- Warne, S.S.J., D.J. Morgan, and A. Milodovski. 1981. Thermal analysis studies of dolomite, ferrous dolomite, ankerite series: Iron content recognition and determination by variable atmosphere DTA. *Thermochim. Acta* 51:105–111.
- Weast, R.C. (ed.). 1980. Constant humidity table. *In* *CRC handbook of chemistry and physics*. 61st ed. CRC Press, Boca Raton, FL.
- Wendlandt, W.W. 1986. *Thermal analysis*. 3rd ed. John Wiley and Sons, New York.
- Wetton, R.E. 1998. Thermomechanical methods. p. 363–399. *In* M.E. Brown (ed.) *Handbook of thermal analysis and calorimetry*. Elsevier, New York.
- Wicks, F.J., and R.A. Ramik. 1990. Vacuum thermogravimetric analysis and evolved gas analysis by mass spectroscopy. p. 160–189. *In* J.W. Stucki et al. (ed.) *Thermal analysis in clay science*. Clay Mineral Society Workshop Lectures. Clay Miner. Soc., Aurora, CO.
- Wiedemann, H.G., and S. Felder-Casagrande. 1998. Thermomicroscopy. p. 473–496. *In* M.E. Brown (ed.) *Handbook of thermal analysis and calorimetry*. Elsevier, New York.
- Xi, Y.F., Z. Ding, H.P. He, and R.L. Frost. 2004. Structure of organoclays- an X-ray diffraction and thermogravimetric analysis study. *J. Colloid Interface Sci.* 277:116–120.
- Xie, W., and W.P. Pan. 2001. Thermal characterization of materials using evolved gas analysis. *J. Therm. Anal. Calorim.* 65:669–685.
- Yariv, S. 1989. Thermal analysis of minerals. *Thermochim. Acta* 148:421–430.
- Yariv, S. 1996. Thermo-IR-spectroscopy analysis of the interactions between organic pollutants and clay minerals. *Thermochim. Acta* 274:1–35.

Petrographic Microscope Techniques for Identifying Soil Minerals in Grain Mounts

WARREN LYNN & J. E. THOMAS, USDA-NRCS National Soil Survey Center,
Lincoln, Nebraska

L. E. MOODY, Earth & Soil Sciences Department, California Polytechnic
State University, San Luis Obispo, California

We dedicate this chapter to Dr. John G. Cady and Dr. H.Y. Tu, who conducted petrographic mineralogical analyses and research with grain mounts and thin sections for many years in the former USDA-SCS Soil Survey Lab in Beltsville, MD. Dr. Cady was a mentor for a generation of mineralogists within and outside the federal service, including the first author. Dr. Tu was a meticulous microscopist with a reputation for leaving no grain unidentified.

The sand and coarse silt fractions of soils represent the skeletal part of the soil, and mineralogical analysis of these fractions facilitates assessment of soil weathering, provides clues to probable source material from which the soil formed, helps identify and explain lithologic discontinuities, provides basic data for specific pedologic studies, and enables placement of soil pedons into the family mineralogy classes of soil taxonomy. Weatherable and resistant minerals (Table 6–1) are used in soil taxonomy (Soil Survey Staff, 1999) in definitions of some family mineralogy classes. Minerals are grouped as weatherable or resistant in the context of relative resistance to weathering in a soil profile. While specific conditions of climate, topography, and drainage at a site influence the weathering processes, if all other soil forming factors are held approximately equal, soil and regolith mineralogy can be indicators of soil age. The Goldich (1938) mineral stability series arranged common primary minerals in a sequence from most to least resistant to weathering—the order was essentially the opposite of crystallization temperatures of common igneous minerals in the Bowen’s reaction series: quartz > muscovite > K feldspars > biotite > Na plagioclase > hornblende > augite > Ca plagioclase > olivine. As an example, mineral composition was used to distinguish between two youngest terraces and two oldest terraces in a chronosequence of alluvial soils in Virginia. With increasing soil age, proportions of quartz increased and proportions of feldspars, mica, and ferromagnesian silicates declined in the sand and silt fractions (Harris et al., 1980). Similarly, in sand dune soils in Indiana, quartz increased, while feldspars, mica, and chlorite decreased with increasing soil age (Esser et al., 1992).

The objectives of this chapter are to present a brief description of the petrographic microscope, review the optical properties of minerals and glasses, describe techniques for preparing and analyzing grain mounts, describe grain counts, and summarize properties of common minerals and glasses found in soils to aid in their identification.

Table 6–1. Weatherable and resistant minerals in coarse silt and sand fractions of soils.

Weatherable		Resistant
Olivine	Hornblende	Magnetite
Gypsum	Andesine	Quartz
Glauconite	Chlorite	Kaolinite
Biotite	Clinozoisite	Iron oxides
Calcite	Zoisite	Sphene
Volcanic ash	Oligoclase	Staurolite
Dolomite	Albite	Tourmaline
Augite	Apatite	Monazite
Aegirine-Augite	Epidote	Sillimanite
Anorthite	Microcline	Andalusite
Diopside	Orthoclase	Kyanite
Enstatite	Sanidine	Spinel
Analcime	Muscovite	Opal
Nepheline		Anatase
Actinolite-Tremolite		Rutile
Labradorite		Zircon

PETROGRAPHIC MICROSCOPE

The petrographic microscope has unique features that allow the study of optical properties of minerals, rocks, and soils. Petrographic microscopy can be used to identify and quantify minerals and glasses in the sand and silt fractions of soils and sediments, the emphasis of this chapter. In addition, researchers can study the relationships among the constituents of rocks and soils using petrographic microscopy with thin sections (Vepraskas and Wilson, 2008, this volume).

Petrographic microscopes vary in specific design, but all have certain components in common (Fig. 6–1). A *light source*, usually consisting of a tungsten bulb, is filtered to bring the light color to that of daylight. Two polarizing filters include one fixed in place (the *polarizer*) and one that can be inserted or removed from the light path (the *analyzer*). The polarizing filters are normally set so that the vibrations directions are at 90° (“normal”) to each other. The polarizer is located above the light source and below the microscope stage. The analyzer is in the light train above the stage and specimen and below the ocular (below). One or two *substage diaphragms* are located above the polarizer. A lower diaphragm is used to increase or reduce light entering the specimen, and an upper diaphragm is used to increase resolution. A *condenser* is a small circular lens that may be inserted into or removed from the light path; its purpose is to change the light from a linear path to a cone that appears to originate from the center of the specimen, and is used to obtain interference figures (below). The microscope *stage* is flat, circular, and can be rotated through 360°. Many microscopes are equipped with *mechanical stages*, each of which is mounted onto the rotating stage, and allow precise counts of mineral or glass components in each specimen. *Objectives* are magnifying lenses mounted on a rotating nosepiece; these usually vary in magnification from 4× to 40× or similar. An *accessory slot*, below the analyzer, allows insertion of accessory plates such as the quartz wedge. A *Bertrand lens* is used to examine interference figures (discussed below), when inserted into the light train along with the use of a condenser. The *ocular* or eyepiece is another magnifying lens, which can be focused to suit the needs of the analyst. Most modern microscopes are binocular and have two oculars, one with crosshairs. The petrographic microscope is focused either by moving the rotating stage up or down or by moving the upper part of the assembly up or down. Coarse and fine focus knobs are provided. The ocular(s) also can be focused.

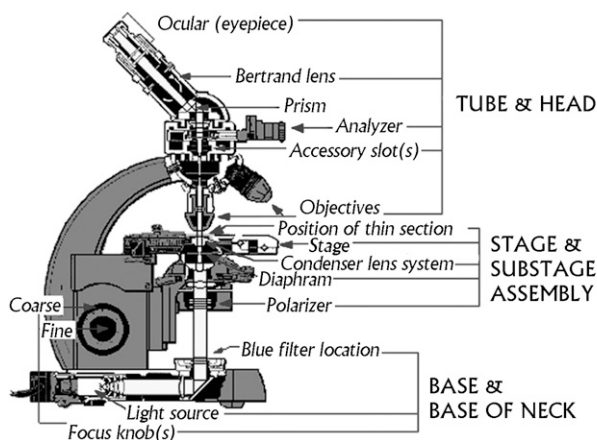


Fig. 6–1. Components of a petroscopic microscope (drawn after a model by Zeiss, Oberkochen, Germany). Courtesy of Michelle Lamberson and University of British Columbia (<http://www.eos.ubc.ca/courses/eosc221>).

In general, then, light is emitted from the source, passes through the diaphragms and lenses to focus it properly, and passes through a polarizing filter, before passing through the specimen. Thus, the light entering the specimen is polarized (the light rays vibrate in one direction or plane). This polarized light (termed *plane-polarized*) enters the specimen. When light enters a crystal, each light ray is split into two light rays. The rays may travel at different velocities, or at the same velocity, depending on the crystal system of the mineral.

Plane-Polarized Light

Under plane-polarized light, minerals have distinctive properties, many of which are unique enough to help identify the minerals. Observations in *plane-polarized light* include color, pleochroism, refractive index, relief, and Becke line. Minerals that are strongly colored in hand sample often show color under plane-polarized light. The color of light transmitted through a grain helps discriminate among the heavy minerals, garnets, and platy minerals (micas and chlorites). *Pleochroism* is the change in color, by variable absorption of the visible light spectrum, when the stage is rotated in plane-polarized light. Pleochroism is diagnostic for many colored minerals. Hornblende and biotite, for example, each commonly exhibit strong pleochroism, and this characteristic aids in their identification.

Refractive index (n) is the ratio of the speed of light in a vacuum to the speed of light in a transparent medium. For practical purposes in optical microscopy, the speed of light in air is assumed to equal the speed of light in a vacuum. In general, the speed of light in a transparent medium decreases as the atomic weight of its elements increase. The refractive index increases accordingly. Water, glass microscope slides, and lenses have relatively low refractive indices. The refractive index of most minerals is between 1.3 and 2.4. The refractive index is the same in all directions for noncrystalline solids (such as natural and manufactured glasses) and for minerals in the isometric (cubic) crystal system; these minerals are called *isotropic*. Minerals in all other crystal systems are *anisotropic*—refractive index is different in different crystallographic directions. In most minerals, there is not a large enough difference in ray velocities to be detected in plane-polarized light. However, in calcite, the difference in ray velocities is great enough that when the microscope stage is rotated, calcite crystals appear to “twinkle.”

Some minerals, such as zircon, with a high refractive index and distinct crystal faces, trap light within the crystal, and the grain’s outer edges appear thick and dark in plane-po-

larized light. The refractive index of a mineral is estimated from relief (explained below), which can be determined by observing the mineral in a series of calibrated immersion oils until a best match is found. When relief is used to estimate refractive index, consider the grain shape, color, thickness, surface texture, and orientation of the grain. The refractive index of thin, platy grains may be underestimated because of the thinness, whereas refractive index of a colored grain or fragment with a rough, hackly surface texture may be overestimated. It is helpful to compare an unknown mineral with known minerals.

Relief expresses the contrast in refractive index between the grain and the mounting medium. The greater the contrast, the greater the relief. The depth of shadows near borders of grains gives the appearance of topographic relief. Grains with high relief are prominent, while grains with low relief appear to fade or blend into the background. The thermosetting epoxy, Petropoxy 154 (Palouse Petro Products, Pullman, WA), used for grain mounts, has a refractive index close to quartz, so quartz has low relief and seems to disappear.¹ Most other minerals stand out by comparison.

Positive relief applies to grains with refractive index greater than that of the mounting medium. In medium of $n = 1.544$, hornblende has fairly high positive relief ($n = 1.61\text{--}1.70$), and zircon has very high positive relief ($n = 1.93\text{--}1.99$). *Negative relief* applies to grains with refractive index less than the mounting medium. In medium of $n = 1.544$, orthoclase has low negative relief ($n = 1.518\text{--}1.526$), and felsic volcanic glass has a moderate negative relief ($n = 1.45\text{--}1.48$).

A *Becke line* is a bright halo of light that forms near the contact between a grain and the mounting medium because of the difference in refractive index between the two. It is best seen in dim light, with the substage diaphragm closed down. As the plane of focus is moved upward through the grain, the Becke line appears to move into the substance with the higher refractive index (Fig. 6–2). For example, in medium of $n = 1.544$, the Becke line moves away from K-feldspar ($n = 1.518\text{--}1.526$), but moves into muscovite ($n = 1.556\text{--}1.611$). A quartz grain ($n = 1.54$) in the same medium may show two halos, a blue halo that moves into the grain and a yellow halo that moves away from the grain, simultaneously. The refractive properties of the quartz grain have split the color spectrum, resulting in the two halos moving in different directions.

Cross-Polarized Light

Inserting the analyzer into the light path reveals more optical properties. Observations in *cross-polarized light* allow the researcher to identify and distinguish isotropic and anisotropic minerals, determine if anisotropic minerals are uniaxial or biaxial, indicate interference colors and birefringence, and determine the angle of extinction.

Crossing the polarizers by inserting the analyzer causes the analyzer to filter out any light rays vibrating 90° to its direction. If the light rays exiting a crystal are vibrating such, the crystal will appear black (*extinct* or *at extinction*) under crossed polarizers. Light rays vibrating at other than 90° relative to the analyzer exit the crystal, traveling at different velocities, and interfere. The result of this interference is to produce what looks, through the microscope with crossed polarizers, like light of some intermediate wavelength, showing some color: gray, white, yellow, orange, red, purple, blue, or a combination of colors. Isotropic substances do not exhibit a difference between refractive indices no matter what crystallographic direction you are observing; therefore, when the polarizers are crossed, the light rays totally interfere, to produce no light. Under crossed polarizers, the isotropic

¹ Brand identification is for technical purposes only and is not a product endorsement.

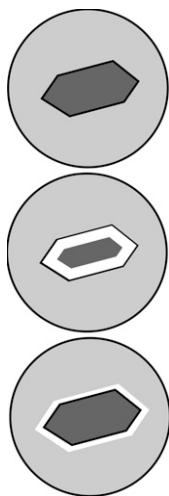


Fig. 6-2. The Becke line moves into the medium with higher refractive index when the microscope ocular assembly is moved upward or the stage is lowered. (upper) Grain in focus; (center) refractive index of grain is higher than the mounting medium (oil or epoxy); (lower) refractive index of grain is lower than the mounting medium.

mineral (or glass fragment) goes to extinction, and stays at extinction through the entire 360° rotation.

Anisotropic minerals, on the other hand, will change color as the stage is rotated with crossed polarizers. They periodically go extinct, then turning various colors as the stage is rotated, four times every 360° rotation. The colors are called *interference colors* and they depend on the size or thickness of the specimen and birefringence of the specific mineral at a particular orientation.

Extinction angle is the angle between the north–south (vertical) or west–east (horizontal) crosshair and the edge of a crystal or the trace of a cleavage plane, when the mineral goes extinct. If the mineral goes to extinction when a crystal edge or cleavage trace is parallel to a crosshair (i.e., parallel to one of the polarizers), it has parallel extinction. Other minerals have inclined extinction; thus the extinction angle can aid in mineral identification. The extinction angle of grains with no cleavage traces, such as a rounded quartz grain, is difficult to determine.

The difference between slow ray refractive index and fast ray refractive index is called *birefringence*. Minerals differ in their birefringence. Typically, quartz has low birefringence, and its interference colors tend to be white, gray, or pale yellow at 0.03-mm thickness (the thickness of a typical thin section). Carbonates have very high birefringence, and their interference colors are so advanced that these minerals have a pastel rainbow appearance under crossed polarizers. Birefringence and resulting interference colors under crossed polarizers depends partly on grain thickness (the thicker the grain, the higher its order of interference colors); this point is important to remember when identifying grains of various size fractions.

Interference colors have been reproduced on a document called the Michel-Levy chart. The chart includes birefringence and specimen thickness in millimeters (thickness of either grain or thin section), presents four orders of interference colors, and gives the ranges of birefringence and interference colors for many common minerals. The optical mineralogist uses the Michel-Levy chart, the thickness of the specimen, and observed interference colors to quantify the birefringence of the specimen and aid its identification. To estimate interference color, observe several grains of a mineral because only a few grains will lie in positions that show the extremes of refractive index. A repeating pattern or sequence of in-

interference colors is divided into units or “orders” of rainbow stripes (red to blue, the range of light visible to the human eye). Three to five orders or sequences of visible light may be observed from a given mineral under cross-polarized light. With more orders, the colors fade to white. Grain size, orientation and thickness affect interference colors. For example, quartz grains in the coarse silt fraction have milky white colors. Very fine sand grains have a gray to pale yellow color, and fine sand-sized quartz grains are likely to have blue, and some have second-order red. A muscovite grain generally lies flat on the slide, generally is thinner than quartz, with interference color nearly always a pale gray in all three size fractions. Calcite has extremely high birefringence, having interference colors of white or pastel pinkish tan (taupe). Colored stripes appear along straight, beveled edges of grains, or as color bands for some rounded grains. Interference colors of calcite can change markedly as the stage is rotated, just like the change in relief.

With respect to their optical properties, transparent substances can be classified into three groups: isotropic, uniaxial, and biaxial. In an isotropic substance, light moves with equal velocity in all directions and in all directions vibrates at right angles to the directions of light ray propagation. Gases, liquids, noncrystalline solids, and minerals in the isometric (cubic) crystal system are isotropic. All other minerals are anisotropic and are either uniaxial or biaxial.

Hexagonal and tetragonal crystals are anisotropic, and they transmit light vibrating at right angles to the propagation direction only in a direction parallel to the *c* crystallographic axis. Another way of stating this is that the light is traveling in the direction of the *c* axis with zero birefringence. In these minerals, the *c* axis is called the optic axis, and since there is only one optic axis, hexagonal and tetragonal minerals are called *uniaxial*. Uniaxial minerals have an optic sign (positive or negative), determined by which of the two light rays travels faster.

Minerals in the orthorhombic, monoclinic, and triclinic crystal systems have two directions along which light travels with zero birefringence. In other words, these minerals have two optic axes. The term for this is *biaxial*. The optic angle, or *angle 2V*, is the angular separation of the two optic axes in biaxial minerals. Biaxial minerals also have an optic sign and are either positive or negative. Any anisotropic mineral with its optic axis (or one of its optic axes if biaxial) oriented perpendicular to the stage will stay at extinction throughout 360° stage rotation.

Observations in conoscopic light are used to distinguish uniaxial and biaxial minerals and to determine the optic sign, and, for biaxial minerals, determine the angle 2V. To view conoscopic light, use an objective of magnification 40× to 50×, insert the substage condenser lens, open the diaphragm wide, and insert the Bertrand lens. If the mineral under observation is anisotropic, you should see an interference figure that varies with type of crystal structure and with grain orientation. The interference figure is a pattern of black bars or bands interspersed with bright areas often containing rings of interference colors.

A uniaxial mineral, oriented to view along its optic axis, gives the best interference figure for determining optic sign. From the array of grains of a given mineral, select the grain with the lowest relief, as those are most likely to show an optic axis interference figure that is centered, and insert the condenser and Bertrand lens and open the diaphragm. The observer will see an orthogonal cross at or near the center of the field with four isogyres (black lines) extending north, east, south, and west to the edge of the field. The background color is gray to neutral if birefringence is low. Blue or red concentric circles appear if birefringence is moderate. Background colors are white if birefringence is high or extreme. If the grain is precisely centered, the cross remains fixed in the center of the field as the stage is rotated. If the optic axis is tilted slightly, the cross is off-center, and appears to rotate in a

circle in the same direction as the stage is turned. If the cross is centered just off stage, one can follow the path of rotation from the sequence that the isogyres sweep across the stage. To determine optic sign, insert a quartz or gypsum plate. With the accessory plate inserted, the northeast quadrant of the cross will be blue for positive minerals or yellow for negative minerals. As a quartz wedge is inserted, colors in the northeast quadrant of the cross move to the center in a positive mineral or away from the center for negative.

A biaxial mineral's two intersecting optic axes gives two acute and two obtuse angles. The acute angle is used to define the "2V," which ranges from near zero to 90°. Isogyres form a cross four times as the stage is rotated 360°. A view that bisects the acute angle is best for determining the optic sign. With a quartz or gypsum plate inserted and the cross in view, its northeast quadrant is blue if the mineral is positive and yellow if the mineral is negative. As a quartz wedge is inserted, colors move to the center of the cross if positive and away from the center of the cross if negative. The crossed isogyres part into two opposing curved segments as the stage is rotated 45° and reform as the stage is rotated 90°. With a small angle 2V, the separation is small. At 30 to 40°, the two opposing arcs are about at the edge of the field at 45° rotation. They move off stage for larger 2V angles. Muscovite, a biaxial negative mineral with a 2V of 30° with plates lying flat on the slide, can be used as a reference for estimating the magnitude of the 2V in other minerals.

PROCEDURES

Site selection, pedon descriptions, sample collection, sample preparation, and particle-size separation should be executed to produce grain-size separates representative of the array of minerals in the soil sample. Quantitative data on minerals normally are reported as percentages of grains counted, which are assumed to be similar to percentage by weight. Statistical methods to be employed in data analysis should be determined before petrographic analysis and grain counts.

Pretreatments and Sample Preparation

Coarse silt (0.02–0.05 mm), very fine sand (0.05–0.1 mm), and fine sand (0.1–0.25 mm) fractions are well-suited for observation and identification of minerals by petrographic microscopy. Fine silts are too small to observe optical properties with confidence, and medium sands are so large that there are too few grains in the microscope field for effective counting. Coatings obscure the passage of light through sand grains. If organic matter coats sand particles, remove it by treatment with sodium hypochlorite adjusted to pH 9.5 (Soukup et al., 2008, this volume). Likewise, if necessary, remove carbonates with sodium acetate (NaOAc) at pH 5, and iron oxides with sodium citrate–bicarbonate–dithionite (Soukup et al., 2008, this volume). Coatings indicate pedogenic history, so the worker may want to examine coatings. Typically one-half the desired fraction is treated to remove coatings, and the other half is kept with coatings intact. Clear glass coatings, commonly present in volcanic ash, indicate a volcanic origin and perhaps a specific source. White coatings often indicate pedogenic accumulations of carbonate. Red or yellow coatings are iron oxides, often adsorbed on clay, and commonly indicate oxidizing conditions, whereas gray coatings could indicate poorly drained, wet conditions.

Typically, sands separated from the whole soil during particle-size analysis are kept and used for petrographic examination. If these fractions have not already been obtained, separate the sands from the whole soil by mixing with deionized or distilled water or dilute sodium solution (to encourage dispersion), sedimentation or centrifugation employing Stokes' Law, and decantation. Several cycles of dispersion, sedimentation, and decanting may be necessary to achieve total separation. After removal of clays and fine and medium

silts, and removal of coatings if necessary, decant the final wash water and dry by evaporation, with or without added heat. Separate sand fractions by dry sieving (Soukup et al., 2008, this volume). Coarse silts may be separated from fine and medium silts and clay by centrifugation or sedimentation, or separated along with the sands and retained in the pan during dry sieving of the sands.

X-ray Diffraction

If sufficient quantity of silt or sand is present, and before petrographic analysis, minerals present in the silt or sand fractions in sufficient quantities (>5%) can be identified by X-ray diffraction (XRD) analysis by crushing or grinding the desired fraction and mounting as a packed powder. This gives the researcher an idea of what major minerals to expect to see under the microscope and will aid identification. See the chapter on XRD techniques by Harris and White (2008, this volume).

Heavy Mineral Separation

Liquids of various densities are commonly used in the laboratory to separate the “light” minerals such as quartz and other silicates from the “heavy” minerals such as iron oxides or ferromagnesian silicates. Techniques for separating minerals by density are well presented in earlier discussions (Cady, 1965; Cady et al., 1986). In the past, organic liquids such as bromoform or tetrabromoethane were used for heavy mineral separation, but they are extremely hazardous. If these chemicals must be used, the procedures must be performed under fume hoods. A new generation of nontoxic, water-soluble heavy liquids, sodium and lithium polytungstates, are now available in similar densities ($>2.8 \text{ g mL}^{-1}$) as the organic liquids. Results from standard grain mounts can guide decisions on appropriate size fractions and amounts of sample needed for analysis of heavy minerals. Kinds and amounts of heavy minerals can help identify source materials or improve “fingerprints” for comparing two different samples, such as two volcanic sources or two events from one source.

The ferromagnetic minerals magnetite and ilmenite can be initially detected by placing a small amount of sample on a sheet of paper and moving a magnet back and forth underneath. Inclusions of magnetite in a rock fragment may cause the fragment to move in response to the magnet.

Slide Preparation

Once the various size fractions in a soil sample have been separated, grain mounts can be prepared for the two most abundant fractions among the fine sand, very fine sand, and coarse silt. Ordinarily, the dominant size fraction is counted. To compare one horizon with another or one pedon with another, it is critical to examine a common particle-size fraction. Avoid using a particle-size separate that is <3 to 5% of the fine earth fraction (<2 mm) because the mineral assemblage is often skewed.

Permanent Mounts

Permanent grain mounts on glass slides (46 by 26 mm) with glass cover slips (18 by 18 mm) can be processed as a batch, stored temporarily before observation and analysis, and later archived for future reference. An epoxy medium with a refractive index similar to quartz ($n = 1.54$) is used to facilitate observation and analysis. A thermal-setting epoxy (e.g., Petropoxy-154) is used to make permanent grain mount slides. The refractive index (n) ranges from 1.545 to 1.540 depending on how long the slide is heated. After heating for 8 min at 125°C, the refractive index for the hardened medium should be close to 1.544,

which closely matches the refractive index of quartz and is similar to Canada balsam ($n = 1.535$), another popular mounting medium (Cady, 1965; Cady et al., 1986).

Mix the epoxy resin and catalyst to form a working medium at least 1 d before use to allow air bubbles to disperse from the solution. Store in a refrigerator when not in use. Prelabel a 27 by 46 mm glass microscope slide with the sample number and grain size. Place a small amount of epoxy mix on the upper-middle of a standard petrographic slide. Use about one-half drop of epoxy for coarse silt, one drop for very fine sand, and two drops for fine sand.

Thoroughly mix the individual sample fractions before removing a subsample for grain counting from the vial. Dip a microspatula about 2 mm into coarse silt, 4 mm into very fine sand, or 6 mm into fine sand to obtain about the right number of grains. Stir the sample into the epoxy slowly with a dissecting needle to avoid introducing bubbles, and work the grains out from the center. Finish stirring with a “figure eight” motion to evenly distribute the grains. Prick noticeable bubbles with the needle to eliminate them. Place one edge of an 18 by 18 mm cover slip next to the edge of the epoxy, and ease the slip onto the sample with the dissecting needle. Align the cover slip to create an equal margin with the top and two sides. Grains should be in close proximity to one another for efficient counting, but not in contact or overlapping. Ideally the epoxy matches the area of the cover slip.

For efficiency, multiple sets of permanent grain mounts should be prepared. For example, four coarse silt samples and six very fine or fine sand samples are a good number of replicates. Do each step in succession for each consecutive slide, so that the sample placed on the epoxy of the first slide has been immersed by the time a sample is placed on the fourth. Place the slides on a hot plate set at 125°C and set a timer for 8 min. After the slides have been on the hot plate for about 30 s, gently tap the cover slip with the dissecting needle to work the epoxy to the edges and orient grains that are lying at an angle and check that the cover slip has not moved. If too little epoxy was used to evenly distribute under the cover slip, introduce a small drop of epoxy next to the edge of the slip so that it wicks under and fills the void. This will bunch some of the grains, but it will secure any sharp edges. If too much epoxy has been applied and extends well outside the cover slip, wait until the epoxy has set and then scrape the excess off with a single edged razor blade along the edge of the cover slip. While these slides are curing, another set can be prepared. Remove slides from the hot plate after 8 min and cool on a wood block or other nonconducting surface. Final slides should contain enough grains to nearly fill the area under the cover slip and still not be packed together.

Temporary Mounts

Oils of various refractive indices serve as mounting media for temporary slides. One advantage of oil mounts is that these are quickly and easily prepared, with no curing time. Another advantage is that oils with a variety of refractive indices can be used sequentially, and relief of various grains compared with that of the oils, to precisely quantify the refractive indices of the mineral components. The disadvantage of oil mounts is that these slides cannot be stored for future use longer than a few days.

Obtain a clean, glass slide, labeled appropriately along one end. Place the slide flat, label side down. Choose an immersion oil with appropriate refractive index (oil of $n = 1.544$ is commonly used). Place three to six drops of oil on the slide, or enough to cover a 1 by 1 cm square in the middle of the slide. With a microspatula, mix the sand grains thoroughly. Scoop out a representative sample with the microspatula, and gently tap the spatula to scatter the grains evenly into the oil on the slide without clumping or overlapping. The

grains should not be heaped in a pile, but should be spread out on the blade of the spatula. A statistically valid sample is generally about 300 to 500 evenly dispersed grains, which is just a light dusting of evenly scattered grains. *Never* wet the microspatula with oil or put the grains on the slide before the oil which could result in contamination of the dropper rod and oil still in the bottle. Carefully place a cover slip over the oil and grains. If the proper amount of oil has been applied it will spread out to cover all of the underside of the cover slip. If insufficient oil has been applied, it may migrate via capillarity between the slide and the cover slip causing the grains to move away even as you view them. Too much oil makes a mess. Practice with several slides to determine the correct amount of oil, number of grains, grain spreading technique, and cover slip mounting.

GRAIN COUNTS

The purpose of grain analysis using petrographic microscopy is to identify and quantify minerals and glassy materials in the sand or coarse silt soil fractions. Systematic identification and tally of 300 to 500 grains in several traverses across the slide will give a reasonable percentage of each mineral present for most purposes.

Many microscopes have a mechanical stage that allows the analyst to traverse a slide in fixed intervals. The analyst should preview a grain slide thoroughly in systematic traverses, starting at one corner, and identify each mineral as it is encountered (Soil Survey Lab Staff, 2004). Count at least 300 grains by moving the stage in systematic traverses across the central portion of a slide and tally the abundance of each mineral. The relative proportion of grains tends to be skewed near the edges of a cover slip. When this is seen, stop the traverse a short distance from the edge. One of the oculars in most petrographic microscopes has a crosshair reticule. Any grain under the crosshair in the direction of traverse is counted. This is termed a *line-count method*. Extend the count past 300 grains in completing the final traverse. For slides with a low density of grains or with larger grain sizes, the area counted can extend a fixed distance (1- or 2- μm marks) on each side of the crosshair. The latter technique is the ribbon method (Drees and Ransom, 1994). For larger fractions, usually medium sand, all grains in each field of view are counted by quadrant.

Several factors may interfere with the accuracy of optical grain counts. Various degrees of weathering and alteration may etch and round a grain to mask its optical properties and hinder identification. Coatings may be present (see "Pretreatments" above). It is important early in the process to categorize similar grains even if unknown. Continue the count until a 300+ grain traverse is complete. If mineral identity is not certain, extra research or consultation is in order. Results should be recorded in a database and reported on data sheets.

Krumbein and Pettijohn (1938), Milner (1962), Brewer (1976), and Drees and Ransom (1994) discussed counting statistics. Many analysts consider a count of 300 grains sufficient for statistical accuracy. Any attempt to improve statistical accuracy would require counting substantially more grains. In general, the more different components present (in other words, the greater the variety of components), the more grains should be counted.

Trace minerals can provide good clues to rock source, provenance of transported material (soil parent material), and the degree of weathering of the soil profile. Usually minerals are listed as trace if the tally is $<0.5\%$. It is left to the individual analyst as to how fine the percentages should be computed according to the purpose of the study. Therefore, it is important to register trace minerals and indicate whether they are resistant or weatherable, even if the count is not statistically reliable.

Mineral Identification

Common soil minerals can be identified under the microscope by color, pleochroism, habit, cleavage, twinning, relief, Becke line, and birefringence. A thorough understanding of these fundamental properties increases directly with experience, which enhances the analyst's ability to interpret observations correctly. Several textbooks and articles describe in detail the physics of light as it passes through a mineral (e.g., Kerr, 1977; Bloss, 1961; Dana, 1959; Nesse, 1986; Phillips, 1971; Phillips and Griffen, 1981). Others illustrate the atomic crystalline structure, and the crystallographic class of minerals (e.g., Klein and Hurlbut, 1985; Nesse, 1986; Phillips and Griffen, 1981; Dana, 1959). Below are brief descriptions of properties most commonly used for mineral identification in transmitted light.

Habit, Cleavage, and Twinning

Habit, cleavage, and twinning are characteristics used to identify minerals in hand sample, and they can be used also in microscopy. For example, the mica group is easily identified by the minerals' platy or foliated habit and by their pinacoidal, (001) cleavage. Acicular habits often will be carried over into silt and sand grains. Weathering intensity (exhibited by pitting or dissolution) may follow crystallographic directions and reflect habit.

In grain mounts, minerals with cleavage commonly rest on a dominant cleavage plane. Other cleavages appear as straight edges or traces in the grains. Under crossed polarizers, minerals resting on a strong cleavage plane may exhibit uniform interference color throughout most of the grain, with narrow color bands at the grain edges, due to the steep margins of the grains. Minerals without cleavage or with poor cleavage will sit on the slide in random orientation.

Twinning is characteristic of certain minerals and can be recognized in grain mounts especially under crossed polarizers (Fig. 6-3). For example, polysynthetic albite twinning is common in the plagioclase feldspars and can be used to distinguish plagioclases from K-feldspars and quartz. Likewise, tartan twinning is used to distinguish microcline from other feldspars.

Reflected Light

Observations in *reflected light* primarily reveal color of opaque minerals (mainly native metals, metallic oxides, and metallic sulfides) and surface features of opaque and transparent minerals. Weathering pits, weathering products, and coatings (if not previously removed) can mask the mineral color, but can also provide clues to the history of the grain. Opaque minerals viewed in reflected light reveal surface features and show differences among ore minerals.

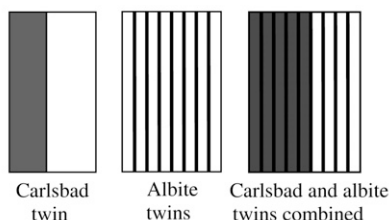


Fig. 6-3. The left Carlsbad twin is at extinction, and in the albite twins, every other twin is at extinction.

MINERAL DESCRIPTIONS

This section is a summary of common soil components and optical properties viewed under the petrographic microscope or (opaque minerals) under reflected light. The reader is also referred to Tables 6–2 through 6–15, which summarize the optical properties of components common in the sand and coarse silt fractions of soil. In reference to the tables, the reader will note that many properties are reported as ranges; this is because the optical properties of minerals vary with chemical composition.

Silica Minerals, Opal, Silica Glass (Lechatelierite), and Volcanic Glass

Quartz (Table 6–2) is the most common mineral in the sand fraction of soils developed in transported materials. Grains commonly are well rounded, pitted, and the pits filled with clays or iron oxides. If the coatings interfere with identification they should be removed (see “Pretreatments” above). Quartz occurs in soils as single crystals and in polycrystalline grains ranging from a few quartz crystals to numerous tiny crystals (microcrystalline) called chert (if crystals are equant) or chalcedony (if crystals are fibrous). The polycrystalline nature is evident by differential extinction of microcrystals in cross-polarized light as the stage is rotated. Chert often has a “salt and pepper” appearance under crossed-polarized light. In a polycrystalline grain with relatively large crystals, the outline of each is evident when it goes extinct as the stage is rotated. Quartzite grains often have an aggregate “snowball” surface appearance. Chalcedony, flint, jasper, agate, and onyx are all forms of microcrystalline quartz.

Tridymite is a polymorph of SiO_2 , but Na, Al, K, and Ca may substitute, giving tridymite a range of optical properties. Tridymite is commonly found in volcanic rocks, felsic to intermediate in composition. Cristobalite is another SiO_2 polymorph, found in volcanic rocks, and like tridymite, Ca, Fe, Al, and Na may substitute, giving cristobalite a range of properties. Cristobalite may be fibrous in habit and may resemble the quartz in chalcedony.

Opal may be biogenic, or not. Nonbiogenic opal may be pedogenic, as in durinodes or duripans, or geological in origin. Geological, nonbiogenic opal commonly is found as cement in sandstones, in altered volcanic ash, or is hydrothermal. Biogenic opal in the form of phytoliths, diatoms, and sponge spicules is present in some soils, usually in small quantities. All are resistant to decay and many retain their original characteristic morphology, which provides means for identification. In a few cases concentrated deposits are soil parent materials. In phytoliths, silica replaces material within plant cells, most commonly associated with stems and leaves grasses in prairie regions such as the mid-continent United States. As the plant tops die and decay, opal “grains,” which retain the conformation of host cells, become incorporated in the soil, where they are resistant to further weathering. Diatoms are aquatic, single-cell microorganisms that use silica to form body structure that outlines internal chambers. As diatoms die, the silica exoskeletons accumulate on the bottom of their habitat (e.g., lake, stream, or ephemeral puddles), such as the depressions in Vertisol gilgai. A concentration of diatoms in strata is called diatomaceous earth or diatomite. Thus, diatoms can be incorporated into soils. The process parallels the incorporation of shells from shell-forming microorganisms into marl. Sponge spicules are sharp-pointed, hair-like structures of silica that protrude from sponges. Broken pieces are tube-shaped with a central hollow core that can usually be discerned in a microscope. Opal commonly exhibits a “hummocky” surface with adamantine luster, and has a low refractive index, similar to volcanic glass. Being poorly crystalline to noncrystalline, opal is isotropic (Table 6–2).

Table 6-2. Optical properties of silica minerals, opal, silica glass (lechatelierite), and volcanic glass.

Formula	Quartz	Tridymite	Cristobalite	Opal	Lechatelierite	Volcanic glass
Crystal system	SiO ₂ hexagonal	SiO ₂ depends on temperature of formation: hexagonal (higher temperature) or orthorhombic (lower temperature)	SiO ₂ isometric (higher temperature) or tetragonal (lower temperature)	SiO ₂ · <i>n</i> H ₂ O poorly crystalline to noncrystalline	SiO ₂ noncrystalline (glass)	Various noncrystalline (glass)
Color	colorless	colorless	colorless	usually colorless, sometimes pale brown or gray	colorless	colorless
Relief in media <i>n</i> = 1.54	neutral to positive, low	negative, moderate	negative, moderate	negative, moderate	negative, moderate	negative, low
Refractive index (<i>n</i>)	1.54–1.55	1.471–1.488	1.482–1.488	1.406–1.460 <i>n</i> decreases with increasing water content	1.457–1.462	1.457–1.61
Birefringence	0.009	0.002–0.004	0.002–0.006	NA	NA	NA
Extinction angle	weak parallel	parallel	parallel	NA	NA	NA
Interference figure	uniaxial (+)	biaxial (+) (uniaxial if hexagonal)	uniaxial (–) or isotropic	isotropic	isotropic	isotropic
Angle 2V (degrees) of biaxial minerals		40–90				

Lechatelierite is SiO_2 glass (Table 6–2). It may occur in pumice, in fulgurites (fused glass from lightning strikes of silica sand), sandstone xenoliths in volcanic rocks, impactites from meteorite strikes, and may occur in soil as fragments of manufactured glass. Lechatelierite, like all glasses, is noncrystalline and isotropic.

Volcanic glass (Table 6–2) includes a range of refractive indices, resulting from a broad chemical composition from felsic through mafic. Felsic volcanic ash is airborne for varying distances from the source volcano, is felsic, and has characteristic vesicular or shardy morphology, as it is the interstices in the gaseous froth ejected from the volcano. Mafic glass (high pH source, mafic rocks) is usually brown or green and has $n > 1.54$. It is common in basalts and as obsidian. *Palagonite* ($\text{SiO}_2\text{--H}_2\text{O}$) is hydrated volcanic ash and is usually amber-brown with an “oolitic” texture. Volcanic glass is isotropic. Counting isotropic grains only can be done more quickly by either positioning the analyzer slightly off “blackout” position or by crossing the polarizers and inserting an accessory plate and increasing the light. The outline of each grain is visible, with the color of an amorphous grain being the same as the epoxy background. Mica grains and garnets may appear isotropic, but will have positive relief (Soil Survey Staff, 1995).

Feldspars

Feldspars (Table 6–3) represent the second most common group of minerals in soils and the most common weatherable minerals. It can be difficult to distinguish individual species of feldspars. Depending on the purpose of the study, the most efficient method is to separate K-feldspars and plagioclase by twinning under crossed polarizers, and refractive index under plane-polarized light. Plagioclases usually exhibit polysynthetic (albite) twinning; K-feldspars never exhibit albite twinning. Both K-feldspars and plagioclases may exhibit Carlsbad twinning. Microcline commonly exhibits “tartan” twinning, a plaid or cross-hatched pattern, and is the only feldspar to do so. Under plane-polarized light, all the K-feldspars have negative relief, while most plagioclases (except albite and oligoclase) have positive relief.

Potassium feldspars are microcline, orthoclase, and sanidine. Microcline is found in igneous and metamorphic rocks. Orthoclase is associated with igneous rocks. It may exhibit simple (linear) Carlsbad twinning of two individuals. Sanidine grains are usually clear phenocrysts, without twinning, and can be distinguished from other K-feldspars by its low 2V. Sanidine is associated with deposits of volcanic rocks or volcanic ash. Perthite is an intergrowth of microcline and albite, formed by exsolution during cooling, and will appear as irregular lamellae in a single grain, best visible under crossed polarizers.

Plagioclase feldspars are albite, oligoclase, andesine, labradorite, bytownite, and anorthite. They increase in the proportion of Ca/Na from sodic albite to calcic anorthite. Refractive index increases accordingly. Albite has negative relief. Andesine, labradorite, and anorthite have positive relief. Oligoclase can have either. Most plagioclase in soils are oligoclase or albite. Some oligoclase can have the same refractive index as quartz, which prevents distinctions by the Becke line. Cleavage is good in two directions parallel to (001) and (010), often producing lath-like or prismatic shapes. Albite is a common plagioclase in the Blue Ridge, Piedmont, and Coastal Plain in the eastern United States. Oligoclase is the only plagioclase common as a vein mineral. Andesine is widely distributed in igneous rocks and occurs in metamorphic rocks. Labradorite is a common mineral in rocks such as augenites, basalts, gabbros, and olivine gabbros. Bytownite occurs in mafic rocks, such as gabbros, anorthosites, and basalts, but is rare in soils. Anorthite is a rare plagioclase that occurs in a few contact metamorphic deposits and lavas.

Table 6-3. Optical properties of feldspars.

	K-feldspars			Plagioclases (Solid solution between albite [NaAlSi ₃ O ₈] and anorthite [An; CaAl ₂ Si ₂ O ₈])					
	microcline KAlSi ₃ O ₈	orthoclase (K,Na)AlSi ₃ O ₈	sanidine (K,Na)AlSi ₃ O ₈	albite An 0–10	oligoclase An 10–30	andesine An 30–50	labradorite An 50–70	bytownite An 70–90	anorthite An 90–100
Formula									
Crystal system	triclinic	monoclinic	monoclinic	triclinic	triclinic	triclinic	triclinic	triclinic	triclinic
Color	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Relief in media $n = 1.54$	negative, low	negative, low	negative, low	negative, low	negative, low to positive, low	neutral to positive, low	positive, low to positive, moderate	positive, low to positive, moderate	positive, low to positive, moderate
Refractive index (n)	1.518–1.530	1.518–1.526	1.517–1.526	1.527–1.542	1.533–1.551	1.543–1.562	1.554–1.573	1.564–1.585	1.573–1.590
Birefringence	0.007	0.008	0.007	0.009–0.011	0.008–0.009	0.008	0.008–9	0.009–0.012	0.012–0.013
Extinction angle	5–15	5–12	parallel to 001	3–5	0–3	0–7 on 001	–7 to –16 on 001	–16 to –32 on 001	–32 to –40 on 001
Interference figure	biaxial (–)	biaxial (–)	biaxial (–)	biaxial (+)	biaxial (+) or (–)	biaxial (+) or (–)	biaxial (+)	biaxial (–)	biaxial (–)
Angle 2V (degrees) of biaxial minerals	77–84	35–85	0–12	3–5	82–90	76–90	76–90	79–88	77–79

Distinguishing feldspars from quartz can also be challenging. With training and experience in examining grain mounts of common soil minerals, the analyst can readily distinguish quartz from untwinned feldspars by cleavage, weathering, tabular habit, relief, and Becke line. The range of refractive index for oligoclase straddles the refractive index of quartz, so relief and Becke line are inadequate for distinguishing oligoclase from quartz. Birefringence in cross-polarized light is greater for quartz—this is a rather easy distinction with fine and very fine sand, but more difficult with coarse silt (shorter path length in grain). It may be necessary to view the grain in conoscopic light to determine if it is uniaxial (quartz) or biaxial (oligoclase).

Phyllosilicates

Muscovite and biotite are the most common micas in soils. Illite, also called clay mica or sedimentary mica, commonly occurs in soils, but is generally concentrated in the clay fraction and is too fine to resolve crystals with the petrographic microscope. In grain slides, mica plates usually orient flat on the slide and have low positive relief and low interference colors. Plates are commonly equidimensional. In thin sections, plates oriented on edge show high-order interference colors, but in ordinary grain mounts, micas rest on the pinacoid and exhibit low-order interference colors in this orientation. Very fine-grained micas (and chlorites) may be sprinkled on gelatin or petroleum jelly before the cover slip is applied, to view the grains in a more “edge-on” orientation.

Biotite (Table 6–4) is one of the most weatherable minerals. Biotite is usually some shade of brown, but can be greenish if interleaved with chlorite, and commonly is pleochroic in shades of brown. Under crossed polarizers and near extinction, biotite commonly shows a rumpled or “birdseye maple” pattern, which is very characteristic, but mostly seen in thin section. The pattern may not be visible in grain mounts.

Paler colors, a lowering of refractive index, and a distortion of the extinction and interference figure indicate weathering to hydrobiotite or vermiculite. In most environments, biotite weathers quickly to hydrobiotite and then vermiculite. Biotite can also alter to iron oxides. Vermiculite looks like biotite with an “accordion” structure (exfoliated books) and higher birefringence. X-ray diffraction of a subsample can detect the presence of vermiculite and possibly semiquantify it. An incident light source will reveal the shiny cleavage planes of micas that may be partly or completely coated with iron oxides, which retain the surface tension of mica. A coarse, matte surface suggests nearly complete alteration to iron oxide (hematite, goethite, lepidocrocite, ferrihydrite, and/or limonite). This distinction is useful for soil engineering purposes and should be noted. In the humid climate of the Piedmont and Coastal Plain of the southeastern United States, biotite alters pseudomorphically to kaolinite (Lynn et al., 2002). It retains the optical properties of biotite, but has no K, and X-ray diffraction indicates kaolinite. Sand grains in thin section commonly exhibit an accordion structure of partly exfoliated books. In the dry climate of southern New Mexico, thin sections of monzonite rock show red crack fillings (ferric iron) radiating from biotite grains as the first evidence of weathering (Gile et al., 1995). With continued weathering, biotite alters to hydrobiotite and then to vermiculite, either in the weathered grus or in the soil.

Talc (Table 6–4), a metamorphic mineral, appears very similar to muscovite but is often fan-shaped like vermiculite. Talc usually is colorless but may be pleochroic to pale yellow or pale green.

The chlorite group (corundophilite, prochlorite, clinochlore [Table 6–4], and penninite) covers a range of chemical modifications, probably associated with various degrees

Table 6-4. Optical properties of biotite, talc, chlorite (clinochlore), and serpentines (chrysotile and antigorite).

	Biotite	Talc	Chlorite (Clinochlore)	Chrysotile	Antigorite
Formula	$K(Mg,Fe)_3AlSi_4O_{10}(OH)_2$	$Mg_3Si_4O_{10}(OH)_2$	$Mg_2(Al,Fe)(Al,Si)_4O_{10}(OH)_8$	$Mg_3Si_2O_5(OH)_4$	$Mg_3Si_2O_5(OH)_4$
Crystal system	monoclinic	monoclinic	monoclinic	monoclinic	monoclinic
Color	yellowish to reddish brown, often pleochroic	colorless, faint yellow, or faint green	green, often pleochroic	colorless, pale green, pale yellow, may be faintly pleochroic	colorless to pale green, may be weakly pleochroic, pale yellow to pale green
Relief in media $n = 1.54$	positive, low to positive, moderate	negative, low to positive, low	positive, low to positive, moderate	negative, low to positive, low	positive, low
Refractive Index (n)	1.541–1.638	1.538–1.590	1.575–1.583	1.493–1.569	1.555–1.573
Birefringence	low in flat-lying grains, strong (0.033–0.059) in tilted grains	very low in flat-lying grains to strong in tilted grains	very low Interference color often an anomalous “Berlin blue”	0.004–0.020, average 0.007	0.004–0.009, average 0.005
Extinction angle	parallel, up to 3	along cleavage traces 0–3	parallel		
Interference figure	biaxial (–)	biaxial (–)	biaxial (+) or (–)	biaxial (–) or (+)	biaxial (–)
Angle 2V (degrees) of biaxial minerals	0–25	6–30	0–20	30–50 interference figure difficult to obtain	20–50 interference figure difficult to obtain

of metamorphism, with corresponding optical variation. They can be green (most common), brown, or red, with moderate positive relief and low to moderate birefringence, which may or may not have a clear extinction angle. They commonly occur as plate-like crystals with anomalous birefringence and pleochroism, generally in shades of green. They commonly have a “mottled” interference color. The chlorites may be confused with glauconite, which is better identified with incident light and knowledge of the source location.

The serpentine group (chrysotile and antigorite, Table 6–4) includes metamorphic minerals common in serpentinite rocks. Chrysotile is usually fibrous or acicular and fine-grained. Optical properties may be difficult to discern because of small crystal size. Antigorite is finely laminar and may also be fibrous. Like chrysotile, antigorite is usually fine-grained, and optical properties may be difficult to study.

Muscovite (Table 6–5) is usually colorless. Under crossed polarizers and in thin section, muscovite may exhibit a birdseye maple pattern similarly to biotite, but is easily distinguished from biotite by the lack of color. Again, the birdseye maple pattern may not be visible in grain mounts. Muscovite is the most resistant of weatherable minerals and is commonly associated with K-feldspars.

Kaolinite (Table 6–5) is rare in size fractions larger than clay in most soils. It occurs as mosaic-like masses or scale-like individual crystals and occasionally as small accordion-like plates. Kaolinite often is present in sand fractions of soils containing feldspars. Because of the small crystal size, optical properties of the kaolinite usually cannot be discerned. Kaolinized feldspars appear cloudy under the petrographic microscope.

Olivine and Pyroxenes

Olivine (Table 6–6) is a high-temperature, ultramafic mineral common in igneous rocks such as basalts, gabbros, peridotites, and dunites. Olivine consists of a solid solution series ranging from forsterite (Mg_2SiO_4) to fayalite (Fe_2SiO_4), with a corresponding range in optical properties (Table 6–6). It is the first silicate to crystallize as igneous magma cools and also the silicate least resistant to weathering in soils. It commonly alters to serpentine minerals such as antigorite. Olivine weathers to iron oxides.

The pyroxenes, enstatite, aegirine-augite (formerly hypersthene), diopside, and augite (Table 6–6) all have positive, moderate to high relief and moderate to high birefringence. They vary from colorless to green and may exhibit slight to moderate pleochroism. Enstatite and aegirine-augite are orthopyroxenes (orthorhombic crystal system), commonly with prismatic habit and cleavage, and show parallel extinction. The clinopyroxenes, augite and diopside, are monoclinic, have good cleavage at angles close to 90° and large extinction angles. Colors usually are shades of green. Pyroxenes generally do not give a good interference figure and are difficult to distinguish from each other. X-ray diffraction may help identify the specific pyroxene. Enstatite is a characteristic mineral of ultramafic igneous rocks, but it also occurs in meteorites. Aegirine-augite is found in mafic to intermediate igneous rocks. The refractive index increases as Fe content increases. Diopside is especially characteristic of contact-metamorphic zones. Augite is a very common mineral in mafic igneous rocks. It is a common detrital mineral and probably the most common pyroxene in soils.

Amphiboles

Hornblende, tremolite-actinolite, and lamprobolite (Table 6–7) are common amphiboles. These amphiboles may be confused with pyroxenes, but generally have lower relief and lower-order interference colors. Hornblende is a common and widely distributed

Table 6-5. Optical properties of muscovite and kaolinite.

	Muscovite	Kaolinite
Formula	$\text{KAl}_2(\text{AlSi}_2\text{O}_{10}(\text{OH})_2)$	$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$
Crystal system	monoclinic	triclinic
Color	colorless to pale green	colorless
Relief in media $n = 1.54$	positive, low to positive, moderate	positive, low
Refractive index (n)	1.556–1.611	1.561–1.566
Birefringence	low in flat-lying grains, strong (0.037–0.041) in tilted grains	weak
Extinction angle	parallel to 3	(usually indistinct)
Interference figure	biaxial (–)	biaxial (–)
Angle 2V (degrees) of biaxial minerals	30–40	interference figure difficult to obtain

Table 6-6. Optical properties of olivine and pyroxenes.

	Orthopyroxenes			Clinopyroxenes		
	Olivine	Enstatite	Aegirine-augite	Diopside	Augite	
Formula	$(\text{Mg,Fe})_2\text{SiO}_4$	MgSiO_3	$(\text{Mg,Fe})\text{SiO}_3$	$\text{Ca}(\text{Mg,Fe})(\text{SiO}_3)_2$	$\text{Ca}(\text{Mg,Fe})(\text{SiO}_3)_2$	$\text{Ca}(\text{Mg,Fe})(\text{SiO}_3)_2$ [(Al,Fe) $_2\text{O}_3$] $_x$
Crystal System	orthorhombic	orthorhombic	orthorhombic	monoclinic	monoclinic	monoclinic
Color	colorless to very pale green	colorless	colorless to pale green or pale red; may be pleochroic	colorless to pale or bright green	colorless to pale green; may be weakly pleochroic	
Relief in media $n = 1.54$	positive, moderate to positive, high	positive, moderate	positive, low to positive, moderate	positive, low to positive, moderate	positive, moderate	
Refractive index (n)	1.651–1.718	1.650–1.665	1.673–1.715	1.650–1.727	1.688–1.712	
Birefringence	0.037–0.041	0.008–0.009	0.010–0.016	0.029–0.031	0.021–0.025	
Extinction angle	parallel	parallel	parallel	–37 to –44	longitudinal sections –36 to –45	
Interference figure	biaxial (+) (Mg rich)	biaxial (+)	biaxial (+)	biaxial (+)	biaxial (+)	
Angle 2V (degrees) of biaxial minerals	Biaxial (–) (others) 70–90	58–80	63–90	58–60	58–62	

mineral in many types of igneous and metamorphic rocks and, by far, the most common amphibole in soils. Crystals are prismatic in habit with a pseudohexagonal cross-section, usually green and often pleochroic. Color darkens and refractive index increase as the Fe content increases. Basaltic hornblende (lamprobolite) is also called brown hornblende and often exhibits cleavage planes at 60 and 120°. Tremolite and actinolite are usually acicular, and colorless to green in plane-polarized light. Glaucophanite is a less common amphibole, found in blueschist, with habit similar to hornblende, and with a distinctive deep blue color in plane-polarized light.

Carbonates

Calcite, dolomite, and siderite, the hexagonal carbonates, have similar optical properties (Table 6–8). Calcite usually dominates in Cretaceous and younger carbonate rocks. Dolomite is a very common mineral, usually dominant in Paleozoic and older carbonate rocks, and very common in soils derived from these rocks. The chief occurrence of siderite is in veins or ore replacement deposits. It is the principal mineral of septarian clay ironstone concretions. Grains are usually clear but may have a pinkish brown or gray tinge. Single crystals of calcite or dolomite in soil are generally of geologic origin. They are easy to identify by their typical rhombohedral cleavage forms, the marked change in relief in plane-polarized light when the stage is rotated, or the bright white interference colors and many bands in cross-polarized light. Calcite and dolomite are not easy to distinguish optically, but can be identified by X-ray diffraction. Siderite (FeCO_3) is the only carbonate with entirely positive relief. In soils, these minerals have other forms—scales and chips, cements in aggregates, microcrystalline coatings or aggregates, and other fine-grained masses that are often mixed with clay and other minerals.

Aragonite, the orthorhombic polymorph of calcite, usually shows a columnar or fibrous habit. It is encountered in soils mainly as fragments of shell and coral.

Pedogenic carbonates nearly always occur in soils as microcrystalline aggregates of calcite. The extreme interference colors provide an identification tool as various tiny carbonate grains in the aggregates will twinkle in cross-polarized light as the stage is rotated. Grains of microcrystalline quartz do not. Increasing the intensity of reflected light reveals a characteristic taupe color. If soil parent material, commonly glacial till, in a leaching environment contains both dolomite and calcite, the dolomite is more resistant to dissolution and remains in the upper calcareous fringe from which calcite has been entirely leached.

Sulfates

Gypsum (Table 6–9) is the chief constituent of gypsum rock, a common evaporite. Gypsum occurs in soils as platy or prismatic flat grains with refractive index approximately equal to orthoclase. It often exhibits a “scratched” surface texture parallel to the extinction angle in plane-polarized light. Pedogenic gypsum can accumulate in arid regions from soluble salts and can form if acid sulfate weathering occurs in the presence of carbonates. If soil samples containing gypsum are dried at 105°C in the laboratory, gypsum partially dehydrates to bassanite ($\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$).

Barite (Table 6–9) is a prominent vein mineral. It also occurs in limestone and as cement in sandstone. It forms pedogenically in some acid soils, as ped coatings in or below the lower solum.

Metamorphic Aluminosilicates

Sillimanite, andalusite, and kyanite (Table 6–10) are anhydrous polymorphs of Al_2SiO_5 with an alumina/silica ratio of 1:1. These minerals are found in medium- to high-

Table 6-7. Optical properties of amphiboles.

	Hornblende	Tremolite-Actinolite
Formula	$\text{Ca}_2(\text{Mg,Fe,Al})_5(\text{OH})_2[\text{Si,Al}]_4\text{O}_{11.2}$	$\text{Ca}_2(\text{Mg,Fe})_3(\text{OH})_2(\text{Si}_4\text{O}_{11})_2$
Crystal system	monoclinic	monoclinic
Color	green or brown to reddish-brown, often pleochroic	colorless to pale green, often pleochroic
Relief in media $n = 1.54$	positive, moderate to positive, high	positive, moderate
Refractive index (n)	1.614–1.701	1.600–1.655
Birefringence	moderate	moderate
Extinction angle	mostly about 12; up to 30	max of 10 to 20
Interference figure	biaxial (+)	biaxial (–)
Angle $2V$ (degrees) of biaxial minerals	52–85	79–85

Table 6-8. Optical properties of carbonates.

	Calcite	Dolomite	Siderite	Aragonite
Formula	CaCO_3	$\text{Ca}(\text{Mg,Fe})(\text{CO}_3)_2$	FeCO_3	CaCO_3
Crystal system	hexagonal	hexagonal	hexagonal	orthorhombic
Color	colorless	colorless to gray	colorless to gray	colorless
Relief in media $n = 1.54$	negative, moderate to positive, moderate	negative, moderate to positive, high	positive, moderate	negative, low to positive, high
Refractive index (n)	1.486–1.658	1.500–1.716	1.596–1.633	1.530–1.686
Birefringence	0.172	0.180–0.190	0.234–0.242	0.156
Extinction angle	symmetrical to cleavage traces	symmetrical to cleavage traces	symmetrical to cleavage traces	parallel
Interference figure	uniaxial (–)	uniaxial (–)	uniaxial negative	biaxial (–)
Angle $2V$ (degrees) of biaxial minerals				18

Table 6-9. Optical properties of sulfates.

	Gypsum		Barite
	CaSO ₄ ·2H ₂ O	BaSO ₄	
Formula	monoclinic	orthorhombic	
Crystal system	colorless	colorless	
Color			
Relief in media <i>n</i> = 1.54	negative, low	positive, moderate	
Refractive index (<i>n</i>)	1.520–1.529	1.636–1.648	
Birefringence	0.009	0.012	
Extinction angle	parallel in sections normal to (010)	parallel to cleavage traces	
Interference figure	biaxial (+)	biaxial (+)	
Angle 2 <i>V</i> (degrees) of biaxial minerals	58	36–37.5	

Table 6-10. Optical properties of high temperature metamorphic silicate minerals.

	Sillimanite	Andalusite	Kyanite	Mullite	Siaurolite
	Al ₂ SiO ₅ (Al ₂ O ₃ ·SiO ₂)	Al ₂ SiO ₅	Al ₂ SiO ₅	Al ₆ Si ₂ O ₁₃	Fe ₂ Al ₆ O ₆ (SiO ₃) ₄ (O,OH) ₂
Formula	orthorhombic	orthorhombic	triclinic	orthorhombic	orthorhombic
Crystal system	colorless	colorless, pale green or reddish, may be pleochroic	colorless to pale blue, may be pleochroic	colorless	pale yellow, may be pleochroic
Color					
Relief in media <i>n</i> = 1.54	positive, moderate to positive, high	positive, moderate	positive, high	positive, moderate	positive, moderate to positive, high
Refractive index (<i>n</i>)	1.657–1.684	1.629–1.647	1.712–1.728	1.642–1.654	1.736–1.747
Birefringence	0.020–0.023	0.007–0.011	0.016	0.012	0.010–0.015
Extinction angle	parallel	parallel	30°	parallel	parallel
Interference figure	biaxial (+)	biaxial (–)	biaxial (–)	biaxial (+)	biaxial (+)
Angle 2 <i>V</i> (degrees) of biaxial minerals	20–30	about 84	about 82	20–50	80–88

grade metamorphic rocks of Al-rich composition. Andalusite, stable at the lowest pressure of the three, converts to sillimanite or kyanite at higher temperatures and pressures. Andalusite and sillimanite have similar fibrous to prismatic forms with parallel extinction. Kyanite looks very similar to zircon but has a 30° extinction angle and is triclinic. The pale blue color of kyanite, the platy, angular cleavage flakes, and the large extinction angles usually can be observed and make identification easy.

Mullite (Table 6–10) is rare and is formed at high temperatures, often from kyanite or sillimanite. Mullite may also be a constituent of bricks and porcelains.

Staurolite (Table 6–10) occurs as porphyroblasts (as well as in the matrix) of metamorphic rocks such as schists, phyllites, and gneisses. Common associates are kyanite, sillimanite, and quartz. Staurolite sometimes contains holes (i.e., a “Swiss cheese” effect) or may have a foggy or milky appearance, which may be caused by colloidal inclusions.

Accessory Minerals

Many minerals, generally those quite resistant to weathering, are fairly ubiquitous in soils in small amounts, generally as coarse silt rather than sand. Zircon (Table 6–11) is one of these common accessory minerals. Zircon is distributed generally in granite and other felsic, coarse grained igneous rocks, and also occurs in schists and gneisses. Zircons usually show excellent prismatic tetragonal habit with pyramidal ends. Zircon has bright, strong interference colors. Grains of zircon are almost always clear and fresh-appearing crystals. However, broken and rounded crystals do occur.

Beryl (Table 6–11) occurs mostly in granitic pegmatites, but also in mica schists and in veins in limestone. It is commonly associated with albite. Grains usually contain gas bubble inclusions. Tourmaline (dravite, Table 6–11) usually occurs in metamorphosed limestone, and in some schists. Other varieties are schorlite (iron tourmaline) and elbaite (alkali tourmaline). These all have similar optical properties, but Fe gives schorlite a somewhat higher refractive index. Colors can be red, green, brown, and sometimes green-blue. Tourmalines usually are strongly pleochroic and have parallel extinction. Interference colors are a characteristic greenish-purple “bronze.” Basal sections show excellent uniaxial negative interference figures and little, if any, pleochroism.

Garnets (pyrope [Table 6–11], almandite, spessartite, uvarovite [Table 6–11], grossularite, and andradite) are characteristic of metamorphic rocks and very common in detrital material. In soils they usually are rounded, equidimensional, isotropic grains of high relief. Cleavage is poorly preserved. Garnet of very fine sand and coarse silt size is often colorless. Pale pink colors are diagnostic in larger grains.

Rutile (Table 6–12) is a widely distributed accessory mineral in metamorphic rocks and in detrital materials. It often appears similar to zircon, except rutile is not invariably prismatic, but commonly occurs as long, acicular crystals. Rutile has an amber color in both plane- and cross-polarized light. Other TiO_2 minerals, anatase (tetragonal) and brookite (orthorhombic), also have very high refractive indices and brown colors, but usually occur as tabular or equidimensional grains. The three minerals may be difficult to distinguish if grains are small.

Cassiterite (Table 6–12) occurs in granite pegmatite, usually associated with quartz, muscovite, schorlite, and topaz. Cassiterite has both extremely high positive relief and extremely high birefringence with interference colors of pastel pinks and blues. It often has a good uniaxial positive interference figure with multiple isochromes.

Sphene (Table 6–12) is a widely distributed accessory mineral in coarse-grained igneous rocks and in gneisses and schists. It is not a common detrital mineral, except locally.

Table 6-11. Optical properties of accessory minerals.

	Zircon	Beryl	Tourmaline(Dravite)	Garnets (Pyrope, Uvarovite)
Formula	ZrSiO ₄	Be ₃ Al ₂ (SiO ₃) ₆	NaMg ₃ B ₃ Al ₃ (OH)(Al ₃ Si ₆ 6O ₂₇)	Mg ₃ Al ₂ (SiO ₄) ₃ ; Ca ₃ Al ₂ (SiO ₄) ₃
Crystal system	tetragonal	hexagonal	hexagonal	isometric
Color	colorless to pale	colorless to pale green, may be pleochroic	gray, slate blue, olive, often pleochroic	colorless, pale reddish, pale to dark brown, greenish gray
Relief in media <i>n</i> = 1.54	positive, very high	positive, low to positive, moderate	positive, moderate	positive, high to positive, very high
Refractive index (<i>n</i>)	1.925–1.993	1.564–1.598	1.613–1.628	1.741–1.887
Birefringence	0.060–0.062	0.004–0.008	0.019–0.025	NA
Extinction angle	parallel	parallel	parallel	NA
Interference figure	uniaxial (+)	uniaxial (–)	uniaxial (–)	NA, isotropic
Angle 2V (degrees) of biaxial minerals				

Table 6-12. Optical properties of titanium minerals and cassiterite.

	Rutile	Cassiterite	Sphene (Titanite)
Formula	TiO ₂	SnO ₂	CaTiSiO ₆
Crystal System	tetragonal	tetragonal	monoclinic
Color	yellowish to reddish brown	colorless to gray, yellowish, reddish, or brown; shows adamantine luster in reflected light	colorless to golden yellow, may be pleochroic
Relief in media <i>n</i> = 1.54	positive, very high	positive, very high	positive, very high
Refractive index (<i>n</i>)	2.603–2.903	1.996–2.093	1.887–2.054
Birefringence	0.286–0.287	0.097	0.092–0.141
Extinction angle	parallel	parallel	parallel
Interference figure	uniaxial (+)	uniaxial (+)	biaxial (+)
Angle 2V (degrees) of biaxial minerals			23–50

It is commonly a rounded or subrounded grain in soil. In reflected light, the grains appear amber, with smooth lustrous cleavage surfaces. Sphene, in some forms, resembles zircon, but has oblique extinction. In cross-polarized light it appears ultra-blue rather than black as the stage is rotated because of its high dispersion. Sphene is the only pale-colored or colorless high-index mineral that provides this effect. The refractive index of sphene is slightly lower than that of zircon, and the grains are often cloudy or rough-surfaced.

Epidote, zoisite, and clinozoisite (Table 6–13) usually are colorless to green (epidote), irregularly shaped or roughly platy grains, with moderate positive relief. Clinozoisite displays an anomalous blue interference color. Zoisite has a very deep bluish-purple interference color. All are colorless, pale-green, or bluish-green. Epidote is common heavy mineral widely distributed in many types of igneous and metamorphic rocks. Typically, epidote has strong yellow and red interference colors, and a pistachio-green pleochroic color. Commonly, grains show an optic axis interference figure with a 2V nearly 90° and a pinwheel with isogyres rotating opposite to rotation of the stage. Weathering or metamorphic alteration modifies epidote to colorless forms with lower birefringence and refractive index.

Zeolites and Phosphates

Zeolites (including analcime and stilbite, Table 6–14) appear similar to K-feldspar but with higher negative relief, lower birefringence, no twinning (but often radiating acicular habit), and adamantine luster. They are highly weatherable, and so not common in soils. Analcime is a secondary mineral in cavities and seams of igneous rocks, usually associated with other zeolites and calcite. Stilbite is a secondary mineral in cavities and seams of igneous rocks. It has a distinctive radiating morphology.

Apatite (Table 6–14) is a common accessory mineral in nearly all igneous rocks. Apatite looks very similar to beryl in plane-polarized light but has higher positive relief, very low birefringence, no gas bubbles and is not pleochroic. It is often “bullet” shaped. Apatite is common in young soil materials, particularly phosphatic limestone. Crystal shapes are common and may appear as prisms. Rounding by solution produces ovoid forms. Apatite is easily attacked by acid and may be lost in pretreatments to remove carbonate coatings. Monazite (Table 6–14) occurs in pegmatite and granite. It occurs as a detrital mineral in sand. It usually gives an excellent 20°–2V positive interference figure with somewhat parabolic isogyres. Interference color changes from purple-blue to gold as the stage is rotated.

Opaque Minerals

Opaque minerals do not transmit light. Observations of color and luster in reflected light, aided by crystal form, provide the best clues for identification. Some grains in soils do not transmit light because of iron oxide and/or clay coatings. Native metals, metallic oxides, and metallic sulfides commonly have metallic luster in silt- to sand-sized crystals. Fine-grained earthy aggregates absorb and disperse light, resulting in variable degrees of opacity.

Magnetite and ilmenite (Table 6–15) look black with a metallic luster in reflected light. Other mineral aggregates (e.g., clachite, leucoxene) are also opaque and appear white in reflected light. Magnetite is common in nearly all igneous and metamorphic rocks. It is a common detrital mineral in sands accumulated along modern or ancient strand-lines of lakes or oceans. A hand magnet can separate magnetite from sand grains readily, but the magnet commonly brings host grains along. Ilmenite is a widely distributed mineral in

Table 6–13. Optical properties of common sorosilicates: epidote, zoisite, clinozoisite.

	Epidote		Zoisite	Clinozoisite
Formula	$\text{Ca}_2(\text{Al,Fe})_3(\text{OH})(\text{SiO}_4)_3$		$\text{Ca}_3(\text{Al,Fe})(\text{OH})(\text{SiO}_4)_3$	$\text{Ca}_2\text{Al}_3(\text{OH})(\text{SiO}_4)_3$
Crystal System	monoclinic		orthorhombic	monoclinic
Color	colorless to yellowish green, commonly pleochroic		colorless to pink, may be pleochroic	colorless
Relief in media $n = 1.54$	positive, high		positive, high	positive, high
Refractive index (n)	1.720–1.779		1.696–1.718	1.710–1.734
Birefringence	0.014–0.045		0.006–0.018	0.005–0.011
Extinction angle	parallel		parallel	parallel
Interference figure	biaxial (–)		biaxial (+)	biaxial (+)
Angle 2V (degrees) of biaxial minerals	69–89		30–60	66–90
	interference colors are strong yellow and red			colors anomalous bright pale blue

Table 6–14. Optical properties of zeolites (analcite and stilbite) and phosphate minerals (apatite and monazite).

	Analcite (Analcite)		Stilbite	Apatite	Monazite
Formula	$\text{NaAl}(\text{SiO}_3)_2 \cdot \text{H}_2\text{O}$		$\text{H}_2(\text{Ca Na}_2)\text{Al}_2(\text{SiO}_3)_6 \cdot 4\text{H}_2\text{O}$	$\text{Ca}_5(\text{PO}_4)_3(\text{OH,F,Cl})$	$(\text{Ce,La,Y,Th})\text{PO}_4$
Crystal system	isometric		monoclinic	hexagonal	monoclinic
Color	colorless		colorless	colorless	colorless
Relief in media $n = 1.54$	negative, moderate		negative, moderate to negative, low	positive, moderate	positive, very high
Refractive Index (n)	1.487		1.494–1.508	1.630–1.651	1.786–1.849
Birefringence	NA		0.006 0.008	0.003–0.004	0.049–0.051
Extinction angle	NA		parallel	parallel	2°–10°
Interference figure	NA, isotropic		biaxial (–)	uniaxial (–)	biaxial (+)
Angle 2V (degrees) of biaxial minerals			33		6–19

Table 6–15. Optical properties of magnetite, ilmenite, hematite, goethite, and pyrite (opaque in transmitted light).

	Magnetite		Ilmenite		Hematite		Goethite		Pyrite	
Formula	Fe_3O_4		FeTiO_3		Fe_2O_3		FeOOH		FeS_2	
Crystal system	isometric		hexagonal		hexagonal		orthorhombic		isometric	
Color in reflected light	black with metallic luster		blue-grey to black may show white or yellowish border due to alteration to leucoxene.		dark gray to black or reddish brown		yellowish brown to dark brown		brassy yellow	
Luster	metallic		metallic; leucoxene has earthy luster		metallic if dark gray in large grains, earthy if reddish and fine grained		adamantine if massive, silky if fibrous, earthy if fine grained		metallic	
Habit	crystals may be octahedra that form triangular, square, or rhombic sections		disseminated tabular crystals		platy to anhedral grains if igneous or metamorphic; anhedral masses or coatings if secondary		usually secondary, as anhedral masses or coatings		cubes or polyhedra, framboids (clusters of cubes or polyhedra)	

some types of igneous rocks, such as diabase and dolorite. Leucoxene is a white opaque microcrystalline substance common as an alteration product of titanium minerals and also is disseminated in various rocks. Pyrite (Table 6–15), the most common sulfide mineral (Klein and Hurlbut, 1985), is brassy yellow with metallic luster in reflected light. Pyrite is common in igneous and metamorphic rocks as a high-temperature mineral. It also is common in sedimentary rocks, both inherited and as a secondary mineral formed under reducing conditions.

Hematite (Table 6–15) is dark gray to black, with metallic luster, if appearing in large crystals such as inherited from igneous and metamorphic rocks. Goethite (Table 6–15) is yellowish brown to dark brown if massive or botryoidal. Coatings or grains of iron oxides or hydroxides appear yellow (goethite), orange (lepidocrocite), or red (hematite) in reflected light (Schwertmann and Cornell, 1991; Schwertmann, 1993). Hematite pigment is relatively intense and commonly masks the color of goethite.

Some grains that appear opaque in plane-polarized light can appear translucent if the substage condenser lens is inserted to increase the light intensity. Many grains are difficult to identify because they are altered by transportation or pitted by weathering.

Rock Fragments

Many soils contain particles of fine-grained parent rocks, for example, basalt, rhyolite, andesite, slate, schist, limestone, or shale, in the sand fractions. Rock fragments are more common in the larger size fractions but can persist through the very fine sands. The fragments often have microcrystalline morphology, variable birefringence, and poor extinction. Schist fragments have multiple areas of high birefringence with preferred orientation and a flat shape reflecting the foliated structure of the rock. Rhyolite fragments contain crystals with low birefringence, a few crystals with high birefringence, a “sprinkling” of opaque crystals, and a rounded shape. Depending on the source region, rhyolite may have a yellowish color due to hydrothermal alteration.

Soil Minerals Related to Source Materials

Optical mineral identification is somewhat subjective, and interpretations may vary among analysts. The best approach is to identify minerals through a process of elimination that narrows the possibilities to the fewest candidates (Cady, 1965). Weathering commonly alters the appearance of minerals compared with geological specimens furnished in the classroom and museum. At times, the decision will rely on the worker’s knowledge of the source parent material, geologic history, genetic processes, degree of weathering, lithologic discontinuities, and soil fertility of the study area. If a result seems to contradict logic, the analyst should consider discarding a selection that does not fit the conditions. For example, olivine in a highly weathered soil, or carbonates in an acidic soil are settings that should make these identifications questionable, but not necessarily impossible. The worker should not lose sight of the possibility of additions of aberrant material by eolian or other sedimentary processes. Eventually, by experience and knowledge of geology, the analyst expects to find particular mineral suites associated with bedrock geology or a likely source of transported deposits. When a combination seems out of place, take extra steps to ensure validity of the data.

Mineral Composition and Particle Size

Generally, successively finer size fractions will be successively depleted in weatherable minerals and rock fragments. In very fine sands and coarse silts, quartz will be most abundant, and resistant (Table 6–1) accessory minerals often are preserved.

Fine sand often contains mostly quartz in many soils and sediments, and well-sorted fine sands of eolian origin typically lack micas and other platy minerals (mica plates or flakes, because of their aerodynamic shape, tend to travel with smaller, lighter, non-platy grains). Rock fragments commonly are absent unless the fragments themselves are very fine-grained and resistant to mechanical and chemical weathering. Coarser sands often include rock fragments in addition to individual minerals. Other than the conditions mentioned above, the expression of mineral diversity in fine sands is pretty good as is the quartz vs. weatherable mineral content and kinds of weatherable minerals. Identification and counting usually are straightforward.

Incidental and Foreign Objects in Grain Mounts

Various foreign items can get into or onto the slide at any time during the collection, transportation, and preparation process. After recognizing them, ignore them.

- Air bubbles can occur during slide preparation by mixing of air into the epoxy or oil, or by degassing of aggregates. They have extreme negative relief and a “fish eye” appearance.
- Fibrous organic matter that has not been removed by oxidation or “floated” off the sample may appear in grain mounts. This material will be noncrystalline and sometimes exhibits cellular structure.
- Pieces of cover slip may mix into the epoxy. These shards usually are long and rectangular and have refractive index slightly greater than volcanic ash.
- Lint often finds its way into slides and appears as fibrous strings. These strings are commonly doubly refractive with high birefringence.
- Some anomalous “dendritic” or “globular” forms may appear in the medium. These probably result from contamination or differential crystallization of impurities in the epoxy.
- Fingerprints can distort the light passing through a slide. Take care to avoid touching flat surfaces of the slide and cover slip.

Technological Advances

Advances in light microscopy have been mainly refinement in manufacturing techniques, computer integration, and aesthetic appearance. Advances in lens grinding technique and lens materials have vastly improved the optical quality and image clarity of today’s petrographic microscopes. Advances in computer software and hardware have allowed increasing amounts of data collection and storage and image storage and analysis. Presentation of images has also improved. Computer technology notwithstanding, mineralogists will probably always carry a hand lens in the field.

The mineralogical analysis of the sand and silt size fraction of soils is still best achieved in the most efficient and economical method by petrographic microscopy. With today’s technology, precise and accurate identification can be achieved using state of the art analytical instruments such as X-ray diffraction, microprobe analysis, and atomic force microscopy. These methods, discussed in accompanying chapters, have enhanced our ability to differentiate the minute variations in related minerals. Yet the art of direct visual mineral identification remains as a personally satisfying, and perhaps, most reliable means.

REFERENCES

- Bloss, F.D. 1961. An introduction to the methods of optical crystallography. Holt, Rinehart and Winston, New York.
- Brewer, R. 1976. Fabric and mineral analysis of soils. Robert E. Krieger Publishing Co., New York.

- Cady, J.G. 1965. Petrographic microscope techniques. p. 604–632. *In* C.A. Black (ed.) *Methods of soil analysis*. Part 1. Physical and mineralogical methods. Agron. Monogr. 9, ASA and SSSA, Madison, WI.
- Cady, J.G., L.P. Wilding, and L.R. Drees. 1986. Petrographic microscope techniques. p. 185–216. *In* A. Klute (ed.) *Methods of soil analysis*. Part 1. Physical and mineralogical methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Dana, J.D. (revised by C.S. Hurlbut, Jr.) 1959. *Mineralogy*. 17th ed. John Wiley & Sons, New York.
- Drees, L.R., and M.D. Ransom. 1994. Light microscopic techniques in quantitative soil mineralogy. *In* J.E. Amonette and L.W. Zelazny (ed.) *Quantitative methods in soil mineralogy*. SSSA Miscellaneous Publication. SSSA, Madison, WI.
- Esser, K.B., J.G. Bockheim, and P.A. Helmke. 1992. Mineral distribution in soils formed in the Indiana Dunes, USA. *Geoderma* 54:91–105.
- Gile, L.H., J.W. Hawley, R.G. Grossman, H.C. Monger, C.E. Montoya, and G.H. Mack. 1995. Supplement to the desert project guidebook, with emphasis on soil micromorphology. Bull. 142. Mew Mexico Bureau of Mines and Mineral Resources, Socorro, NM.
- Goldich, S.S. 1938. A study of rock weathering. *J. Geol.* 46:27–58.
- Harris, W.G., S.S. Iyengar, L.W. Zelazny, J.C. Parker, D.A. Lietzke, and W.J. Edmonds. 1980. Mineralogy of a chronosequence formed in New River alluvium. *Soil Sci. Soc. Am. J.* 44:862–868.
- Harris, W., and N. White. 2008. X-ray diffraction techniques for soil mineral identification. p. 81–116. *In* A.L. Utery and L.R. Drees (ed.) *Methods of soil analysis*. Part 5. Mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Kerr, P.F. 1977. *Optical mineralogy*. 4th ed. McGraw-Hill, New York.
- Klein, C., and C.S. Hurlbut, Jr. 1985. *Manual of mineralogy* (after J.D. Dana). 20th ed. John Wiley & Sons, New York.
- Krumbein, W.C., and F.J. Pettijohn. 1938. *Manual of sedimentary petrography*. Appleton-Century-Crofts, New York.
- Lynn, W.C., R.J. Ahrens, and A.L. Smith. 2002. Soil minerals, their geographic distribution, and soil taxonomy. p. 691–709. *In* J.B. Dixon and D.G. Schulze (ed.) *Soil mineralogy with environmental applications*. SSSA Book Ser. 7. SSSA, Madison, WI.
- Milner, H.B. 1962. *Sedimentary petrography*. 4th ed. Macmillan Co., New York.
- Nesse, W.D. 1986. *Introduction to optical mineralogy*. Oxford Univ. Press, New York.
- Phillips, W.R. 1971. *Mineral optics, principles and techniques*. W.H. Freeman and Company, San Francisco.
- Phillips, W.R., and D.T. Griffen. 1981. *Optical mineralogy, the nonopaque minerals*. W.H. Freeman and Company, San Francisco.
- Schwertmann, U., and R.M. Cornell. 1991. *Iron oxides in the laboratory. Preparation and characterization*. VCH Publishers, New York.
- Schwertmann, U. 1993. Relations between iron oxides, soil color and soil formation. p. 51–69. *In* J.M. Bigham and E.J. Ciolkosz (ed.) *Soil color*. SSSA Spec. Publ. 31. SSSA, Madison, WI.
- Soil Survey Staff. 1999. *Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys*. Agric. Handb. 436. 2nd ed. U.S. Gov. Print. Office, Washington, DC.
- Soil Survey Staff. 1995. *Soil survey laboratory information manual*. Soil survey Investigations Rep. 45. Version 1.0.
- Soil Survey Staff. 2004. *Soil survey laboratory methods manual*. Soil Survey Investigations Rep. 42. Version 4.0.
- Soukup, D.A., B.J. Buck, and W. Harris. 2008. Preparing soils for mineralogical analyses. p. 13–32. *In* A.L. Utery and L.R. Drees (ed.) *Methods of soil analysis*. Part 5. Mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Vepraskas, M.J., and M.A. Wilson. 2008. Soil micromorphology: Concepts, techniques, and applications. p. 191–226. *In* A.L. Utery and L.R. Drees (ed.) *Methods of soil analysis*. Part 5. Mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI.

Soil Micromorphology: Concepts, Techniques, and Applications

M. J. VEPRASKAS, North Carolina State University, Raleigh

M. A. WILSON, USDA-NRCS, Lincoln, Nebraska

Micromorphology is the branch of earth science that describes, interprets, and measures the components, features, and fabrics of soils, regolith materials, and prehistoric/historic artifacts at the microscopic and submicroscopic levels (Goldberg, 1983; Stoops, 2003). Undisturbed samples are required to see the arrangement and relationship of individual soil particles or components as they occur in nature. Both mineral and organic materials (including organisms and roots) are considered. The magnifications used for observation range from that obtained with a simple hand lens to the high magnifications produced by transmission electron microscopes (van Oort et al., 1994). Micromorphological concepts can also be applied using the naked eye. This has been done in the case of hydric soil field indicators (USDA-NRCS, 2002), and also with redoximorphic features that are used in the soil classification system *Soil Taxonomy* (Soil Survey Staff, 1999). Microscopic examinations are usually performed on thin sections at magnifications beginning at 10× (for examination of microstructure) and followed by increasingly higher magnifications. Thin sections are slices of soil or sediment that have been impregnated with a resin, fixed to a glass microscope slide, and then ground to a precise thickness so that the optical properties of minerals and pedological features are evident.

The objective of this chapter is to present an overview of the concepts and techniques used in micromorphology. Particular emphasis will be given to sampling and preparation of thin sections for examination of mineral soil materials. Preparation of organic soil materials was described by Fox and Parent (1993) and won't be included here. Procedures for describing thin sections will be discussed briefly following the description system presented by Stoops (2003).

APPLICATIONS

Micromorphology is now an established analytical tool that provides unique information for any discipline where soil particles, pores, and organisms play a role. It can be used simply as a descriptive tool, or as a quantitative one when descriptions are backed by morphological measurements or chemical and mineralogical analyses. The oldest application of micromorphology has been to enhance work in soil morphology, genesis and classification. The major textbooks on soil micromorphology were written primarily by pedologists and geologists interested in these topics (Brewer, 1964, 1976 [reprint of 1964 edition with

supplementary material]; Bullock et al., 1985; FitzPatrick, 1984, 1993; Kubiena, 1938; Stoops, 2003).

Applications of micromorphology can be found in studies of virtually all aspects of soil science. Micromorphological techniques have been widely used to study soil genesis (Bullock 1983; Douglas and Thompson, 1985; Stoops and Eswaran, 1986). Micromorphology was used to develop the definitions of cambic, oxic, spodic, and argillic diagnostic horizons in *Soil Taxonomy* (Soil Survey Staff, 1999; Wilding and Flach, 1985). Recent studies related to soil genesis can be found in the work of Nordt et al. (2004), Fauzi and Stoops (2004), Driese et al. (2004), and Scarciglia et al. (2005). Micromorphology also contributed to studies of fragipan degradation and duripan genesis (Chadwick et al., 1987; Lindbo et al., 2000), gypsum in soils (Artieda and Herrero, 2003; Dultz and Kuhn, 2005; Herrero and Porta, 2000), interpretation of paleosols (Alonso et al., 2004; Kemp et al., 1995), and clay movement (Chadwick and Nettleton, 1994; Holliday and Rawling, 2005; Khormali et al., 2003). The varied depositional forms of Fe in soils are documented in studies involving ortstein (Kaczorek et al., 2004; Horbe et al., 2004), placic horizons (Chadwick and Nettleton, 1990; Wu and Chen, 2005; Hseu et al., 1999), and laterite (Fritsch et al., 2002).

Micromorphology has frequently been used to describe soil structure, normally by evaluating the sizes and shapes of soil pores. For example, Trowse (1971) used photographs of thin sections to show how wheel compaction reduced the number of macropores in trafficked compared with nontrafficked rows. Computer image analysis can be used to describe the abundance of pores of specific size classes when more detailed data are needed. Norton and Schroeder (1987) used this technique to compare porosity and pore diameters among moldboard-plow, chisel-plow, ridge-till, and no-till tillage treatments. Kapur et al. (1997) used image analysis to describe the development of spheroidal microstructure (discontinuous concentric curved planar voids) in basaltic Vertisols. Pachepsky and Rawls (2003) evaluated the influence of soil structure on water retention, and concluded that future research requires quantitative characterization of soil structure.

Surface crusts affect soil use and management by reducing both water infiltration and seedling emergence. At least two kinds of crusts have been identified, and these were termed *structural crusts* and *depositional crusts* (Chen et al., 1980; Chiang et al., 1994). Structural crusts form by water drops striking and modifying bare soil, causing a destruction of aggregates (Fox et al., 2004; Lado and Ben-Hur, 2004). This is similar to the process of collapse of seedbeds as a result of overburden pressures rather than rainfall kinetic energy (Bresson and Moran, 2004). Depositional crusts develop from a build-up of translocated fine particles carried onto the surface by flowing water. Numerous studies have shown that crust characteristics are influenced by a variety of soil properties, including texture, degree of aggregation, mineralogy, and dispersibility of the soil (Onofriok and Singer, 1984; West et al., 1990; Miedema, 1997; Lado and Ben-Hur, 2004). For example, in soils that are easily dispersed, fine particles that are stripped off coarse particles or aggregates are washed into soil and plug pores. This "washed-in" zone was first described by McIntyre (1958) but has not been observed in all structural crusts (Evans and Buol, 1968, Onofriok and Singer, 1984).

Archaeologists use micromorphology as well (Courty et al., 1989; Kooistra and Kooistra, 2003; Arpin and Goldberg, 2004). Charcoal, coprolite, pottery fragments, or bone can be identified through thin section examination, and the effects of burning and physical disturbance can be seen as well (Goldberg, 1983). Pottery and ceramics develop unique microstructural patterns and minerals at high temperature, and these features can be used in archaeological studies as indicators of furnace firing and also to understand how

raw materials were prepared for building (Kapur et al., 1995, 1998; Sakarya et al., 2004). Micromorphology has also been used to integrate interpretations among artifacts, climate, geomorphology, and soils excavated in archaeological sites (Courty et al., 1989, 2004; Çambel et al., 1996; Mermut et al., 2004). The depositional history of the sediments covering the floors of caves can often reveal if and approximately when humans (or animals) occupied the site, as well as the amount of activity (Angelucci, 2003). Human and animal activity is revealed by the presence of bone, fecal material, or other artifacts (Schiegl et al., 2003; Shahack-Gross et al., 2004). In thinly bedded sediments, the history of the site can sometimes be reconstructed by identifying sedimentary layers and comparing them with the surrounding geological materials. Lima et al. (2002) studied a toposequence of anthropogenically influenced soils in western Amazonia and combined total elemental analysis, mineralogy, particle size, and micromorphological data to evaluate origins of soil material, pottery, and to estimate population density in the precolonial era.

Micromorphology has been applied to the study of biological materials in soils. Classification systems for soil organic residues have been developed (Barratt, 1969; Babel, 1985; Bal, 1973). Degens (1997) reviewed biological mechanisms of soil aggregation and concluded that microscale observations, as opposed to whole soil analyses, are needed to understand the fundamental processes that create aggregates. Protz and vanden Bygaart (1998) indicated that an analysis of soil fabric at multiple scales would provide an improved understanding of these mechanisms. Recently, Jongmans et al. (2003) and Pulleman et al. (2005) quantified the effects of earthworm activity on aggregation, soil structure, compaction, and decalcification, and documented the abundance of earthworm activity by identification of fecal pellets, calcite spheroids, and worm-worked groundmass in thin sections. Bruneau et al. (2004, 2005) used image analysis of stained thin sections to quantify bacterial populations and void space in grassland soils. Use of thin sections in the science of soil palynology has recently been demonstrated (Kooistra and Kooistra, 2003; van Mourik, 2003). Buurman et al. (2005) used micromorphology in combination with other techniques to study organic matter in podzols (Spodosols) and to better understand humus chemistry in these soils.

Bouma and coworkers pioneered the use of micromorphology to estimate hydraulic conductivity and water movement through soil materials (Bouma and Anderson, 1973; Bouma, 1977; Bouma et al., 1977; Vepraskas et al., 1991b). The water-conducting pores in the soil can be easily seen with dyes or fluorescence microspheres (Droogers et al., 1998; Driese and McKay, 2004). This technique has been used to determine whether quartz veins in saprolite conduct water rapidly (Williams and Vepraskas, 1994). Saprolite, or weathered bedrock, can contain quartz veins that are planar-shaped bodies of quartz gravels. They appear capable of conducting water and wastewater quickly. Septic system drainlines that are placed over quartz veins could cause untreated sewage to flow quickly to groundwater and contaminate it. Vepraskas et al. (1991b) used dyes and measurements of pore diameters in thin sections to identify the pores that conducted water at soil water pressures ranging from 0 to -100 cm. They showed that 93% of the water moving through the saprolite flowed through root channels at saturation. When these drained, the hydraulic conductivity was reduced to one-hundredth of the original value. Thin section examinations showed that fractures and veins in saprolite did not conduct water quickly because they were plugged with clay and Fe or Mn oxides. McKay et al. (2005) found similar decreases in saturated hydraulic conductivity related to macropore infilling and concluded this relationship was a common feature in many saprolites found in humid, temperate regions.

SAMPLING

Sampling is a critical part of all micromorphological studies. Any mistakes made in sampling will affect what is observed in the thin section and may cause erroneous conclusions to be made about the soil material being studied. It is essential that all samples collected represent the soil material as it exists in the field. Collecting such representative samples is difficult because soils are very heterogeneous and even the largest thin sections contain only a very small volume of soil. In addition, special care must also be taken in collecting ceramic and other building materials such as mortar, cement, and concrete for archeological studies.

To collect representative materials, the following aspects must be carefully considered before collecting samples:

- purpose of the investigation
- site selection and supplementary data
- sizes of the samples and thin sections
- orientation of samples
- number of replicate samples needed
- time of sampling
- sampling method
- documentation, transport, and storage of samples

Each of these aspects will be briefly reviewed.

Purpose of the Investigation

Most thin sections are made from undisturbed blocks of soil of known orientation. There is no single way to collect such samples that can be applied to all investigations. Therefore, the sampling techniques one decides to use must be selected to meet the objectives of a specific study, and in some cases novel techniques may have to be developed.

The purpose of the study will dictate how samples will be collected, the number needed, and what portion of a soil needs to be sampled (Fig. 7–1). For example, studies of soil genesis require samples be collected from each horizon, in contrast to studies of soil crusting focus only on the upper few millimeters of soil. While samples for bulk soil analyses are collected throughout the entire horizon, samples for micromorphology focus on specific locations within or between horizons. The boundary between two horizons may yield the most information regarding soil processes. The investigator must also decide whether the results of the analyses need to be quantitative or descriptive. Descriptive studies require fewer replicate samples to meet an objective than studies where specific features need to be quantified and a specific degree of precision achieved.

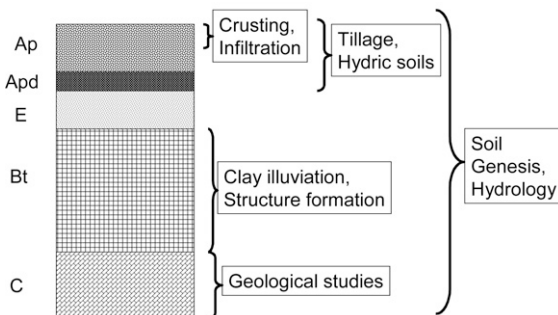


Fig. 7–1. Locations where samples are typically collected in a soil for the topics shown.

Site Selection and Supplementary Data

In general, the sampling site should not be on the border between different soils, experimental treatments, or fields. If samples are going to be collected from a soil profile, then the soil should first be divided into relatively homogeneous horizons and a profile description completed. Soil structure should be described in detail and unique features noted. Bulk samples must also be collected for laboratory analyses that are related to the purpose of the investigation. In the final analysis, the thin section observations should be compared with the profile description and supplementary data to be sure the features seen in the thin section are likely to be found in a greater volume of soil than that seen in the thin section.

Sizes of Samples and Thin Sections

The size of the sample needed depends on (i) the size of the feature of interest and its distribution, (ii) the size of the desired thin sections, (iii) the number of thin sections that will be made from each sample, and (iv) the drying technique selected. For example, examining voids between large prismatic peds will require larger samples and larger thin sections than examinations of the minerals in sandstone. From a practical standpoint, the dimensions of the glass slides available to an investigator are limited, and are determined by the equipment on hand to prepare the thin sections. Such equipment limitations must also be considered when selecting a sample size.

Glass slide dimensions range from about 25 by 48 mm to more than 100 by 200 mm (termed *mammoth size*). Larger thin sections are normally preferred over smaller ones because they show more features and show how the features are interrelated. However, glass slides larger than 76 by 110 mm are cumbersome to use with polarizing microscopes. These slides are too large for most mechanical stages and they usually strike the microscope body during stage rotation. This problem then limits the use of the polarizing microscope for mineral and void examinations unless special modifications are made to the microscope body (FitzPatrick, 1984). In general, laboratories specializing in micromorphology prefer slides of about 28 by 48, 25 by 75, or 50 by 75 mm. Laboratories specializing in examinations of soil structure or soil voids use glass slides of about 76 by 110 mm or larger. Relatively few laboratories specialize in the use of glass slides as large as 100 by 200 mm.

An appropriate sample size can be determined on the basis of the size and distribution of the features of interest and the glass slide to be used. In some cases, large samples may have to be collected in the field and subdivided later in the laboratory to fit the glass slides. This is advisable when large structural aggregates are present that could crumble or separate while being cut into smaller pieces. In addition, it is possible to make more than one thin section from the same sample, but doing this will require collecting larger samples than when only one thin section is made.

Sample size needs to be considered when a particularly drying technique is preferred. Freeze-drying works best when samples are <2 cm thick (Murphy, 1986). While large samples could be collected in the field, they will have to be subdivided in the laboratory before freeze-drying. Oven-drying samples subject to shrinkage can create dense clods that are difficult to impregnate completely. Sizes for such should probably be kept small (e.g., <125 cm³). Sample size is less important when acetone exchange is used for drying because this method of water removal does not cause pores to shrink and close. Open pores are needed to allow the resin solution to penetrate the sample completely.

Sample Orientation

The orientation of the sample can be critical to some investigations, and in all cases it must be known. An example of how orientation affects the appearance of a feature in thin section is shown in Fig. 7–2. Generally the sample orientation will be either horizontal or vertical. Vertically oriented samples are useful to examine soil horizon boundaries, bedding planes, void patterns, or other features that change with depth. Horizontally oriented sections are useful for studies on clay illuviation, water movement, and root growth. In cases where the best orientation is not known in advance, both horizontal and vertical samples should be taken.

Both horizontal and vertical samples should be taken of major horizons for soil genesis studies. This has the advantage of providing both orientations as well as a duplicate sample of the horizon should one sample be damaged. If sufficiently large blocks are impregnated, both vertical and horizontal thin sections can be made from one block. Two thin sections can be easily related to one another when both are cut from the same block.

Number of Replicate Samples

Replicate samples should be collected whenever possible because a single sample will not represent the variation in structure, voids, or numbers of features present in the object of interest. Furthermore, single samples may be destroyed in transit or during thin section preparation. Replicate samples must be collected in studies requiring a statistical analysis of the data. The number of replicates needed is estimated by considering how variable the feature of interest is in the soil. Unfortunately, few studies have been conducted on the variability of micromorphological features (Milfred et al., 1967; Murphy and Banfield, 1978). Additional information on this problem was provided by Brewer (1964), Webster (1977), Wilding and Drees (1983), and Vepraskas et al. (1991a) for sampling soils. Sampling schemes for geological studies were described by Birks and Birks (1980), Griffiths (1967), and Krumbein and Graybill (1965).

From a practical standpoint, the number of replicate samples selected will be determined by the time, fiscal, and personnel resources available. At least two replicate samples per horizon should be collected from a pit even if only one thin section will be produced. A crude estimate of the statistical variability of the features of interest may require between 4 and 10 replicates per horizon. It is probably more feasible to prepare larger thin sections (e.g., 50 by 75 mm) and use fewer replicates than to collect a large number of replicates and prepare smaller thin sections. The soil seen in the larger sections will be more variable and will show the relationships among features better than in smaller thin sections.

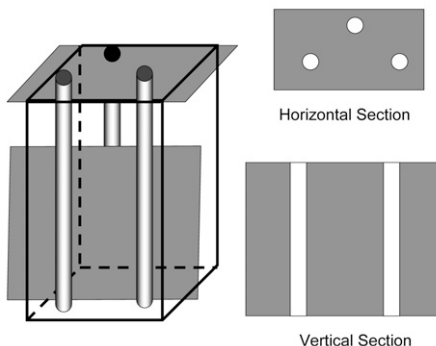


Fig. 7–2. Sample orientation must be known to interpret features seen in thin section correctly. Channels through a sample can appear differently in thin sections depending on how the sections are oriented.

Time of Sampling

Many soil features change with the season of the year. At various times during the year, structural cracks can be open or closed, soluble minerals can crystallize or dissolve, and organic materials can undergo various stages of decomposition. Accordingly, the investigator must anticipate the potential seasonal changes in the features of interest to select the most appropriate sampling time, although sampling in two different periods can provide much information regarding soil dynamics. Excessively dry soils create special problems because cracks can be created when the sampling container is forced into the soil. Such cracks are artifacts that confound observations. When such cracks form, the soil is too dry to sample and sampling should be postponed until the soil becomes wetter.

Sampling Methods

Friable Soils

Most soils and sediments can be sampled using metal or cardboard boxes, or by simply collecting natural soil clods or peds. “Kubiena tins” are metal boxes consisting of three parts: sides (one piece) and two removable lids (Fig. 7–3). These boxes are usually custom-made to the desired size out of rigid aluminum, galvanized iron, or stainless steel. The sides of the box consist of a strip of metal folded into a rectangular frame (Fig. 7–4). The ends of the strip can be taped together, or a special hinge can be constructed for fastening. These boxes can be made out of rigid cardboard or plastic electrical outlet boxes (Josephs and Bettis, 2003). Specially constructed steel holders are needed to protect the cardboard box while it is being pushed into the soil. An alternative for some situations is to use a soil moisture can. They are readily available in convenient sizes and come with a tight-fitting lid.

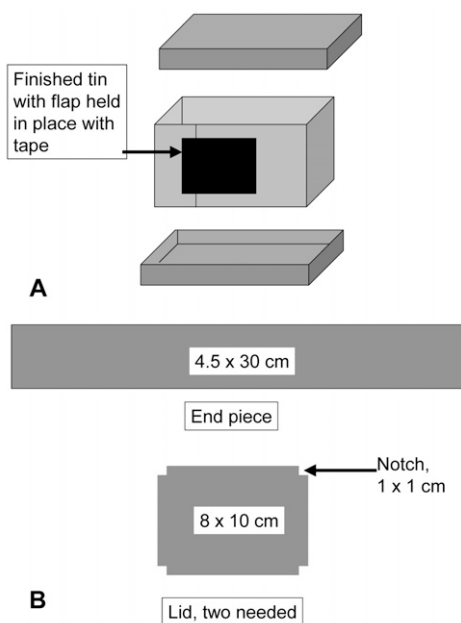


Fig. 7–3. (A) Kubiena tins are boxes having a removable top and bottom. They are easily constructed from metal. (B) Sizes of pieces needed to construct a tin that is 4.5 by 6 by 8 cm (H by W by L) are indicated.

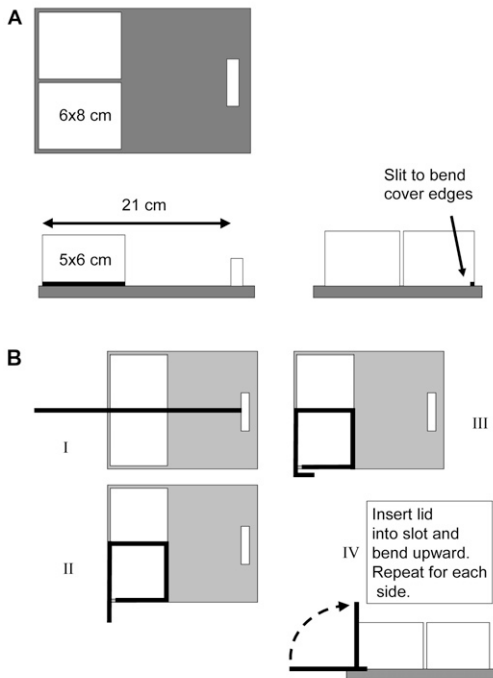


Fig. 7-4. (A) Kubiena tins can be constructed using a mold made out of rigid metal (e.g., steel) so that aluminum pieces can be bent around the blocks. The blocks are made to the size needed for the tins. A slit is cut into the bottom of one of the blocks to bend the edges of the lids. (B) The four steps needed to construct a tin.

The boxes should have a length and width slightly larger than the thin sections that are needed. The depth of the box should be at least one-half its width to prevent it from deforming when filled (Murphy, 1986). Many researchers prefer the boxes to be inexpensive and disposable so samples can be dried and impregnated with resin in the box to lessen the chance of disturbing the sample during these operations.

To collect a sample, a flat surface is first prepared at the desired depth and orientation. The box, with one lid removed, is placed on the surface and is gently pushed into the soil with one hand, while a knife is used with the other hand to clear soil away from around the sides of the box. When full, the box is carefully cut and pried out of the soil using a knife or trowel. The exposed sample surface is then cut flat and the box covered with a lid. All soil surfaces in the final sample should be cut or trimmed so void openings are not smeared shut.

Immediately after it is removed from the soil, each box should be labeled along the side (not on a removable lid) with the sample's orientation, depth, replicate number, and profile number. Labels should be able to withstand any heat or solvents that will be applied to the samples later. Writing on aluminum tins in pencil is faint, but has the advantage that the markings will not dissolve in acetone or other solvents that may be used to remove water from the samples. Some researchers label the boxes on a lid first and photograph them in place before removing them from the soil so a complete record of sample location and orientation is made (Murphy, 1986). Before sealing samples for transport, a few drops of a fungicide or formalin can be added to each sample to kill earthworms and insects to prevent them from burrowing throughout a sample during storage. All samples should then be wrapped in aluminum foil or placed in plastic bags to prevent them from drying out. This must be done for *all* samples, because even air-dry materials from arid regions can absorb water during storage in the laboratory.

Cemented and Cohesive Materials

Undisturbed specimens of cemented materials can be obtained without boxes by carving or chiseling pieces out of the soil. Ceramic materials can be collected from surfaces and/or predetermined settlement levels of excavation sites for archeological studies. Large cohesive peds can also be removed intact without the aid of boxes, but these should be placed in boxes to prevent breakage or separation of the structural units. Orientation can be identified by pressing thin staples, or thumbtacks, into the top of the sample. All such samples should be wrapped in protective material such as foil or plastic and labeled as described previously.

Loose Materials

Some materials may be too loosely consolidated to sample with boxes without disturbing the samples during their removal from the soil. Particularly sensitive samples are sands, gravelly soils, some organic soils, and soils recently tilled. These loose materials can be partially hardened in place, or they can be encased in protective materials before being removed from the soil. Murphy (1986) and Camuti and McGuire (1999) gave step-by-step instructions for such procedures. The sample is first surrounded by a frame while it is in the soil. It is then covered and partially impregnated with a cementing agent such as cellulose acetate, plaster of Paris, sodium silicate, a polyester resin, or similar material. After the cement has hardened, the sample can be removed, labeled, and wrapped as previously described. Goldberg and MacPhail (2003) discussed a variety of materials that can be used for collecting intact archeological samples, including use of masking tape, plastic wrap, and rain-gutter downspouting. These same concepts can be applied in routine soil investigations.

Of the impregnating materials available, cellulose acetate has the advantage of dissolving in the polyester resin that is used to make the thin section. This allows the sample to be dried and impregnated as a regular sample in the laboratory. Use of the other materials can prevent further impregnation with polyester resin. This may not be a problem for some studies where finished thin sections are not needed. For example, plaster of Paris can be used to impregnate materials containing numerous large pores such as found in recently tilled Ap horizons (FitzPatrick et al., 1985). Most pores will be filled with plaster, and its white color seen against the darker soil makes pore patterns clearly visible.

Sample Documentation, Transport, and Storage

Before leaving a sampling pit, all samples should be checked to be sure they are properly labeled and bagged. The profile description should be reviewed for any omissions. Sketches of the profile wall should be made (or photographs taken), showing sample locations with respect to horizons and special features. Site data may also be required, such as detailed geographic location, landscape position, land use, etc. Before transport, samples should be placed in boxes and packed with newspaper or other suitable material to prevent them from bumping against or being crushed by other samples.

In the laboratory, samples should be stored in a cool, dry place, but not frozen. Freezing could alter particle arrangements and form cracks. A temperature of 4°C may be needed to retard the growth of plants or organisms unless other precautions were taken.

PREPARATION OF THIN SECTIONS

Making a thin section requires three basic operations. First, the sample must be free of water. Second, it is impregnated with a resin. Third it is ground to a standard thickness

(frequently 30 μm or less) while bound to a glass slide. Making thin sections is time-consuming and requires expensive sawing and grinding equipment. It is frequently easier to have thin sections made commercially. Before beginning any of these operations, however, it is essential to assign each sample a laboratory number that will be used to identify the finished thin section.

Water-Removal Techniques

Before samples can be fully impregnated with a resin, water must be removed from all soil voids. Water and resin won't mix in most cases, so moist samples cannot be completely impregnated unless samples are dried. Water is commonly removed from micromorphological samples in one of three ways: (i) air- or oven-drying, (ii) acetone replacement, and (iii) freeze-drying. Researchers should be familiar with the advantages and disadvantages of each technique to select the best method to use for a specific study. No single method is suitable for all soils or all investigations.

Air- and Oven-Drying

Samples for many investigations can be dried at room temperature with adequate ventilation or in an oven set at a temperature of 40°C or less, although some laboratories will use temperatures up to 60°C. Air-drying is relatively slow and may require 5 to 8 wk before a constant sample weight is reached in samples high in clay. As the water is removed from pores, the soil water tension increases to the point that soil particles are pulled closer together, and shrinkage cracks may form. The slow drying at room temperature will minimize cracking (FitzPatrick, 1984), but more particles could reorient as drying time is increased (Smart and Tovey, 1982). Oven-drying is faster and works best in sandy-textured soils (Murphy, 1986), but various types of sample alterations may occur at temperatures above 40°C, including sample shrinkage (most severe in soils high in expanding layer clays and organic matter), formation of cracks, clay particle rearrangement and fracture, void closure, crystallization of soluble minerals, dehydration of some salts, such as mirabilite, and increased oxidation of organic matter (Jongerius and Heintzberger, 1975; Smart and Tovey, 1982). Some of these changes can occur at temperatures below 40°C as well, but it is generally believed that slow drying at low temperatures reduces problems such as sample shrinkage and crack formation. It should be noted that when air-drying is used, samples will still need to be dried at 40°C for about 48 h to remove hygroscopic water (unless hygroscopic salts such as mirabilite are present). Air- and oven-drying are probably adequate techniques to use for samples that are low in expanding clays or organic matter, and where soil voids or soluble minerals are not being investigated. The following methods should be used when air- or oven-drying are not appropriate.

Acetone Replacement

Instead of removing water by evaporation, the water can be replaced with acetone through a process of liquid or vapor exchange. In liquid acetone exchange, samples are submerged in an acetone bath, allowing the water to diffuse out of the soil voids and into the acetone solution surrounding the sample (Chartres et al., 1989; Moran et al., 1989; Murphy, 1986). As this solution is changed periodically, all the water in the soil voids will eventually be replaced by acetone. Most polyester-type plastics used to impregnate soils readily mix with acetone, so a sample that is saturated with acetone can be fully impregnated without the acetone in the voids having to be removed. Acetone replacement may be the best technique to use to minimize shrinkage and crack formation and maintain void dimensions. However, soil aggregates that are held together by organic cements may fall

apart when placed in acetone because the cements will be dissolved. Not all organic matter dissolves in acetone, and the method has been used to replace water in peats (Miedema et al., 1974).

Various methods for performing acetone replacement were reviewed by Murphy (1986) and Fox et al. (1993). The fastest method is liquid acetone exchange, in which samples are submerged in a 100% acetone solution. The acetone is slowly poured into the container holding the soil to submerge about one-half of the sample. This lets the soil absorb the mixture by capillary action and wet to the top. Once the acetone has been drawn up into the sample, more acetone is poured into the container to completely cover the samples. The solution is best agitated or stirred, and should be replaced every 3 to 7 d with fresh acetone.

The process is complete when no water is found in the acetone solution. This is checked by placing 20 mL of solution in 80 mL of solvent (e.g., turpentine) and shaking. Any water present will produce bubbles or turbidity in the spirit because the two substances do not mix. A more exact method of detecting the presence of water is to measure the specific gravity of the solution using a pycnometer or hydrometer. Acetone replacement may require anywhere from 2 to 8 wk depending on the amount of clay and organic matter present in the sample, the amount of stirring done, the frequency of solution replacement, and the volume of acetone in the bath relative to the volume of sample. Fox et al. (1993) found that water should be reduced to 1 to 2% in the acetone for the sample to be suitable for impregnation.

Some micromorphology laboratories use a series of graded water–acetone mixtures (e.g., 75:25%, 50:50%, 25:75%, finishing with 100% acetone; FitzPatrick, 1993). Despite the length of time this procedure takes and the difficulty in observing the drying threshold as found in the 100% acetone application, the procedure can prevent the disintegration of the soil structure (S. Kapur and E. Akça, Univ. of Çukurova, Dep. of Soil Science, Adana, Turkey, personal communication, 2005).

Vapor exchange is the most suitable technique to use for soils with large amounts of organic matter or soluble salts. This procedure is performed in a closed desiccator. The sample is held on a metal rack above a pool of liquid acetone that also contains a desiccant, such as anhydrous CaCl_2 . The sample absorbs the acetone vapor and displaces the water that subsequently drips from the sample (Fox et al., 1993; FitzPatrick, 1993).

Special precautions have to be taken during water removal when live tissues (e.g., roots or microorganisms) are present and need to be examined. Fresh roots in soil samples will shrink noticeably if placed directly in a 100% acetone solution (Tippkötter et al., 1986). This problem is minimized if the first bath consists of a 70% acetone solution, and a subsequent bath consists of a 90% solution, before a 100% acetone solution is used. Tippkötter et al. (1986) developed a reliable method for using acetone replacement to dehydrate biological materials. These authors felt that acetone replacement is the best technique to use for drying biological materials. In addition, clayey soils containing expansive clay minerals have had their aggregates disintegrate when exposed to acetone (Chartres et al., 1989). This has been attributed to “massive” interlayer expansion occurring when water is present in the acetone (Brindley et al., 1969). The problem is reduced by use of methanol or dioxane (Chartres et al., 1989). Fox and Parent (1993) described the sampling of organic soils and use of tetramethylene oxide for dehydration.

Freeze-Drying

Freeze-drying begins by dipping the sample into liquid nitrogen (N_2 , -196°C) so the water in the sample is quickly frozen. Large ice crystals are prevented from forming within

the sample under these conditions, and thus the chances of the sample cracking are reduced. After freezing, the samples are transferred to an ice condensator (freeze drier) where the ice is removed by sublimation. The process takes about 1 wk. The ice condensator is an expensive, specialized piece of equipment that may not be available in all laboratories.

Complete step-by-step instructions for the freeze-drying technique were presented by Jongerius and Heintzberger (1975). They note that most samples will crack to some degree, particularly in the center of the sample where the rate of freezing is slower than at the edges. The samples should be <2 cm thick to ensure rapid freezing. Jongerius and Heintzberger recommend freeze-drying for samples that would shrink considerably with air- or oven-drying, and which contain organic materials that might dissolve in acetone. Freeze-drying is probably impractical for materials that can be more easily dried by evaporation or acetone replacement.

Impregnation and Hardening

Impregnating Resins

Materials such as friable soils and sediments must first be hardened by impregnating them with a plastic resin before they can be ground to a thickness of 30 μm or less. The types of plastics commonly used for impregnation include unsaturated polyester resins, epoxies, and methyl methacrylate. The use of each has been thoroughly discussed by Murphy (1986). The resin of choice should be transparent, have a refractive index of about 1.54, be isotropic, and have low viscosity to penetrate the smallest pores, be dimensionally stable, and become hard without becoming brittle. Certain safety precautions are necessary when using resins. Protective clothing and eyewear must be worn during the impregnation process, which must be conducted in a fume hood. Samples must also be stored in the fume hood during the hardening phase that follows impregnation, which might require several weeks.

In simplest terms, an unsaturated polyester resin is a viscous liquid solution of a polyester that consists of chains of carbon and oxygen atoms (multiple ester groups $[\text{CO}-\text{O}-\text{C}]$ with additional reactive sites) in a monomer that is typically styrene. The gelling and subsequent hardening of the resin is the result of the cross-linking of the molecular chains of the polyester by the styrene and formation of macromolecules that form a rigid structure (Jongerius and Heintzberger, 1975; Smart and Tovey, 1982). Polyester resins have a limited storage life because they will gradually polymerize or gel with time. To speed this polymerization process, a hardener is added to the resin. A *hardener* is a general term for any compound added to resins that promotes the curing process. Hardeners often consist of two substances, a catalyst that is added to the resin to initiate the polymerization process, and an accelerator that is added to speed the process. The two components are added to the resin separately because they are explosive when mixed together outside the resin. Many different types of unsaturated polyester resins and hardeners are available, as these products have a wide usage in the industrial and medical fields. In some polyester resins, no accelerator is used to speed the hardening process because hardening is an exothermic reaction and rapid hardening may cause the sample to crack, or not allow enough time for the resin to penetrate the sample.

Polyester resins are too viscous to seep into small soil voids quickly, so they must be diluted with acetone or styrene monomer to lower the viscosity to impregnate materials completely. The quantities of resin, dilutant, and hardener used in an impregnation mixture must be adjusted to allow time for the resin to move into the soil pores before the resin hardens. Specific mixtures are best determined by trial and error to suit a particular mate-

rial. Commonly, 1:1 or 2:1 volumes of resin to dilutant (e.g., acetone) are used with enough hardener to allow approximately 4 to 6 wk before complete hardening occurs. Heating can be used to speed the curing time of resins, although this can leave insufficient time for the resin to completely impregnate the sample. The temperature can be increased after hardening appears complete to harden the plastic further by increasing the cross linking of the polymer molecules. The general rule of thumb (as with many chemical reactions) is that the reaction rate will double for each 10°C increase in temperature.

Although not common, some samples contain chemicals that either prevent hardening or accelerate it (Grieve et al., 2005). Certain dyes, salts, or heavy metals have prevented hardening for up to 1 yr. When this occurs, samples can be hardened overnight by exposing them to about 5 Mrad of gamma radiation (Bisdorf et al., 1983). Other samples contain substances that may cause the resin to harden so fast the plastic is literally fractured to pieces, ruining the sample. If this happens, epoxy resins should be tried. The chemistry of polyester resins is not fully understood, and it is difficult to predict when these hardening problems will occur. For this reason, it is advisable to test a resin mixture on a spare sample first to ensure that proper hardening will occur before the good samples are impregnated.

Epoxy resins are relatively expensive, and partly for this reason they have been used much less than polyester resins in micromorphological studies. One advantage of epoxy resins is that some of the chemicals that delay hardening of polyester resins won't affect epoxy resins to the same extent. Other advantages include a low amount of shrinkage upon curing, a short curing time (48 h) that allows thin sections to be prepared rapidly, and better stability during exposure to electron beams during microprobe studies. The chemistry of epoxy resins is complex and was briefly reviewed by Smart and Tovey (1982). Epoxy resins don't mix with acetone, so samples can't be dried by acetone replacement. However, other solvents such as methanol may be used instead. The viscosity of some epoxies is reduced by heating to 60 to 80°C. The hot resin is then poured directly into the container holding the sample, which is at the same temperature. These temperatures can affect the structures of organic materials and crystallization of salts, so samples containing these components probably should not be impregnated with epoxies requiring high temperatures.

Methacrylate is a third type of material that has been used to impregnate soils. It is used commercially to make hard, clear plastics known as acrylics (e.g., Perspex and Plexiglas; Palle et al., 1985). Use of methyl methacrylate is similar to polyester resins in that a catalyst must be added to begin polymerization. Methyl methacrylate is less viscous than water and does not have to be diluted. It has a long shelf life and is stable once thin sections are prepared. These advantages are offset by high cost, strong foaming, harmful vapors, shrinkage on hardening (which can be up to 20%), and instability in electron beams (Glauert, 1974). In addition, an inhibitor may have to be removed before use, and this is done by washing the methyl methacrylate with sodium hydroxide and water (Murphy, 1986). These disadvantages have resulted in infrequent use of methyl methacrylate, so it won't be discussed further.

Method of Impregnation Using Polyester Resin

Each micromorphology laboratory seems to have its own method of impregnating samples. All methods are similar in principle, but details vary. A procedure will be given here for using unsaturated polyester resin, and additional details can be found in FitzPatrick (1984), Jongerius and Heintzberger (1975), and Murphy (1986). Users should try different techniques until one is found that works best for the samples of interest. Resins produced by different manufacturers (as well as different lots or batches from the same manufacturer) may require differing proportions of hardener. A vacuum chamber is used to aid in

removal of air and allow full penetration of the sample with resin. Vacuum chambers are typically desiccators with a vacuum port. It may not be necessary to use vacuum during the process with sandy samples because pores are large and interconnected enough to allow the plastic to enter without entrapping air (P. Goldberg, personal communication, 2005). Not using a vacuum will also reduce stress on fragile soil pedis.

Impregnation Procedure—Polyester Resin

Materials

- **Unsaturated polyester resin.** General purpose, orthophthalic resin with properties including low viscosity, minimal shrinkage, unpromoted (no accelerator added), nonthixotropic, pale color (no pigments added), good clarity, and a refractive index similar to quartz. Available from many sources including: Advance Coatings, Inc., 42 Depot Rd., Westminister, MA 01473; phone: 1-800-247-1117.
- **Hardener.** Usually consists of both a catalyst (e.g., Methyl Ethyl Ketone Peroxide) and accelerator. It is important not to mix these compounds together before adding to resin. They are explosive when mixed alone. (Note: Some manufacturers may have added the accelerator to the resin).
- **Container.** 250- or 400-mL disposal plastic beaker for intact clods, or disposable aluminum or plastic loaf pans for Kubiena tins (available at supermarkets). Containers should be acetone-resistant and leak-proof. Closely match size of clod or Kubiena tin with container to avoid excess use of resin.
- **Magnetic stirrer and stir bar**
- **Acetone.** Technical grade
- **Vacuum chamber or desiccator with cover attached to vacuum source**
- **Miscellaneous.** Beaker or pitcher, aluminum foil, marking pens, and thin wire.

Safety: Materials used in this procedure may be hazardous if they are inhaled or come in contact with skin. Perform all procedures in a ventilated fume hood, using lab coat, rubber gloves, and safety glasses. Glass desiccators under vacuum can implode and puncture skin with glass fragments. Desiccators should be surrounded by wire mesh or netting. Eye protection must be worn, and face shields are recommended when using vacuum desiccators.

Procedure

1. Carefully unpack the sample. Both the sample container (Kubiena tin or soil fabric) and outside of the container (beaker) should be labeled to show sample number and field orientation. Acetone may dissolve the ink used to label Kubiena tins and containers, and so a pencil should be used.
2. It may be necessary to wrap thin wire around the bottom of the Kubiena tin to keep the sample contained. Transfer the sample to a plastic beaker or container to check the fit. It may be necessary to trim the sample to fit, such that the top of the sample is 2 to 4 cm below the top of the beaker. Also, it may be necessary to line the bottom of the beaker or container with sand or gravel to fill excess space. A layer of plastic may be placed over this coarse layer before transferring the clod or Kubiena tin.
3. Samples should be air- or oven-dry ($\leq 40^{\circ}\text{C}$), or saturated with acetone.
4. The unsaturated polyester resin is diluted into acetone in a 1:1 or 2:1 mixture. Cover the container and thoroughly mix by stirring until there is a uniform appearance. Let the mixture stand or place under low vacuum until air bubbles have escaped. If the

mixture contains clumps, it likely is old and has begun to polymerize, and should be discarded.

5. Add an appropriate quantity of hardener compounds. The catalyst is typically added first, followed by stirring, before the accelerator is added. Use a different pipette for each material. Amounts of hardener are determined in advance to provide adequate time for the resin to fully penetrate the soil.
6. Continue stirring until the solution is well mixed and appears uniform. Avoid incorporation of air during mixing.
7. Place sample container into vacuum chamber or desiccator. Slowly add the resin down the side of the sample container without pouring directly onto the sample. Fill the container until approximately the lower one-half of the sample is submerged.
8. Hold the sample under vacuum for 15 to 30 min to help remove air from the pores. When acetone is used in the resin mixture, increase the vacuum until the acetone begins to boil and then reduce it slightly to stop the boiling. Care should be taken to prevent the acetone boil from disturbing the sample. The resin level will drop around the sample as it penetrates the soil.
9. Repeat the process of adding resin and applying the vacuum treatment two to three additional times, slowing increasing the level of the resin around the sample. After the final addition, the sample should be covered by approximately 2 cm of resin.
10. Keep the sample under a vacuum for a minimum of 2 h and overnight if possible.
11. Remove the sample from the vacuum chamber, add additional *undiluted* resin (with hardener added) to maintain the solution to a depth of 2 cm above the level of the sample.
12. Tightly cover the sample with foil and leave undisturbed for 3 to 7 d in a well-ventilated fume hood. During this period the sample may be placed under vacuum for 30 min to 2 h each day during the week to help remove air.
13. After 1 wk, uncover the samples, allowing acetone to evaporate and hardening to occur. As the solution level falls from evaporation, refill the container with undiluted resin (with catalyst and accelerator added) to keep the resin level above the sample surface.
14. Samples should be left in the hood until hard (4–6 wk). Then transfer them to a vented oven to cure at 40°C for 2 to 3 d.
15. When the sample is hard, remove it from the container. Sticky surfaces can be hardened by exposure to sunlight or ultraviolet (UV) light. Check to ensure that all labels (sample number and orientation) remain.

Method of Impregnation Using Epoxy Resin

Many kinds of epoxy resins are commercially available. As discussed above, they have advantages over polyester resins in that they have a shorter curing time, their viscosity can be reduced by heating, and many epoxy resins shrink little with curing. Their disadvantage is that they are more expensive than polyester resins. It is especially important to lower the moisture content of samples (to <0.1%) for successful embedding and curing of epoxy resins. These resins are hygroscopic and are best used at relative humidity <50% (Pearson, 1977). The process of curing (gel formation to hardness) is exothermic. The amount of heat generated during the curing process is greater in polyester resins than in epoxy resins. Unlike polyester resins, the speed of cure in epoxy resins is controlled by the type, not quantity of accelerator or hardening agent.

Impregnation Procedure—Epoxy Resin

Materials

- **Resins.** Two choices that are currently available include:

3M Scotchcast 3 Electrical Resin (a two-component system, resin and hardener, that are mixed thoroughly before impregnation). Retail suppliers are available at <http://3M.com> (3M, St. Paul, MN) (verified 12 June 2006).

Spurr Low Viscosity Embedding Media (a four-component system, vinylcyclohexene oxide, flexibilizer that functions as the resin, a hardener, and an accelerator), available from Polysciences, Inc. (Warrington, PA) at <http://www.polysciences.com> (verified 12 June 2006).
- **Container.** 250- or 400-mL disposal plastic beaker for intact clods, or disposable aluminum or plastic loaf pans for Kubiena tins (available at supermarkets). Containers should be chemical-resistant and leak-proof. Closely match size of clod or Kubiena tin with container to avoid excess use of resin.
- **Convection oven**
- **Vacuum chamber or desiccator with cover attached to vacuum source**
- **Miscellaneous.** Beaker or pitcher, aluminum foil, marking pens, and thin wire.

Safety: Materials used in this procedure may be hazardous if inhaled or in contact with skin. Perform all procedures in a ventilated fume hood, using lab coat, rubber gloves and safety glasses. Glass desiccators under vacuum can implode and puncture skin with glass fragments. Desiccators should be surrounded by wire mesh or netting. Eye protection must be worn, and face shields are recommended when using these desiccators.

Procedure

1. Carefully unpack the sample. The sample (Kubiena tin or soil fabric) and the outside of the container should be labeled to show sample number and field orientation.
2. It may be necessary to wrap thin wire around the bottom of the Kubiena tin to keep the sample contained. Transfer to a plastic beaker or container to check fit. It may be necessary to trim the sample such that the top of the sample is 2 to 4 cm below the top of the beaker. Also, it may be necessary to line the bottom of beaker or container with sand or gravel to fill excess space.
3. Allow sample to dry for 1 to 2 wk at room temperature. Oven-dry the sample at 60°C for 12 h or more immediately before impregnation. (An alternative to drying at room temperature or oven drying is to dry the samples in a dehydrator at 50 to 60°C for 2 wk.)
4. Stir each resin component in their respective containers before combining. (Note: Do not use acetone to reduce viscosity of epoxy resins.)

Scotchcast 3 epoxy resin: Weigh parts A and B in separate containers in a weight ratio of two parts A to three parts B. Heat both parts separately for 1 to 2 h at 60°C to reduce viscosity, and then mix in a fume hood immediately before impregnation. (Scotchcast resin may be heated up to 95°C to reduce viscosity.)

Spurr's resin: Combine the four components at ratios recommended by the manufacturer. Use exact weight measurements to ensure consistent results. The user may modify the exact amounts to combine to meet their specifications. Combine the first three ingredients and mix well before adding the accelerator (dimethylaminoethanol). Heat the mixture at 50 to 60°C to reduce viscosity before impregnation.

5. Place the sample container into the vacuum chamber or desiccator. Slowly pour the resin down the side of the sample container without pouring directly onto the sample. Fill the container until the lower part of the sample is covered with at least 1 to 2 cm of resin. Allow the resin to penetrate the sample for a few minutes at room temperature and add additional resin to keep sample submerged at least 2 cm.
6. Place the sample in a vacuum desiccator and immediately evacuate to help remove air from the pores. Maintain vacuum for at least 30 min. Vacuum should not be high enough to cause the resin mixture to boil.
7. Turn off the vacuum source and slowly release vacuum. Add an additional aliquot of resin, increasing the depth of the resin in the container to about 3 cm. Return to desiccator, and initiate vacuum for an additional 30 min.
8. Repeat the previous step until sample is 90% covered and air has ceased to be released from the fabric under vacuum (i.e., bubbles entering the resin).
9. Remove samples from the desiccator. Add sufficient epoxy to cover the sample.
10. Place samples back into the oven at 60°C for 12 to 24 h to promote hardening. Remove and check to ensure that all labels (sample number and orientation) remain.

Cutting and Lapping

After hardening, the blocks must be cut to the appropriate size using a rock saw that contains a diamond-impregnated blade and liquid cooling and lubricating system to keep samples from heating excessively during cutting (Fig. 7–5). Use commercial oil-based coolant. Do not use water or water-based coolant, unless required by health and safety regulations, because water can react with the soil material and damage the sample (Murphy, 1986). The first step in sawing samples is to mark the top of each sample with a shallow cut of the saw blade so that the orientation of the sample is known. The sample is then sliced into slabs (about 15-mm thick) having the desired orientation, which are then trimmed to fit the area of the glass slides. Each slab should be examined carefully for sampling-induced cracks and other artifacts, as well as for poor impregnation.

The cut samples must be fully impregnated before they can be polished. A sample may be re-impregnated by cleaning the slab, spreading an undiluted resin mixture over the surface, and placing it under vacuum until the resin seeps into the sample's pores. The resin mixture should have enough hardener added to allow overnight curing, because at this stage of the process the resin does not have to penetrate deeply into the slab.

Once a slab is cut to fit the glass slide, one side of the slab is ground until it is flat and smooth. This grinding can be done by hand or with special machines made for the purpose (Fig. 7–6). The procedure involves polishing a face using progressively finer powders or papers until the scratches are removed and the surface has a mirror-like finish. Grinding powders composed of corundum are considered best, followed by carborundum or silica carbide (G. Stoops, personal communication, 2005). The final grinding is done with a powder of 600 grit (about 9 μm) or finer. In all cases, a sample must be kept well lubricated with oil, water, or methanol during the grinding operation. Water can only be used on some samples because it can cause expanding clay minerals to swell and soluble materials to dissolve, which will ruin the finish on the slab. When grinding is complete, the face is cleaned using a brush and solvent (ethanol) or an ultrasonic cleaner.

Cement the slab to a glass slide using either an epoxy or undiluted polyester when both slab and slide are clean and dry. After the adhesive is applied and the glass set onto the slab, allow it to cure under pressure to produce a uniformly thin bond. The pressure can be applied by weights or a special press (Fig. 7–7). Curing of the glue may require several

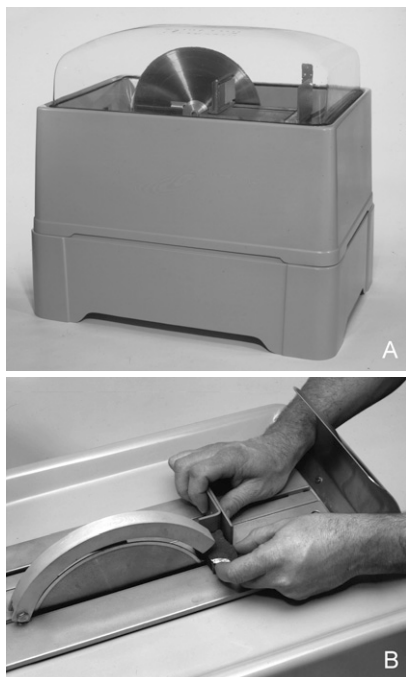


Fig. 7-5. (A) Slab saw used to cut large samples down to size that fit on glass slides. (B) Smaller trim saws are used to cut the chips to the dimensions needed to fit on the glass slide. Photographs courtesy of Hillquist, Inc., Denver, CO.

days for large slabs. As with the impregnating resin, the bonding epoxy should have a refractive index of about 1.54 and be isotropic.

When fully cured, the slide is placed into a special jig and the slab is sawn to a thickness of 1 to 2 mm (Fig. 7-8). The slide is next ground to a uniform thickness of about 40 μm using either a mechanical grinder or hand grinding. A final thickness between 20 and 30 μm is achieved by hand grinding on diamond plates or with powders on a glass plate. A lubricant must be used in both cases. Initial grinding should be done with a powder of about 240 grit (78 μm), with subsequently finer materials (600 grit or finer) used to grind the sample until the target thickness is achieved. The final thickness is reached when the quartz grains show white interference colors between crossed polarizers.

The final step is to either polish the surface of the section to a mirror-like finish using special powders or to overlay it with a cover slip or other protective surface. Certain analytical techniques such as elemental analyses by electron microprobe require polishing and leaving the thin section surface uncovered. Thin sections should be covered when viewed



Fig. 7-6. Polishing machine used to smooth surfaces of chips that will be glued to glass slides. Depending on the model used, grinding materials can be powders or papers that are attached to the wheel. All grinding is done under a slurry, which can be water or a solvent. Photograph courtesy of Hillquist, Inc., Denver, CO.

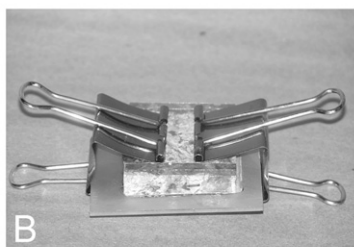
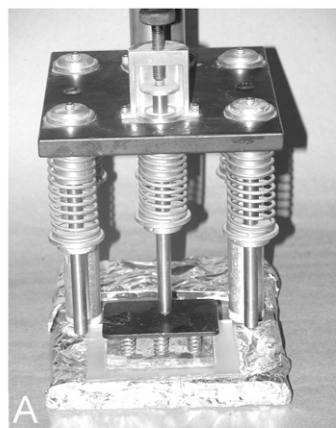


Fig. 7–7. Chips that have been ground smooth on one surface are glued to glass slides. The glued materials cure under pressure to ensure a uniform coating of glue or epoxy covers the entire chip. (A) Curing jigs can be specially designed for this purpose, (B) but simple binding clips are surprisingly effective.

with a light microscope to improve image quality. They can be coated with a thin film of oil, sprayed with a lacquer, or have thin glass sheets (cover slips) glued over them (Murphy, 1986), using the same resin as used for mounting the slab on the support slide. Cover slips come in various sizes and thicknesses. Objective lenses work best with a specific cover slip thickness, and this required thickness is commonly stamped on the objective lens.

DYES AND STAINS

A variety of stains and dyes can be used to help identify and quantify porosity, bacteria, and specific minerals. Some dyes and stains can be mixed with the resins or applied to the uncovered thin section and examined under either short or long-wavelength UV light (10–380 nm). When stained with an aqueous solution of Calcofluor white M_r (Nunan et al., 2001), bacteria will absorb the UV light and fluoresce or exhibit luminescence (Bruneau et al., 2005).

Dyes that exhibit blue, red, or yellow colors under UV radiation can be mixed with resin. This dye-colored resin will illuminate even the smallest pores, allowing pore quantification by image analysis of the fabric (Bresson and Moran, 2004). Droogers et al. (1998) and Minh et al. (1997) used methylene blue (without UV light) to stain pore linings to illustrate flow pathways in columns. Use of fluorescent monodispersed polystyrene microspheres is common in column experiments related to colloid transport and retention (Driese and McKay, 2004). These microspheres are available in different sizes, and the color of fluorescence under long-wave UV light is related to the size of the spheres. They are available from Polysciences, Inc. as Fluoresbrite BB Carboxylate Microspheres and range in size from 0.1 to 6.0 μm . Natural fluorescence in calcite is related to the presence

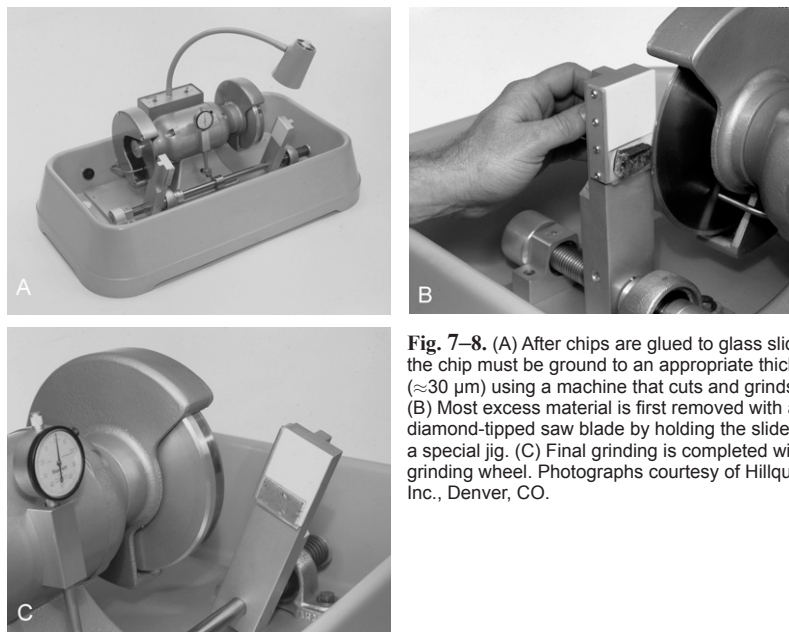


Fig. 7–8. (A) After chips are glued to glass slides, the chip must be ground to an appropriate thickness ($\approx 30 \mu\text{m}$) using a machine that cuts and grinds. (B) Most excess material is first removed with a diamond-tipped saw blade by holding the slide in a special jig. (C) Final grinding is completed with a grinding wheel. Photographs courtesy of Hillquist, Inc., Denver, CO.

of humic substances and possibly trace elements. Khormali et al. (2006) was able to separate pedogenic from geogenic sources of carbonates by examining this natural fluorescence with a specific microscopic light source and filters.

Some chemicals react with specific minerals within the matrix of the thin section and produce a distinctive color or stain that can be used for their identification (Friedman, 1959, 1971; Dickson, 1965; Lindholm and Finkelman, 1972; Houghton, 1980). The dyes and stains are applied to clean, uncovered, etched thin sections and react with specific mineral or organic components. Common chemicals used include (i) sodium cobaltinitrite (stains potassium feldspars bright yellow); (ii) BaCl_2 used with the stain rhodizonate, whereby Ba replaces Ca in the plagioclase structure and the Ba reacts with rhodizonate to produce a red precipitate with the intensity of the color proportional to the amount of Ca in the structure; (iii) alizarin red S, which stains calcite red while dolomite remains unstained; and (iv) potassium ferricyanide, which when applied before alizarin red S will react with Fe in calcite to identify ferrous carbonates.

THIN SECTION DESCRIPTION

Researchers use written text, tables, graphs, figures, or pictures to describe thin sections and summarize observed objects. Some examples of tabular descriptive formats with associated photomicrographs are provided in Alonso et al. (2004), Jongmans et al. (1991), Kemp et al. (1995), and Khormali et al. (2003). The presentations need to be clear, simple, logical, and systematic so other specialists with little knowledge about micromorphology can use the information. The level of detail needed in the description varies with the investigation. A uniform terminology is needed so that specialists from around the world can understand the description. Four major systems proposed over the years have developed terminology and concepts for describing thin sections (Kubiena, 1938; Brewer, 1976; Bullock et al., 1985; FitzPatrick, 1984, 1993; Stoops, 2003). The system presented

in *Guidelines for Analysis and Description of Soil and Regolith Thin Sections* by Stoops (2003) will be used here to illustrate the basic description process. This system represents the latest terminology used to describe soil fabrics and features and is based on the already widely used system of Bullock et al. (1985).

Relationships Between Field and Microscopic Observations

Micromorphological studies span the range between morphological observations in the field and observations of the same fabric at the microscopic level (Scarciglia et al., 2005). Too often gaps exist between observations made in the field and those made under the microscope, with little attempt to bridge these gaps. Field descriptions of, for example, particle-size distribution, structure, mottles, and features of pedogenic origin can also be observed in detail in thin sections. In addition, laboratory analysis of bulk samples of soil materials provides additional data that will increase the understanding of microscopic observations.

To help bridge the gap between the field and microscope, a detailed field (site and pedon) description should be made at the time the soil is sampled so the context of the soil fabric samples can be understood. It is important that the field description of the site and soil, including the supporting laboratory analyses, be available to the micromorphologist when the thin sections are examined. Description and interpretation should be kept separate. A full description of a thin section must precede interpretation of the processes. Most previous systems of description had a genetic bias and have mixed description and interpretation.

Basic Concepts

A basic description of a thin section contains information on the following: general data, microstructure, groundmass, and pedofeatures. The general data section identifies the thin section, site location, landscape position, and other information relative to the specific thin section's location within the set of samples. Even a detailed thin section description is useless without this general information that ties the thin section to a specific site and landscape.

Soil structure is the property formed by the size, shape, and arrangement of primary particles and voids in both aggregated and nonaggregated materials. Structure also includes the size, shape, and arrangement of any aggregates present. Using this concept, no material is considered structureless (Stoops, 2003). The term *microstructure* is used for structures described at magnifications greater than 5 $\times$. The types of microstructure seen in thin section are similar to those commonly described in profile descriptions (Table 7–1). Stoops (2003) has defined additional types that are variations on those that can be seen with the naked eye. The grade of structure frequently described in soil profiles is identified in thin section using a concept called “degree of separation.” Weakly separated peds do not have planes of weakness completely around the peds, while highly separated peds do. Soil horizons with highly separated peds may also be described as having a “strong” grade of structure.

Voids include packing voids, vesicles, channels, vughs, and planes, among others (Fig. 7–9). Sizes of voids are difficult to measure realistically because the thin section only cuts through a portion of the void. For example, unless a tubular channel is cut perpendicular to its long axis it is not possible to measure its horizontal diameter accurately. As was shown in Fig. 7–2, a section perpendicular to the long axis of a channel will produce a circular

Table 7–1. Examples of microstructure commonly seen in thin section. Terminology and definitions are those of Stoops (2003). Many other kinds of microstructure are possible.

Microstructure type	Definition
Single grain	Almost exclusively sand-sized grains; little or no fine material in intergranular spaces, corresponding to a monic <i>c/f</i> related distribution pattern.
Bridged grain	Almost entirely sand-sized grains bridged by fine material, usually clay or organic matter, corresponding to one of the <i>gefuric c/f</i> related distribution patterns.
Channel	Apedal or intrapedal material with channels as dominant voids.
Vughy	Apedal or intrapedal material with noninterconnected vughs and occasional channels and chambers.
Granular	Granules are separated by compound packing voids and do not accommodate each other. They contain few or no voids or recognizable smaller units.
Subangular blocky	Aggregates are separated by short planar voids on all or most sides; aggregate faces largely accommodate each other.
Platy	Stacks of aggregates generally horizontally elongated and separated by planar voids
Massive	No separated peds; few, if any, visible voids

void. Oval voids result when sections through the channel are oriented at directions that are not perpendicular to the long axis.

Groundmass is a general term used for the coarse and fine material forming the base material (matrix) of the soil. *Micromass* is a general term for the finer groundmass. It is used in preference to “matrix,” which has been defined in several different ways in the literature. In addition to the micromass, coarse mineral grains are usually present in the groundmass. Coarser grains can be seen individually and therefore they are described separately from the micromass. The size limit between coarse and fine material (micromass) must be defined for a given thin section, and this boundary has been termed the *c/f limit* by Stoops (2003). Commonly the *c/f* limit is set based on the smallest grain size that can be seen at a given magnification. Values of 2, 10, and 20 μm might be used depending on the

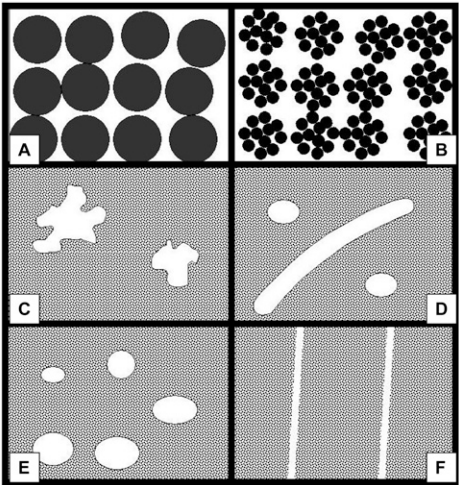


Fig. 7–9. Examples of voids that can be seen in thin section: (A) simple packing voids, (B) compound packing voids, (C) vughs, (D) channels, (E) vesicles, and (F) planes (Stoops, 2003).

soil material. Examples of the related distributions that can be observed between the coarse and fine material are shown in Fig. 7–10.

Individual particles can't usually be seen in the micromass, but if the smaller clay particles are aligned by compression, they form anisotropic zones (visible under cross-polarized light) that appear as a group of particles oriented the same way. The phenomenon is termed the *birefringence fabric* or *b-fabric* (Stoops, 2003). Such b-fabrics are common in the groundmass of soils that contain swelling clays, for example, and develop when swelling pressures in the soil orient the fine material in the groundmass. Other causes of clay orientation result from mass movement, creep, slump, and root pressure (Blevins et al., 1970).

Description of the coarse particles in the groundmass can include their size, shape, and mineralogy. Size measurements have to be interpreted carefully because they are based on a section through a mineral grain that is rarely through the exact center of the grains. As a result, if the size distribution of the material is of interest, then it is probably best determined from a standard particle-size distribution procedure. The degree of weathering of mineral grains can also be described (Stoops et al., 1979; Delvigne, 1998).

Pedofeatures are discrete units that are recognizable from adjacent groundmass by a difference in concentration of one or more components, or by a difference in internal fabric. Pedofeatures are, in short, objects that formed in the soil. Examples of some kinds of pedofeatures are shown in Plate 6–1 (see the color insert). Coatings of clay along voids, nodules of iron oxides, and infilled animal burrows are examples of pedofeatures. Stoops (2003) identified the following principle types of pedofeatures: coatings, infillings, crystals, nodules, intercalations, and excrements. Coatings form by material moving onto a surface. Clay coatings along voids are one of the most common examples of pedofeatures. This translocated clay can be distinguished from residual, stress-oriented clay by location, composition, size homogeneity, and laminar appearance (Nettleton et al., 1969; Nettleton and Wilson, 2004). When soluble material moves across the surface of a channel and precipitates in the matrix, it will form a hypocoating if the newly precipitated material lies adjacent to the void in the matrix, while a quasicoining is formed if the precipitated material lies in the matrix and parallel to the void but not directly in contact with it (Stoops, 2003).

Infillings form by material moving into channels or cracks and filling them, either completely or in part. These can form when illuvial clay fills a channel or crack rather than creating a thin coat along the void's surface. Earthworms moving through channels leave casts behind them that may also create infillings. Crystals and crystal intergrowths refer to material that has precipitated in the soil to form distinct crystals that are usually larger than 20 μm . Nodules and excrements have been frequently described in soils because they can

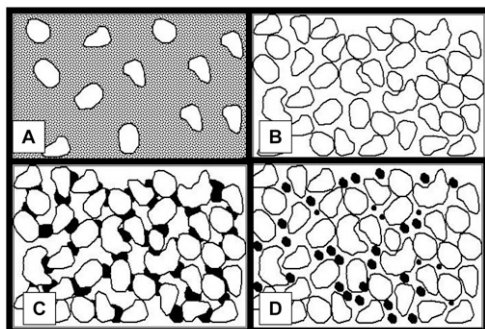


Fig. 7–10. Examples of the related distributions that can be found between coarse and fine material in the groundmass of soils: (A) porphyric (course grains in matrix of finer material), (B) monic (single grain material), (C) gefuric (e.g., grains cemented by fine material), and (D) enaulic (fine material occurs as aggregates in simple packing voids) (Stoops, 2003).

be seen with the naked eye in some cases. Intercalations are concentrations of material in the groundmass that form elongated features; examples of these relatively rare features are given in Stoops (2003).

Description Procedure

Thin section descriptions should begin with an examination of the section with the naked eye. Homogeneous zones can then be identified and examined separately under the microscope. At this stage it is sometimes useful to study the field description to see if items noted earlier are visible in the thin section. Some micromorphologists complete a macromorphological description of the thin section first. This approach helps bridge the gap between the field relationships and micromorphology. The need for maintaining continuity of observations at different scales (macro to submicroscopic) via an established framework was discussed by Protz et al. (1987). These authors suggested using digital images to store data and compare data at different scales, using the pixel unit as the connecting bridge between scales.

It is useful to scan the entire section using different magnifications to see the variation in microstructure, groundmass, and pedofeatures before describing the thin section. When soil profiles have been sampled, it is also useful to quickly scan the thin sections from adjacent horizons to gain an overall view of the material. Begin the description at low magnification (e.g., 2× objective lens) to identify the microstructure and any peds that are present. Set a coarse to fine limit and record the c/f-related distribution; then describe the groundmass within peds. Identify the mineralogy of the coarse grains and characteristics of the coarse organic materials when possible. Describe the micromass by its b-fabric. Describe pedofeatures such as clay coatings. These are usually the first features to catch the eye, but are not necessarily the most important features in the soil. Keys to the identification of the common pedofeatures can be found in the appendices of Stoops (2003), and these should be used when identifying pedofeatures as well as the other components mentioned above.

QUANTIFICATION

Two basic types of data, global data and feature-specific data, can be measured on thin sections (Russ, 1988). Global data pertain to the entire thin section and do not give any information about the sizes of specific features or their orientation. Modal analysis provides one type of global data where the percentages (based on area) of different types of soil components are determined (Table 7–2). Feature-specific data provide detailed information about individual objects such as the sizes or shapes of soil pores. These data can be

Table 7–2. An example of global data obtained for a granite. Five thin sections of the same rock were examined by one analyst, and mean values were determined for the types of mineral present. Mineral amounts are percentages by volume (modified from Chayes, 1956).

Slide	Mineral						
	Quartz	Microcline	Plagioclase	Biotite	Muscovite	Opakes	Nonopakes
	%						
1	24.7	35.6	33.3	3.3	2.0	0.6	0.6
2	26.8	35.7	32.6	3.5	0.4	0.6	0.4
3	28.8	34.2	32.1	3.4	1.1	0.7	0.4
4	27.8	35.0	31.5	3.3	1.0	0.9	0.5
5	26.6	34.5	33.6	3.0	1.4	0.6	0.3
Mean	26.8	35.0	32.6	3.3	1.2	0.7	0.4

summarized in the form of frequency distributions, where the characteristics of an entire population are portrayed (Fig. 7–11).

Different techniques are normally used to obtain global and feature-specific data. Global data are collected by counting objects of different types. This is usually done manually by an operator trained to distinguish the features of interest. On the other hand, feature-specific data are collected by making a large number of detailed measurements on the same type of object present in the thin section (e.g., soil pores). These measurements may be too tedious to do reliably by eye, and so image analysis software is frequently used to make the measurements.

Stereological Relationships

Stereology is the study of three-dimensional objects using only measurements made on two-dimensional sections through the objects. Mathematical equations are normally used to take the data obtained from a two-dimensional section, such as a thin section, and compute a characteristic that relates back to the three-dimensional object that could not be completely studied. The stereological equation that is most commonly used in soil science determines the volume fraction (global data) of objects in a soil sample as shown in Table 7–1. The relationship is

$$V_o/V_t = A_o/A_t \quad [1]$$

where V_o is the volume of the object of interest, V_t is the total volume of the sample, A_o is the area of the object as seen in a thin section, and A_t is the total area of the sample seen in the thin section.

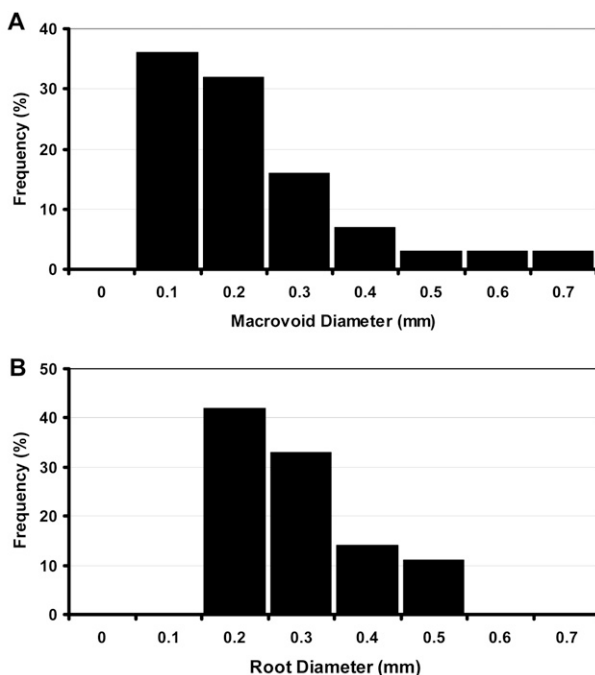


Fig. 7–11. An example of feature-specific data showing distributions of macrovoids and roots that were observed in thin sections made from saprolite.

The equation states that the volume of an object per unit volume of sample is equal to the cross-sectional area of the object per unit area of the sample as seen in a random section through the sample. The shape of the object does not affect its volume fraction. The equation applies to any object, whether it is a large single feature such as a piece of gravel or a large number of quartz sand grains. The derivation of the equation was discussed by Chayes (1956).

Because measuring the total area of an object is tedious, the areas have been reliably estimated by counting points or intersections with lines. Equation [1] can be expanded to:

$$V_o/V_t = A_o/A_t = P_o/P_t = L_o/L_t \quad [2]$$

where P_o is the number of regularly spaced points falling on the object, P_t is the number of regularly spaced points over the sample, L_o is the length of a group of lines intersecting the object, and L_t is the total length of lines drawn over the sample.

Thus, the data obtained from counting points or measuring lines or areas can all be related back to a specific volume. The major requirements for soils and sediments are that (i) the points, lines, and areas are measured over a representative portion of the thin section and (ii) that the section be a random section through the sample. Many features may be assumed to have a random orientation in the soil; however, features along vertically oriented structural cracks will have their volumes underestimated in horizontal thin sections. In a newer approach, Protz et al. (1987) suggested the use of automated section polishing equipment to produce multiple sections from the same block. Through the use of computer imaging, it would be possible to locate and analyze three-dimensional units in the soil.

There are many other stereological relationships available, but few have been used for soils research. A full discussion was provided by Weibel (1979) and Underwood (1970). The major point is that the relatively simple measurements that can be made on thin sections have a fundamental and well-understood relationship to the original sample.

Measurement Techniques

Two measurement techniques that are frequently used to quantify soil features will be discussed. Point counting is a manual method used to acquire global data, while computer image analysis is being used increasingly to make feature-specific measurements.

Point Counting Procedure

Point counting is the most common technique in use to determine the volume fraction of soil components. It is commonly used to verify quantification of a feature via image analysis (Bruneau et al., 2004; Aydemir et al., 2004). The technique consists of placing a grid of regularly spaced points over the sample and then identifying the types of features under each point. There are different ways of forming the point grid. When small slides are examined, they can be placed in a mechanical stage that moves the slide along a line below the objective. The points are formed by a cross-hair in the eyepiece. Features are identified at regular or randomly spaced intervals along each line transect. Numerous parallel line transects of points are normally counted to sample a representative portion of the thin section.

Larger slides are too big for most mechanical stages and alternative procedures for point counting have been developed. FitzPatrick (1984) placed a reference grid on the thin section itself, and then used it to position a grid of intersecting lines in the eyepiece. The eyepiece grid forms the points used to select the features that will be counted. The reference grid on the thin section is used only to position the eyepiece grid.

The locations of the points counted can be selected systematically or at random. Systematic methods using line transects or reference grids are better for soils because they ensure uniform or representative coverage of the sample without favoring one portion of the sample or certain prominent features. It can usually be assumed that the features in the sample are randomly distributed. If they are not, then systematic counting could give misleading results by consistently neglecting certain types of features. Line transects or reference grids can still be used in such cases, but the spacing of the points along the line should be chosen randomly so the points are not equally spaced across the entire sample. For example, a grid could be used to select preliminary sampling positions at 1-cm intervals along the thin section, and then the final point location selected by adding an additional distance, ranging from 0.0 to 0.9 cm, that is chosen by some random means (e.g., drawing these numbers out of a box).

When points are positioned systematically, they should be spaced far enough apart so two adjacent points don't fall within the same feature. Thus, point spacing should be greater than the size of the larger features present. Points that fall on the boundary between two objects can be counted as one-half, each half being given to one of the two objects.

Numbers of Counts

The number of objects counted influences the accuracy of the point counting results. The purpose of point counting is to estimate the true percentage (volume fraction) of a feature in the sample. How close an estimated percentage is to the actual percentage is expressed by the probable error (E) of the count. Probable errors are estimated using the following formula (Galehouse, 1971):

$$E_x = X[P(100 - P)/N]^{1/2} \quad [3]$$

where E_x is the probable error for a given level of confidence, X is a number related to the desired level of confidence, P is the percentage of a given component in the sample, N is the total number of points counted. The value of X is derived from standard tables of critical values of the t distribution for degrees of freedom $= \infty$ (e.g., Snedecor and Cochran, 1989); for example, $X = 2$ for a 95% level of confidence and $X = 0.67$ for a 50% level of confidence.

The use and meaning of the equation are best illustrated with an example. Suppose 300 points are counted in a thin section, and it was found that 30 of the points fell on quartz sand grains. Quartz sand would then comprise 10% of the sample volume. The probable error for the estimate of quartz sand is

$$E_{95} = 2[10(90)/300]^{1/2} = 3.5\% \quad [4]$$

The final estimate for quartz sand is then expressed as $10 \pm 3.5\%$ for the 95% level of confidence. This means that if the slide were examined 100 times, with 300 counts made each time, then the estimate for quartz sand would fall between 6.5 and 13.5% in 95 of the estimates. We assume that the true percentage of quartz sand in the sample also lies somewhere between 7 and 13%. Note that the accuracy of the point counts depends on both the total number of counts made (N) and on the percentage of the component in the sample (P).

In some cases, the probable error is expressed as a relative error, meaning as a proportion of the estimated percentage (P). Brewer (1964) termed this the *probable percentage error*. In the above example, the relative error for quartz sand is $35\% = [(3.5\%/10\%) \times 100]$. The relative errors can be estimated using a modified form of Eq. [3] (Brewer, 1976).

A graph was developed based on Eq. [3] (van der Plas and Tobi, 1965) to allow investigators to estimate the probable errors and relative errors of their point count estimates (Fig. 7–12). Frequently investigators use the graph to determine in advance how many points need to be counted to achieve a given level of accuracy. To do this, the approximate percentage of the feature of interest must be known or estimated in advance. This can be determined approximately from a preliminary count of 300 points or less. Jongmans et al. (1991) used point counts on mineral grains, rock fragments, and illuvial clay coatings in thin sections and counted between 500 and 800 points for each feature for quantification.

Use of Replicate Thin Sections

If replicate thin sections have been prepared, then the total number of counts needed for the desired level of precision can be divided evenly among the replicates (Grossman, 1964; Kelley, 1971). For example, if four replicate slides are prepared from a B horizon to estimate illuviated clay, and 1000 counts are needed to achieve the desired precision, then 250 points should be counted on each slide. The results from all slides can be combined to obtain the final result.

Operator Errors

The accuracy of point counting ultimately depends on the proper identification of features before they are counted. In some cases, two different operators may classify the same feature differently. McKeague et al. (1980) reported that for one thin section of a B horizon the estimates of the percentage of illuvial clay ranged from approximately 2 to 18% among 10 different operators. This gross difference was exceptional and is prob-

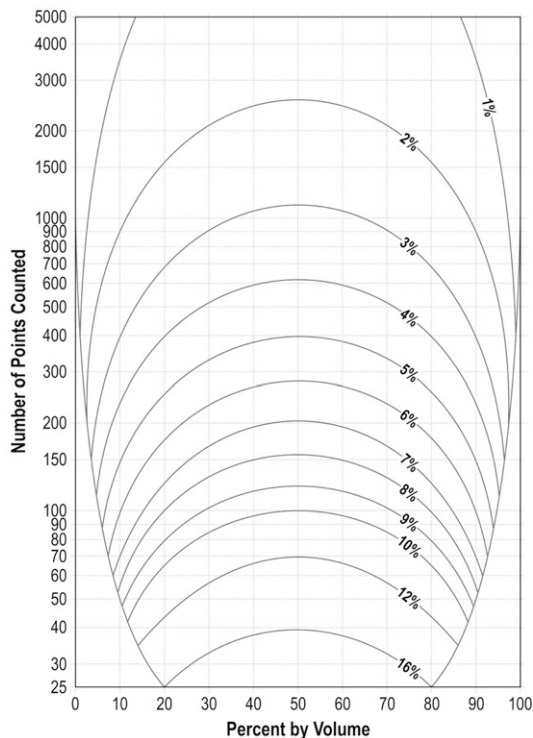


Fig. 7–12. Chart for judging the reliability of point counting results. Curves show absolute percentages for a 95% confidence interval. For example, if 900 points were counted and the estimated concentration of a mineral was 10%, then the actual concentration must be between 8 and 12% (modified from van der Plas and Tobi, 1965).

ably due to the operators disagreeing on what is illuvial clay in a slide that was difficult to interpret. Whatever the actual cause, it is clear that results of point counting by different operators cannot be compared reliably in all cases. The problem can be reduced if special precautions are taken among operators before they make the point count estimates or if the operators agree to criterion as to how to identify features. McKeague et al. (1980) noted that groups of operators who interacted with each other regularly tended to make similar independent estimates of illuvial clay. In short, they identified similar features the same way. A study on quantification of clay illuviation at the University of Gent showed that the magnification used plays a very important role and that only thin sections analyzed at the same magnification can be compared (G. Stoops, personal communication, 2005).

Van der Plas and Slager (1964) recognized that even trained operators that interact with one another develop “bad habits” while point counting that will bias their results. They proposed three rules that should help standardize point count results among operators:

1. Do not let an untrained operator work alone without adequate and frequent control.
2. Let two operators change places while counting, and let them compare their results frequently.
3. Provide a number of standard sample areas that are measured frequently.

Computer Image Analysis

Overview

A detailed discussion of imaging equipment is presented by Vandenbygaert et al. (2008, this volume), and it will only be summarized here. Capturing images of thin sections has always been important for documenting fabric and presenting research information. Features can be identified and measured using differences in their form, shape, and color as seen by comparing photographs taken under both plane and polarized light. Digital images can be used for archiving as well as quantitative analysis. Multiple images of the entire thin section taken under reflected, transmitted, and polarized light can be created using flatbed scanners and stored for future reference (Arpin et al., 2002; Aydemir et al., 2004; Protz and VandenBygaert, 1998). Arpin and Goldberg (2004) discussed the importance of such images at very low magnification in geoarchaeological studies to study mesoscopic features and providing a bridge between field and microscopic observations.

Studies of the size distribution of soil pores frequently use measurements of pore area and perimeter. The shape factor ($\text{area}/\text{perimeter}^2$) has been used to separate voids into different groups such as channels, vughs, and planar voids because all have different shape factors. Jongerius and Bisdom (1981) have also used Feret diameters to further subdivide these void groups. Applications of these and additional measurements for characterizing voids were presented by Bouma et al. (1977), Ismail (1975), Murphy et al. (1977), and Ringrose-Voase and Bullock (1984).

Specimen Preparation for Pore Studies

Soil pore size distribution is frequently quantified using image analyzers. Image analysis works best when the pores are made easily identifiable on impregnated blocks of soils or thin sections prepared from the blocks (also see earlier discussions in “Applications” and “Dyes and Stains” sections). It is essential that the voids be made to contrast with the soil particles as much as possible to distinguish the two features before using an image analyzer. A fluorescent dye can be added to the impregnation solution to achieve high contrast

under UV light. Polished blocks can be photographed with high contrast film under UV light such that thin sections are unnecessary (Murphy, 1986). Because most current camera lenses absorb UV energy, quartz or fluorspar lenses should be used for UV photography. Some digital cameras are available with near UV (320–400 nm) capability, although use of such cameras may require long exposure times, and the images can be affected by atmospheric scattering and image noise.

In soils containing large amounts of organic matter, the fluorescent dyes may be absorbed by the organic material, and so another procedure has to be used to develop contrast between voids and particles (Ismail, 1975). For such conditions, a thin section is first prepared and inserted into a special stage that literally sandwiches the thin section between polarizer and analyzer filters. The thin section is photographed twice, first under plane polarized light and then again under crossed polarizers. In the latter case, the thin section is left in place and not touched, while the polarizer and analyzer are rotated together around the thin section to illuminate all anisotropic minerals. By overlaying and aligning the images from both exposures, the voids are seen to be transparent and the remaining features are either black or gray. This image can also be obtained by simply using circularly polarized light.

In the above procedures, pores that are visible on the photographic images all have diameters $>30\ \mu\text{m}$. To examine smaller pores, photographs must be taken with a scanning electron microscope using a backscattered electron mode that will make the voids appear black (Jongerius and Bisdom, 1981).

REFERENCES

- Alonso, P., C. Dorronsoro, and J.A. Egido. 2004. Carbonation in palaeosols formed on terraces of the Tormes river basin (Salamanca, Spain). *Geoderma* 118:261–276.
- Angelucci, D.E. 2003. Geoarchaeology and micromorphology of Abric de la Cativera (Catalonia, Spain). *Catena* 54:573–601.
- Arpin, T.L., and P. Goldberg. 2004. Using optical microscopy to evaluate human history. *Microsc. Anal.* 18(4):13–15.
- Arpin, T.L., C. Mallol, and P. Goldberg. 2002. Short contribution: A new method of analyzing and documenting micromorphological thin sections using flatbed scanners: Applications in geoarchaeological studies. *Geoarchaeology* 17(3):305–313.
- Artieda, O., and J. Herrero. 2003. Pedogenesis of Lutitic Cr horizons of gypsiferous soils. *Soil Sci. Soc. Am. J.* 67:1496–1506.
- Aydemir, S., S. Keskin, and L.R. Drees. 2004. Quantification of soil features using digital image processing (DIP) techniques. *Geoderma* 119:1–8.
- Babel, U. 1985. Basic organic components, p. 74–87. In P. Bullock et al. (ed.) *Handbook for soil thin section description*. Waine Research Publ., Wolverhampton, UK.
- Bal, L. 1973. Micromorphological analysis of soils. Lower levels in the organization of organic soil materials. Soil Survey Paper 6. Soil Survey Inst., Wageningen, The Netherlands.
- Barratt, B.C. 1969. A revised classification and nomenclature of microscopic soil materials with particular reference to organic components. *Geoderma* 2:257–271.
- Birks, H.J.B., and H.H. Birks. 1980. Quaternary paleoecology. Edward Arnold, Ltd., London.
- Bisdom, E.B.A., A. Boekstein, P. Curmi, P. Lagas, J.P.G. Loch, R. Nauta, and C.B. Wells. 1983. Submicroscopy and chemistry of heavy-metal contaminated precipitates from column experiments simulating conditions in a soil beneath a landfill. *Geoderma* 30:1–20.
- Blevins, R.L., N. Holowaychuk, and L.P. Wilding. 1970. Micromorphology of soil fabric at tree root-soil interface. *Soil Sci. Soc. Am. Proc.* 34:460–465.
- Bouma, J. 1977. Soil survey and the study of water in unsaturated soil. Soil Survey Paper 13. Soil Survey Inst., Wageningen, The Netherlands.
- Bouma, J., and J.L. Anderson. 1973. Relationships between soil structure characteristics and hydraulic conductivity. p. 77–105. In R.R. Bruce et al. (ed.) *Field soil water regime*. SSSA Spec. Publ. 5. SSSA, Madison, WI.
- Bouma, J., A. Jongerius, O. Boersma, A. Jager, and D. Schoonderbeek. 1977. The function of different types of macropores during saturated flow through four swelling soil horizons. *Soil Sci. Soc. J.* 41:945–950.

- Bresson, L.M., and C.J. Moran. 2004. Micromorphological study of slumping in a hardsetting seedbed under various wetting conditions. *Geoderma* 118:277–288.
- Brindley, G.W., K. Wiewora, and A. Wiewora. 1969. Intracrystalline swelling of montmorillonite in some water-organic mixtures. *Am. Mineral.* 54:1635–1644.
- Brewer, R. 1964. *Fabric and mineral analysis of soils*. John Wiley and Sons, New York.
- Brewer, R. 1976. *Fabric and mineral analysis of soils*. Robert E. Kreiger Publ. Co., Huntington, New York.
- Bruneau, P.M.C., D.A. Davidson, and I.C. Grieve. 2004. An evaluation of image analysis for measuring changes in void space and excremental features on soil thin sections in an upland grassland soil. *Geoderma* 120:165–175.
- Bruneau, P.M.C., D.A. Davidson, I.C. Grieve, I.M. Young, and N. Nunan. 2005. The effects of soil horizons and faunal excrement on bacterial distribution in an upland grassland soil. *FEMS Microbiol. Ecol.* 52:139–144.
- Bullock, P. 1983. The changing face of soil micromorphology. p. 1–18. *In* P. Bullock and C.P. Murphy (ed.) *Soil micromorphology*. Vol. I. Techniques and applications. A.B. Academic Publishers, Berkhamsted, U.K.
- Bullock, P., N. Fedoroff, A. Jongerius, G. Stoops, and T. Tursina. 1985. *Handbook for soil thin section description*. Waine Research Publ., Wolverhampton, U.K.
- Buurman, P., P.F. van Bergen, A.G. Jongmans, E.L. Meijer, B. Duran, and B. van Lagen. 2005. Spatial and temporal variation in podzol organic matter studied by pyrolysis-gas chromatography/mass spectrometry and micromorphology. *Eur. J. Soil Sci.* 56:253–270.
- Çambel, H., S. Kapur, C. Karaman, E. Akça, G. Kelling, M. Şenol, Z. Yeğingil, and S. Yaman. 1996. Source determination of the Late Hittite basalt sculptures, reliefs, orthostats and inscriptions at Karatepe–Aslantaş and Domuztepe, Cilicia-southern Anatolia. p. 575–584. *In* The Proceedings of the 29th International Symposium on Archaeometry, Ankara. 9–14 May 1994. TUBITAK, Ankara, Turkey.
- Camuti, K.S., and P.T. McGuire. 1999. Preparation of polished thin sections from poorly consolidated regolith and sediment materials. *Sediment. Geol.* 128:171–178.
- Chadwick, O.A., D.M. Hendricks, and W.D. Nettleton. 1987. Silica in duric soils: I. A depositional model. *Soil Sci. Soc. Am. J.* 51:975–982.
- Chadwick, O.A., and W.D. Nettleton. 1990. Micromorphologic evidence of adhesive and cohesive forces in soil cementation. p. 207–212. *In* L.A. Douglas (ed.) *Soil micromorphology: A basic and applied science*. Proc. VIII Int. Working Meeting on Soil Micromorphology. San Antonio, TX, July 1988. Developments in Soil Science 19. Elsevier, Amsterdam, The Netherlands.
- Chadwick, O.A., and W.D. Nettleton. 1994. Quantitative relationships between net volume change and fabric properties during soil evolution. p. 353–359. *In* A.J. Ringrose-Voase and G.S. Humphreys (ed.) *Soil micromorphology: Studies in management and genesis*. Developments in Soil Science 22. Elsevier, Amsterdam, The Netherlands.
- Chartres, C.J., A.J. Ringrose-Voase, and M. Raupach. 1989. A comparison between acetone and dioxane and explanation of their role in water replacement in undisturbed soil samples. *J. Soil Sci.* 40:849–863.
- Chayes, F. 1956. *Petrographic modal analysis. An elementary statistical appraisal*. John Wiley and Sons, New York.
- Chen, Y., J. Tarchitzky, J. Brouwer, J. Morin, and A. Banin. 1980. Scanning electron microscope observations on soil crusts and their formation. *Soil Sci.* 130:49–55.
- Chiang, S.C., L.T. West, and D.E. Radcliffe. 1994. Morphological properties of surface seals in Georgia soils. *Soil Sci. Soc. Am. J.* 58:901–910.
- Courty, M.A. 2004. Archives in soil microfabrics of environmental perturbations produced by the 4 KYR BP impact in various regions of the world. p. 138–141. *In* Extended Abstracts of the 12th Int. Meeting of Soil Micromorphology, Adana, Turkey. 20–26 Sept. EC-DG-JRC Publication, Ispra, EUR21275 EN/2.
- Courty, M.-A., P. Goldberg, and R. Macphail. 1989. *Soils and micromorphology in archaeology*. Cambridge Univ. Press, Cambridge, U.K.
- Degens, B.P. 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: A review. *Aust. J. Soil Res.* 35:431–459.
- Delvigne, J.E. 1998. *Atlas of micromorphology of mineral alteration and weathering*. Canadian Miner. Spec. Publ. 3. Mineral. Assoc. of Canada, Ontario.
- Dickson, J.A.D. 1965. A modified staining technique for carbonates in thin section. *Nature* 205:587.
- Douglas, L.A., and M.L. Thompson (ed.) 1985. *Soil micromorphology and soil classification*. SSSA Special Publ. 15. SSSA, Madison, WI.
- Driese, S.G., G.M. Ashley, Z.H. Li, V.C. Hover, and R.B. Owen. 2004. Possible late Holocene equato-

- rial palaeoclimate record based upon soils spanning the Medieval Warm Period and Little Ice Age, Loboi Plain, Kenya. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 213:231–250.
- Driese, S.G., and L.D. McKay. 2004. Epi-fluorescence micromorphology of saprolite reveals evidence for colloid retention in microscale pore systems. *Geoderma* 121:143–152.
- Droogers, P., A. Stein, J. Bouma, and G. de Boer. 1998. Parameters for describing soil macroporosity derived from staining patterns. *Geoderma* 83:293–308.
- Dultz, S., and P. Kuhn. 2005. Occurrence, formation, and micromorphology of gypsum in soils from the Central-German chernozem region. *Geoderma* 129:230–250.
- Evans, D.D., and S.W. Buol. 1968. Micromorphological study of soil crusts. *Soil Sci. Soc. Am. Proc.* 32:19–22.
- Fauzi, A.I., and G. Stoops. 2004. Reconstruction of a toposequence on volcanic material in the Honje Mountains, Ujung Kulon Peninsula, West Java. *Catena* 56:45–66.
- FitzPatrick, E.A. 1984. *Micromorphology of soils*. Chapman and Hall Ltd., London.
- FitzPatrick, E.A. 1993. *Soil microscopy and micromorphology*. John Wiley and Sons, New York.
- FitzPatrick, E.A., L.A. Mackie, and C.E. Mullins. 1985. The use of plaster of Paris in the study of soil structure. *Soil use and management* 1:70–72.
- Fox, C.A., R.K. Guertin, E. Kickson, S. Sweeney, R. Protz, and A.R. Mermut. 1993. Micromorphological methodology for inorganic soils. p. 683–709. *In* M.R. Carter (ed.) *Soil sampling and methods of analysis*. Lewis Publ., Boca Raton, FL.
- Fox, C.A., and L.E. Parent. 1993. Micromorphological methodology for organic soils. p. 473–485. *In* M.R. Carter (ed.) *Soil sampling and methods of analysis*. Lewis Publ., Boca Raton, FL.
- Fox, D.M., R.B. Bryan, and C.A. Fox. 2004. Changes in pore characteristics with depth for structural crusts. *Geoderma* 120:109–120.
- Friedman, G.M. 1959. Identification of carbonate minerals by staining methods. *J. Sediment. Petrol.* 29:87–98.
- Friedman, G.M. 1971. Staining. p. 511–530. *In* R.E. Carver (ed.) *Procedures in sedimentary petrology*. John Wiley and Sons, New York.
- Fritsch, E., C.R. Montes-Laur, R. Boulet, A.J. Melfi, E. Balan, and P. Magat. 2002. Lateritic and redoximorphic features in a faulted landscape near Manaus, Brazil. 2002. *Eur. J. Soil Sci.* 53:203–217.
- Galehouse, J. 1971. Point counting. p. 385–407. *In* R.E. Carver (ed.) *Procedures in sedimentary petrology*. Wiley-Interscience, New York.
- Glauert, A.M. 1974. The high voltage electron microscope in biology. *J. Cell Biol.* 63:717–748.
- Goldberg, P. 1983. Applications of micromorphology in archaeology. p. 139–150. *In* P. Bullock and C.P. Murphy (ed.) *Soil micromorphology*. Vol. I. Techniques and applications. A.B. Academic Publ., Berkhamsted, U.K.
- Goldberg, P., and R.I. MacPhail. 2003. Short contribution: Strategies and techniques in collecting micromorphological samples. *Geoarchaeology* 18:567–570.
- Grieve, I.C., D.A. Davidson, and P.M.C. Bruneau. 2005. Effects of liming on void space and aggregation in an upland grassland soil. *Geoderma* 125:39–48.
- Griffiths, J.C. 1967. *Scientific method in the analysis of sediments*. McGraw-Hill, Inc., New York.
- Grossman, R.B. 1964. Composite thin sections for estimation of clay-film volume. *Soil Sci. Soc. Am. Proc.* 28:132–133.
- Herrero, J., and J. Porta. 2000. The terminology and the concepts of gypsum-rich soils. *Geoderma* 96:47–61.
- Holliday, V.T., and J.E. Rawling III. 2006. Soil-geomorphic relations of lamellae in eolian sand on the high plains of Texas and New Mexico. *Geoderma* 131(1–2):154–180.
- Horbe, A., M. Horbe, and K. Suguio. 2004. Tropical Spodosols in northeastern Amazonas State, Brazil. *Geoderma* 119:55–68.
- Houghton, H.F. 1980. Refined techniques for staining plagioclase and alkali feldspars in thin section. *J. Sediment. Petrol.* 50:629–631.
- Hseu, Z., Z. Chen, and Z. Wu. 1999. Characterization of placic horizons in two subalpine forest Inceptisols. *Soil Sci. Soc. Am. J.* 63:941–947.
- Ismail, S.N. 1975. Micromorphic soil-porosity characterization by means of electro-optical image analysis (Quantimet 720). *Soil Survey Paper* 9. Soil Survey Inst., Wageningen, The Netherlands.
- Jongerius, A., and E.B.A. Bisdorff. 1981. Porosity measurements using the Quantimet 720 on back-scattered electron scanning images of thin sections of soils. p. 207–216. *In* E.B.A. Bisdorff (ed.) *Submicroscopy of soils and weathered rocks*. Centre for Agricultural Publication and Documentation, (Pudoc), Wageningen, The Netherlands.
- Jongerius, A., and G. Heintzberger. 1975. Methods in soil micromorphology. A technique for the

- preparation of large thin sections. Soil Survey Inst., Wageningen, The Netherlands.
- Jongmans, A.G., T.C.J. Feijtel, R. Miedema, N. van Breemen, and A. Veldkamp. 1991. Soil formation in a Quaternary terrace sequence of the Allier, Limagne, France. Macro- and micromorphology, particle size distribution, chemistry. *Geoderma* 49:215–239.
- Jongmans, A.G., M.M. Pulleman, M. Balabane, F. van Oort, and J.C.Y. Marinissen. 2003. Soil structure and characteristics of organic matter in two orchards differing in earthworm activity. *Appl. Soil Ecol.* 24:219–232.
- Josephs, R.L., and E.A. Bettis. 2003. Short contribution: A practical alternative to Kubiena boxes for the collection of samples for micromorphological analysis. *Geoarchaeology* 18:567–570.
- Kaczorek, D., M. Sommer, I. Andruschkewitsch, L. Oktaba, Z. Czerwinski, and K. Stahr. 2004. A comparative micromorphological and chemical study of “Raseneisenstein” (bog iron ore) and “Ortstein”. *Geoderma* 21:83–94.
- Kapur, S., C. Karaman, E. Akca, M. Aydin, U. Dinc, E.A. FitzPatrick, M. Pagliai, D. Kalmar, and A.R. Mermut. 1997. Similarities and differences of the spheroidal microstructure in Vertisols from Turkey and Israel. *Catena* 28:297–311.
- Kapur, S., N. Sakarya, E.A. FitzPatrick, M. Pagliai, G. Kelling, E. Akca, C. Karaman, and B. Sakarya. 1998. Mineralogy and micromorphology of Iznik ceramics. *Anatolian Studies. J. Brit. Inst. Archaeol.* Ankara 48:181–189.
- Kapur, S., N. Sakarya, C. Karaman, E.A. FitzPatrick, and M. Pagliai. 1995. Micromorphology of basaltic ceramics. *British Ceramics Trans.* 94:33–37.
- Kelley, J.C. 1971. Mathematical analysis of point count data. p. 409–425. *In* R.E. Carver (ed.) *Procedures in sedimentary petrology*. Wiley-Interscience, New York.
- Kemp, R.A., E. Derbyshire, M. Xingmin, C. Fahu, and P. Baotian. 1995. Pedosedimentary reconstruction of a thick loess-paleosol sequence near Lanzhou in north-central China. *Quat. Res.* 43:30–45.
- Khormali, F., A. Abtahi, S. Mahmoodi, and G. Stoops. 2003. Argillic horizon development in calcareous soils of arid and semiarid regions of southern Iran. *Catena* 53:273–301.
- Khormali, F., A. Abtahi, and G. Stoops. 2006. Micromorphology of calcitic features in highly calcareous soils of Fars Province, Southern Iran. *Geoderma* 132:31–46.
- Krumbein, W. C., and F. A. Graybill. 1965. *An introduction to statistical models in geology*. McGraw-Hill, Inc., New York.
- Kooistra, M.J., and L.I. Kooistra. 2003. Integrated research in archaeology using soil micromorphology and palynology. *Catena* 54:603–617.
- Kubiena, W.L. 1938. *Micropedology*. Collegiate Press, Ames, IA.
- Lado, M., and J. Ben-Hur. 2004. Soil mineralogy effects on seal formation, runoff, and soil loss. *Appl. Clay Sci.* 24:209–224.
- Lima, H.N., C.E.R. Schaefer, J.W.V. Mello, R.J. Gilkes, and J.C. Ker. 2002. Pedogenesis and pre-Colombian land use of “Terra Preta Anthrosols” (“Indian black earth”) of Western Amazonia. *Geoderma* 110:1–17.
- Lindbo, D.L., F.E. Rhoton, W.H. Hudnall, N.E. Smeck, J.M. Bigham, and D.D. Tyler. 2000. Frigipan degradation and nodule formation in Glossic Fragiudalfs of the lower Mississippi River valley. *Soil Sci. Soc. Am. J.* 64:1713–1722.
- Lindholm, R.C., and R.B. Finkelman. 1972. Calcite staining: Semiquantitative determination of ferrous iron. *J. Sediment. Petrol.* 42:239–242.
- McIntyre, D.S. 1958. Permeability measurements of soil crusts formed by raindrop impact. *Soil Sci.* 85:185–189.
- McKay, L.D., S.G. Driese, K.H. Smith, and M.J. Vepraskas. 2005. Hydrogeology and pedology of saprolite formed from sedimentary rock, eastern Tennessee, USA. *Geoderma* 126:27–45.
- McKeague, J.A., R.K. Guertin, K.W.G. Valentine, J. Belisle, G.A. Bourbeau, A. Howell, W. Michalyna, L. Hopkins, F. Page, and L.M. Bresson. 1980. Estimating illuvial clay in soils by micromorphology. *Soil Sci.* 129:386–388.
- Mermut, A.R., L. Montanarella, E.A. FitzPatrick, H. Eswaran, M. Wilson, E. Akca, M. Serdem, B. Kapur, A. Öztürk, T. Tamagnini, M.A. Çullu, and S. Kapur. 2004. *Soil micromorphology excursion book*. 12th Int. Meeting of Soil Micromorphology. EC-DG-JRC Publication, Ispra, EUR 21275 EN/1.
- Miedema, R. 1997. Applications of micromorphology of relevance to agronomy. *Adv. Agron.* 59:119–169.
- Miedema, R., T. Pape, and G.J. van der Waal. 1974. A method to impregnate wet soil samples, producing high quality thin sections. *Neth. J. Agric. Sci.* 22:37–39.
- Milfred, C.J., F.D. Hole, and J.H. Torrie. 1967. Sampling for pedographic modal analysis of an argillic horizon. *Soil Sci. Soc. Am. Proc.* 31:244–247.

- Minh, L.Q., T.P. Tuong, H.W.G. Booltink, M.E.F. van Mensvoort, and J. Bouma. 1997. Bypass flow and its role in leaching of raised beds under different land use types on an acid sulfate soil. *Agric. Water Manage.* 32:131–145.
- Moran, C.J., A.B. McBratney, A.J. Ringrose-Voase, and C.J. Chartres. 1989. A method for the dehydration and impregnation of clay soil. *J. Soil Sci.* 40:569–575.
- Murphy, C.P. 1986. Thin section preparation of soils and sediments. A.B. Academic Publ., Berkhamsted, U.K.
- Murphy, C.P., and C.F. Banfield. 1978. Pore space variability in a sub-surface horizon of two soils. *J. Soil Sci.* 33:156–166.
- Murphy, C.P., P. Bullock, and R.H. Turner. 1977. The measurement and characterization of voids in soil thin sections by image analysis. Part I. Principles and techniques. *J. Soil Sci.* 28:498–508.
- Nettleton, W.D., K.W. Flach, and B.R. Brasher. 1969. Argillic horizons without clay skins. *Soil Sci. Soc. Am. Proc.* 33:121–125.
- Nettleton, W.D., and M.A. Wilson. 2004. Procedure for description and interpretation of soil micromorphology as seen in thin sections. *In* R. Burt (ed.) *Soil survey laboratory methods manual*. Soil Survey Investigative Rep. 42. Ver. 4.0. USDA-NRCS, Washington, DC.
- Nordt, L.C., L.P. Wilding, W.C. Lynn, and C.C. Crawford. 2004. Vertisol genesis in a humid climate of the coastal plain of Texas, USA. *Geoderma* 122:83–102.
- Norton, L.D., and S.L. Schroeder. 1987. The effects of various cultivation methods on soil loss: A micromorphological approach. p. 431–436. *In* N. Fedoroff et al. (ed.) *Soil micromorphology*. French Assoc. Soil Studies, Plaisir.
- Nunan, N., K. Ritz, D. Crabb, K. Harris, C. Wu, J.W. Young, and I.M. Young. 2001. Quantification of the in situ distribution of soil bacteria by large-scale imaging of thin sections of undisturbed soil. *FEMS Microbiol. Ecol.* 36:67–77.
- Onofiok, O., and M.J. Singer. 1984. Scanning electron microscope studies of surface crusts formed by simulated rainfall. *Soil Sci. Soc. Am. J.* 48:1137–1143.
- Pachepsky, Y.A., and W.J. Rawls. 2003. Soil structure and pedotransfer functions. *Eur. J. Soil Sci.* 54:443–451.
- Palle, S., D. Chappard, C. Alexandra, and J.B. Orsini. 1985. Friable rock embedding in methyl methacrylate for thin-sectioning in geological preparations. *J. Microsc.* 141:193–198.
- Pearson, C.B. 1977. Better epoxy resin embedding for electron microscopy at low relative humidity. *Stain Technol.* 52:5.
- Protz, R., M.J. Shipitalo, A.R. Mermut, and C.A. Fox. 1987. Image analysis of soils—Present and future. *Geoderma* 40:115–125.
- Protz, R., and A.J. van der Bygaart. 1998. Towards systematic analysis in the study of soil micromorphology. *Sci. Soils* 3:4. Available at <http://hintze-online.com/sos/> (verified 7 June 2006).
- Pulleman, M.M., J. Six, A. Uyl, J.C.Y. Marinissen, and A.G. Jongmans. 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. *Appl. Soil Ecol.* 29:1–15.
- Ringrose-Voase, A., and P. Bullock. 1984. The automatic recognition and measurement of soil pore types by image analysis and computer programs. *J. Soil Sci.* 35:673–684.
- Russ, J.C. 1988. Computer assisted microscopy (The measurement and analysis of images). North Carolina State Univ., Engineering Extension Education Div., Raleigh.
- Sakarya, N., E.A. FitzPatrick, G. Kelling, E. Akça, B. Sakarya, and S. Kapur. 2004. Microstructural peculiarities in historical Turkish ceramics. p. 197–199. *In* Extended Abstracts of the 12th Int. Meeting of Soil Micromorphology. EC-DG-JRC, Publication, Ispra, EUR 21275 EN/2.
- Scarciglia, F., E. Le Pera, and S. Critelli. 2005. Weathering and pedogenesis in the Sila Grande Massif (Calabria, South Italy): From field scale to micromorphology. *Catena* 61:1–29.
- Schiegl, S., P. Goldberg, H. Pfrezschner, and N.J. Conrad. 2003. Paleolithic burnt bone horizons from the Swabian Jura: Distinguishing between in situ fireplaces and dumping areas. *Geoarchaeology* 18:541–565.
- Shahack-Gross, R., F. Marshall, K. Ryan, and S. Weiner. 2004. Reconstruction of spatial organization in abandoned Maasai settlements: Implications for site structure in the pastoral Neolithic of East Africa. *J. Archaeol. Sci.* 31:1395–1411.
- Smart, P., and N.K. Tovey. 1982. *Electron microscopy of soils and sediments: Techniques*. Clarendon Press, Oxford, U.K.
- Snedecor, G.W., and W.G. Cochran. 1989. *Statistical methods*. 8th ed. Iowa State Univ. Press, Ames.
- Soil Survey Staff. 1999. *Soil taxonomy: A basic system for making and interpreting soil surveys*. 2nd ed. USDA-NRCS Agric. Handb. 436. U.S. Gov. Print. Off., Washington, DC.
- Stoops, G. 2003. *Guidelines for analysis and description of soil and regolith thin sections*. SSSA, Madison, WI.

- Stoops, G., H.-J. Altemüller, E. B. A. Bisdorf, J. Devigne, V. V. Dobrovolsky, E. A. FitzPatrick, G. Paneque, and J. Sleeman. 1979. Guidelines for the description of mineral alterations in soil micromorphology. *Pedologie* 29:121–135.
- Stoops, G., and H. Eswaran (ed.) 1986. *Soil micromorphology*. Van Nostrand Reinhold Co., New York.
- Tippkotter, R., K. Ritz, and J.F. Darbyshire. 1986. The preparation of soil thin sections for biological studies. *J. Soil Sci.* 37:681–690.
- Trouse, A.C., Jr. 1971. Soil conditions as they affect plant establishment, root development, and yield. p. 265–267. *In* K.K. Barnes et al (ed.) *Compaction of agricultural soils*. ASAE, St. Joseph, MI.
- USDA-NRCS. 2002. Field indicators of hydric soils of the United States. Version 5.0. G.W. Hurt, P.M. Whited, and R.F. Pringle (ed.) USDA-NRCS in cooperation with the National Technical Committee for Hydric Soils, Fort Worth, TX.
- Underwood, E.E. 1970. *Quantitative stereology*. Addison-Wesley, Reading, MA.
- van der Plas, L., and S. Slager. 1964. A method to study the distribution of biopores in soils. p. 411–419. *In* A. Jongerius (ed.) *Soil micromorphology*. Proceedings of the second international working-meeting on soil micromorphology, Arnhem, The Netherlands. 22–25 Sept. 1964. Elsevier, Amsterdam.
- van der Plas, L., and A.C. Tobi. 1965. A chart for judging the reliability of point counting results. *Am. J. Sci.* 263:87–90.
- Vandenbygaert, A.J., R. Protz, and C. Duke. 2008. Digital image analysis of soil micromorphology. p. 227–234. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis*. Part 5. Mineralogical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- van Mourik, J.M. 2003. Life cycle of pollen grains in modern humus forms of young acid forest soils: A micromorphological approach. *Catena* 54:651–663.
- van Oort, F., A.G. Jongmans, and A.M. Jaunet. 1994. The progression from optical light microscopy to transmission electron microscopy in the study of soils. *Clay Miner.* 29:247–254.
- Vepraskas, M.J., M.T. Hoover, and J. Bouma. 1991. Sampling strategies for assessing hydraulic conductivity and water-conducting voids in saprolite. *Soil Sci. Soc. Am. J.* 55:165–170.
- Vepraskas, M.J., A.G. Jongmans, M.T. Hoover, and J. Bouma. 1991. Hydraulic conductivity of saprolite as determined by channels and porous groundmass. *Soil Sci. Soc. Am. J.* 55:932–938.
- Webster, R. 1977. *Quantitative and numerical methods in soil classification and survey*. Clarendon Press, Oxford, U.K.
- Weibel, E.R. 1979. *Stereological methods*. Vol. 1. Academic Press, London.
- West, L.T., J.M. Bradford, and L.D. Norton. 1990. Crust morphology and infiltrability in surface soils from the southeast and midwest U.S.A. p. 107–113. *In* L.A. Douglas (ed.) *Soil micromorphology: A basic and applied science*. Developments in Soil Science 19. Elsevier, Amsterdam, The Netherlands.
- Wilding, L.P., and L.R. Drees. 1983. Spatial variability and pedology. *In* L.P. Wilding et al. (ed.) *Pedogenesis and soil taxonomy*. I. Concepts and interactions. Developments in Soil Science 11A. Elsevier, Amsterdam, The Netherlands.
- Wilding, L.P., and K.W. Flach. 1985. Micropedology and soil taxonomy. p. 1–16. *In* L.A. Douglas and M.L. Thompson (ed.) *Soil micromorphology and soil classification*. SSSA Spec. Publ. 15. SSSA, Madison, WI.
- Williams, J.P., and M.J. Vepraskas. 1994. Solute movement through quartz-diorite saprolite containing quartz veins and biological macropores. *J. Environ. Qual.* 23:810–815.
- Wu, S., and Z. Chen. 2005. Characteristics and genesis of Inceptisols with placic horizons in the sub-alpine forest soils of Taiwan. *Geoderma* 125:331–341.

Digital Image Analysis of Soil Micromorphology

A. J. VANDENBYGAART, Agriculture & Agri-food Canada, Ottawa, Ontario

R. PROTZ, deceased, formerly University of Guelph, Ontario, Canada

C. DUKE, Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada

Micromorphology in soil and earth sciences can be broadly defined as the study of undisturbed soils and sediments at the microscopic level (Courty et al., 1989). In soil science micromorphology is “concerned with the description, interpretation and, to an increasing extent, the measurement of components, features and fabrics in soils at a microscopic level” (Bullock et al., 1985). As a result of advances in digital imaging, remote sensing tools, and computer processing power in the past few decades, quantitative digital image analysis of soil micromorphology has progressed such that entire systems for analysis can be readily built. With respect to soil micromorphology, *digital image analysis* can be defined as a system or series of operations performed on a digital image acquired from soil thin sections or three-dimensional blocks to quantify characteristics of the image, detect and measure the morphology and structure, and/or adjust the image in such a way to allow easier interpretation. This chapter briefly describes a complete system developed for the quantitative image analysis of soil micromorphology.

BACKGROUND

The first uses of automated image analysis of soil thin sections occurred in the early 1970s with the work of Jongerius and coworkers on the Quantimet 720 (Cambridge Instruments Limited, Royston, Herts, UK) (Jongerius et al., 1972). Much work since then has focused on measurements of the size, shape, and distribution of soil pores on thin sections and soil blocks (Ringrose-Voase and Bullock 1984; Drees et al., 1994; VandenBygaart et al., 1999). Image analysis techniques have also been useful for quantifying faunal excremental pedofeatures (Pawluk 1987; VandenBygaart et al., 2000; Bruneau et al., 2004). Terribile and Fitzpatrick (1992) used digital image analysis to distinguish and quantify pedofeatures such as clay coatings, along with pores and quartz grains. Sweeney et al. (1992) described the pedological components of redoximorphic pedofeatures in a soil profile using quantitative analysis multispectral images. They were able to quantify faint and prominent mottling and manganiferous concretions diagnostic of Gleysolic soils in Ontario, Canada. Dekayir and El Maataoui (2000) used digital image processing techniques to characterize the weathering of Quaternary alkali basalts from Morocco. They used backscattered electron image analysis to characterize and quantify proportions of primary minerals, clays,

and pore space in different stages of weathering. Knight et al. (2002) utilized energy-dispersive X-ray spectrometry image analysis for quantitative mineralogical analysis of soil samples from Canada. Ten elements (Si, Al, K, Mg, Na, Ca, Fe, Ti, Mn, and S) were used to represent key components of common rock-forming and secondary soil minerals. Each of the elements was mapped as a gray-scale image and quantified by image analysis.

As imaging and computer technologies have evolved, multispectral and multisource image analysis of soil thin sections has also progressed (Protz et al., 1992; Protz and VandenBygaart 1998; Aydemir et al., 2004). The great advantage of such systems is the ability to interpret soil properties and processes on the basis of multiple spatially registered data sources. These sources include (not exclusively) spatially registered data from transmitted, reflected, and circularly polarized visible light (Protz and VandenBygaart, 1998), ultraviolet transmitted and reflected light (Mooney et al., 1998; VandenBygaart et al., 2000), electron backscatter images (Deleporte et al., 1997), and X-ray maps (Cossio and Borghi, 1998). In this chapter we describe an image analysis system built for the multispectral and multisource image analysis of soils in thin section or blocks situated at the University of Guelph, Canada.

Multiscale Analysis and Applications

Scales of analysis in imaging of soils can range from submicroscopic (e.g., Wierzbos, 1997) under soil thin section to the macroscopic (e.g., VandenBygaart et al., 1999) on soil blocks. Tovey et al. (1992a, 1992b, 1994) quantitatively assessed the microfabric and microporosity of soil thin sections. Ringrose-Voase and Bullock (1984) introduced the first automated method for identifying soil macropore types by image analysis in soil, while Pagliai et al. (1981, 1983a, 1983b, 1989) were the first to use the micromorphology of soil pores quantified by image analysis as a tool for assessing management impacts on soil structure.

More recently, multispectral assessment of soils has become more easily attainable through the decreasing costs of imaging and computer equipment (Protz and VandenBygaart 1998). Adderley et al. (2002) developed a system to convert colors quantified by the image analyzer into soil Munsell color values. They emphasized that a uniform soil thin section and accurate thickness are critical for universal application of such a system. Aydemir et al. (2004) used a single illumination source on soil thin sections and unsupervised classification to automatically measure pedofeatures. When compared with point counting, the automated system was very accurate in quantifying calcite, voids, sesquioxides and plasma (>95% accurate). However, quartz was only accurately identified and measured 80% of the time (Aydemir et al., 2004). An obvious next step after building an accurate imaging system is to assess the spatial distribution of soil minerals for studies of soil processes.

METHODS

Soil Block and Thin Section Preparation

Undisturbed soil samples are typically collected in Kubiena tins from carefully prepared soil pit walls (McKeague et al., 1987). In the laboratory, the samples are usually impregnated with polyester or epoxy resin which on curing hardens to a solid block for cutting and thin section mounting (Murphy 1986). The hardened blocks are then cut into sections (usually approximately 1 cm thick) with a rock saw and prepared as polished sections with successively finer grades of aluminum oxide grit on a lapping wheel (Protz et al., 1992). In some circumstances the use of resins containing ultraviolet fluorescent dye (e.g.,

methylene blue or Uvitex-OB dye) can be useful for aiding the computer in distinguishing pores from matrix and quartz grains.

Precautions are required when using polyester or epoxy resin impregnation and acetone. When handling and working with resins and, in particular, using acetone to liquefy the resin, users should wear clean, long-sleeve, long-leg clothing, rubber gloves, and safety glasses. The work area should have adequate ventilation and preferably work should be completed under a fume hood. The applicable Material Safety Data Sheet must be followed. Additional information about sample preparation is available in Chapter 7 (Vepraskas and Wilson, 2008, this volume).

Building the Lighting and Imaging System

A simple imaging system for soil thin sections can be built from readily available materials. It can consist of a digital camera, a camera mount for bird's-eye-view imaging, a uniform light-emitting table, light-polarizing sheets, and thin section stabilizing materials (Fig. 8-1). Thin sections can be imaged with a high-resolution, commercially available digital camera. Figure 8-1 shows the apparatus for imaging soil thin sections and blocks, the arrangement of transmitting and reflecting light sources, the location of the linear polarizer plus 1/4 wavelength (circular polarizers), and the camera. Once a thin section is in place it remains static while an external switch changes the light source. This ensures that any multiple images taken will be completely registered between one another. A fiber-op-

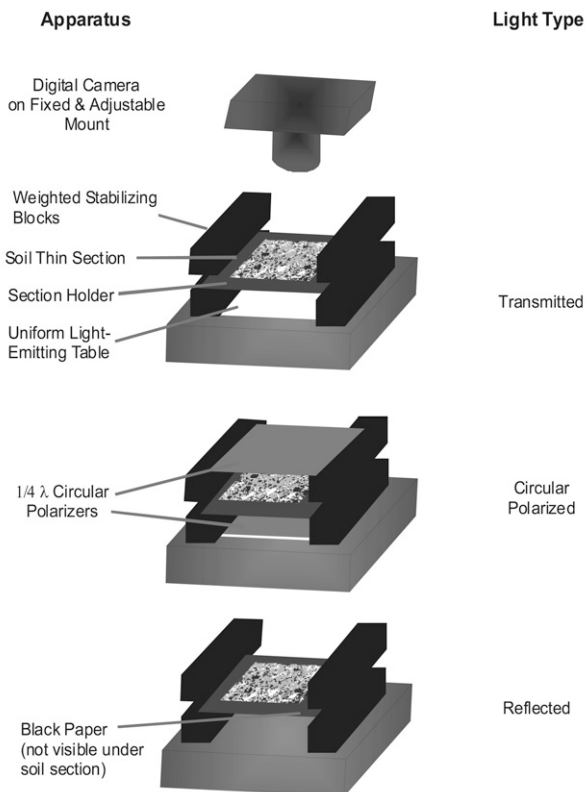


Fig. 8-1. Apparatus for acquisition of images from multiple illuminations of a soil thin section.

tic light box is used to obtain the most uniform light possible so as to minimize the effect of the lighting system on the analysis. A polarizer can be slipped into position above and below the thin section for acquiring images in circular polarized light for distinguishing voids from minerals in the section (Ruark et al., 1982). Transmitted wavelengths of light will reflect the optical properties of the pore spaces and primary and secondary minerals as generally seen under the microscope. The reflected wavelengths further aid in discrimination among soil components. The added information assists the computer in recognition of unique organic, primary and secondary minerals in section. This arrangement results in spatially registered data sets among the three illuminations (transmitted, reflected, and circularly polarized). The distance from the thin section to the camera and the magnifying power of the camera lens control the size of the digitized area.

Steps in Image Acquisition

Soil thin sections are placed in a fixed position on the light table. Light is passed through the section from below, and the camera captures the image (Fig. 8-1). The 1/4 wavelength circular polarizers are slipped above and below the section and light is passed through. The camera captures another image. Then a constructed black body is slipped beneath the fixed thin section-holding apparatus, and the circular polarizing sheets are removed. Diffuse light from dual fluorescent light sources is reflected off of the section, and an image is captured as reflected light. The result after this sequence is three RGB images stored on the camera's memory card. The nine channels are then transferred to the computer for import into the Geomatica (PCI Geomatics, Richmond Hill, ON, Canada) image analysis system.

Segmentation Procedure for Multiple Illuminations

In the procedure the initial characterization of pedofeatures is made by an unsupervised classification with the KCLUS algorithm in Geomatica. KCLUS performs a *K*-means cluster analysis on all channels of the file simultaneously, and as such uses all spectral data to derive similar classes of pixels. Although we use the *K*-means cluster analysis for differentiation of pedofeatures, the user is open to utilize other algorithms, such as isodata clustering. Other software packages in the geomatics industry perform similar operations. Geomatica can separate up to 255 classes using the KCLUS algorithm. Although the number of classes for separation using this procedure is somewhat arbitrary, the initial segmentation of the images into 30 classes for transmitted, reflected, and circular-polarized light has been sufficient for adequate pedofeature differentiation (Protz and VandenBygaart 1998). The classes are then aggregated to an "acceptable" number for the purpose of the research question. For example, if only soil voids are required for segmentation, then only two classes are required (void and solid) such that the 30 classes are aggregated into the two classes by the supervised aggregation procedure (Fig. 8-2). This procedure involves sequentially "grouping" classes of pixels by visual inspection of the sample until a reasonable representation of voids versus solid is obtained. The result is an image with two distinct classes (void and solid), which can be exported to a single-channel image with two pixel color values (or sometimes referred to as a "binary image") with soil matrix "on" (pixel color 255 or white) and voids "off" (pixel color 0 or black) (Fig. 8-2).

There are some shortcomings of the KCLUS unsupervised classification in soil micromorphology. Statistical clustering uses clustering algorithms to determine the natural groupings of pixels with similar spectral properties in an image (Lillesand and Kiefer 1994), but the quality of the classification is contingent on the quality of the image and the

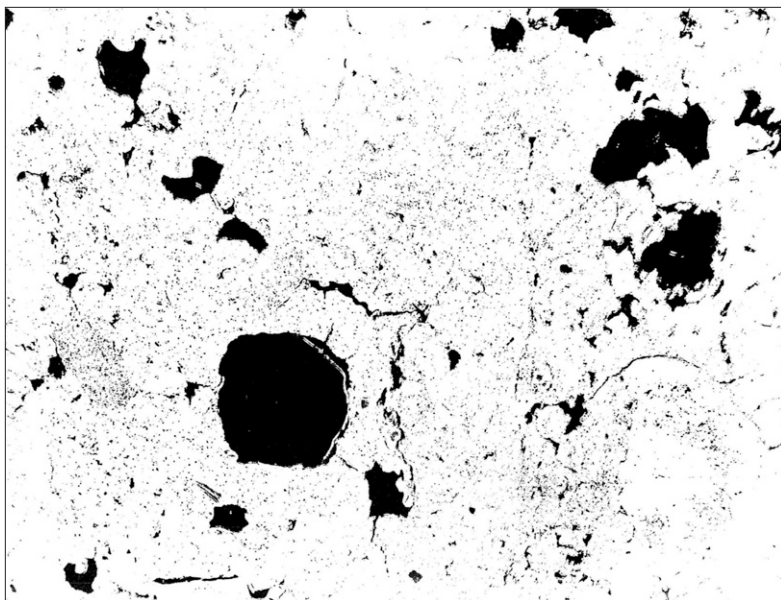


Fig. 8–2. Binary image of horizontally oriented thin section showing voids as black and soil matrix as white. Large circular void to the left of center is the cross section of an earthworm burrow. Width of image is approximately 4 cm.

material from which the image is captured. Problems with impregnation of the soil, such as heterogeneous resin and dye distribution, can result in artifacts in the acquired image, which can cause objects to be classified inappropriately by the clustering method. This can lead to erroneous interpretation and quantification of pedofeatures or soil minerals. Digital artifacts such as noise can also cause problems and can be dependent on the quality and resolution of the digital imaging system.

Morphometric Analysis

After deriving a binary image, measurements of objects in the image can be made; this is termed *morphometric analysis*. There are a number of commercial and noncommercial software packages that facilitate measurement of objects in images. NIH Image is a public domain image processing and analysis program that can be used for measurements such as object area, perimeter, and major and minor axis length. It is available at <http://rsb.info.nih.gov/nih-image/> (verified 30 Mar. 2007).

EXAMPLES

There are a number of applications of multispectral image analysis in soil micromorphology including soil genesis, archeology, and soil use and management. Below we give two examples of how image analysis may be useful to understand genetic processes occurring in the soil.

C_{ca} Horizon

Color Plate 8–1 (see the color insert section) shows the analytical procedure for the quantitative assessment of pedogenic calcite accumulation in a C_{ca} horizon in a soil from

southern Ontario. Reflected light image data can be very useful for the accurate differentiation of pedogenic minerals. The complex processes of secondary calcium carbonate accumulation are readily apparent after only a general separation of different zones of the thin section (Plate 8–1). It is evident from the results that there is a spatial relationship between the secondary calcite containing higher amounts of organic matter, and the roots in the soil thin section. It can be interpreted from the results that there is a spatial relationship between the precipitation of calcite and the roots passing through the soil. This is reflected in the image analysis process having the ability to distinguish relative degrees of coprecipitation of root exudates and calcite. This may not have been obvious by observation with the human eye since the bare eye can only adequately process the information from three channels (i.e., red, green, and blue wavelengths), whereas the imaging system can decipher nine channels of information from the various illuminations.

B_{gcc} Horizon

Increasing our understanding of redoximorphic pedofeatures is not only useful for the study of soil genesis but also for understanding the role of secondary minerals in environmental soil chemistry. The analytical sequence for differentiation of secondary amorphous and cryptocrystalline Fe–Mn concretions in a B_{gcc} horizon of a fine-textured soil of southern Ontario is given in Color Plate 8–2. The possibility of separating many classes of Fe, Mn, and Fe–Mn concretions is evident from the procedure. Spatial relationships of the reprecipitation of the reduced elements relative to voids in the thin section are evident from the resulting classification (Plate 8–2).

QUESTIONS TO CONSIDER

What problems will be solved or questions answered about the soil? Is the scale of measurement adequate to answer the question?

The researcher must question the level of repetition for statistical analysis. For example, is it adequate to take one intact soil block from the soil and extract a single thin section to answer a question? Would it be adequate to take only one soil block, yet take multiple thin sections from the same block of soil? Or would it be more appropriate to take one thin section from multiple soil blocks within a traditional replicated block-type design? These questions must be considered before embarking on a sampling program to answer a research question using soil micromorphology as a tool.

Is the thin section representative of the soil? Are the pedofeatures within the section representative of the soil

Consideration must be given to the representativeness of pedofeatures in a thin section. Micromorphologists must determine the minimum area on a soil thin section or block that is required to represent the pedofeature of interest based on its distribution in soil space. The representative elementary area (VandenBygaart and Protz 1999) must be attained for the feature if adequate analysis is to be performed.

What is the quality of the thin sections? Are the thin sections uniform in thickness?

If light transmitted through soil sections is captured, it is critical that thin sections be uniform in thickness within and among thin sections. This is because variability in thin section thickness will affect the nature of transmission of light, which will in turn result in inaccurate image analysis.

REFERENCES

- Adderley, W.P., I.A. Simpson, and D.A. Davidson. 2002. Colour description and quantification in mosaic images of soil thin sections. *Geoderma* 108:181–195.
- Aydemir, S., S. Keskin, and L.R. Drees. 2004. Quantification of soil features using digital image processing (DIP) techniques. *Geoderma* 119:1–8.
- Bruneau, P.M.C., Davidson D.A. and Grieve, I.C. 2004. An evaluation of image analysis for measuring changes in void space and excremental features on soil thin sections in an upland grassland soil. *Geoderma* 120:165–175.
- Bullock, P., N. Fedoroff, A. Jongerius, G. Stoops, T. Tursina, and U. Babel. 1985. Handbook for soil thin section description. Waive Research Publications, Albrighton, Wolverhampton, UK.
- Cossio, R., and A. Borghi. 1998. Petromap: MS-DOS software package for quantitative processing of X-ray maps of zoned minerals. *Comput. Geosci.* 24:805–814.
- Courty, M.A., P. Goldberg, and R. Macphail. 1989. Soils and micromorphology in archaeology. Cambridge Univ. Press, Cambridge, UK.
- Dekayir, A., and M. El Maataoui. 2000. Contribution of image analysis to the study of weathering process in alkaline basalt (Middle Atlas, Morocco). *Eng. Geol.* 56:325–334.
- Deleporte, S., V. Hallaire, and P. Tillier. 1997. Application of image analysis to a quantitative micromorphological study of forest humus. *Eur. J. Soil Sci.* 33:83–88.
- Drees, L.R., A.D. Karathanasis, L.P. Wilding, and R.L. Blevins. 1994. Micromorphological characteristics of long-term no-till and conventionally tilled soils. *Soil Sci. Soc. Am. J.* 58:508–517.
- Jongerius, A., D. Schoonderbeek, A. Jager, and S. Kowalinski. 1972. Electro-optical soil porosity investigation by means of Quantimet B equipment. *Geoderma* 7:177–198.
- Knight, R.D., R.A. Klassen, and P. Hunt. 2002. Mineralogy of fine-grained sediment by energy-dispersive spectrometry (EDS) image analysis—A methodology. *Environ. Geol.* 42:32–40.
- Lillesand, T.M., and R.W. Kiefer. 1994. Remote sensing and image interpretation. John Wiley & Sons, New York.
- McKeague, J.A., C.A. Fox, J.A. Stone, and R. Protz. 1987. Effects of cropping system on structure of brookston clay loam in long-term experimental plots at Woodslee, Ontario. *Can. J. Soil Sci.* 67:571–584.
- Mooney, S.J., N.M. Holden, S.M. Ward, and J.F. Collins. 1998. The rapid preparation of structural images from undisturbed, non-cohesive material (milled peat). *Geoderma* 86:159–168.
- Murphy, C.P. 1986. Thin section preparation of soils and sediments. AB Academic Publishers, Berkhamsted, Herts, UK.
- Pagliai, M., E.B.A. Bisdom, and S. Ledin. 1983a. Changes in surface structure (crusting) after application of sewage sludges and pig slurry to cultivated agricultural soils in northern Italy. *Geoderma* 30:35–53.
- Pagliai, M., G. Guidi, M. La Marca, M. Giachetti, and G. Lucamante. 1981. Effects of sewage sludges and composts on soil porosity and aggregation. *J. Environ. Qual.* 10:556–561.
- Pagliai, M., M. La Marca, and G. Lucamante. 1983b. Micromorphometric and micromorphological investigations of a clay loam soil in viticulture under zero and conventional tillage. *J. Soil Sci.* 34:391–403.
- Pagliai, M., B. Pezzarossa, E. Bonari, and M. Mazzoncini. 1989. Effects of tillage on porosity and microstructure of a loam soil. *Soil Technol.* 2:345–358.
- Pawluk, S. 1987. Faunal micromorphological features in moder humus of some Western Canadian soils. *Geoderma* 40:3–16.
- Protz, R., S.J. Sweeney, and C.A. Fox. 1992. An application of spectral image analysis to soil micromorphology. I: Methods of analysis. *Geoderma* 53:275–288.
- Protz, R., and A.J. VandenBygaert. 1998. Towards systematic image analysis in the study of soil micromorphology. *Sci. Soils* 3:34–44.
- Ringrose-Voase, A.J., and P. Bullock. 1984. The automatic recognition and measurement of soil pore types by image analysis and computer programs. *J. Soil Sci.* 35:673–684.
- Ruark, G.A., P.L.M. Veneman, D.L. Mader, and P.F. Waldron. 1982. Use of circular polarization on soil thin sections to distinguish voids from mineral grains. *Soil Sci. Soc. Am. J.* 46:880–882.
- Sweeney, S.J., R. Protz, and C.A. Fox. 1992. An application of spectral image-analysis to soil micromorphology. II. Comparison of two soil profiles. *Geoderma* 53:341–355.
- Terribile, F., and E.A. Fitzpatrick. 1992. The application of multilayer digital image-processing techniques to the description of soil thin-sections. *Geoderma* 55:159–174.
- Tovey, N.K., D.L. Dent, and D.H. Krinsley, and W.M. Corbett. 1994. Quantitative micromineralogy and microfabric of soils and sediments. p. 541–547. In A.J. Ringrose-Voase and G.S. Humphreys (ed.) *Soil micromorphology*. Elsevier, New York.

- Tovey, N.K., D.H. Krinsley, D.L. Dent, and W.M. Corbett. 1992a. Techniques to quantitatively study the microfabric of soils. *Geoderma* 53:217–235.
- Tovey, N.K., P. Smart, M.W. Hounslow, and X.L. Leng. 1992b. Automatic domain mapping of some types of soil fabric. *Geoderma* 53:179–200.
- VandenBygaart, A.J., C.A. Fox, D.J. Fallow, and R. Protz. 2000. Estimating earthworm-influenced soil structure by morphometric image analysis. *Soil Sci. Soc. Am. J.* 64:982–988.
- VandenBygaart, A.J., and R. Protz. 1999. The representative elementary area REA in studies of quantitative soil micromorphology. *Geoderma* 89:333–346.
- VandenBygaart, A.J., R. Protz, and A.D. Tomlin. 1999. Changes in pore structure in a no-till chronosequence of silt loam soils, southern Ontario. *Can. J. Soil Sci.* 79:149–160.
- Vepraskas, M.J., and M.A. Wilson. 2008. Soil micromorphology: Concepts, techniques, and applications. p. 191–226. *In* A.L. Ulery and L.R. Drees. (ed.) *Methods of soil analysis. Part 5. Mineralogical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Wierzbos, J., M.T. García-González, and J. Boixadera. 1997. Structure and structure-related characteristics of dryland and long-term irrigated Xerochrept soils. *Arid Soil Res. Rehabil.* 11:127–138.

Transmission Electron Microscopy for Soil Samples: Preparation Methods and Use

FRANÇOISE ELSASS, Institut National de la Recherche Agronomique,
Centre de Géochimie de Surface, Strasbourg, France

CLAIRE CHENU, AgroParisTech, BIOEMCO, Thiverval Grignon, France

DANIEL TESSIER, Institut National de la Recherche Agronomique, PESSAC
Unit, Versailles, France

Soil material consists mainly of particles with dimensions ranging from a few nanometers to several centimeters. Under the climatic conditions that prevail at the surface of Earth, soils are subjected to biological activities, chemical reactions, and physical processes, including phase dissolution, authigenesis of new components, and the concentration and reorganization of soil particles. These processes are important for understanding the evolution of soils in the long term, but they are also essential to our understanding of the mechanisms that control their properties, as well as enabling prediction of soil behavior in response to anthropogenic activity.

Among soil components, finely divided clay minerals, oxides, organic matter, and microorganisms are of major importance because of their highly reactive surfaces for solutes and water and because they control the physical properties of the soils.

Among the tools available for the study of very small particles, transmission electron microscopy (TEM) is perfectly adapted to the characterization of soils fine particles, which range from a few nanometers to a few micrometers.

A major feature of soils is the heterogeneity of their components. Indeed, many different mineral species occur in the clay-size fractions of soils ($<2 \mu\text{m}$), and these species cannot be easily separated from each other by physical and/or chemical methods. Transmission electron microscopy is especially useful because it allows characterization of this heterogeneity and identifies individual soil components. For instance, a better understanding of the retention of heavy metals by contaminated soils requires the identification of heavy metal species and their associations with soil components. Transmission electron microscopy is particularly well suited to studying the speciation of heavy metals in soils, and especially the nature and location of specific mineral phases containing metals, such as oxides, phosphates, sulfides, sulfates, and carbonates, which can be associated with clay minerals, organic matter, and bacteria.

Another feature of soils is the association of their colloidal components into complex mineral and organic associations at very small scales, which determine many soil structures and physical properties that can be observed at larger scales. Transmission electron

microscopy offers the necessary spatial resolution and chemical sensitivity to describe and analyze such associations.

Many universities and research institutes are equipped with TEM. The conditions at which the microscope can be used on soil samples are not different, generally speaking, from those developed for geological or biological materials. However, soils are characterized by coexisting mineral and organic phases, including microorganisms such as bacteria and fungi. Preparation methods as well as observation conditions should be monitored in the study of both mineral and organic phases. Because of the variety of soil mineral and organic components and their fragility, successful TEM on soil samples is strictly dependent on the methods and techniques used in specimen preparation that preserve the integrity of the soil components and their organization, as well as minimizing any disturbance. This is especially important for samples containing organic components. The preparation methods chosen to observe organic phases need to avoid their dissolution and also enhance their very low contrast because of the low atomic number of their constituting elements (i.e., H, C, N, O).

Another constraint with soils is that they are hydrated and consist of soft materials that may shrink and swell with variations in water content and potential, especially when humidity ranges between field capacity and wilting point. Because fine soil particles are very reactive, their water content and the stress history determine part of their microstructure. For instance, B horizons of some soils are often subject to wet conditions, while a more variable atmosphere in most A horizons allows more consolidated soil microstructures. Hence, characterization of microstructures should be performed as closely as possible to the hydration state and history of the soil samples under their natural conditions.

In addition, the chemical composition of solutions has a great influence on dispersion–aggregation processes, as well as crystal structure changes of fine particles of soils. Precipitation and dissolution processes, for instance those related to pH changes or redox potentials, are often observed. Special attention needs to be given to control the composition, pH, and redox status of soil constituents and solutions.

Transmission electron microscopy studies also require having sufficient information to adequately select samples for efficient investigations. Because of high maintenance costs and because of time-consuming preparation procedures, the number of samples that can be studied is generally restricted. Actually, TEM is often used after preliminary investigations have been performed, including optical microscopy for petrographic and textural studies (micromorphology), X-ray diffraction for mineralogical studies, scanning electron microscopy observations and electron microprobe analysis for determination of the chemical composition, and other spectroscopic methods, for example, infrared spectroscopy.

The following is intended to provide a broad outline of 20 yr experience in the field of the application of TEM to soil samples, especially to clay minerals and to clay–organic matter associations, when prepared in their hydrated state with different chemical environments. This experience was developed while the authors were at the Unité de Science du Sol INRA, Versailles. Until the 1990s, studies on soil microstructure were based on previous experience gained from reference clays of geological deposits (Ben Rhaïem et al., 1986, 1987; Tessier and Pédro, 1987; Tessier, 1990; Hetzel et al., 1994) or on synthetic clay–organic matter associations (Chenu and Jaunet, 1992; Chenu, 1993). The samples were saturated with monoionic compensating cations, equilibrated with controlled salt concentrations, and subjected to various chemical or physical stresses. The contribution of such simplified systems made it possible to understand the possible impact of these different factors on soil samples due to the preparation techniques. The addition of organic compounds to pure clays (Chenu and Jaunet, 1992; Uhlík et al., 1999; Dudek et al., 2002) and their preparation in reducing media (Stucki and Tessier, 1991) were essential steps toward

studying soil samples. High resolution TEM is dedicated to the study of nanostructure and nanotexture of fine clay particles and has been used extensively to understand their evolution through degradation and aggregation processes (Elsass and Robert, 1992; Aoudjit et al., 1996; Vicente et al., 1997; Hardy et al., 1999). However, it should be noted that electron microscopy provides the greatest value when coupled with chemical microanalysis to associate the chemical composition, crystal structure, and morphology of the soil components (Romero et al., 1992; Aoudjit et al., 1995; Gillot et al., 2000). The aspects concerning chemical microanalysis will be treated in another chapter.

While extremely powerful, TEM is also a complex technique. It is not our intention to present the physical theory of TEM in detail, this part having been extensively developed in a chapter of a preceding book in the same SSSA series (Gilkes, 1994). Furthermore, abundant literature is available on electron microscopy theory. Detailed descriptions of the theoretical physics underlying TEM applied to the study of clay minerals have been given by Drits (1987), Eberhart (1981, 1989), Buseck (1992), and Kogure (2002). Güven (1990) and Elsass (2006) each dedicated a chapter to the state of the art in the study of clay minerals by TEM, with different applications in the fields of geology and surface weathering. The focus of this chapter is to provide basic information useful for applying TEM in appropriate conditions for soil sample examination, especially with the goal of interpreting the relationships between soil components and their properties, such as surface properties, microstructures, and the nature of soil component associations.

GUIDELINES FOR SAMPLING

This section and the following review the main questions that arise in sampling and sample preparation when TEM is applied to the study of soil samples.

Transmission electron microscopy can be used on a wide variety of soil samples, ranging from undisturbed wet or dry samples to infiltrating waters. Because TEM is particularly well suited to the study of fine soil fractions, these fractions are often separated from soils before their processing for TEM. In addition, coarse quartz grains can damage the diamond knives used to prepare ultrathin sections of soils, disturb the sample integrity during cutting, and distort observations since they remain opaque. Hence soil fractionation is also used to discard such components. The specific research situation will dictate whether the process of removing these components is a greater or lesser problem than their presence in thin section.

Sampling Undisturbed Specimens

Because fine-particle assemblages of soils are very sensitive to the soil cultivation and fertility history, as well as to the geochemical environment in which they were formed and exist presently, sampling is a major concern for TEM studies. It generally requires several steps, with the first one involving the identification of the factors influencing soil heterogeneity at different scales. Also, as stated previously, the number of samples to be analyzed is often limited because of cost and time. To avoid misinterpretations, any TEM study should thus be based on a rigorous analysis of the characteristics of the soils from the point of view of their texture and the nature of their components.

- **Spatial context.** The lateral and vertical variations of the nature and organization of the soil, for instance according to topography or parent material distribution, has to be taken into account. It is essential, as a preliminary to any TEM study, to collect generic soil data to gain information on the representative nature of the soil. At the scale of any soil profile, one refers generally to soil architecture, changes in color, and the local texture. In addition, any sampling should be made so that the selected samples are representative of the studied soil.

- **Human activity.** Major variations of the soil microstructure are often related to human activity, such as tillage, fertilization, irrigation, or organic matter management (Chenu et al. 2001; Favre et al., 2002; Pernes-Debuyser et al., 2003). Local concentrations may occur, such as salt precipitation due to fertilizers or climatic events, which generally concentrates at the edges of the fine soil components. The sampling period should thus be suited to the specificity of the study.
- **Geochemical context.** The geochemical context during sampling should be characterized, for example, by measuring Eh, pH, CEC at soil pH, exchangeable cations, salinity, organic matter content, and determining mineralogy.
- **Biological activity.** Wet samples are sensitive to biological activity, which may cause changes in redox potential, C content, and pH and may result in unexpected growth of microorganisms. Also, desiccation can irreversibly modify the nature of soil components: this is the case for K-saturated swelling clay minerals, halloysites, allophanes, and iron oxides, as shown by X-ray diffraction.

It should be kept in mind that routine sample preparation and storage can change soil components at the scale of their microstructure and thus be inappropriate for TEM studies. Special caution must be taken if the effects of physical and chemical parameters have to be determined in different environments.

Sampling Soil Fractions

Several methods allow for the separation of soil primary particles obtained after complete dispersion of aggregates based on particle-size or density or the separation of aggregate size fractions (after incomplete dispersion of soil). The method of separation should be selected in light of the specific objectives of the study. For example, clay mineral studies are best performed on particle-size fractions separated after removal of organic matter and some of the mineral components (carbonates, oxyhydroxides). The organic matter is oxidized, and carbonate phases are dissolved in acid solutions. The destruction of amorphous and crystalline metallic oxide phases (Al, Fe) can be performed using a variety of treatments (Tamura, 1957; Mehra and Jackson, 1960; de Endredy, 1963; Schwertmann, 1964; Jackson, 1965; Holmgren and George, 1967).

In contrast, the dissolution of associated phases should be avoided for studies on soil aggregation, and it may then be imperative to not separate clay and silt from sand-size aggregates. For the study of undisturbed soil samples, one may cut a fragment from a soil sample and discard coarse particles such as sand grains, organic matter fragments or, alternatively, select special features under a binocular microscope (van Oort et al., 1994). Šucha et al. (2002), selecting Fe-rich nodules for embedment in resin and ultramicrotomy, confirmed the coexistence of different types of Fe oxyhydroxides having different morphologies: 3-line ferrihydrite with a “mohair” structure, 5-line ferrihydrite with a “massive” structure and goethite in submicrometric grains. The three types of Fe oxyhydroxides appear arranged in concentric rings, crystallinity increasing toward the core of the nodule (unpublished data, Fig. 9–1).

Sampling Clay Size Fractions for Microstructural Studies

Although chemical treatments for soil dispersion are still accepted as routine methods in most soil laboratories, care is needed to avoid or minimize the deleterious effects of chemical treatments on organic and mineral components of soils, and on their organization. For instance, the treatments applied to remove soluble salts and polyvalent cations adsorbed on the surface of components can promote changes in particle size and arrangement, and in the relationships between the components (minerals, organominerals, and

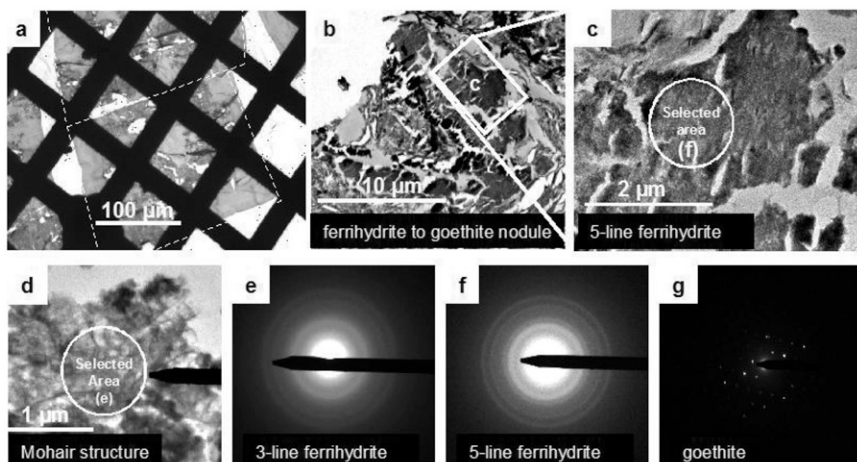


Fig. 9-1. Iron nodule in a soil affected by acid mine drainage: (a) general view of the nodule at low magnification in a suite of ultrathin sections forming a ribbon; (b) medium magnification, nodule formed from concentric precipitation of iron oxyhydroxides, gray ferrihydrite and dark goethite; (c) enlargement of a ferrihydrite area with a granular texture; (d) area of ferrihydrite with a mohair texture, external to nodule; (e) microdiffraction pattern of 3-line ferrihydrite in (d) showing diffuse rings typical of very limited short-range order; (f) microdiffraction pattern of 5-line ferrihydrite in (c) showing modulations at high angles; (g) microdiffraction pattern of goethite grains in (b) showing dots attesting for three-dimensional ordered structure. Sucha et al. (2002).

organic components). To visualize components associated with clay minerals, we recommend physical methods to separate clay-size fractions, such as the use of mechanical agitation and ultrasonic vibration (Edwards and Brewner, 1967; Watson, 1971; Balesdent et al., 1991).

Using such physical fractionation methods, Pernes-Debuyser (2002) evidenced the impact of fertilizers on the clay fraction microstructure in a long-term experiment (Fig. 9-2). Abundant organic features present in the 70-yr-old reference soil (Fig. 9-2a) are no longer observed in the present day untreated soil (Fig. 9-2b), whereas similar features are observed in the soil fertilized with manure (Fig. 9-2c). Organic matter fills up the space between mineral particles but does not aggregate clay mineral particles. An aggregation can be observed in basic and acidic conditions, for example, in basic slags (Fig. 9-2d) and NH_4 -phosphate (Fig. 9-2e), respectively. Dissolution affects the very fine particles in acidic conditions (pH 3.5). With Na as exchangeable cation (Fig. 9-2f), a densification of the clay fabric is observed, in accordance with physical properties (Pernes-Debuyser et al., 2003).

It should be kept in mind that only a complete dispersion of the sand-size and silt-size fractions of soils releases a clay-size fraction that is representative of all clay-size particles and aggregates. Incomplete dispersion of aggregates releases a so-called water-dispersible clay fraction, which usually has a lower C content and a slightly different mineralogy than the bulk clay fraction (Nelson et al., 1999; Chenu et al., 2001). Hence the fractionation method should be adapted to the specific objectives of the study. Comparison between untreated and chemically treated soil samples, for instance after iron oxide dissolution or hydrogen peroxide treatment, can be an interesting approach in the investigation of soil microstructures. For example, the role of organic matter in the organization of fine clay particles was observed in the clay-sized fraction isolated from a Cambisol developed on

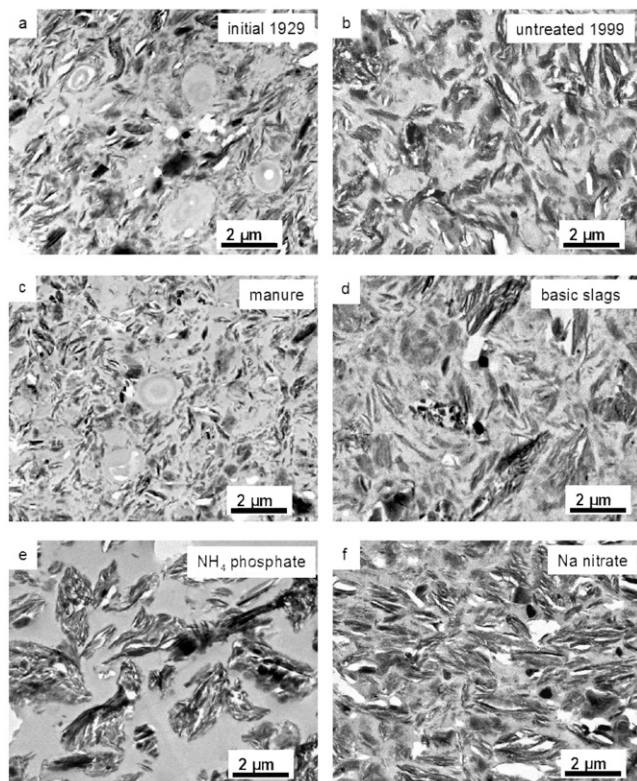


Fig. 9–2. The impacts of soil management on the clay fraction, particle-size distribution, and aggregation: (a, b) effect of time—note that organic features observed in the initial 1929 sample are no longer observed in the untreated 1999 sample; (c) effect of organic amendment—note the abundance of organic and biota particles after 70 yr of manure addition; (d, e, f) effect of mineral fertilizers—note aggregation of the clay in basic (slags) and acidic (phosphate) conditions and dissolution of fine particles in acidic conditions; (f) densification of the clay fabric with Na⁺ fertilizer.

loess in a temperate region (France; Chenu, unpublished data). While oxidation of organic matter is indicated by the disappearance of bacteria, organic debris, and other organic features, clay mineral particles appear smaller (Fig. 9–3); the removal of organic matter leads to a disaggregation of the inorganic phases and a global densification of the bulk material.

Sampling Clay-Size Fractions for Mineralogical Studies

Individual mineral particles can be isolated from soil samples, by, for instance, gentle mechanical stirring or reciprocal shaking overnight with or without an ultrasonic vibration treatment of the soil suspensions and with minimal or no chemical treatment. The main advantage of this separation procedure of elementary clay particles is the characterization of the chemical composition of individual phases, i.e., homogeneous crystals or fundamental particles. It may be important for characterizing the crystal structure and establishing the structural formulae of clay minerals. Nevertheless, it must be kept in mind that the most efficient dispersion of the clay requires a preliminary destruction of carbonates and the saturation of clay minerals by a monovalent cation, for instance Na⁺, as an exchangeable cation. Furthermore, an optimal visualization of the layer stacking and loose particles will be obtained after oxidation of the organic matter.

Examples of ultrastructural organization of the clay-sized fraction are shown in Fig. 9–4. The first three examples (Fig. 9–4a through 9–4f), dealing with clay-rich soils developed on sedimentary rocks in a temperate climate (France), show the general phenomenon of textural interstratification in such materials. Sequences of clay layers 100 nm thick or

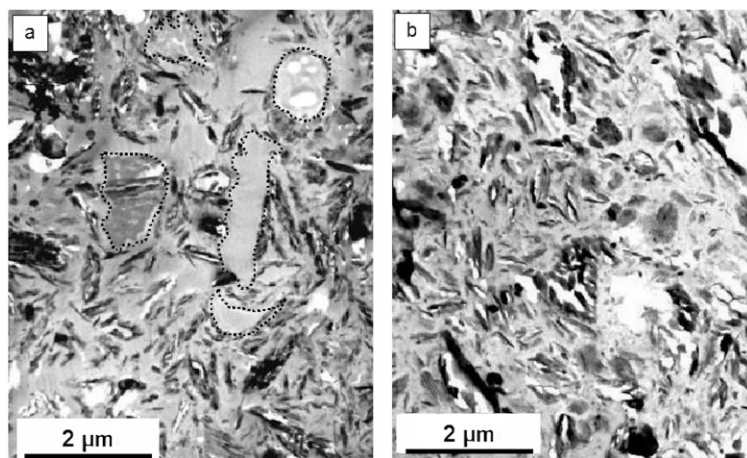


Fig. 9-3. Clay size fraction (<2 μm) isolated from a temperate Cambisol: (a) without treatment and (b) after treatment with H_2O_2 to oxidize organic matter. Note that organic features (outlined with dotted line) are no longer observed and that clay particles are smaller due to the removal of aggregating organic matter (Chenu, 2006, unpublished data).

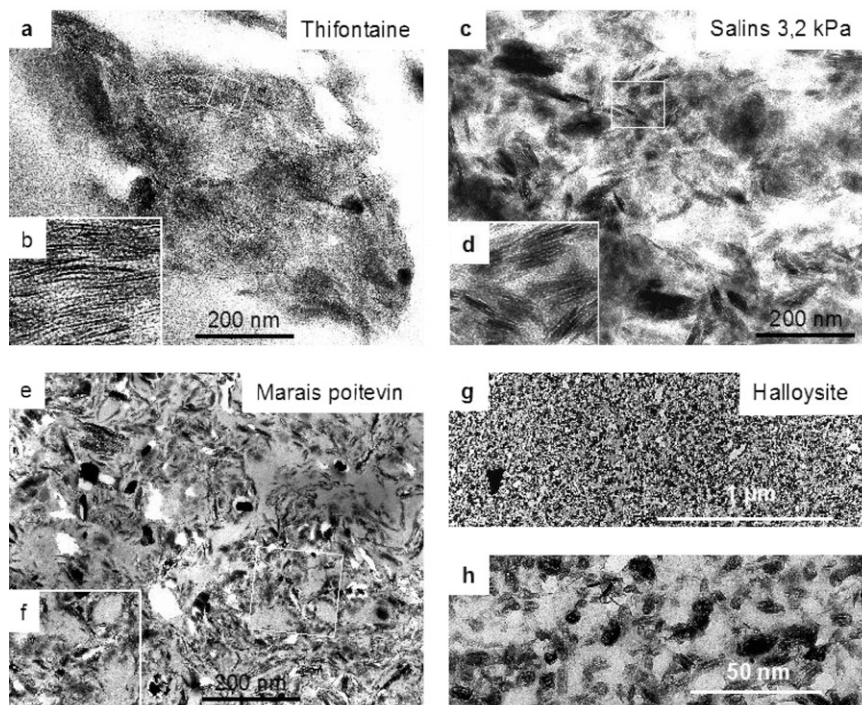


Fig. 9-4. Ultrastructural organization in soils: (a, b, c, d) clay fractions from soils on sedimentary rocks under a temperate climate (Robert et al., 1991); (a) textural mixed-layer clay of soil smectite; (b) internal ultrastructure of the nanoaggregate—note the short lateral extension of the layers; (c, d) structure and ultrastructure of a mixed-layer clay mineral of soil illite; (e, f) microstructure of an undisturbed soil sample (Djéran-Maigre et al., 1998); (g, h) organization of halloysite clay affected by the presence of smectite (Delvaux et al., 1992).

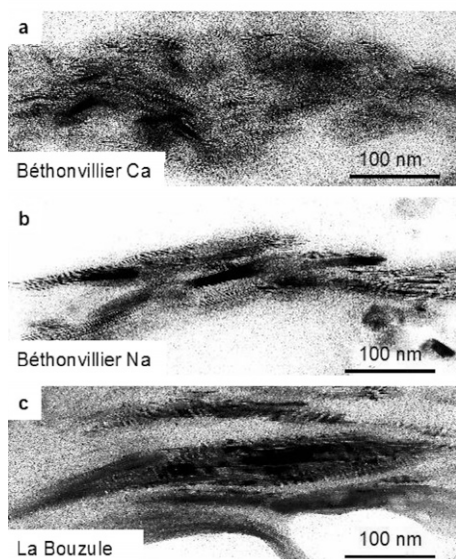


Fig. 9-5. Soil smectites commonly found in soils: (a, b) high charge smectite from Bethonvillier Ca^{2+} - and Na^+ -saturated—note the large lateral extension of quasicrystals and increased thickness with Ca^{2+} (Tessier and Pédro, 1987); (c) typical appearance of a soil illite.

more are formed by the stacking of individual particles made of one to three layers and generally having lateral extension of less than 100 nm (Robert et al., 1991; Djéran-Maigre et al., 1998). The next example (Fig. 9-4g and 9-4h) shows a typical arrangement of halloysitic material developed in a tropical climate, with spherical or tubular particles having an homogeneous size without any evidence of preferential aggregation (Delvaux et al., 1992). Smectites having high layer charges are commonly found in soils (Fig. 9-5); Na^+ -clays, such as Béthonvilliers smectite (Fig. 9-5a), occur as aggregates similar to those formed by illite. The crystals within the aggregates contain about 10 layers. Lateral extension in the (a,b) plane is usually 100 to 500 nm. Exchange from Na^+ to Ca^{2+} cations (Fig. 9-5b) results in regrouping of individual crystals (Tessier and Pédro, 1987). Figure 9-5c shows a typical shape of a Ca-illitic soil clay as found in the loessic formations largely spread North of Europe.

Sampling Mobile Colloidal Fractions

The colloidal components of soils represent a reactive soil phase, which can also be mobile in the soil and thus play an important role in soil genesis (eluviation) as well as in the mobility and bioavailability of pollutants. Such fractions, generally termed *colloidal fractions* can be collected in water or extracted with appropriate methods from soil samples. Infiltration (gravitational) water can be collected, for instance, in soils under different land uses and managements, such as forests, crops, or heavily contaminated areas (e.g., Denaix et al., 2001; Citeau et al., 2003, 2006).

Gravitational water is usually collected with a zero tension lysimeter. Coarse particles can be eliminated by filtering through a 5- μm filter (fine silt fraction) or a 2- μm filter (clay fraction), whereas a 0.45- μm -diameter filter is required to separate particulate matter (mainly clay minerals and associated compounds) of the colloidal matter. To separate the dissolved and colloidal fractions, ultracentrifugation ($t = 270$ min, $270,000 \times g$) may be used to concentrate colloidal particles with a theoretical size larger than 5 nm for mineral colloids and 11 nm for organic colloids, onto TEM grids (Perret et al., 1991; Citeau et

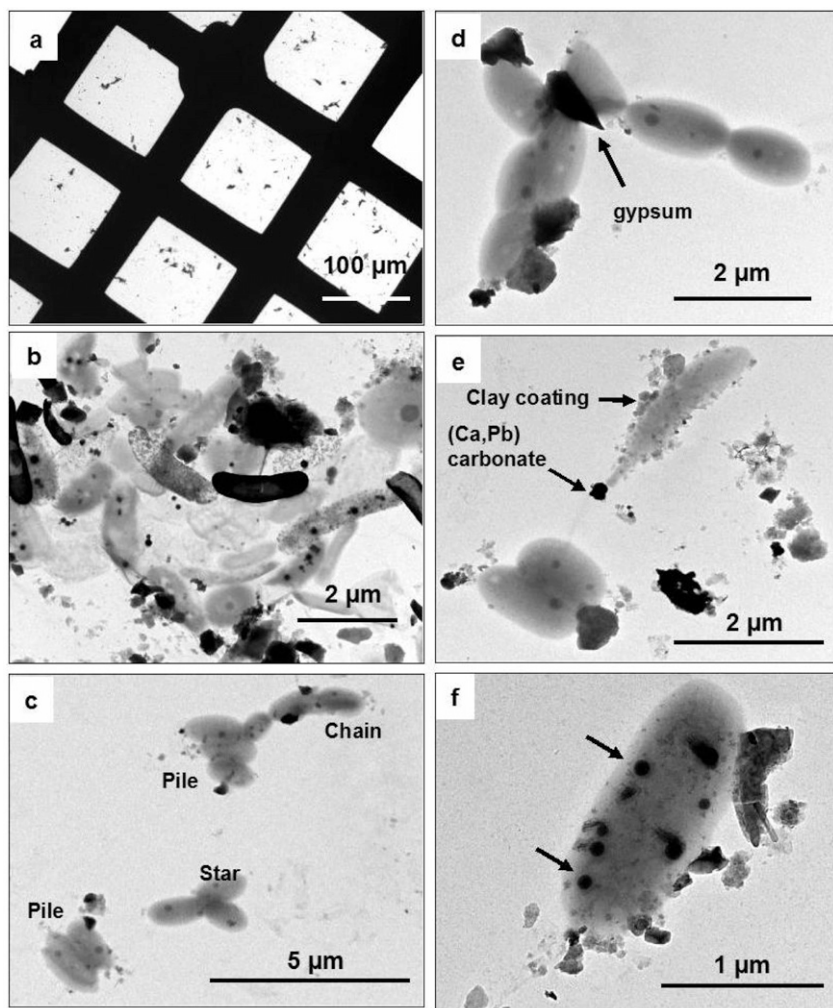


Fig. 9-6. Organic colloids from soils polluted by waste water: (a) view of the grid at low magnification—groups of bacteria can constitute large aggregates; (b) the morphological diversity of bacteria may reflect the diversity of the structure of the population; (c) characteristic assemblages of bacteria in chains, piles, or stars; (d) association of a gypsum grain with bacteria; (e) exopolymeric secretions secreted by a bacteria entraps clayey and carbonate colloids within the microorganism's biofilm; and (f) vacuoles containing Pb, indicated by arrows.

al., 2003, 2006). Soil solutions as well as rainwater can also be filtered in real time in the field using filtration boxes mounted as lysimeters for the collection of the fine suspended matter directly on microscope grids (Perdrial et al., 2008). This in situ procedure allows for the preservation of microbial entities and the conservation of their arrangement with respect to the mineral fraction (Fig. 9-6). The structure of the population is reflected by the morphological diversity of the bacteria (Fig. 9-6b). A great variety of bacteria assemblages (Fig. 9-6c) and associations of bacteria with minerals was observed (Fig. 9-6d and 9-6e). Figure 9-6f shows Pb-rich nodules included in bacteria, a common feature in

polluted soils. Such features can elucidate the specific role of bacteria in the presence of toxic elements.

SPECIMEN PREPARATION

Specimen preparation depends largely on the initial physical state of the samples given that, whatever the type of sample to be examined by TEM, the specimen must be very thin. Preparation techniques are adapted to the physical state of the samples (i.e., suspension, wet material, or dry material; Fig. 9–7). A schematic curve of soil hydration shows that different domains are distinguished corresponding to the main rheological characteristics (Fig. 9–8). The water content decreases rapidly as gas pressure is applied to the material. The most convenient to handle are clay pastes with a consistency of firm gel (32 kPa, $pF = 1.5$) for smectitic soils or soft plastic solid (320 kPa, $pF = 2.5$) for kaolinitic soils. The following sections detail the main sample preparation methods encountered in TEM for various sorts of samples. These include:

- **Dilute suspensions of clay-size fractions or effluents from gravitational waters.** Grid deposits (from evaporation) are routinely used, especially when very low amounts of material are available.
- **Clay-size fractions or clay gels and soil pastes.** One of the key points in discussing particle shape, size, and arrangement of swelling materials is their full expansion in a water-saturated state. Such work requires a specific preparation to avoid changes in soil texture and arrangement.
- **Dry materials.** These samples are rigid solid materials prepared under vacuum or in equilibrium with air dry conditions. Such samples are subjected to specific methods of embedding.

In addition to the preparation procedure for samples listed above, specific methods must be used for the preparation of samples containing organic matter, and for the preparation

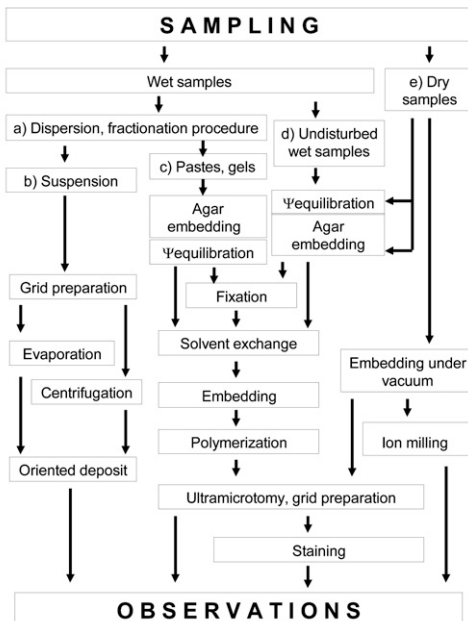


Fig. 9–7. Procedures of sample preparation for transmission electron microscopy observations. Arrows indicate where sample- or experiment-specific decisions must be made, such as whether samples (a) require dispersion procedures, (b) are suspensions, (c) are gels or pastes separated by fractionation procedures, (d) are undisturbed wet soil aggregates, and (e) are dry soil fractions or aggregates.

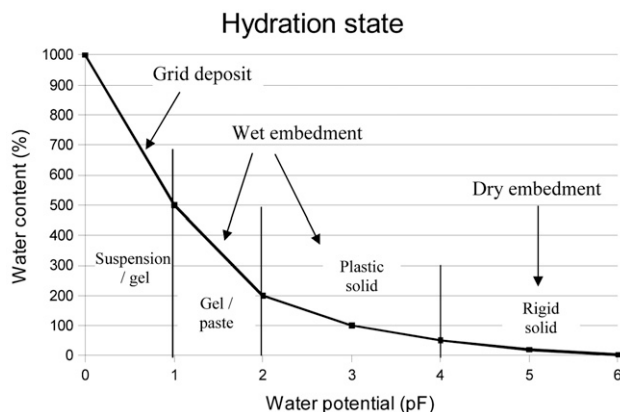


Fig. 9-8. Relationship between the stress and the clay water content controlling the changes: (a) suspension state (500–10,000% water), (b) paste/gel (200–500% water), (c) a solid plastic (50–200% water), and (d) solid rigid state (0.5–50% water).

of reduced samples. Furthermore, when post-staining of organic matter is to be performed or microanalysis is to be coupled with images, the type of grid support must be carefully chosen.

Wet Samples

The advantage of studying hydrated samples is the preservation of particle assemblages that prevail in the field. Two types of samples may be distinguished: natural undisturbed samples and samples prepared in the laboratory.

In the case of soils, the *in situ* state is not always the most appropriate for study of microstructure. Indeed, the variations in water content with time are such that it is generally preferable to compare different samples in an identical hydration state, so samples are preferably prepared under a controlled suction pressure before embedding and cutting the ultrathin sections (Tessier, 1990). Obviously, the question has less importance for the study of the components themselves than for the study of their texture, two- and three-dimensional arrangement, and parent–daughter relationships.

In addition, it is generally preferable to embed hydrated samples that have an open microstructure because the resin penetrates more easily in a continuous pore network than in narrow pores of a discontinuous pore system (Tessier, 2006, unpublished data). A further advantage of having highly hydrated samples is to allow easier identification and analysis of the individual particles. The high water contents obtained in the range of high water potentials are also more favorable to the study of the role of physicochemical factors, such as exchangeable cations, salt concentration, pH, or Eh on soil microstructures, because the impact of these factors is greater under these conditions than in more dry states.

Ion Exchange for Mineral Studies

Clay samples are prepared according to the well-known procedure of extraction and separation by sedimentation and/or centrifugation techniques (Laird and Dowdy, 1994). Depending on the objectives, the clay fraction can be extracted by dispersion in water with or without H_2O_2 pretreatment and Na^+ saturation.

Dispersion in water can preserve cation saturation and thus some of the features characterizing soil clays. When the dispersion is performed after Na^+ saturation, the extracted clay can be resaturated with a given cation, K^+ , Mg^{2+} , or Ca^{2+} , using a chloride salt solution. The suspension is further washed with dilute solutions (10^{-3} M) according to classical

preparation methods (Robert and Tessier, 1974). Washing with a $\sim 10^{-3}$ M solution is needed to mimic soil ionic strength. If drying of these samples is required, they should be further washed with deionized water, as drying will concentrate salts. The suspensions are then centrifuged, and the clay slurries homogenized by mechanical stirring and stored in the refrigerator (4°C). It must be mentioned here that freezing and freeze-drying (lyophilization) can cause major changes in soil microstructures (Chenu and Tessier, 1995).

Selection and Preparation of the Sample Support

A large choice of sample supports exists in the catalogs of consumables and small devices for electron microscopy provided by the various suppliers.

Choice of Grids

- **Single hole grids.** Used with ion milling, a circular thin slice of rock is glued on the grid and further thinned down by an argon beam.
- **Circular holes grids.** Used for the ultracentrifugation of organic lysis products, these grids are especially resistant and nonflexible during ultracentrifugation. Unfortunately, the holes are of small size and strongly limit the field of observation.
- **Mesh grids.** These are the most commonly used grids in soil science. Some are inexpensive. They come in different mesh sizes (100, 200, 300) and are made of various metals (e.g., Be, C, Si, Al, Ti, Ni, Cu, Au).

Metal grids have the drawback of producing a characteristic X-ray background signal. Therefore, the choice of the metal must be adapted to the elements of interest when microanalysis is performed, taking into account the whole characteristic emission spectrum of the grid metal to determine whether it will interfere with the characteristic X-rays of interest.

Beryllium and carbon grids are dedicated to the microanalysis of trace elements, providing the minimum background emission when used with a beryllium sample holder. Silicon and aluminum grids should not be used for the chemical analysis of soil components. Titanium grids are of poor quality because of the electrolytic process used in their fabrication and because the bars have irregular borders and a rough surface. The titanium grids must be carefully cleaned just before use to wash out the numerous scales that peel off the bars. However, they are recommended for the analysis of trace elements because they produce no interference with other metals except barium. Nickel grids are of excellent quality; their thin bars have a smooth surface, allowing a strong adherence of the organic membrane support. The drawback of nickel is that the grids are highly magnetic; Fe-rich components are attracted by the bars and thus disappear from the square holes between the bars, which are the observation fields for TEM. Copper grids are also of excellent quality and are nonmagnetic. They are the most popular and least expensive. Nylon grids also exist and present the advantage of being free of metallic elements. They may appear ideal at a first glance for metallic elements analysis purposes as long as the sample holder is not of brass, which is the composition of standard sample holders. Using nylon grids requires having a beryllium sample holder. The disadvantage is that nylon grids are woven, which results in a wavy surface. The organic membrane support adheres irregularly to the nylon threads and presents an uneven surface. Furthermore, nylon deforms when heated under the electron beam. The type of grid to be used should be specified in the preparation and analytical protocols.

Caveat: Any metal sensitive to oxidization (namely Cu) must be avoided when specific reagents are required for post-staining using contrast agents for organic matter or

intercalation of clay interlayers with alkylammonium. Gold or Ni grids are commonly used in such cases, but any analyst must be aware that the characteristic emission spectrum of gold presents a number of interferences with major elements present in soil components.

Recommendations

1. Rinse the grids with acetone before covering them with an organic membrane support.
2. Use a beryllium sample holder for microanalysis to lower the background signal.

Membrane Support

Two organic materials have long been used for coating grids: Formvar (Ted Pella, Inc. and PELCO International, Redding, CA) and Collodion (Mavidon, Lake Worth, FL). Preparation of the organic membranes requires a special set of laboratory equipment and some skill.

Formvar is thick and has good mechanical stability suitable for handling with tweezers, under high vacuum, and when exposed to the heat of the electron beam. It is provided in powder to be dissolved. Follow manufacturer instructions to prepare the solution. Grids are deposited on a grid holder at the bottom of a glass-crystallizing dish with a drain cock and filled with pure water. Two drops of the formvar solution are deposited on the water surface, spreading a thin organic film on the water surface, and allowed to polymerize for 2 min. Then the water is drained until the organic membrane settles on the grids. The grid holder is then stored in a dust free area to dry overnight.

Collodion (or parlodion) is available as a ready-to-use solution. Collodion can be prepared following the protocol described for formvar, using a 2% dilution. A simplified procedure is to fill a classical glass-crystallizing dish completely with water, with the surface making a convex meniscus. Two drops of collodion are deposited on the water surface, spreading a thin organic film on the water surface. The grids are then deposited on the organic film and a paper filter is laid over the grids and left until wet. The paper filter is then rapidly removed using clean tweezers to maintain the organic film sticking to the paper filter and deposited in a Petri dish with the grids upward to dry in the ambient air.

Cautions: Formvar and collodion solutions contain hazardous chemicals, the use of which is regulated by Material Safety Data Sheets of the products (Table 9–1). They must be manipulated under an exhaust hood using gloves, a mask, and eye protection. Carefully follow the appropriate risk and safety protocols specified by the manufacturer.

Recommendations

After preparation of the sample support, grids stored in a dark, cool, clean place can be kept for decades. Storage boxes are well adapted for the archiving of grids.

Preparation of Oriented Samples from Dilute Suspensions

Deposition by Evaporation

The very simple method to prepare oriented deposits of clay fractions is the deposition of a drop of dilute suspension directly on a microscope grid. A dilution of 25 mg L⁻¹ will result in a dispersed deposit where individual particles can be observed and analyzed separately. Such dispersed deposits enable one to record a complete data set on a single particle: image, diffraction pattern, chemical composition, and hk0 lattice fringes for clay minerals. To obtain a diffraction pattern representing the bulk oriented sample or a spectrum repre-

Table 9-1. Selected chemicals in use in a TEM laboratory, hazardous designations (R-code) and safety advices (S-code). Access to Safety Data Sheets through website <http://www.quick-fds.com/> (verified 13 Dec. 2007).

Concern	Substance	R code†	S code‡
Detergent agent	TFD4 solution	34	26-37/39-45
Grid support	Formvar (polyvinyl formal) 1,2-dichloroethane (solvent) Collodion	No regulation 11-22-36/37/38-45 12-19-22-66-67	No regulation 45-53 9-16-29-33
Sample dehydration	Isoamyl acetate (solvent) Acetone Methanol	10-66 11-36-66-67 11-23/24/25-39	23-25 9-16-26 53-7-16-45-36/37
Resins	Propylene oxide Spurr resin kit LRW/white medium, hard	45-46-12-20/21/22-36/37/38 45-20/21/22 36/37/38	53-45 53-23-26-45-36/37/39 16-24-26-36-37
Fixative	Accelerator Glutaraldehyde Uranyl acetate Lead citrate Osmium tetroxide Phosphate buffer Cacodylate buffer	33-23/24/25 22-23-34-42/43 26/28-33-51/53 61 26/27/28-34 No regulation 23/25-50/53	53-28-45-36/37 26-36/37/39-45-61 20/21-45-61 7/9-26-45 No regulation 20/21-28-45-60-61

† R 10, flammable; R 11, highly flammable; R 12, extremely flammable; R 19, may form explosive peroxides; R 20/21/22, harmful by inhalation, in contact with skin, and if swallowed; R 23/24/25, toxic by inhalation, in contact with skin and if swallowed; R 26/27/28, very toxic by inhalation, in contact with skin and if swallowed; R 33, danger of cumulative effects; R 34, causes burns; R 36/37/38, irritating to eyes, respiratory system and skin; R 39, danger of very serious irreversible effects; R 41, risk of serious damage to eyes; R 42/43, may cause sensitization by inhalation and skin contact; R 45, may cause cancer; R 46, may cause heritable genetic damage; R 50, very toxic to aquatic organisms; R 51, toxic to aquatic organisms; R 53, may cause long-term adverse effects in the aquatic environment; R 61, may cause harm to the unborn child; R 66, repeated exposure may cause skin dryness or cracking; R 67, vapors may cause drowsiness and dizziness.

‡ S 7/9, keep container tightly closed and in a well-ventilated place; S 16, keep away from sources of ignition—no smoking; S 20/21, when using do not eat, drink, or smoke; S 23, do not breathe gas/fumes/spray (appropriate wording to be specified by the manufacturer); S 25, avoid contact with eyes; S 26, in case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S 28, after contact with skin, wash immediately with plenty of water; S 29, do not empty into drains; S 33, take precautionary measures against static discharges; S 36/37/39, wear suitable protective clothing, gloves, and eye/face protection; S 45, in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible); S 53, avoid exposure—obtain special instructions before use; S 60, this material and its container must be disposed of as hazardous waste; S 61, avoid release to the environment. Refer to special instructions/Safety Data Sheets.

senting its chemical composition, the dilution should be at a concentration of particles of about 100 mg L^{-1} . Multiple overlaps of particles should be avoided. Furthermore, multiple overlaps will result in blurred diffraction patterns and impair resolution of lattice fringes.

Recommendations

Grip the extreme border of the grid with reverse tweezers and hold the tweezers over a clean ashless filter paper. Deposit a drop of suspension on the grid using a micro pipette calibrated for $10 \text{ }\mu\text{L}$ drops. Let dry under the cover of a Petri dish turned upside down.

Deposition by Centrifugation

Centrifugation is used when suspensions are too diluted, so that a $10\text{-}\mu\text{L}$ drop of suspension gives an insufficient number of particles on the grid after evaporation. A tube filled with 4 mL of suspension concentrates the deposition of the matter on the grid by $\times 400$. Ultracentrifugation is necessary to force the deposition of colloids on the grid. Colloids by definition form a stable suspension and do not sediment under normal gravitational forces. Mavrocordatos and Perret (1995) used grids placed at the bottom of the centrifuge tube before centrifugation. They used an ultra centrifuge during 270 min at $270,000 \times g$ at 20°C equipped with a swing rotor for horizontal centrifugation. During centrifugation, the colloidal particles sediment onto the grid and when the supernatant is discarded the grid can be rapidly air-dried before TEM observation. Citeau et al. (2001, 2003) used Teflon tubes of 4 mL and centrifuged for 4 h . On the other hand, it might be necessary to dilute the suspensions to obtain well-dispersed particles on the grid. The turbidity of the suspensions is a reliable control of the dilution so that the deposit on the grid can be optimized to one layer of particles. Even after dilution, the presence of aggregates is common in untreated soil solutions or clay separates of soil samples, thus reflecting the natural behavior of fine particles. An appropriate dispersion is nevertheless essential for a precise chemical analysis and size measurement of individual particles (Fig. 9–9). The condition of perfect dispersion is not absolutely necessary to obtain the chemical composition of aggregated monophasic particles (Fig. 9–9b and 9–9c). For particle size measurement, this condition can be bypassed by selecting particles with well-defined edges (Fig. 9–9d).

Advantages and limitations to the use of different procedures for collection in the field, storage, and preparation of fragile particles for TEM observations are discussed by Perret et al. (1991), Mavrocordatos and Perret (1995), and Mavrocordatos et al. (2007).

Preparation of Embedded Samples from Hydrated Pastes and Gels

Water Potential Equilibration

With soil clay pastes (Tessier and Pédro, 1987; Tessier, 1990) as well as with undisturbed soil samples (Tessier et al., 1991), a filtration device is used to control the suction pressure and the salt solution in contact with the sample. Various alternatives of this kind of equipment were also used, for instance by Stucki and Tessier (1990), to control the matrix and osmotic components of the water potential and to avoid contact with oxygen.

In the equipment described by Tessier and Berrier (1979), the filtration cell used to control water potential is immersed in a beaker containing a solution that is in contact with the sample through a membrane filter. This device allows the samples to be subjected to a range of gas pressures between 1 kPa and 1 MPa , which are realistic for soils prepared under wet conditions. For soil samples and clay samples, the porous membrane filter generally used is at least $0.45\text{-}\mu\text{m}$ size, but better if $<0.20 \text{ }\mu\text{m}$, which makes it possible to obtain

oriented particles in the section (i.e., cut along the *c* axis) makes it possible to obtain images of clay stacking and determine interlayer spaces at high resolution much more easily.

Agarose Coating Procedure

The embedding procedure for hydrated samples consists of replacing water by solvents and finally by an epoxy resin. This procedure requires the preservation and knowledge of the sample geometry. It is thus necessary to avoid the dispersion or the fragmentation of the samples during the rehydration step. This is especially important if one wants to discuss the effect of parameters influencing the soil microstructure, such as wetting rate gradients or dispersion ability in water compared with other dispersing media. Samples are thus first coated with agarose. The advantage of agarose is to confine the sample in a rigid and porous envelope, which prevents any possible migration of particles toward the outside.

The procedure presented in Elsass et al. (1998) consists of immersing the samples in an agarose gel. Agarose powder is poured into pure water at a 2% concentration and heated just above the melting point (70°C) for 1 h. Ceramic cups are used to prepare agarose cakes 3 cm large and 5 mm thick. The samples are introduced in the cakes when the agarose gel is cooled close to the solidification point (~30 to 60°C, see specifications given by the manufacturer). Simple tube caps are also convenient to prepare agarose cakes.

Agarose can be used in two ways:

- **For coating the samples before water potential fixation.** This procedure is adapted for liquid and gel samples that cannot be handled with tweezers. It is also suitable for small fragments, such as those extracted under a binocular microscope or microdrillings from thin sections and rock chips having uneven surfaces. Agarose cakes ensure a perfect contact with the porous membrane of the filtration cell and homogenous rehydration from the periphery to the center of the sample pellet (Środoń et al., 1990). When hydrated according to the protocol described, the agarose coating protects the sample throughout the whole embedding procedure.
- **For coating samples after water potential fixation.** This procedure is adapted for large samples, for instance pastes and gels or undisturbed soil samples. Fragments are cut out and rapidly covered with agarose. Then agarose preserves the samples from dehydration by contact with the ambient air for a few hours when the embedding procedure must be delayed, and further during the embedding procedure, that is, during the solvent and resin exchange. The agarose limits the leaching of mobile phases during the embedding procedure.

In both cases, it is recommended that the size of the specimen to be embedded is reduced to 1 mm in diameter to ensure rapid and complete exchange of liquids and impregnation of the sample. Such a size is also consistent with the optimal slicing of ultrathin sections of soil samples with a diamond knife. At least three to five replicates of each sample are preformed to enable a check of the homogeneity of the samples. Replicates are also an insurance against the risks related to the preparation procedure, such as bad polymerization, loss of orientation in the resin block, ultrathin sectioning, and staining.

Organic Matter Fixation

Organic constituents are more fragile than minerals during TEM preparation techniques. Several constituents, such as low molecular weight lipids, carbohydrates, and polyphenols, may be soluble in the solvents used for dehydration and embedding, or may be denatured by them, like proteins. Moreover, organic constituents are very sensitive to the electron beam. For these reasons, organic constituents are usually chemically fixed before dehydration and embedding, and the methods used by biologists (Spurr, 1969; Glauert, 1975) have been extended or adapted to soil samples.

Common fixatives are aldehydes, such as glutaraldehyde [$\text{CH}_2(\text{CH}_2\text{CHO})_2$], which establishes bridges between neighboring peptide chains through amino groups, and osmium tetroxide (OsO_4), which is a strong oxidant and reacts with unsaturated bonds (aliphatic $\text{C}=\text{C}$, aromatic and heterocyclic C) to cross-link them. The use of glutaraldehyde and then of osmium tetroxide ensures a satisfactory fixation of most organic structures in biological samples as well as in soils (Foster, 1981; Villemin and Toutain, 1987; Chenu et al., 2001).

A classical procedure is first to embed the samples in agarose as described above. Then immerse them in 2% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8–7.2) for 1 h. After several washings with the phosphate buffer, transfer them to 1% osmic acid in phosphate buffer for 16 h and rinse them again extensively with the buffer rinsing solution. Guidelines to prepare the fixation and rinsing solutions are presented in Table 9–2. Two buffer solutions are available, phosphate and cacodylate, their major difference being the presence of phosphate in the former and of arsenate in the latter. Both contain Na. In the case of incomplete rinsing, these elements (e.g., P or As) may affect energy dispersive X-ray elemental analysis of the sample. The pH of the fixative solutions, 7.2, may be modified to better reflect soil conditions, especially when studying acid soils.

An alternative to immersion is to wet the samples with the fixatives by capillarity under controlled matrix pressure using small filtration devices as suggested by Villemin and Toutain (1987).

Caution: Always be aware of the encountered risks for health. Operate all the way under a hood and carefully follow the safety rules (Table 9–1).

Solvent Exchange and Embedding in Resin

Originally developed for curing biological specimens (Spurr, 1969), the embedding technique, which is used for hydrated samples, has been refined by soil scientists and clay mineralogists at the Soil Science Laboratory of INRA at Versailles (France) (Tessier and Pédro, 1987; Elsass et al., 1998; Elsass, 2006). The process starts with a sample saturated with an aqueous solution, in which the initial pore pressure is controlled.

Table 9–2. Ingredients needed for the preparation of buffer, fixative and rinsing solutions.

Buffers						
Solution	Concentration		Volume	pH		
Sörensen's phosphate buffer: (stock solution 0.1 M, pH 7.2)						
Na ₂ HPO ₄ , anhydrous	0.2 M		36 mL			
NaH ₂ PO ₄ , H ₂ O	0.2 M		14 mL			
Excipient	H ₂ O		50 mL			
Total	0.1 M		100 mL			7.2
Cacodylate buffer: (stock solution 0.1 M, pH 7.2)						
Na(CH ₃) ₂ AsO ₂	0.4 M		25 mL			
HCl	0.2 M		4 mL			
Excipient	H ₂ O		71 mL			
Total	0.1 M		100 mL			7.2
Fixative solutions						
Fixative	Buffer			Excipient		pH
Glutaraldehyde 25%	5 mL	Phosphate 0.1 M	25 mL	H ₂ O	20 mL	7.2
Glutaraldehyde 25%	5 mL	Cacodylate 0.1 M	25 mL	H ₂ O	20 mL	7.2
Rinsing solutions (0.05 M buffer solutions)						
Fixative	Buffer		Excipient		pH	
		Phosphate 0.1 M	50 mL	H ₂ O	50 mL	7.2
		Cacodylate 0.1 M	50 mL	H ₂ O	50 mL	7.2

- Spurr resin.** Spurr resin (Polysciences Inc., Warrington, PA) is a very low viscosity multicomponent epoxy resin for superior specimen penetration. Curing rate, viscosity, and block hardness are all adjustable. Ultrathin sections are stable under the electron beam without the need of a supporting film. On wet samples, the embedding procedure consists of successive exchanges as described by Elsass (2006).
 - The first step of exchange requires replacing the imbibing solution by immersing the sample in mixtures of water and alcohol having successively higher alcohol contents, and finally pure methanol or ethanol. Elsass et al. (1998) immersed the samples directly in pure alcohol, but recommended limiting the number of such exchanges to four.
 - The methanol is then progressively replaced by 1,2 epoxy-propane (four baths with four different proportions of methanol and 1,2 epoxy-propane: 1:4, 1:2, 3:4, and 1:1).
 - Finally the 1,2 epoxy-propane is replaced by pure resin and allowed to sit overnight. A last bath of pure resin is done for 3 h before polymerization.
- LRWhite resin.** The LRWhite acrylic resin (London Resin Co.) has an extremely low viscosity, and this property can be exploited by using short infiltration times. A graded methanol series is the method of choice when embedding in LRWhite resin (Table 9-3). The use of 1,2 epoxy-propane can be avoided. A 1 mm³ sample will be adequately infiltrated in about 2–3 h if 4–6 changes of resin at 60°C are employed. However, an overnight infiltration at room temperature, followed by two short changes of resin will often be more convenient and results in good imbedding. When using the LRWhite embedding resin for dedicated electron microscopy, very few changes need to be made to the procedure used for epoxy resin embedding. No change for normal fixation should be made. Because of its extremely low viscosity, LRWhite resin is well suited to the impregnation of rock chips of very fine pore size (Środoń, et al., 1990; Kim et al., 1995; Elsass et al., 1997).
- Polymerization.** Each specimen is placed in a mold and if necessary carefully oriented (and the orientation noted) to obtain the appropriate direction during ultrathin section preparation. Rectangular molds are preferred for Spurr resin. However, gelatin capsules are necessary for the LRWhite resin to avoid the oxidation of the resin by contact with air. If flat embedding is required for cutting orientation, the surface of the LRWhite resin must be covered to prevent contact with oxygen. The samples

Table 9-3. Solvent mixtures and time table for resin exchange procedures with LRWhite resin that have proven useful.

Exchange	Water	Methanol	Resin	Time	Temperature
	%				°C
Initial state	100				25
Exchange 1	75	25		10 min	25
Exchange 2	50	50		10 min	25
Exchange 3	25	75		10 min	25
Exchange 4		100		20 min	25
Exchange 5		100		20 min	25
Exchange 6		100		20 min	25
Exchange 7		75	25	20 min	25
Exchange 8		50	50	20 min	25
Exchange 9		25	75	20 min	25
Exchange 10			100	16 h	25
Exchange 11			100	1 h	25
Exchange 12			100	1 h	25
Polymerization			100	24 h	60
Cooling			100	24 h	25

must be heated at $60 \pm 2^\circ\text{C}$ (LRWhite resin) for 24 h or 70°C (Spurr resin) for 16–20 h to enable resin polymerization.

Caveat: With this procedure, all exchanges are made at air pressure and room temperature. Thus the samples prepared at a given suction are quickly dipped in methanol and in the successive solvents. It is important to notice that during solvent or resin exchange, the sample *must* be completely immersed to avoid volume change and reorganization due to the action of capillary forces at air–solid–liquid interface. It was shown by Elsass et al. (1998) that it is possible to check the extent of possible shrinking or swelling by monitoring the apparent volume and solution volume during solvent exchange.

Recommendations

The embedding process consists in the whole of 12 successive exchanges (Table 9–3). For pastes equilibrated under low gas pressure (3.2 kPa), when the size of samples is reduced to <1 mm in diameter the time for exchanges can be reduced to 10 min each, especially when possible leaching of adsorbed elements or mobilization of colloids are suspected. For compact samples with fine pore size, like rock chips, microdrills of thin sections, and soil samples equilibrated under relatively high gas pressure (e.g., 100 kPa), the time for exchanges must be at least 20 min. This time can be extended to 1 h with no major risk of dispersion since the sample is molded in agarose.

Caution: Most of the chemicals used in the course of the embedding procedure pose health risks, especially epoxy-propane and hardeners mixed with the resin (Table 9–1), so the complete embedding procedure must be conducted under a hood. Until 2005, Spurr resin was of the carcinogenic R45 type; its composition has since been changed to lower its danger. Metalcrylate resins on the whole present lower risks. The ready-to-use bottles are easy to handle and can be stored for years in a refrigerator.

Specific Conditions for Reduced Samples

A procedure was first developed for pure clay and clay–bacteria systems (Stucki and Tessier, 1991; Gates et al., 1998) and then developed for soils to study the effect of reducing conditions on the soil microstructure and properties (Favre et al., 2002). Reduction and oxidation can induce rapid and very spectacular changes in the soil organization and can lead to the dissolution of iron-rich components, and resultant changes in the clay texture and crystal structure. The location of bacteria, the concentration of salts, and the precipitation of iron oxides at their periphery, are also characteristic of this process.

Sampling is preferably performed during a wet period when the soil is in its reduced state, for instance, during the submersion period of paddy soils. According to Favre et al. (2002, 2004), it is important to have precise data on the physicochemical state of the soil and in particular the Eh state. Mössbauer spectroscopy for the characterization of iron oxidation states and coordination environments is also advised (Dainyak et al., 1992; Komadel et al., 1998).

Soil samples can be reduced experimentally by the dithionite method based on the procedure of Mehra and Jackson (1960), as modified by Roth et al. (1969) and described by Komadel et al. (1990). Clays or soils can also be reduced by bacteria (Stucki et al., 1987; Gates et al., 1998; Dong et al., 2003; Kim et al., 2003). Studying reduced samples requires a very rigorous procedure for minimizing changes to the reduced state. Wet specimens should be collected from a freshly excavated soil profile (Ernstsen et al., 1998). Soil samples must be quickly transferred into sealed tubes to avoid oxygen diffusion from the atmosphere. Using a double sealed envelope filled with an inert gas or maintaining

the samples under vacuum is recommended to limit diffusion of oxygen with time. The embedding procedure does not differ from that of other samples, except that the entire procedure must be performed in the absence of oxygen (i.e., under oxygen-free nitrogen, argon, or helium gas) during the water potential equilibrium phase.

One of the best indicators of the success of the impregnation of reduced soil samples is the color and the appearance of the samples. After embedding, the samples should have their original color, form, and reduced aspect in the block of resin (Stucki and Tessier, 1991).

Only water saturated with inert gas is used, and the procedure is performed in a glove box under a small excess of gas pressure compared with that of the atmospheric pressure. Of utmost importance is advanced preparation—have all necessary materials for sample impregnation and embedding prepared and at hand. A checklist should be used to avoid any delay due to missed steps or unprepared solutions and to avoid any exposure of the sample to oxygen. To avoid oxidation of the sample in water and in the resin components used during the experiment, all liquids need to be deoxygenated before use by outgassing with an inert gas. Also for LRWhite resin, if any oxygen remains in the gel, the resin does not fix properly and forms a grainy texture that does not cut well. For the preparation of the ultrathin sections, the ultra microtome should be placed in a glove bag filled with nitrogen (Stucki and Tessier, 1991; Gates et al., 1998; Favre et al., 2002). The grids also should be carefully desiccated and prepared in a glove box. The grids can then be placed in sealed tubes filled with inert gas for transfers to the column of the TEM. It has been shown that when the samples were carefully desiccated in this way (Stucki et al., 1984), there was little risk of electron transfer and thus of soil component oxidation. However, we recommend that all observation and analysis of the sample be done in only one TEM session, and therefore it is important to ensure sufficient time is available on the instrument for thorough characterization.

Ultrathin Sectioning

Ultrathin sections of samples are required for TEM observations. These are obtained using ultramicrotomes and diamond knives. The resin block is first worked into a pyramid shape in a pyramidotome using a diamond mill, then truncated in the microtome using a glass knife, and finally surfaced using a diamond knife. Until recently, diamond knives were mounted with a 45° inclination angle with respect to the surface of the resin block. Diamond knives with a 52° inclination angle are now available and are better performing on hard samples with compact textures or samples that are coarse grained. Diamond knives are available in different sizes, but the 1-mm size is usually sufficient for soil samples. Because of the hardness of soil minerals, ultramicrotomy of soil samples can most often produce ultrathin sections that rarely exceed a few tenths of a millimeter in diameter. The thin sections floating at the water surface in the knife beaker are trapped on the grid by simple contact of the grid on the water surface.

For low electron dense samples such as organic matter, the optimum thickness of ultrathin sections ranges between 70 and 100 nm. For crystalline materials such as layer silicates, a convenient thickness to visualize the silicate layers ranges between 50 and 70 nm, although the theoretical maximum thickness allowed to visualize lattice fringes should not exceed 30 nm. Such thicknesses are almost impossible to obtain by ultramicrotomy, which is limited to about 50 nm in the most favorable cases. This limit necessitates the use of ion milling, especially when thinner thicknesses (10–15 nm) are needed for ultra-high resolution of lattice fringes.

Staining of Organic Components

Organic components are largely composed of elements of low atomic number (e.g., H, O, C, N). As a consequence, they are nearly transparent to electrons and their features cannot be distinguished from that of the background (i.e., the grid support for deposits and resin for embedded samples). Some organic materials strongly complexed with Fe or Mn may have some natural contrast, but usually the contrast of organic components must be enhanced by staining with high-atomic number (Z) elements. The basic principle of the staining of organic components in soil ultrathin sections is always to perform a chemical reaction that is unambiguously between the organic components and a high- Z containing reagent. The reaction results in the organic-specific deposition of high atomic number material on the ultrathin section. The high Z elements can be precipitated, bound by electrostatic interactions, or covalently bound to the soil organic matter. In addition to increasing contrast between the organic matter and the background, staining enables differentiation of organic matter from amorphous colloidal mineral material and perhaps even helps distinguish between different kinds of organic matter. Two main categories of staining methods for soils exist: classical cytochemical methods and immunocytochemical methods. The former can be specific for dissociated acidic groups of the organic matter or for specific molecules, such as polysaccharides or proteins, whereas the latter have a much narrower specificity. Here we cover general cytochemical methods. Immunocytochemistry has seldom been used to study soil samples by TEM. For studies with immunocytochemistry, one should refer to the work of Foster (1981) and should perform cautious preliminary experiments. Unspecific binding of the antibodies can be expected due to the tendency of proteins to adsorb to clay minerals.

Another difference between the staining methods is that they can be applied either before embedding or on samples already embedded, ultrathin-sectioned, and deposited on grids. Staining soil samples enables visualization and an often identification of the organic components. Staining does have some drawbacks. First, very small amounts of heavy element are usually deposited on the thin section; hence, optimal operating conditions for observing the organic matter are a fairly low acceleration voltage (e.g., 80 keV), whereas a higher acceleration voltage (120 keV) is better to study clay minerals with a high resolution. Second, the inherent granularity of the metals deposited on the organic matter can limit the detection of very fine organic or mineral structures. A wide variety of cytochemical methods allowing viewing of different cytochemical groups are precisely described in biology transmission electron microscopy manuals (e.g., Hayat, 1970; Lewis, 1992). Several of these methods have been applied to soil samples, or could be. However, one should keep in mind that stains may react with soil minerals, giving nonspecific staining. Specificity of new stains should be checked for such reactions before conducting studies. Energy dispersive spectroscopy microanalysis can be used for this.

- **Staining with Osmium.** Osmium tetroxide is used as a fixative. Since osmium has a high atomic number ($Z = 76$), it brings additional contrast to the aliphatic unsaturated organic compounds and to the aromatic ones to which it binds. Use it in the standard procedure as mentioned above.
- **General staining with U ($Z = 92$) and Pb ($Z = 82$).** Uranyl acetate ($\text{UO}_2[\text{CH}_3\text{COO}]_2 \cdot 2\text{H}_2\text{O}$) reacts at acidic pH, with dissociated organic anions such as carboxylic acids. Uranium salts or U^{2+} are then deposited on the organic constituents. A weak contrast is generally achieved with U alone on soil samples. Staining with lead citrate needs to be performed at a high pH, and Pb cations react with anionic organic groups (phenol, phosphate, sulphydryl, carboxyl). Lead provides a much stronger contrast on soil samples, which can be of rather fine granularity (Fig. 9–10). However, its fixation is largely nonspecific. Extensive washings after staining can

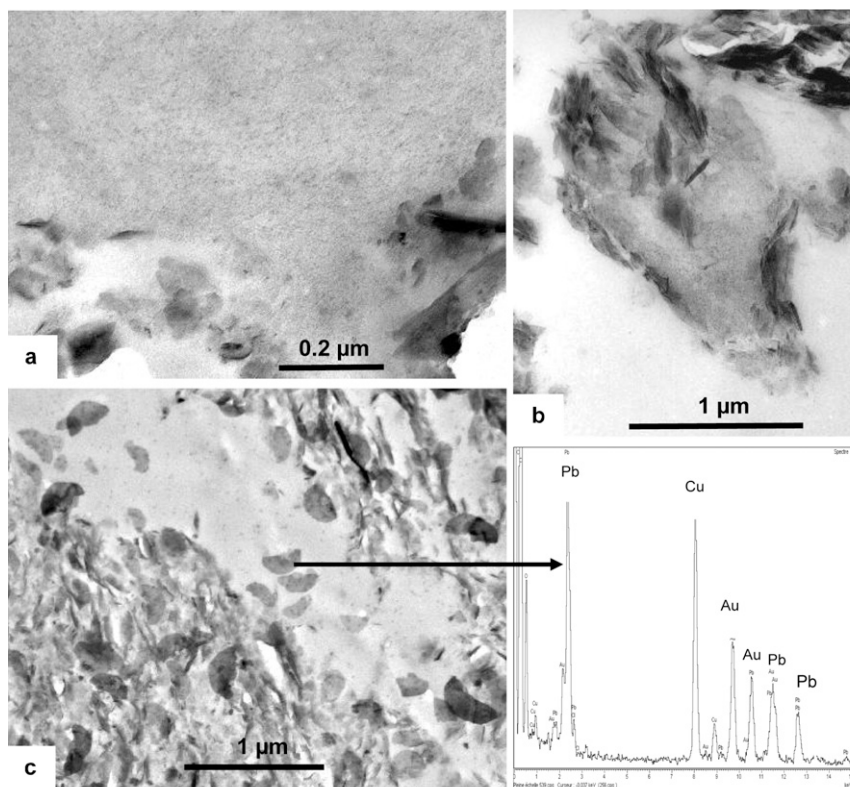


Fig. 9–10. Staining of organic matter in soil thin sections: (a) amorphous organic matter successfully stained with U-Pb—note the very thin grains; (b) clay organic matter microaggregate stained with U-Pb; (c) artifact of staining with Pb—note the half moon—shape lead deposits; (d) corresponding energy dispersive spectroscopy spectrum (Au and Cu X-rays are produced by the specimen grid and the sample holder).

remove Pb from clay minerals, but it remains fixed to iron and aluminum oxides and hydroxides (Chenu, 2006, unpublished data).

The following protocol may be used. Thin sections deposited on grids are floated successively on the different reagents, the thin section being in contact with the solution. First the grids are deposited for 30 min on a 2% uranyl acetate solution prepared in 1% acetic acid, in darkness and filtered at 0.45 μm. After treatment, the grids are washed three times with deionized and filtered water. A 0.5% aqueous solution of lead citrate is prepared, alkalized with a few drops of NaOH, and filtered at 0.45 μm. For staining, grids are floated for 10 min on the lead citrate solution in a closed small vessel in which dissolved CO₂ is trapped and removed with alkali. Carbon dioxide must be trapped to prevent the formation of lead carbonate precipitates. Afterward, the grids are rinsed first in 0.01 N NaOH and then with deionized water. This can be done by placing the grids on a filter paper and rinsing directly. Absence of the alkali trap or insufficient rinsing lead to extensive precipitates of lead (Fig. 9–10).

- **Staining with KMnO₄.** Potassium permanganate is a strong oxidant. It has been used extensively as a stain for lignin because it oxidizes the aromatic groups. Although it is not specific for these groups, it has been successfully used to visualize organic components rich in aromatic carbon (Bourdon et al., 1997; Lichtfouse et al., 1996; Poirier et al., 2006).

To stain soil samples with potassium permanganate, prepare a staining solution of 1% KMnO_4 in deionized water. After agitation and dissolution, filter the solution at $0.45\ \mu\text{m}$. Deposit the grids for 1 h on the surface of a drop of the staining solution, and then remove and rinse extensively with deionized water. The possible exchange of K with clay exchangeable cations or the possible change of clay crystal structure and texture due to oxidation of ferrous into ferric iron form should be taken into account if performing mineralogical analyses on the same samples.

- Specific staining of polysaccharides.** Specific staining of polysaccharides is derived from the periodic acid Schiff method of classical histology and is conducted in two steps (Roland, 1978). First, a specific treatment with 1% periodic acid for 20 to 30 min oxidizes vicinal glycol groups to aldehydes. Second, the aldehyde groups are visualized by reacting the grids with 0.2% thiocarbohydrazide in a 20% acetic acid aqueous solution for 4 h and are rinsed with a series of 20%, 10%, 5%, and 2% acetic acid for 10 min each and then with deionized water. Third, the grids are deposited on an 1% aqueous solution of silver proteinate in darkness for 25 min and then rinsed repeatedly with deionized water (Thierry, 1967). A positive reaction is indicated by the deposition of coarse silver grains on the polysaccharide, and in some cases polysaccharide fibers can be recognized by the alignment of the silver grains (Fig. 9–11), (Chenu and Jaunet, 1992; Chenu and Plante, 2006). Not all polysaccharides react to this method, as pairs of free vicinal glycol groups are required. In addition, a few vicinal glycol groups may be present in other molecules (proteins) but give faint staining. There was no staining of pure clay minerals or pure Fe and Al oxides with this protocol (Chenu, 2006, unpublished data). To perform the Thierry reaction, the samples must be deposited on gold or platinum grids because periodic acid dissolves copper grids.

Cautions: Staining solutions contain hazardous chemicals (such as U acetate). They must be manipulated under an exhaust hood using gloves, a mask, and eye protection. Carefully follow the appropriate risk and safety protocols specified by the manufacturer.

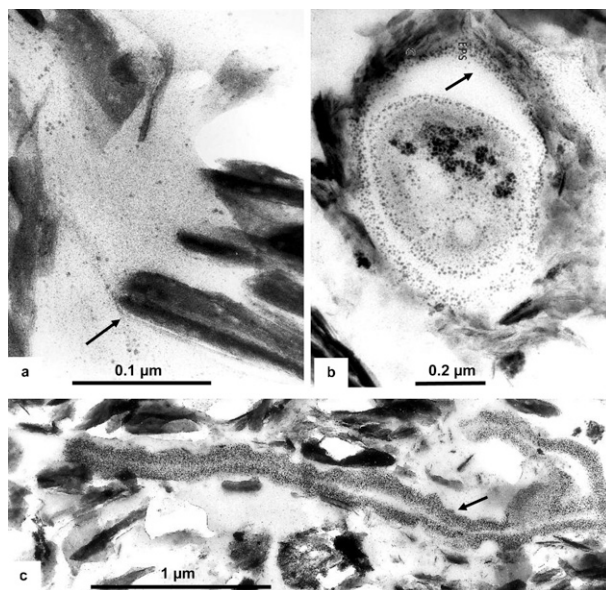


Fig. 9–11. Staining of organic matter in thin sections. Polysaccharide stained according to Thierry (1967) appears as alignments of silver grains (arrows). (a) Polysaccharide strands adsorbed to kaolinite, (b) bacteria surrounded by extracellular polysaccharides and clay particles, and (c) wall remnant (probably fungal). (Chenu, 2006, unpublished data).

Recommendations

The duration of staining steps should be tested when possible to adapt them to samples. However, the rinsing steps should always be maximized because most problems associated with staining come from insufficient rinses.

OBSERVATIONS IN THE TRANSMISSION ELECTRON MICROSCOPE

The purpose of this section is to guide new users, following the graduated steps required in the knowledge and application of theoretical physics. The first section summarizes the basic optical principles of the transmission electron microscopy technique (TEM). The second section describes the most common uses of this technique by soil scientists and explains interpretation of crystalline and amorphous phases, including how to avoid forecasted artifacts that lead to biased interpretations. The third part addresses advanced uses to optimize and validate operating modes for sample preparation, microscope settings, collection of images, and further unbiased interpretations. Finally we provide an overview of the different image supports available and their advantages and limitations.

Principles of Image Formation

The ideal imaging system of the transmission electron microscope is illustrated in Fig. 9–12. The sample is placed in the focal plane of the objective lens, which is called the eucentric position. A parallel beam of electrons illuminates the specimen. The incident electron beam is scattered by the crystalline lattice of the sample and passes through the magnetic field of the objective lens. In the process of forming the primary image, the objective lens produces a diffraction pattern at its back focal plane. This diffraction pattern approximates the Fourier transform of the scattered electron wave. In turn, the primary image approximates the inverse Fourier transform of the diffraction pattern. The primary image is subsequently magnified by additional lenses to form a highly magnified final image. This two-step process is the basis of image formation during TEM.

In high-resolution transmission electron microscopy (HRTEM) experiments, the image is formed by a direct (central) beam and a number of diffracted beams (coherent scattering) that surround the central beam. The high-resolution image formed at the image plane of the objective lens is, in fact, an interference pattern of the transmitted and diffracted beams. To select the beams to be imaged, an objective aperture of appropriate size is placed in the back focal plane of the objective lens.

Due to the small wavelength of the electron beam HRTEM provides direct structural information on individual crystals with an atomic resolution; that is, it permits the imaging of arrays of atoms (lattice images) as well as some details within individual unit cells (Buseck and Iijima, 1974). Using this technique it is possible to obtain point to point resolution of 2 to 5 Å (Eberhart, 1981).

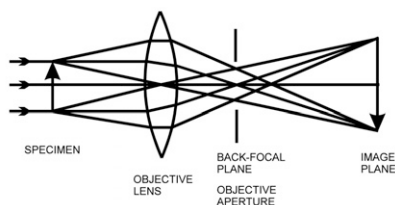


Fig. 9–12. Ideal imaging system in the transmission electron microscope (from Veblen, 1990).

Application of TEM to Soil Science

Texture

Under proper focusing conditions, two-dimensional bright-field images produced by the transmitted beam (coupled or not with diffracted beams) can be directly interpreted in terms of texture, whatever the magnification of the images. Regions of relatively high atomic density (aggregates, particles, clay layers) appear dark, whereas regions of relatively low density (pores, organic matter, clay interlayer spaces) appear white on the microscope screen and on the photographic prints. Images formed by selecting the direct beam and discarding the scattered beams, thus enhancing the atomic density contrast, are usefully adapted for noncrystalline material like colloidal and organic matter as studied in biology.

Crystal Structure

The principles of electron diffraction are similar to those of X-ray diffraction, but large differences in the much smaller wavelengths of electron beams govern the types of specimen that can be examined and the nature of the results that can be derived. The diffraction pattern is formed in the back focal plane of the objective lens and magnified on the microscope screen. It is possible to visualize one family of reticular planes at a time, that is oriented parallel to the optical axis. The beam emerging from the condenser lens has an aperture angle of about 10^{-4} to 10^{-5} rad, which depends on the first condenser aperture and the excitation of the second condenser (intensity button). The angle aperture is adjusted to produce diffracted beams following Bragg's Law, distributed symmetrically around the optical axis. The optimal aperture is a compromise, with the objective to provide the smallest diffracted spot sizes of sufficient intensity for measurement. When diffraction diagrams are not strictly symmetric according to spot positions and intensity, the reticular planes are not properly aligned along the optical axis and the projections of Fourier transforms are distorted. In such cases, measurement of spots positions will lead to erroneous calculations of lattice periodicities.

Electron-diffraction patterns can be readily recorded from a selected area that contains an isolated crystal of theoretical size as small as 10 nm in diameter. In soils, selected area electron diffraction is used for studying the crystallinity of small single crystals, but because of the generally low crystallinity of the very fine soil particles, the diffraction intensity is often so weak that exposure times as long as 1 min or more are needed. A minimum size of 100 nm for the selected area diameter is more realistic to obtain clear diffraction patterns in a reasonable exposure time.

Lattice Fringe Images

To obtain useful and characteristic structure projection in HRTEM, crystals should be oriented so that their major zone axis (e.g., for [001] in clay minerals) is perfectly parallel to the microscope axis, thereby permitting strong diffraction by many beams because rows of atoms in alignment with the beam give large phase changes (Eberhart, 1981). Consequently, for visualizing the layer-stacking of clay minerals, layers must be parallel to the electron beam. This condition can best be achieved for particles sectioned normal to their basal planes. The proper orientation of basal planes can be checked either in the electron diffraction pattern (i.e., symmetrical dots of equal intensities along the [001] row) or by the contrast in the images (i.e., lattice fringes of the silicate layers show the maximum contrast). The image of the structure is projected on the image plane of the objective lens. The HRTEM contrast results from phase difference of the imaged beams, which, in

the kinematic model, is a function of the spherical aberration of the objective lens and the optical defocus.

To perform measurement of layer periodicity, for particles properly focused (i.e., where the separate layers are clearly distinguished), the number of layers is obtained by dividing the total thickness by the interlayer spacing, which is 10 Å for micaceous minerals (Środoń, et al., 1990; Veblen et al., 1990). Special attention is required to recognize and exclude any image taken when focus of the microscope is not set properly, that is, when the microscope is overfocused or too underfocused. In both cases artifacts are introduced to the images, which make the interpretation and measurements erroneous. In overfocus conditions, the contrast is reversed as compared with underfocused conditions (i.e., interlayer spaces are white and layers are dark) so that monolayers could be mistaken for bilayers. This artifact can be recognized by examining the external fringes of particles, which should have borders darker than the background, when the microscope is properly underfocused. When underfocus is too large, the resolution is poor and, especially important, bilayers could be mistaken for monolayers because the bilayers look like one single line. This artifact can easily be recognized because lattice fringes are wide and blurred.

Operating Conditions

The most important parameters characterizing the operating conditions that have a direct effect on the resolution and interpretation of structural images are the following: the accelerating (operating) voltage, the sample thickness, the optical settings of the objective lens (spherical aberration, defocus and objective aperture), and the orientation of the sample with respect to the optical axis.

Accelerating (Operating) Voltage

In electron microscopes, electrons are produced by an electron gun, and gain high energy by acceleration within a high-voltage field (V). The wavelength of the electron beam varies with the accelerating voltage, decreasing with increasing voltage (i.e., energy). The imaging resolution is determined by the wavelength of the electron beam in accordance with the Raleigh formula: $R = 0.61\lambda/\alpha$, with R the resolution limit, λ the wavelength, and α the effective aperture size (Mackinnon, 1990). The resolution of the image is an important factor for crystalline and small objects, when diffracted beams (coherent scattering) can be used to visualize short periodic features: the higher the accelerating voltage and the larger the objective aperture, the better the resolution of the image. For noncrystalline objects, such as colloidal and organic matter, high resolution is no longer a concern since scattering is diffuse and can be eliminated in the image by an objective aperture discarding scattered beams.

On the other hand, scattering is proportional to Z^2 (coherent) and to Z (diffuse) and is inversely proportional to I^2 . Low-energy beams thus favor atomic density contrast, and increasing the accelerating voltage of the microscope is not the best choice for the observation of light structures such as clays and organic matter.

Sample Thickness

Thickness of the sample is a critical factor for TEM measurement that requires diffracted beams to contribute to the signal. To obtain good structural information about crystalline matter, the thickness of the examined objects should not exceed 10 nm (Eberhart, 1981) to 15 nm (Buseck and Iijima, 1974). Such thin sections minimize the effect of multiple scattering (called dynamical diffraction) and permit direct structural interpretation of images

according to the kinematic theory of electron–crystalline substance interactions (if other criteria like optimal defocalization, aperture size and proper orientation of crystallites are fulfilled). The *kinematic theory* is based on the assumption that incident electrons are scattered by the specimen only once, and incident and scattered electron beams have the same wavelength and therefore do not interact with each other. If these assumptions are fulfilled, then the intensity of the diffracted beam is related simply to the structure factor, which represents the amplitude of scattering. Therefore, diffraction intensities give detailed and reliable information on the structure of the specimen. *Dynamical scattering* occurs when electrons are multiply diffracted, which results in alteration of diffracted intensities and consequently disturbs and complicates the interpretation of the structure image. If the examined objects are too thick, direct interpretation of images in terms of structure–projection is no longer reliable. This is the case for ultrathin sections with thickness of 70 nm, as is advised for soil samples. However, clay crystallites, due to their naturally small size ($<2\ \mu\text{m}$) and anisomorphy (thin thickness vs. lateral extension), when deposited on grids along their (a,b) plane, commonly fulfill the thickness criterion (10 layers $\approx 10\ \text{nm}$) of the kinematic theory.

On the other hand, for TEM measurements that do not require diffracted beams, the thickness is not a limiting factor as long as the direct beam can be transmitted through the sample to form an image. Moreover, extremely low atomic density samples like organic matter (mostly formed of H and C) are better visualized on sections that are 100 nm thick.

Optical Settings of the Objective Lens

Because the microscope is not perfect, the electronic lenses have optical aberrations. The spherical aberration of the objective lens (C_s) must be taken into account because it introduces a phase shift that must be compensated for. C_s is a fixed value for each given experimental setup and is provided by the factory. Varying the focus of the image compensates for spherical aberration. If the objective current is varied, the focus also changes (Δz) and introduces an additional phase delay, which is proportional to the focus change (Δz). The minimum phase delay to compensate for the spherical aberration is the Scherzer defocalization value, which can be easily calculated using $\Delta z = 1.25\sqrt{C_s\lambda}$, where λ is the wavelength of the electrons. The value of defocus must be optimized for clays to obtain the proper phase relationship with respect to the central beam to contribute “good” contrast (Elsass, 2006). As a result, the total phase shift of the electron beams contributing to the image, which accounts for a proper contrast seen in HRTEM, thus depends on the spherical aberration of the objective lens plus the optical defocus in the image plane, i.e., a large deviation from the ideal Gaussian focus. For the purpose of studying the stacking of clay layers, the optimal conditions require an underfocus of about 150 nm for imaging both smectite and illite stacking periodicities simultaneously, with a good transfer efficiency in a microscope operated at a 120-kV accelerating voltage. Visualization of kaolinite layers requires strict adherence to the Scherzer defocus; however, this defocus has the disadvantage of showing second-order basal periodicities of chlorite. Chlorite is more easily recognized in images taken at the same defocus as smectite. The zero focus is obtained on the carbon support that covers the grid, when the contrast of carbon grains is minimized. The Scherzer defocus is obtained when the contrast of carbon grains is maximized. Computer simulations show that image details are highly sensitive to changes in defocus (Guthrie and Veblen, 1989). The astigmatism correction is set at the Scherzer defocus. An objective aperture limits the number of diffracted beams of higher orders contributing to the image, which results in reducing the background noise (Buseck and Iijima, 1974).

Orientation of the Sample with Respect to the Optical Axis

Most clay minerals are very sensitive to extended exposure by the electron beam and can be damaged (become amorphous) within the matter of seconds. This short time does not allow the operator to accurately orient the sample and consequently, the crystals almost always deviate slightly from perfect orientation with respect to the electron beam axis. Computer simulations for crystals that were tilted so that their layers were not perfectly parallel to the electron beam exhibit simple white and dark fringes (Guthrie and Veblen, 1989), which while not directly related to the ultra structure, could be generally interpreted in terms of the number of layers and interlayer spaces.

Plates, Low and High Resolution Cameras, and CCD Cameras

Plates

Plates filled with traditional silver film are stored in the camera compartment of the microscope at the level of the microscope screen. They are placed in the path of the electron beam for exposure during 1 to 2 s for direct images of the sample and for 10 to 60 s for diffraction patterns. The films require chemical processing by developing and fixing solutions before data can be exploited for printing or scanning during preparation of documents, or for measurements. The size of the silver grains in the electron image films allows a resolution in the images compatible with the resolution of the TEM optic.

Low- and High-Resolution Camera

Low-resolution cameras are placed at the bottom of the column just after the last projector lens. They scan large fields of the sample, equivalent to the plates and at the same magnification as viewed on the microscope screen. The resolution in the image is directly related to the resolution of the camera (500–2000 lines or pixels).

High-resolution cameras are placed at the bottom of the observation chamber under the microscope screen. They scan reduced fields of the sample to proportionally increase the resolution in the image with respect to full field cameras.

CCD Cameras

Raster cameras use charge-coupled device (CCD) technology, delivering digital images. Their resolution has increased dramatically during the last decade by about double every 2 yr, but a 2000-pixel resolution is still insufficient to visualize silicate layers (e.g., 1 nm) at moderate magnifications such as 50 to 100,000 $\times$. This limit is dramatic for most of the soil clay minerals sensitive to high electron fluxes required at high magnifications around 300 to 400,000 $\times$. The state of art in 2007 requires the coupling of two CCD cameras, one in low-resolution and the other in high-resolution mode. Image analysis of the raster images can be performed by image analysis software to extract geometrically quantitative data (Fig. 9–8).

The benefit of CCD cameras is their capability of allowing recording a large number of pictures within a short time and to perform image analysis, while ensuring representativeness (Elsass, 2006).

Images can be processed with the Adobe Photoshop CS software or with the ImageJ program developed by the National Institute of Health, the latter being a free software available online. This program returns data on length, area, and apparent perimeter for each individual particle. Due to the resolution of the images, the minimal particle size taken in account corresponds to a combination of minimum 5 pixels on the image. For each

grid chemical analyses of individual particles enable the identification of the different mineral or organic components and eventually the localization of the main pollutants. From the gray-scale image, the shape, and the chemical composition of the particles, it is possible to derive a multicriterion classification and a modal analysis of the components. Taking about 20 images on three different grid meshes (i.e., ~60 images per sample) makes it possible to make statistics well representative of the whole sample. The measured parameters qualifying the size of particles or aggregates allow one to establish the size distribution equivalent to that obtained by laser granulometry (Dur et al., 2004).

CONCLUSIONS

The studies of the fine soil constituents and soil solutions that have been conducted in the last two decades reflect a general evolution of concepts in soil science and pedology. They point out the importance of fine particles in the evolution of soils and in the transfer of pollutants in the environment. Furthermore, the improvements in imaging and analysis at the scale of TEM have led to the realization that the techniques classically used in research often were insufficient for the investigation of the finest soil fractions, which are the most reactive, and that bulk analysis had to be coupled with finer-scale investigation methods.

The methods used in sampling, conditioning, and preparing soil samples also benefited from developments brought about by biologists in the 1970s and researchers in aquatic environments in the 1980s. These advances enable the study of soil units in their natural complexity and fragility, which means preserving all of the different mineral and organic phases as well as their assemblages, and this within the original physicochemical conditions of the medium.

Transmission electron microscopy is an invaluable tool in soil science in the study of the crystallochemical evolution of fine terrestrial particles with respect to environmental factors and physicochemical parameters. It also enables taking into account the role of living organisms, fungi and bacteria, their affinity for chemical elements, and their capacity to modify their chemical form.

The knowledge of the surface reactivity of solid phases at molecular scale and of the dynamic character of interfacial mechanisms will help us understand interaction processes and link observed phenomena to sets of bulk parameters of the soil.

A major aspect of the composition and evolution of soils is thus now coming to light, leading us to new developments in research in line with today's demands regarding environmental health and sustainable development.

Acknowledgments

The authors have developed the expertise presented herein while working together at the Unité de Science du Sol, INRA, at Versailles. They are grateful to A.M. Jaunet and M. Robert, who contributed to this development.

REFERENCES

- Aoudjit, H., M. Robert, F. Elsass, and P. Curmi. 1995. Detailed study of smectite genesis in granitic saprolites by analytical microscopy. *Clay Miner.* 30:135–147.
- Aoudjit, H., F. Elsass, D. Righi, and M. Robert. 1996. Mica weathering in acidic soils by analytical electron microscopy. *Clay Miner.* 31:319–332.
- Balesdent, J., J.P. Pétraud, and C. Feller. 1991. Effets des ultrasons sur la distribution granulométrique des matières organiques des sols. *Science du Sol* 29:95–106.
- Ben Rhaiem, H., C.H. Pons, and D. Tessier. 1987. Factors affecting the microstructure of smec-

- tites. Role of cation and history of applied stresses. p. 292–297. *In* L.G. Schultz et al. (ed.) *Proceedings of the International Clay Conference, 1985, Denver*. The Clay Minerals Society, Bloomington, IN.
- Ben Rhaiem, H., D. Tessier, and C.H. Pons. 1986. Comportement hydrique et évolution structurale et texturale des montmorillonites au cours d'un cycle de dessiccation-humectation. Partie I: Cas des montmorillonites calciques. *Clay Miner.* 21:9–29.
- Bourdon S., Laggoun-Défarge F., Chenu C. 1997. Effets de la diagenèse précoce sur la matière organique sédimentaire d'origine palustre. Exemple du Lac Tritrivakely (Madagascar). *Bull. Soc. Géol. France, Ser. I* 168:565–572.
- Buseck, P.R. 1992. Minerals and reactions at the atomic scale: Transmission electron microscopy. *Reviews in Mineralogy*, Vol. 27. Mineralogical Society of America, Washington, DC.
- Buseck, P.R., and S. Iijima. 1974. High resolution electron microscopy of silicates. *Am. Mineral.* 59:1–21.
- Chenu, C. 1993. Clay- or sand-polysaccharides associations as models for the interface between microorganisms and soil: Water-related properties and microstructure. *Geoderma* 56:143–156.
- Chenu, C., and A.M. Jaunet. 1992. Cryoscanning electron microscopy of microbial extracellular polysaccharides and their association with minerals. *Scanning* 14:360–364.
- Chenu, C., and D. Tessier. 1995. Low temperature scanning electron microscopy of clay and organic constituents and their relevance to soil microstructures. *Scanning Microsc.* 9:989–1010.
- Chenu, C., M. Arias, and E. Besnard. 2001. The influence of cultivation on the composition and properties of clay-organic matter associations in soils. p. 207–213. *In* R.M. Rees et al. (ed.) *Sustainable management of organic matter*. CABI International, Wallingford, UK.
- Chenu, C., and A.F. Plante. 2006. Clay-sized organo-mineral complexes in a cultivation chronosequence: Revisiting the concept of the “primary organo-mineral complex”. *Eur. J. Soil Sci.* 57:596–607.
- Citeau, L., I. Lamy, F. van Oort, and F. Elsass. 2001. Nature des sols et nature des colloïdes circulant dans les eaux gravitaires: Une étude in situ. *C. R. Acad. Sci. Paris* 332:657–663.
- Citeau, L., I. Lamy, F. van Oort, and F. Elsass. 2003. Colloidal facilitated transfer of metals in soils under different land use. *Colloids Surf., A: Physicochem. Eng. Aspects* 217:11–19.
- Citeau, L., F. Gaboriaud, F. Elsass, F. Thomas, and I. Lamy. 2006. Investigation of physico-chemical features of soil colloidal suspensions. *Colloids Surf., A: Physicochem. Eng. Aspects* 287:94–105.
- de Endredy, A.S. 1963. Estimation of free iron oxides in soils and clays by a photolytic method. *Clay Miner. Bull.* 29:209–217.
- Dainyak, L.G., V.A. Drits, and L.M. Heifits. 1992. Computer simulation of cation distribution in dioctahedral 2:1 layer silicates using IR data: Application to Mössbauer spectroscopy of a glauconite sample. *Clays Clay Miner.* 40:470–479.
- Delvaux, B., D. Tessier, A.J. Herbillon, G. Burtin, A.M. Jaunet, and L. Vielvoye. 1992. Morphology, texture, and microstructure of halloysitic soil clays as related to weathering and exchangeable cations. *Clays Clay Miner.* 40:446–456.
- Denaix, L., R.M. Semlali, and F. Douay. 2001. Dissolved and colloidal transport of Cd, Pb and Zn in a silt loam soil affected by atmospheric industrial deposition. *Environ. Pollut.* 114:29–38.
- Djéran-Maigre, I., D. Tessier, D. Grünberger, B. Velde, and G. Vasseur. 1998. Evolution of microstructures and of macroscopic properties of some clays during experimental compaction. *Marine Pet. Geol.* 15:109–128.
- Dong, H.G., R.K. Kukkadapu, J.K. Frederickson, J.M. Zachara, D.W. Kennedy, and H.M. Kostandarithes. 2003. Microbial reduction of structural Fe(III) in illite and goethite. *Environ. Sci. Technol.* 37:1268–1276.
- Drits, V. 1987. Electron diffraction and high resolution electron microscopy of mineral structures. Springer-Verlag, Berlin.
- Dudek, T., J. Srodon, D.D. Eberl, F. Elsass, and P. Uhlik. 2002. Thickness distribution of illite crystals in shales. I: X-ray diffraction vs. high-resolution transmission electron microscopy measurements. *Clays Clay Miner.* 5:562–577.
- Dur, J.C., F. Elsass, V. Chaplain, and D. Tessier. 2004. The relationship between particle-size distribution by laser granulometry and image analysis by transmission electron microscopy in a soil clay fraction. *Eur. J. Soil Sci.* 55:265–270.
- Eberhart, J.P. 1981. High resolution electron-microscopy studies applied to clay minerals. p. 31–50. *In* J.J. Fripiat (ed.) *Advanced techniques for clay mineral analysis*. Developments in Sedimentology. Elsevier Scientific Publishing Company, New York.
- Eberhart, J.P. 1989. Analyse structurale et chimique des matériaux. Diffraction des rayons X, électrons et neutrons. Spectrométrie des rayons X, électrons et ions. Microscopie électronique. Dunod, Paris.

- Edwards, A.P., and J.M. Brewner. 1967. Dispersion of soil particles by sonic vibrations. *J. Soil Sci.* 18:47–63.
- Elsass, F. 2006. Transmission electron microscopy. p. 939–978. *In* F. Bergaya et al. (ed.) *Handbook of clay minerals*. Elsevier, New York.
- Elsass, F., A. Beaumont, M. Pernes, A.M. Jaunet, and D. Tessier. 1998. Changes in layer organization of Na- and Ca-exchanged smectite materials during solvent exchanges for embedment in resin. *Can. Mineral.* 36:1475–1483.
- Elsass, F., and M. Robert. 1992. Application of high resolution electron microscopy to soil clay origin and organization in temperate climate. *Geol. Carpathica, Ser. Clays* 2:55–61.
- Elsass, F., J. Środoń, and M. Robert. 1997. Illite–smectite alteration and accompanying reactions in a pennsylvanian underclay studied by TEM. *Clays Clay Miner.* 45:390–403.
- Ernstsen, V., W.P. Gates, and J.W. Stucki. 1998. Microbial reduction of structural iron in clays—A renewable source of reduction capacity. *J. Environ. Qual.* 27:761–766.
- Favre, F., A.M. Jaunet, M. Pernes, M. Badraoui, P. Boivin, and D. Tessier. 2004. Changes in clay organization due to structural iron reduction in a flooded vertisol. *Clay Miner.* 39:123–134.
- Favre, F., D. Tessier, J.M. Génin, W. Gates, and P. Boivin. 2002. Iron reduction and cation exchange capacity in intermittently and waterlogged soils. *Eur. J. Soil Sci.* 53:1–9.
- Foster, R.C. 1981. Localization of organic materials in situ in ultrathin sections of natural soil fabrics using cytochemical techniques. p. 309–319. *In* E.B.A. Bisdom (ed.) *Submicroscopy of soils and weathered rocks. 1st Workshop of the International Working Group on Submicroscopy of Undisturbed Soil Material (IWGSUM)*. Wageningen, The Netherlands.
- Gates, W.P., A.M. Jaunet, D. Tessier, M.A. Cole, H.T. Wilkinson, and J.W. Stucki. 1998. Swelling and texture of iron bearing smectites reduced by bacteria. *Clays Clay Miner.* 46:487–497.
- Gilkes, R.J. 1994. Transmission electron microscope analysis of soil materials. p. 177–204. *In* J.E. Amonette and L.W. Zelazny (ed.) *Quantitative methods in soil mineralogy*. SSSA, Madison, WI.
- Gillot, F., D. Righi, and F. Elsass. 2000. Pedogenic smectites in Podzols from Central Finland: An analytical electron microscopy study. *Clays Clay Miner.* 48:655–664.
- Glauert, A.M. 1975. *Practical methods in electron microscopy*. Vol. III. Part I. North-Holland Publ., Amsterdam.
- Guthrie, G.D., and D.R. Veblen. 1989. High-resolution transmission electron microscopy of mixed-layer illite/smectite: Computer simulations. *Clays Clay Miner.* 37:1–11.
- Güven, N. 1990. Electron diffraction of clay minerals. p. 42–68. *In* I.D.R. Mackinnon and F.A. Mumpton (ed.) *Electron-optical methods in clay science. CMS Workshop Lectures, Vol. 2*. The Clay Minerals Society, Evergreen, CO.
- Hardy, M., M. Jamagne, F. Elsass, M. Robert, and D. Chesneau. 1999. Mineralogical development of the silt fractions of a Podzolvisol on loess in the Paris Basin (France). *Eur. J. Soil Sci.* 50:443–456.
- Hayat, M.A. 1970. *Principles and techniques of electron microscopy. Biological applications*. Van Nostrand Reinhold, New York.
- Hetzel, F., D. Tessier, A.M. Jaunet, and H. Doner. 1994. The microstructure of three Na⁺ smectites: The importance of particle geometry on dehydration and re-hydration. *Clays Clay Miner.* 42:242–248.
- Holmgren, G.G., and S.S. George. 1967. A rapid citrate-dithionite extractable iron procedure. *Soil Sci. Soc. Am. Proc.* 31:210–211.
- Inigo-Inigo, A.C., D. Tessier, and M. Pernes. 2000. Use of X-ray transmission diffractometry for the study of clay particle orientation at different water contents. *Clays Clay Miner.* 48:682–692.
- Jackson, M.L. 1965. Free oxides, hydroxides, and amorphous aluminosilicates. p. 578–603. *In* *Methods of soil analysis*. Agron. Monogr. 9. ASA, Madison, WI.
- Kim, J., Y. Furukawa, T.E. Daulton, D. Lavoie, and S.W. Newell. 2003. Characterisation of microbial Fe(III) reduced nontronite: Environmental cell-transmission electron microscopy study. *Clays Clay Miner.* 51:382–389.
- Kim, J.W., D.R. Peacor, D. Tessier, and F. Elsass. 1995. A technique for maintaining texture and permanent expansion of smectite interlayers for TEM observations. *Clays Clay Miner.* 43:51–57.
- Kogure, T. 2002. Investigations of micas using advanced transmission electron microscopy. p. 281–312. *In* A. Mottana et al. (ed.) *Micas: Crystal chemistry and metamorphic petrology. Reviews in Mineralogy* 46. Mineralogical Society of America, Washington, DC.
- Komadel, P., T. Grygar, and H. Mehner. 1998. Reductive dissolution and Mössbauer spectroscopic study of Fe forms in the fine fraction of Slovak Fe-rich bentonites. *Clay Miner.* 33:593–599.
- Komadel, P., P.R. Lear, and J.W. Stucki. 1990. Reduction and reoxidation of nontronite: Extent of reduction and reaction rates. *Clays Clay Miner.* 38:203–208.
- Laird, D.A., and R.H. Dowdy. 1994. Preconcentration techniques in soil mineralogical analyses. p.

- 236–266. In J.E. Amonette and L.W. Zelazny (ed.) *Quantitative methods in soil mineralogy*. SSSA, Madison, WI.
- Lewis, P.R. 1992. Cytochemical staining methods for electron microscopy. Elsevier, Amsterdam.
- Lichtfouse, E., C. Chenu, and F. Baudin. 1996. Resistant ultralaminae in soils. *Org. Geochem.* 25:263–265.
- Mackinnon, I.D. 1990. Electron-optical methods in clay science. CMS Workshop Lectures, Vol. 2. The Clay Minerals Society, Evergreen, CO.
- Mavrocordatos, D., and D. Perret. 1995. Non-artifacted specimen preparation for transmission electron microscopy of submicron soil particles. *Commun. Soil Sci. Plant Anal.* 26:2593–2602.
- Mavrocordatos, D., D. Perret, and G.G. Leppard. 2007. Strategies and advances in the characterization of aquatic colloids by electron microscopy. p. 345–404. In K.J. Wilkinson and J.R. Lead (ed.) *Environmental colloids: Behaviour, structure and characterization*. IUPAC series on Analytical and Physical Chemistry of Environmental Systems. Vol. 10. Wiley, New York.
- Mehra, O.P., and M.L. Jackson. 1960. Iron oxide removal from soils and clays by dithionite-citrate system buffered with sodium carbonate. *Clays Clay Miner.* 7:317–327.
- Nelson, P.N., J.A. Baldock, P. Clarke, J.M. Oades, and G.J. Churchman. 1999. Dispersed clay and organic matter in soils: Their nature and associations. *Aust. J. Soil Res.* 37:289–315.
- Perdrial, N., F. Elsass, and N. Liewig. 2008. New technique for in situ sampling of particulate matter and colloids in soil and atmospheric fallout. *Colloids Surf. A: Physicochem Eng. Aspects*. doi:10.1016/j.colsurfa.2007.09.027.
- Pernes-Debuyser, A., M. Pernes, B. Velde, and D. Tessier. 2003. Soil mineralogy evolution in the INRA 42 plots experiment (Versailles, France). *Clays Clay Miner.* 51:577–584.
- Pernes-Debuyser, A., and D. Tessier. 2002. Influence de matières fertilisantes sur les propriétés des sols. Cas des 42 parcelles de l'INRA à Versailles. *Etude Gestion Sols* 9:177–186.
- Perret, D., G.G. Leppard, M. Mueller, N. Belzile, R. De Vitre, and J. Buffle. 1991. Electron microscopy of aquatic colloids: Non-perturbing preparation of specimens in the field. *Water Res.* 25:1333–1343.
- Poirier, N., S. Derenne, J. Balesdent, C. Chenu, G. Bardoux, A. Mariotti, and C. Largeau. 2006. Dynamics and origin of the non-hydrolysable organic fraction in a forest and a cultivated temperate soil, as determined by isotopic and microscopic studies. *Eur. J. Soil Sci.* 57:719–730.
- Robert, M., M. Hardy, and F. Elsass. 1991. Crystallochemistry, properties and organization of soil clays derived from major sedimentary rocks in France. *Clay Miner.* 26:409–420.
- Robert, M., and D. Tessier. 1974. Méthode de préparation des argiles des sols pour des études minéralogiques. *Annal. Agron.* 25:859–882.
- Roland, J.C. 1978. General preparation and staining of thin sections. p. 1–60. In J.L. Hall (ed.) *Electron microscopy and cytochemistry of plant cells*. Elsevier, New York.
- Romero, R., M. Robert, F. Elsass, and C. Garcia. 1992. Evidence by transmission electron microscopy of weathering microsystems in soils developed from crystalline rocks. *Clay Miner.* 27:21–33.
- Roth, C.B., M.L. Jackson, and J.K. Syers. 1969. Deferration effect on structural ferrous-ferric iron ratio and CEC of vermiculites and soils. *Clays Clay Miner.* 17:253–264.
- Schwertmann, U. 1964. Differenzierung des Eisenoxides des Bodens durch Extraction mit Ammonium Oxalat Lösung. *Z. Pflanzenernährung* 105:194–202.
- Spurr, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26:31–43.
- Środoń, J., C. Andreoli, F. Elsass, and M. Robert. 1990. Direct high-resolution transmission electron microscopic measurements of expandability of mixed-layer illite/smectite in bentonite rock. *Clays Clay Miner.* 38:373–379.
- Stucki, J.W., D.C. Golden, and C.B. Roth. 1984. Preparation and handling of dithionite-reduced smectite suspensions. *Clays Clay Miner.* 32:191–197.
- Stucki, J.W., P. Komadel, and H.T. Wilkinson. 1987. Microbial reduction of structural iron(III) in smectites. *Soil Sci. Soc. Am. J.* 51:1663–1665.
- Stucki, J.W., and D. Tessier. 1991. Effects of iron oxidation state on the texture and structural order of Na-nonttronite gels. *Clays Clay Miner.* 39:137–143.
- Šucha, V., M. Dubíková, P. Cambier, F. Elsass, and M. Pernes. 2002. Effect of acid mine drainage on the mineralogy of a distric Cambisol. *Geoderma* 110:151–167.
- Tamura, T. 1957. Identification of the 14 Å clay mineral component. *Am. Mineral.* 42:107–110.
- Tessier, D. 1989. Behavior and microstructure of clay water systems. p. 73–81. In *Management of Vertisols for improved agricultural production*. ICRISAT, Patancheru, India.
- Tessier, D. 1990. Behaviour and microstructure of clay minerals. p. 387–416. In M.F. De Boer et al. (ed.) *Soil colloids and their associations in aggregates*. Plenum Press, New York.

- Tessier, D., and J. Berrier. 1979. Utilisation du microscope électronique à balayage dans l'étude des sols. Observations de sols humides à différents pH. *Sci. Sol* 1:67–82.
- Tessier, D., A. Bruand, and Y.M. Cabidoche. 1991. Vertisols of France. p. 227–231. *In* J.M. Kimble (ed.) *Characterization, classification and utilization of cold Aridisols and Vertisols*. USDA/SMSS, Soil Conservation Service, Washington, DC.
- Tessier, D., and G. Pédro. 1987. Mineralogical characterization of 2:1 clays in soils: Importance of the clay texture. p. 78–84. *In* L.G. Schultz et al. (ed.) *Proceedings of the International Clay Conference*, Denver, CO. 1985. The Clay Minerals Society, Chantilly, VA.
- Thierry, J.P. 1967. Mise en évidence de polysaccharides sur coupes fines en microscopie électronique. *J. Microsc.* (Paris) 6:987–1017.
- Uhlík, P., V. Šucha, F. Elsass, and M. Čaplovičová. 1999. High resolution transmission electron microscopy of mixed layer clays dispersed in PVP-10: A new technique to distinguish detrital and authigenic illitic material. *Clay Miner.* 35:781–789.
- van Oort, F., A.G. Jongmans, and A.M. Jaunet. 1994. The progression from optical light microscopy to transmission electron microscopy in the study of soils. *Clay Miner.* 29:247–254.
- Veblen, D.R. 1990. Transmission electron microscopy: Scattering processes, conventional microscopy, and high-resolution imaging. p. 15–40. *In* I.D.R. Mackinnon and F.A. Mumpton (ed.) *Electron-optical methods in clay science*. The Clay Minerals Society, Boulder, CO.
- Veblen, D.R., G.D. Guthrie, K.J.T. Livi, and R.C.J. Reynolds. 1990. High-resolution transmission electron microscopy and electron diffraction of mixed-layer illite/smectite: Experimental results. *Clays Clay Miner.* 38:1–13.
- Villemin, G., and F. Toutain. 1987. Method to stabilize organo-mineral soil samples for transmission electron microscopy. p. 43–48. *In* N. Fedoroff et al. (ed.) *Soil micromorphology*. Proceedings of the 7th International Working Meeting on Soil Micromorphology, Paris. July 1985. Association Française pour l'Etude des Sols Publ., Plaisir, France.
- Vicente, M.A., F. Elsass, E. Molina, and M. Robert. 1997. Paleoweathering in slates from the Iberian Hercynian Massif Spain: Investigation by TEM of clay mineral signatures. *Clay Miner.* 32:435–451.
- Watson, J.R. 1971. Ultrasonic vibration as a method of soil dispersion. *Soils Fert.* 34:127–134.

REFERENCES FOR LABORATORY SAFETY

- NIOSH International Safety Cards**—The Occupational Safety and Health Act of 1970 created both NIOSH and the Occupational Safety and Health Administration (OSHA). OSHA is in the U.S. Department of Labor and is responsible for developing and enforcing workplace safety and health regulations.
- MSDS-Search**—The purpose of a Material Safety Data Sheet (MSDS) is to inform industrial purchasers and users of hazardous chemicals of the reasonably foreseeable physical and chemical hazards that may arise from the use of those chemicals.
- Safety Data Sheets**—Available at <http://www.quickfds.com> (verified 19 Oct. 2007). Quick-FDS, Paris.

Scanning Electron Microscopy

G. NORMAN WHITE, formerly Texas A&M University, currently Texas Commission on Environmental Quality, Austin

The scanning electron microscope (SEM) has very diverse uses in the study of soils. Only those solids that decompose in a very weak electron beam or are unstable in even the slightest vacuum cannot be examined with scanning electron microscopy. The SEM has a large magnification range, allowing examination of solids with almost no magnification to imaging at well over 100,000 times. Another important feature is the large depth of field of SEM images, which appears three-dimensional and provides much more information about a specimen's topography and surface structures than light microscopy at the same magnification. In most instruments, the specimen can be moved in the x , y , and z directions as well as rotated about the vertical axis and tilted almost 90° . These movements facilitate obtaining quality images and optimum sample information.

A very close relative of the SEM is the Electron Probe Microanalyzer (EPMA). The basic optical design of an EPMA is very similar to an SEM, but the EPMA is arranged to optimize X-ray signal detection, while the SEM is optimized for visual information and only secondarily for X-rays and other electron interactions.

In this chapter, the basics of scanning electron microscopy are presented, with special emphasis on maximizing the quality of data obtained from the instrument. This includes the identification of minerals with an SEM and recognition of soil features that may not otherwise be detectable. As EPMA is discussed elsewhere in this book (Guillemette, 2008), this chapter covers only examination of a sample using visual characteristics and compositional characteristics that can be detected by energy dispersive X-ray spectrometry (EDS), an accessory that is available on most SEM instruments and is almost vital for proper identification of minerals with the instrument. There are several treatises on the background and theory of scanning electron microscopy (e.g., Goldstein et al., 1992, 2003). For more detail about electron optics and SEM use, check these books or the chapter on EPMA in this volume (Guillemette, 2008, this volume).

While the X-ray signal from EDS is often vital to the correct identification of minerals in soils and sediments, SEMs are not optimized to obtain quantitative compositional data. Such data can only be obtained with an SEM for the higher atomic number elements if they have a significant concentration and then only after making assumptions that need not be made with an EPMA. Obtaining good quantitative compositional data is easier with an EPMA (Guillemette, 2008, this volume).

The environmental scanning electron microscope (ESEM) is a recent modification of traditional SEM. The major difference between ESEM and traditional SEM is the much

higher specimen chamber pressures that are allowed in the ESEM. A differential pumping system allows pressures of several hundred Pa in the specimen chamber while maintaining a pressure of 1.3×10^{-11} MPa near the electron gun. The high vacuum near the electron gun makes it possible to use high intensity filaments such as a LaB_6 filament and get a significant signal despite the higher sample chamber vacuum. The ESEM allows the introduction of various gases and allows the partial pressure of the gases in the chamber to reach up to 0.0027 MPa. With the addition of a peltier cooler, this pressure is sufficient to condense water on a specimen. The higher gas pressure serves the additional purpose of transmitting heat and excess electrons away from the specimen, with the result that sample coating is not needed.

The ESEM exhibits its full potential in studies involving gas interactions or effects of temperature on a sample. The helpful addition of a heating stage allows the control of temperature up to 1000 °C. The ability to control atmospheres permits the study of surface–gas interactions. As the effects of heating or reactions with gases are kinetically controlled, video output allows the storage of video data for future use, in addition to photography. Instruments are only recently gaining sufficient control over the atmosphere to obtain reliable data on soil wetting because the range of humidity involved in soil wetting is too small; thus, the full potential of these instruments is to be determined in the future.

SAMPLE PREPARATION FOR USE IN SOIL MINERALOGY

The Importance of Coating a Sample

When a sample is examined by SEM, a large number of electrons are striking the surface. If these electrons are not removed, the sample may be damaged by heating and an area with a high electric charge may result. In practice, this is most often observed as a dark area surrounded by a brightly glowing area that increases in light intensity with time during examination. This phenomenon is termed *charging*. The presence of sample areas that are charging is more than an inconvenience; particles may become loose from the sample and affect the valves in the vacuum system. The particles may also decompose chemically. It is impossible to examine the sample near the charging areas because the beam is affected by the electrical charge. Although examination of a sample with no conductive coating is possible at very low beam voltages, the low beam voltage renders use of EDS impossible, limits the amount of signal available for visual examination, and limits examination to relatively low magnifications. A lack of signal strength translates into a lack of contrast in the output. For that reason, most samples are coated with a thin layer of conducting material to allow removal of excess electrons to ground, thereby preventing charging.

Sample coating involves the addition of a very thin layer (about 10 nm depending on the sample) of a conductive material to the surface of a sample. This layer is added in a sputter coater, a specially designed device in which high voltage is applied to a negative cathode constructed of the target material to be sputtered (typically gold or gold–palladium) in a vacuum. A diffuse cloud of positively charged ions results and they impinge on the specimen from all directions. A coating of ions condenses evenly on the specimen, placed on the anode, which is kept at ground potential. Carbon coating prepares specimens by evaporating high purity carbon under vacuum onto the specimen in a manner similar to the metal coating method. A thin layer uniformly covering the specimen is the desired result. The coating should be sufficiently thick to transmit the excess electrons, reducing problems with charging, but not so thick as to obscure morphological or chemical features on the sample. The choice of coating is very important because it affects the quality of the images produced and may interfere with some elements during EDS. Coating the sample

Table 10–1. Commercially available elements used for coatings and elements with EDS peaks that would overlap to some degree with the coating.

Coating	X-ray peaks keV	Elements affected
Al	1.487	Mg, Al, Si
Cr	5.411	V K β
	5.947	Mn K α
Cu	8.041	Ni K β
	8.907	Zn K α
Au	2.1–2.2	P K α , S K α
	9.7	Zn K β
Au/Pd	2.1–2.2	P K α , S K α , Y L β , Zr L β
	2.838	Cl K β
	2.99	
	3.172	
	3.328	K K α
	9.7	Zn K β
Ir	1.9–2.1	P K α , Y L β , Zr L β
	9.2	Zn K β
Ni	7.472	Co K α
	8.265	Cu K α
Pd	2.838	Cl K β
	2.99	
	3.172	
	3.328	K K α
Pt	2.0–2.1	P K α , S K α , Y L β , Zr L β
	9.44	Zn K β
Pt/Pd	2.0–2.1	P K α , S K α , Y L β , Zr L β
	2.838	Cl K β
	2.99	
	3.172	
	3.328	K K α
	9.44	P K α , S K α , Y L β , Zr L β
Ag	2.984	I L α
	3.348	K K α , Ca L α , In L α , In L β
Ta	1.775	Si K α
	3.149	K K α
	8.145–9.341	Ni K α

with carbon will result in the best EDS data, but charging is potentially more of a problem and the micrographs do not have the contrast and resolution of metal-coated samples.

Many metals are used for the conductive coatings. Equipment catalogs show numerous choices, including aluminum, chromium, copper, gold, gold–palladium, iridium, nickel, palladium, platinum, platinum–palladium, silver, and tantalum. The user must be careful to choose a coating that does not interfere with the interpretation of the EDS patterns if used. Table 10–1 lists potential peak interferences by coating materials in EDS. The most common metal coatings are gold, a mixture of platinum and palladium, and gold and palladium. Gold has X-ray peaks in the region just above 2 keV and between 9 and 10 keV. The second peak does not interfere with the principle peaks for any important elements, but the location of the first peak interferes with the peaks from P and S and would not be a good choice for a coating if those elements are important in your study. Platinum interferes with the same elements as gold, while palladium interferes with chlorine and potassium. Potassium is very important in the identification of micas and feldspars, so the use of gold for a metallic coating is advised for work involving those minerals.

The metallic coatings usually produce better quality micrographs, but when minerals containing S or P are to be examined, carbon coating is the best choice. Carbon does not have EDS peaks in the region above 1 keV and thus does not interfere with EDS detection of higher atomic number elements, but carbon coatings are not as efficient at removing the electrons, which may reduce the quality of the micrographs, especially at high magnifications. In addition, while the use of a sputter coater to apply metallic coatings is relatively easy even for a beginner, application of an adequate carbon coating requires experience.

Sample Preparation: Peds vs. Grains vs. Thin Sections

The first step in examining samples with an SEM is preparation of the sample. This step is often time-consuming and should be finished before the time scheduled for SEM examination of the sample. Do not assume that the sample can be prepared for examination in a negligible amount of time.

To a great degree the type of information desired determines the method used to examine a sample with an SEM. In general, soil is prepared for SEM examination in three ways, as individual grains, peds, or as thin sections. Each method can yield different information about the sample. Examination of individual grains allows the user to obtain qualitative information about grain morphology and relative concentrations of different minerals in a similar manner to that used in petrographic microscopy for grain counts. With each method, the sample must be strongly adhered to a sample holder and coated with a metal or carbon to transmit the excess electrons and heat from the area examined. Which method is appropriate for use is the first decision to be made by an SEM user.

Examination of Single Mineral Grains

Examination of single grains is best done on individual size fractions. See Soukup et al. (2008, this volume) for information on particle size separation and pretreatment. More information with less preparation can be obtained by examining the finer sand fractions and the silt. These fractions have more mineralogical variability, and more grains can be placed on one sample holder. Examination of single mineral grains in a size fraction allows one to see the variability in grain morphologies. Surface morphology may reveal information about weathering intensity, environment of deposition, parent material, or past history of a soil (e.g., the discussion on Decatur soil in White and Dixon, 1998). The observation of a mineral that is present only in trace quantities within a sample is more likely during examination of grain mounts of individual grains, especially when the mineral has a morphology that contrasts strongly with the minerals that make up the majority of the sample.

To prepare a sample fraction for examination:

1. Place a conducting adhesive material on the surface of the sample holder. The standard material for such examinations in the past was double-sided tape, but an apparent change in manufacturing resulted in the tape becoming unstable in the electron beam. Several manufacturers produce metal or carbon conducting tapes or tabs that are specifically designed for this use. Liquid adhesives should be used with caution because the thickness of the adhesive is often significant when compared with the thickness of the grains, and capillary action may obscure portions of the sample.
2. Sprinkle a small number of grains onto the adhesive. Use a spatula with as small a surface as possible to scoop up about 1 mm³ of grains. Sprinkle the grains as evenly as possible over the adhesive surface by gently tapping the side of the spatula while holding it above the adhesive. Be careful not to add too much material. Sand grains should be well separated. When working with silt fractions, you can examine the stub with a binocular microscope to check for loading. Experience will allow you

to make this judgment by differences in the appearance of the adhesive. Too many particles can result in problems from a shadow effect.

3. Make sure that the material is adequately adhered to the surface. Softly pushing sand grains down increases adhesion with the sample holder, but care must be taken not to avoid marring the surface of the grain. There may be features not readily observed by other methods that could be disturbed by frequent handling or manipulation, resulting in artifacts. Adhesion is rarely a problem with silt because of the higher surface area. Blow loose grains from a silt fraction SEM mount with a gas duster or remove them by tapping the mount while it is inclined at a high angle. Failure to remove poorly adhered grains will result in areas that are obscured by sample charging and the possibility of sample loss within the microscope column. In a worst case scenario, a grain lost from the sample may get caught in one of the vacuum valves, rendering the microscope inoperable. In any case, loose grains will result in areas that cannot be examined due to sample charging because the excess electrons cannot be removed when grains are poorly adhered to a conducting mount.
4. Coat the sample for use with either carbon or a metal coating using a sputter-coater following the manufacturers instructions. The removal of the electrons is the purpose of sample coating.

Examination of Peds

Examination of peds also requires careful sample preparation. The sample needs to be dried and fixed to the sample holder, but choosing the area to be examined requires care. Peds should be examined on a fresh fracture surface to avoid artifacts due to handling or smoothing. Examination of peds allows observation of morphological features of the minerals and soil matrix itself, how peds or particles are arranged relative to each other, the presence of coatings and cutans, and the void sizes and arrangements.

There are several potential problems that may be encountered when examining peds. Ideally, the ped or portion of ped examined must be sufficiently cohesive to remain intact during sample preparation and under examination in the microscope. This requires the presence of a certain amount of clay or natural cementing agent to bind the ped into a cohesive unit. As the clay content decreases, a problem with grain charging is often observed because the grain contacts are insufficient to allow the heat and electrons to diffuse from the area examined. The particles begin to become brighter with time, may begin to move, and possibly fly off the sample holder. Minerals may or may not be identifiable when viewed because areas visible by imaging are not necessarily unobstructed by other grains for the detector used for determination of composition and because the beam may penetrate the particle being observed, resulting in an apparent composition with contributions from underlying grains. It is important to recognize that the EDS characteristics observed might not entirely result from the particle that you are examining. Electrons penetrate from 2 to 5 μm or more depending on the beam kilo electron voltage. As a result, EDS patterns for very thin grains may have significant contributions from underlying grains.

A less obvious problem is due to the liquid adhesive used to bind the specimen to the sample holder. This adhesive may wick up through the pores of the peds by capillary action and appear in the region that is to be examined. Without EDS, features resulting from the adhesive are hard to distinguish from features of the ped. Most conducting adhesives are either organic or contain a metal such as Ag. The researcher should always know what the EDS characteristics are for the sample adhesive to prevent mistaking the adhesive for sample features.

When preparing a ped for examination, the problem arises as to the effect of water on the fabric of the ped. If the sample is to be examined using ESEM or in an environmental cell, no drying step is needed. In a normal SEM, sample instability is likely to result from drying of the sample due to thermal expansion of the water resulting from the vacuum and the heat produced by the beam.

To prepare a ped for examination:

1. Dry the ped in an oven at 105°C or remove the free water in some other manner such as acetone displacement and drying.
2. Trim the ped to fit the appropriate stub size. Remove any loose grains by tapping or using a gas duster or other source of a light wind. Be careful, as many gas dusters produce too high a flow rate for this use. A smaller ped is less likely to break off the sample holder and is much less likely to have charging problems. Most microscopes have some limitations on sample size both vertically and horizontally. Too large a sample may not fit into the microscope for examination. In addition, a sample that is too tall may damage the detectors by contact or may limit the working distance.
3. Adhere the sample to the sample holder (stub). The method used could include a conducting adhesive tab or some type of liquid adhesive. If using a liquid adhesive, apply it only to the sample holder and allow it to begin to dry slightly before adding the sample. Check to make sure that the sample is adequately attached to the stub. Except when used in an ESEM, the sample must be strongly adhered to the holder with a conducting adhesive. Low adhesion results in more problems with charging, as does use of a nonconducting adhesive.
4. Coat the sample for use with either carbon or a metal coating.

Examination of a Sample Prepared as a Thin-Section or Polished Block

A thin section or polished block is by nature smooth and will lack topographic relief that is common in peds or single grains. Thus, thin sections or polished blocks are often more suited to backscatter electron imaging or X-ray analysis and imaging. The benefit of using a thin section ground to a thickness of 25 to 30 μm is that the images observed in a petrographic microscope can be correlated with SEM or EDS images. Optically, a polished block can be viewed by reflected light, but it lacks the detailed fabric and mineralogical information observed in a petrographic thin section.

Use of thin sections or polished blocks is more often used with EPMA than an SEM but there may be occasions when the SEM may be used to answer questions about the thin-section interpretation, for example, comparison of the distribution of Fe and Mn in Fe-Mn nodules. Thin section analysis with SEM is also useful to search for obscured sedimentary features by mapping for elements that occur in highly stable, high-specific gravity minerals such as Ti and Zr, or as a quantitative analysis tool using the EDS system.

Free water in the sample must be removed before impregnation. To prepare a thin section or polished block for examination:

1. Consolidate the sample with an impregnating agent. Epoxies are used because other resin impregnation agents such as polyester resins are unstable under the electron beam. In selecting an impregnating resin, there are several factors to consider. The first is the stability of the epoxy under the vacuum and electron beam conditions used in the microscope. All epoxies are somewhat unstable under the beam, but some are better than others. The manufacturer should provide this information. The second factor is the composition of the epoxy. Many epoxies contain elements that will show up on the EDS pattern. For example, many products contain trace amounts of Cl or S and some marine epoxies contain Si. Another factor is the viscosity of the

fixative. If the epoxy is too viscous, it may not penetrate into the finer pores and polishing the sample may be difficult. Diluting with acetone may decrease the viscosity, but this increases the length of time needed for the epoxy to harden. A harder epoxy polishes more evenly. It is best to select an epoxy especially formulated for sample impregnation, ability to transmit electrons, and stability in an SEM.

2. Once the sample is impregnated, produce a flat face that bisects the features of interest by cutting with a diamond saw.
3. Make a second cut parallel to the first to produce a 3- to 5-mm thick block. Trim the edges to accommodate the SEM sample holder. The thickness of the block is not as critical as when producing thin sections for petrographic use because only the surface will be examined. If the sample is too thick, however, there is the risk of damage to the signal detectors in the microscope.
4. Polish the face to be examined with increasingly smaller grit size polishes until the final polish is with a grit size much smaller than will be resolved in the sample. At the minimum, polishing should continue to $<1\text{-}\mu\text{m}$ grit size because a high spot on a flat surface may absorb the beam from the area examined. This step is more essential for proper examination with this technique than with light microscopy because of the effects that small variations in surface topography may have on EDS data.
5. Attach the sample to a sample holder and coat the sample with either carbon or a metal coating.

Advanced SEM Techniques in Soil Mineralogy

Finding a Mineral with a Low Concentration in a Sample

In some studies using an SEM, it is desirable to be able to find certain minerals that do not occur at high concentrations even in individual size fractions. Reasons might be that the morphology of the mineral may indicate a distinctive parent material, or the mineral may be an important weathering product. X-ray images are useful for finding a mineral that contains a distinctive element, for example, Ti in titanium oxide minerals. The most effective method to perform such searches is to obtain an image for an area at a low magnification and resolution. Then, increase the magnification in areas where characteristic X-rays show elevated concentrations of the elements in the sample. This method is useful to determine the morphology of a certain mineral in a size fraction when the mineral comprises only a small fraction of the total sample or mineral. The mineral need not be distinctive in a single element as you can simultaneously map several elements at once and look for certain combinations of elements, for example, Fe and Ti to find ilmenite (FeTiO_3) or Ca, Ti, and Si to find sphene (CaTiSiO_5). Both minerals are often found in soils but are usually present at low concentrations.

Staining for SEM

Hillier and Clayton (1992) used a CsCl_2 cationic “stain” to examine the heterogeneity of clays in a thin section using an electron microprobe. The premise was that changes in mineralogy, especially the decrease in smectite content, would be detectable because of the change in cation exchange capacity (CEC). They chose Cs^+ for the exchangeable cation because it was not found in significant quantities in most geological samples and it forms a one-layer water complex, thereby alleviating concern for the clay swelling out of the thin section during the exchange process. They tested no other cations but were of the opinion that Sr^{2+} and Ba^{2+} would also be suitable. A common exchange cation always helps in the interpretation of EDS patterns. This method could be expanded to include anionic “stain-

ing.” but problems occur with the choice of anion as the most obvious choices, PO_4^{3-} and SO_4^{2-} , are hampered by the energies for the P and S K-lines overlapping a peak from the Au coating. The use of AsO_4^{3-} is a problem if Mg data is required because the As L-series peaks overlap Mg K-peaks, but still has potential if the beam voltage is sufficiently high to excite the As $\text{K}\alpha$ and $\text{K}\beta$ peaks at 10.5 and 11.7 KeV, respectively. Most of the other anions are not held with sufficient strength for use. The potential for use of CuCl_2 as a “stain” for organic material has yet to be examined.

To stain the thin section with CsCl_2 :

1. Prepare the thin section as described above, except do not coat the sample.
2. Immerse the thin section in a 1 M CsCl_2 solution overnight.
3. Rinse the thin section thoroughly several times with deionized water.
4. Allow the sample to dry thoroughly.
5. Coat the sample for use.

CHOICE OF OPERATING PARAMETERS FOR SEM USE

This chapter can in no way replace the experience obtained from operating an SEM, and assumes that the reader is either trained in the use of an SEM or accompanies an operator of an SEM. The author advises anyone who desires SEM data be present when the sample is examined. It is extremely rare that a person who operates an SEM for others knows what the researcher is looking for or wishes to determine. Therefore, it is important that the researcher be present during sample examination. This section explains how the choices in operating conditions may affect the results obtained from SEM examination of a sample.

The method used for specimen magnification in SEM differs considerably from that used in light microscopy and transmission electron microscopy (TEM). In light microscopy, a condensed light beam passes through a specimen, is refracted by sets of objective and projector optical lenses, and focused on the eye. The optical system used in TEM is very similar. In TEM, a condensed electron beam passes through a specimen and is focused by magnetic objective and projector lenses such that a magnified image is formed on a fluorescent screen. In SEM, a highly focused electron beam scans the surface of the specimen in a raster mode (Fig. 10–1). The interaction of the beam with the surface results in the formation of several types of signals (Fig. 10–2). The desired signal is collected and converted to electron pulses that are then used to reconstruct the image on a cathode ray tube (CRT) spot by spot (Goldstein et al., 1992).

The electron beam used in SEM is much smaller than that used in TEM. Like TEM, the electron beam is generated by a voltage difference between a filament (cathode) and an anode. The magnetic condenser and objective lens concentrate the beam into a very small spot about 5 nm or less in diameter and focuses the beam onto the specimen surface. The scanning coil shifts the beam in horizontal directions at increments such that the beam scans a rectangular area during a scanning cycle. Focusing is an adjustment of the objective lens to make the sharpest section of the beam fall on the specimen. The desired signal given off by the specimen is collected and shown on the CRT screen as a point whose brightness is proportional to the original signal strength.

The choices of beam voltage, aperture size, operating distance, and imaging method define the quality of the results that are obtained. In most cases, certain combinations of these settings are required to obtain suitable data. In some cases, compromises must be made as the settings that optimize one type of data reduce the quality of another.

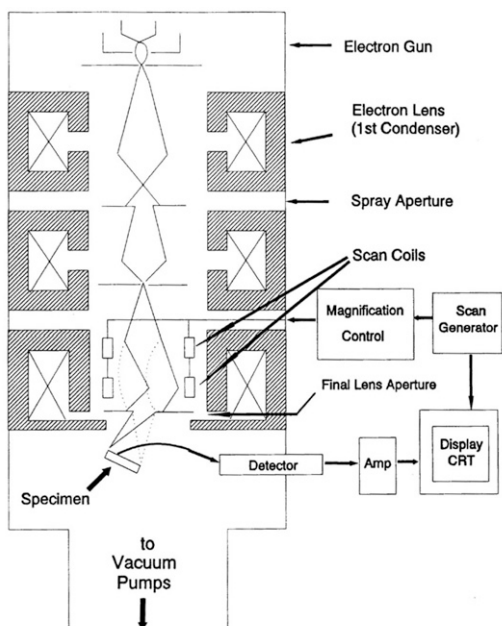


Fig. 10-1. Schematic representation of a scanning electron microscope. (a) Basic geometry. (b) Detail showing the locations for some signal detectors relative to the sample in a SEM.

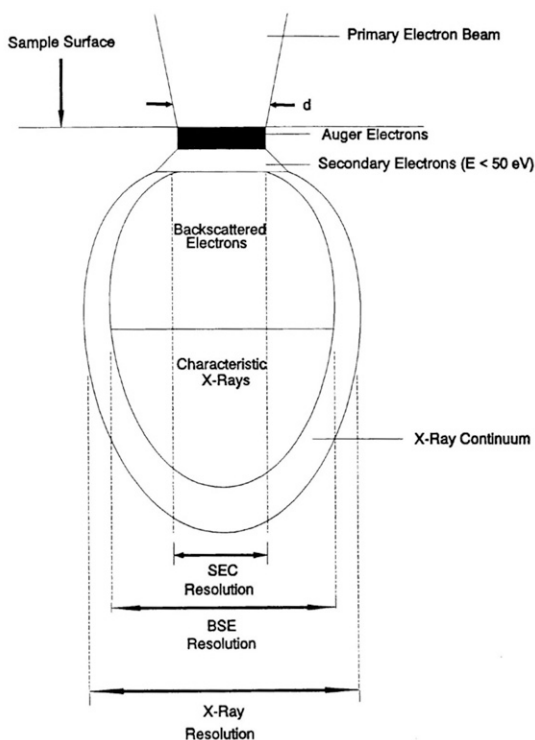


Fig. 10-2. Signals produced by interactions of an electron beam with a solid that are potentially detectable by SEM. Other signals may be produced that are observable with other techniques, such as electron probe microanalysis.

Choice of Imaging Method

As discussed above, the SEM beam is not stationary as in a TEM but is rastered across the specimen. A rastered beam starts at one corner of the area being viewed and moves across the top of the image area, then drops down a distance equal to the size of the viewing area divided by the vertical pixel resolution of the monitor and moves horizontally across the specimen in a straight line again repeating until the bottom of the viewed area is reached. The rastered beam interacts with the surface of a specimen, producing a variety of signals. To produce images, these signals are sampled in some manner, with the results displayed on a CRT, producing an image much like that used by a common television. A rastered beam is time resolved, since the signal is recorded almost instantaneously as compared with the rate of the beam movement. The contrast results from the differences in signal captured for each pixel. The differences in signal strength may result from specimen relief or changes in composition of the specimen.

Each of the signals described above need to be detected to produce an image. Most signals are initially analog; that is, the signal is captured continuously as the beam is rastered. For viewing on a cathode ray tube or photography, this manner of signal sampling is more than adequate. Many applications such as image processing or computer data storage require a digital image. In a digital image, the signal is averaged over the time it takes for the beam to move horizontally across the viewing area divided by the horizontal pixel resolution. This averaging results in a grid of signal values. The viewed image is the result of arranging the signal pixels into a grid corresponding to the position of the beam at the time that the signal was captured. One advantage of digital image capture is that averaging many individual images can produce an image that is superior to the analog image by reducing the signal/noise ratio, as the noise is unlikely to occur at the same position in each scan. This average, however, assumes that the material being imaged remains stationary throughout the averaging process, hence the importance of proper adhesion of the specimen to the sample holder.

Image contrast in SEM images is the visualization of relative differences in the number of image signal counts that were obtained for an individual pixel as compared with other pixels in a view. The actual number of counts in a signal is not as important as the differences, as setting the contrast on an SEM is the result of assigning a lower limit of counts to be black and an upper number of counts to be white. This is usually done with a set of controls that set the brightness and contrast. The brightness control sets all channels with a count rate below a certain level to be black in the image. The contrast controls the count rate above that level setting the count rate that is white and the shape of the curve that converts intermediate counts to screen brightness. This conversion is most often linear, but there is the ability to apply various nonlinear functions to the brightness conversion, allowing the experienced user to emphasize specific features, such as small relief changes, that normally lack enough contrast to be observed.

Image contrast in the SEM depends somewhat on what type of signal is being collected, but in general it can be considered the result of sample composition and topography. The expression of these two factors varies with the type of signal being collected. If the difference between the highest number of counts and the lowest number of counts is low, the image will appear grainy due to noise becoming significant relative to the total difference. Digital signal collection methods can overcome this problem to some degree by averaging the results over several scanning cycles. Any digital signal storage method assumes a maximum signal (e.g., 8-bit, 16-bit, 32-bit) and scales the data to this result. In any situation, better results are always obtained if the total strength of the difference is greater.

Several types of output are available for imaging from an SEM equipped with an EDS detector (Fig. 10–2). The most commonly used are backscattered electrons, secondary electrons, and X-rays. Each method has advantages and disadvantages.

Backscattered Electron Images

Backscattered electrons are those electrons from the incoming beam that have been scattered by interacting with the nuclei of the atoms within the sample and coating. The energy of these electrons can be as high as the beam energy and as low as a few electron volts depending on their path through the sample. Images from backscattered electrons are usually formed from electrons captured with a solid-state detector, normally surrounding the last lens opening at the bottom of the microscope column. Because backscattered electrons have higher energy than secondary electrons, an electrical bias cannot be used to draw the electrons to the detector. Therefore, backscattered electron images are the result of the collection of only those electrons that are scattered in the direction of the detector. Because the electrons cannot be gathered magnetically or by use of an electrical bias, better results are obtained using relatively large detectors arranged geometrically as close to the specimen as possible. The higher energy of backscattered electrons allows these electrons to represent interactions with the sample from a greater depth. As a result, backscattered electron images are generally less sharp than secondary electron images, especially at higher magnifications. For that reason, secondary electron images (discussed below) are usually preferred over backscattered images. One instance where backscattered electron images are preferred is in the examination of thin sections using an SEM (Fig. 10–3).

Backscatter images have two sources of contrast, topographic contrast and atomic number contrast. Areas of a surface perpendicular to the beam will not produce as many backscattered electrons as areas toward the detector. Topographic contrast uses a combination of detectors with different geometries relative to the specimen to determine the topography of the specimen. Increasing atomic number increases the quantity of backscattered electrons by reducing the depth of penetration of the impinging electrons. This reduces the path between collisions of the impinging electrons and specimen atoms. There is also an increase in the number of electrons that have single scattering events (collisions), with collisions closer to the surface (Goldstein and Yakowitz, 1975). As a result, all other factors

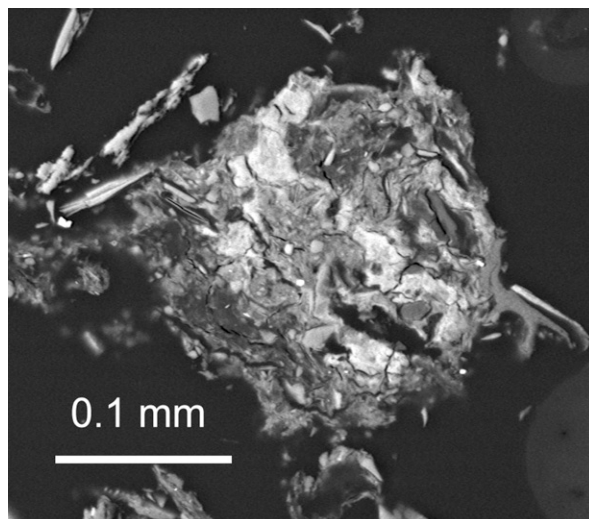


Fig. 10–3. An example of a backscattered electron image of a rock fragment undergoing spheroidal weathering from a thin section of grains from a Bangladesh rice soil. The brighter region forming a ring in the central region of the particle is due to atomic number contrast resulting from an increased concentration of a higher average atomic number in that region because of the precipitation of Fe oxides in that portion of the particle along the spheroidal weathering front.

being equal, a particle with higher mean atomic number will appear brighter than a particle with a lower mean atomic number when observed using backscattered electrons. This is a great advantage when examining thin sections with their lack of topographic relief.

To differentiate topographic and atomic number contrast, many solid-state backscatter detectors are radially divided into several sectors. With the detector surrounding the beam, contrast resulting from topography will be removed if the signals from opposite sectors are combined. Conversely, subtracting the signal for one sector from that of the opposite detector can emphasize topographic contrast.

Secondary Electron Imaging

Secondary electron imaging is the most commonly used imaging method in SEM. Secondary electrons are produced through the interaction of electrons with the atoms in the sample when inelastic collisions occur between the loosely bound outer electrons and the incoming beam. These collisions result in an energy loss from the beam electrons and the ejection of the loosely bound electrons with energy typically <50 eV. If the secondary electrons are produced close to the surface (<10 nm) and have sufficient energy to escape the surface (2–6 eV), then they may escape and be detected. That these electrons can only escape from close to the surface makes the image produced sharper since it is from a very small sample volume.

The most widely used detector for secondary electrons is the scintillator-photomultiplier detector as developed by Everhart and Thornley (Goldstein et al., 1992). The basic component of the detector is a scintillation material that emits light when struck by high-energy electrons (Fig. 10–4). A low positive voltage serves to collect the low-energy secondary electrons from the specimen by attracting them to the vicinity of the cage, where they respond to the potential on the scintillator. The light produced by the scintillator follows the light guide to the photomultiplier tube where it is converted to energy. This system is very efficient as a very large gain in amplitude is obtained. The light guide can be bent, which allows the detector to be optimally positioned relative to the specimen. The surface producing the signal does not require an unobstructed field of view to the detector, resulting in images with a three-dimensional quality (Fig. 10–5). It must always be remembered, however, that this detector will also collect that part of the backscattered electron beam that strike the detector. This quantity is relatively small when the working distance (discussed below) is long, and the detector represents only a small portion of the total area of the sphere around the specimen that represents the directions in which electrons can be backscattered.

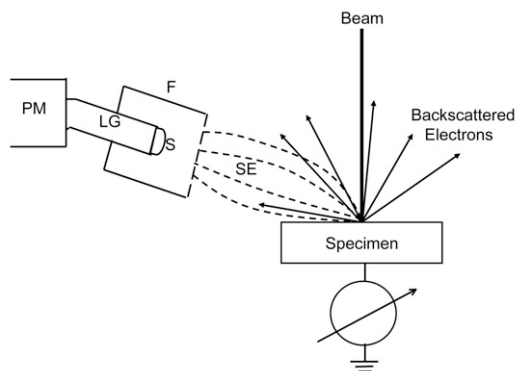


Fig. 10–4. A schematic of an Everhart–Thornley detector modeled after Goldstein and Yakowitz (1975). The specimen holder is shown in the level position as it would normally be used in SEM unlike the original figure. Solid arrows show backscattered electrons. SE, secondary electrons (dashed lines); F, Faraday cage; S, scintillator; LG, light guide; PM, photomultiplier.

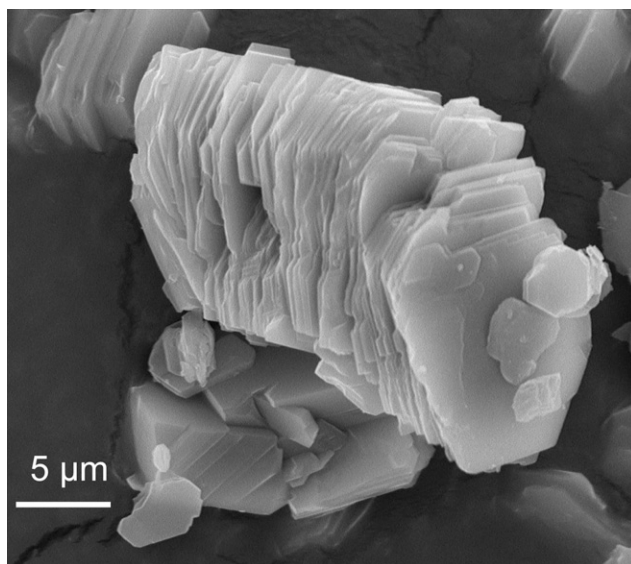


Fig. 10-5. An example of a secondary electron image of kaolinite vermiculite from Keokuk, Iowa. Notice the three-dimensionality of the image.

X-ray Signal Capture for Mineral Identification and Mapping

When the electron beam strikes the sample, X-rays are produced from interactions of the beam with the sample. The X-rays produced can be placed into two broad categories, white X-rays and characteristic X-rays. When an electron beam is inelastically scattered by the nucleus, the electrons lose energy, resulting in the formation of white X-rays. These X-rays do not have energies characteristic of the sample composition, but instead have a variety of energies ranging up to that of the incident beam. However, when the beam electron strikes an inner shell electron, the primary electron beam loses energy equivalent to the binding energies of the K, L, or M shells, and the electrons are ejected. This difference in energy results in either the production of characteristic X-rays or the ejection of an Auger electron. Characteristic X-rays are called such because the energy of the X-rays produced can be used to identify the elements present in the sample. The relative probability of X-ray versus Auger electron production is higher with increasing atomic number.

X-rays can be collected in several ways. Most commonly, an X-ray spectrum is collected for an area of a specimen using an energy dispersive X-ray analyzer (EDS) or multiple X-ray goniometers. These spectra can be used to obtain an estimate of the chemical composition of the selected area and will be discussed in more detail later in the chapter. It is also possible to obtain images based on the characteristic X-rays given off by the sample by setting the detector electronics to image using only those X-rays with energies in a set range. These images are termed *X-ray maps* and can be obtained for any element detectable by the X-ray detector in the SEM. The most common method of detecting X-rays in a SEM is by use of EDS. The EDS detector is usually pointed toward the sample from a position above and to one side of the sample. Due to the cost of solid-state detectors and the requirement that they are kept at extremely cold temperatures, most detectors are only 1 to 1.5 cm in diameter. The detector is protected from physical damage by being recessed in a tube that is most often covered by a thin Be or mylar window, although windowless detectors are also used. The EDS detector itself is a solid-state device that is capable of discriminating the energy of the X-rays hitting the device.

X-ray detectors are limited to those X-rays that are released in the direction of the detector and not absorbed by any solid between the area producing X-rays and the detector. As X-rays are produced isotropically, the proportion of the total X-rays detected is related to the inverse cube of the distance between the specimen and the detector and the size of the detector. Considering the small size of most detectors, even when the distance between the specimen and detector is minimized, the X-rays detected are only a small proportion of the total produced; thus, longer times are required to produce a usable X-ray image than an electron image.

The chemical composition of the area impacted by the electron beam can be estimated using EDS data by viewing a graph with the energy of the X-rays produced on the x axis and the number of X-rays counted at that energy on the y axis (Fig. 10–6). This graph is limited by the number of channels in which X-ray energy is discriminated. The regions appearing as peaks are the result of characteristic X-ray production, while the intensity in the regions between peaks is the result of white X-rays. In general, the relative intensity of the EDS peaks is proportional to the relative composition on a molar basis. The individual points on EDS graphs represent the number of counts captured by the detector with energies within a channel. For example, an EDS system may discriminate 1920 channels. If a range of 0 to 10 KeV is chosen for examination, each band would represent $10 \text{ KeV}/1920$, or 0.0052 KeV . To use X-ray data for mapping purposes, areas of the EDS pattern are chosen in which the signal resulting from a predetermined element is much greater than the background. Multiple regions, often called “windows,” can be chosen for mapping purposes and the counts from the windows can be gathered simultaneously allowing for the mapping of several elements at once. It is best to set the windows as narrowly as possible, corresponding to those channels that correspond to the greatest intensity for that element.

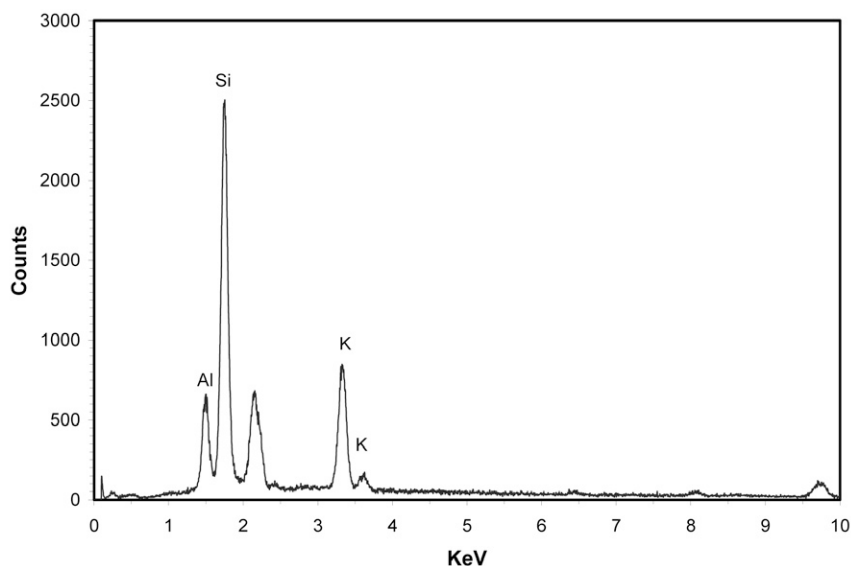


Fig. 10–6. Energy dispersive spectroscopy (EDS) data for a K-feldspar grain (KAlSi_3O_8) obtained with a 20-kV beam. Notice the general relationship between the EDS peak intensity and composition with the Si peak about three times as intense as the Al and K peak. The K $\text{K}\alpha$ peak is larger than the Al peak because of the increased efficiency of the detector and the increased proportion of X-rays produced per collision. The peak between 2 and 2.5 KeV and the peak between 9.5 and 10 KeV are the result of the Au coating, and the two small peaks near 6.5 and 8 KeV are artifacts from scattering within the SEM.

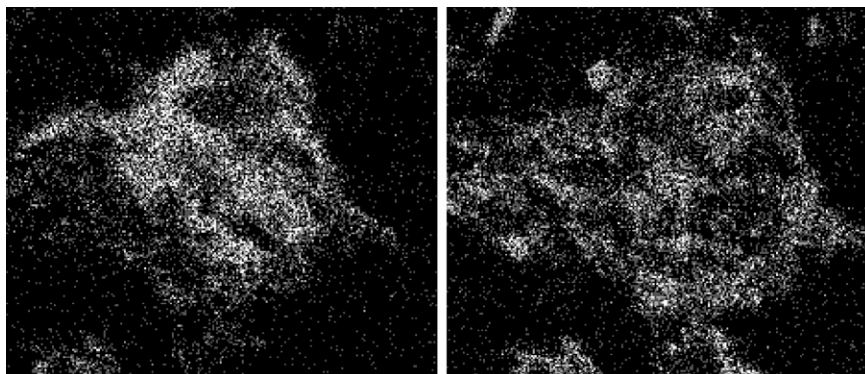


Fig. 10–7. Figure produced using (left) Fe and (right) Si X-rays for the particle shown in Fig. 3. The brighter regions correspond to higher concentrations of those elements.

In an X-ray map, the number of X-rays counted for each pixel in a micrograph is counted for all elements for which element windows are set. If the mapping is performed for a sufficient time, an image resembling a topographic map with the high points representing the regions with more of the element will result. Counting for several minutes or hours is not usually economical with an SEM (a microprobe is better setup for this type of work). More commonly with an SEM, the resulting micrograph will show an increased density of dots in regions with the element. Usually contrast is set in such a manner that the background counts are subtracted from the map before display. Background counts are more easily removed in maps representing elements that have a high peak/background ratio in the EDS pattern (i.e., those elements in high concentrations) than those with lower ratios.

All X-ray images are digital, and resolution is limited by the rate of production of the X-rays and the number of pixels in the image. The rate of production of X-rays and the line-of-sight requirement in X-ray detection cause the formation of X-ray images to be much slower than other image types. As a result, X-ray images are rarely used, except for two purposes: (i) for finding minerals with distinctive elements at the microscopic level and (ii) at a more macroscopic scale for showing the distribution of elements or different mineral phases.

The contrast in the X-ray maps is the result of the number of X-rays collected as the sample is scanned. Images from the characteristic X-rays of an element with low concentrations may be poor because the noise resulting from the white X-rays (background) may be sufficient to mask the element. For that reason, it is best to collect the X-ray signals for a considerable time (minutes) to improve the signal/noise ratio. Care should be taken to have a very high vacuum because a low vacuum may result in the formation of a contamination spot on the sample from condensing particulates and pump oil and the absorption of low energy X-rays. With the increased number of counts in each pixel, it is possible to use a thresholding technique in which a set number of counts equal to the background are subtracted from each pixel, resulting in a better image.

Photomicrographs formed from X-ray maps are useful, but the method is rarely acceptable as an imaging method (Fig. 10–7). Problems can arise in the use of X-ray maps when examining grains because of the line-of-sight requirement for X-ray collection. Surface relief may prevent the observation of X-rays from nearby grains or other parts of the same grain (Fig. 10–8).

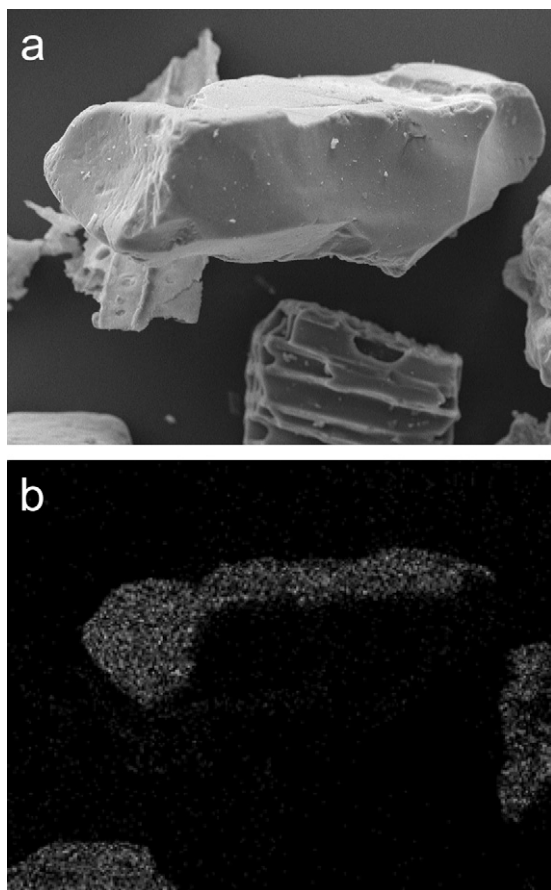


Fig. 10-8. Example of the problem that can arise from the line of sight requirements for EDS. (a) Secondary electron image of a quartz grain; (b) Si EDS map of the same region. The EDS detector is to the left and above the field of view. As a result, Si X-rays from part of the quartz grain are blocked from detection by the grain itself. Note that the area mapped by EDS is not the exact shape of the area in the video.

Choice of Beam Voltage

Beam voltage greatly affects the type of data obtainable from SEM examination of a sample. There are two principle ways in which the beam voltage affects the data collectable by SEM examination, by affecting depth of penetration of the beam into the sample and through the production of X-rays by interaction with the sample.

When an electron beam impinges on a solid surface, the beam is scattered and absorbed as it interacts with the specimen. Each type of signal used for imaging is characteristic of a different type of specimen-beam interaction, with each signal resulting from a unique sample emission volume. These emission volumes are strongly affected by the beam accelerating voltage, the average atomic number, and the density of the specimen. For example, an electron beam will penetrate almost twice as far into quartz ($\rho = 2.65 \text{ g cm}^{-3}$) than it will into hematite ($\rho = 5.25 \text{ g cm}^{-3}$). In general, electrons in an SEM penetrate 1 to 4 μm at 20 KeV but only 0.3 to 0.75 μm at 10 KeV (Fig. 10-9). When you consider that this calculation is a determination of the absorption of beam energy along the path of the electrons, there can be some unintended consequences. One consequence is a distortion of the appearance of a secondary electron image along changes in relief due a large contribution of backscatter electrons escaping from certain places on a specimen such as at particle edges (Fig. 10-10).

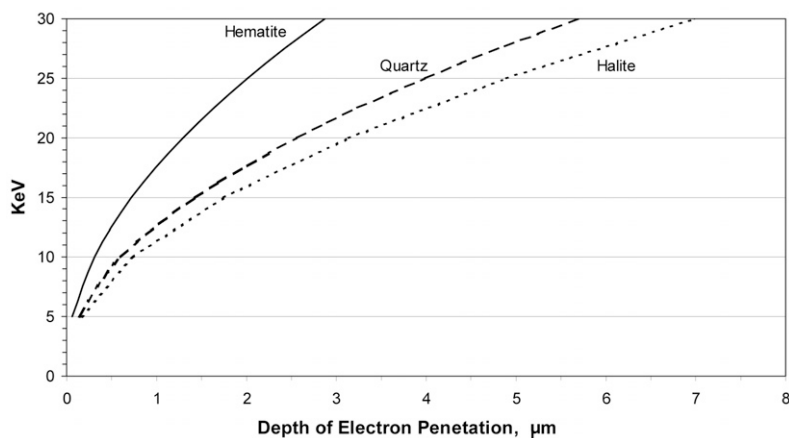


Fig. 10-9. The depth of penetration of electrons of a given beam energy as affected by specimen density. The densities, ρ , of halite, quartz, and hematite are 2.16, 2.65, and 5.25, respectively.

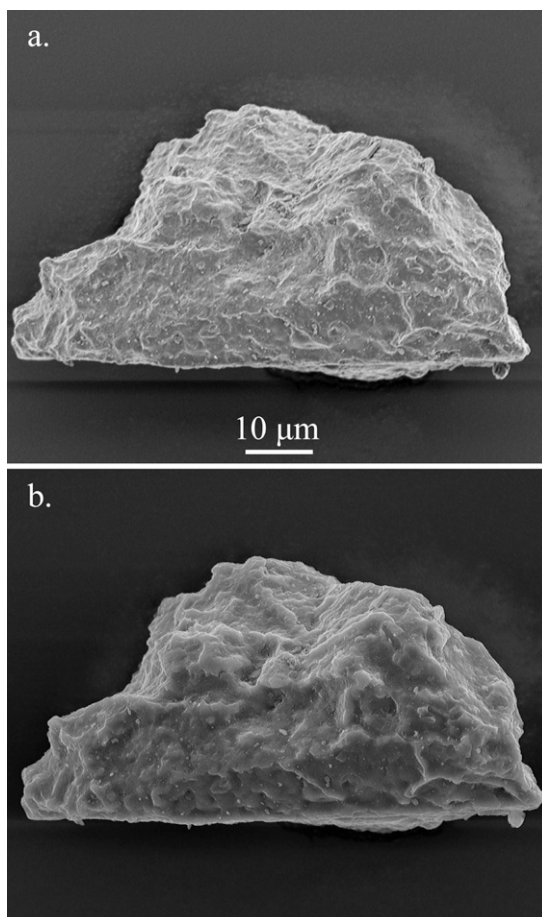


Fig. 10-10. An example of the effects that beam voltage has on a secondary electron image. (a) Image at 5 KeV. (b) Image at 20 KeV. Notice the false appearance of overgrowths on the 20-KeV image.

Choice of Operating Distance

Operating distance may indirectly affect the imaging results in many ways. Some effects result from the preset geometry of various imaging methods. Many SEMs can only obtain good backscatter or EDS results from certain working distances because of the angles between the surfaces and the detectors. Aside from these geometric effects, the most important effect of changing the operating distance is the effect it has on depth of field obtained in a micrograph.

The depth of field refers to the depth, or vertical distance, in the specimen plane that is in focus. One of the most important advantages of the SEM is the large available depth of field. At comparable magnifications, the depth of field of the SEM is more than 100 times greater than that of the light microscope. The increased depth of field allows for the production of an image that appears three-dimensional and can give important information that would not be available with light microscopy. The large depth of field in SEM images allows for the characterization of grain and ped surfaces at a level of detail that is not possible with a light microscope.

The depth of field in an electron micrograph is affected by several geometric factors. The SEM beam is very small and goes through small apertures that are separated by a large distance when compared with most other optical devices. This results in a very small focal angle for the electron beam impinging on the specimen. This small focal angle causes the distance along the axis of the beam that is in focus to be greater, thus resulting in a greater depth of field.

There is a correlation between depth of field and image quality. A deeper depth of field can be obtained by increasing the working distance, the distance from the center of the lens to the specimen plane (Fig. 10–11). Increasing working distance decreases the focal angle by increasing the distance between the lens and the surfaces. As a result, in focus depth of the specimen is increased. Increased working distance is advantageous at lower magnifications but reduces image quality at higher magnifications by reducing the total signal captured by the detector. Image quality is increased by reducing working distance at higher magnifications, but with a decreased depth of field. Thus, there are compromises required when optimizing depth of field and image quality.

Choice of Beam Size

The aperture setting is the principle factor affecting the size of the beam that hits a specimen. A larger beam delivers more signal, assuming all other settings are constant. A larger beam is advantageous for compositional analysis by EDS. A more intense beam also delivers more signal to the image detector, allowing for better contrast in the image. A less intense beam may result in only a few electrons or X-rays hitting the detector for each pixel in a micrograph. As a result, the difference between the pixel with the most electrons or X-rays and the pixel with the least number of electrons or X-rays is reduced. With less signal difference between pixels, the contrast range is lessened and the micrograph quality is degraded with the appearance of random dark spots within an area that should have been uniformly bright. To reduce this potential image degradation, slower scan rates can be used to increase the amount of count time per pixel, or the results of several scans can be averaged. Continuous use of large beam sizes would seem to be an obvious way to decrease the problems that arise from the lower signal strength of a smaller beam, but the larger beam can cause charging or in rare cases, destruction of the specimen by the heat resulting from the collisions of the electrons on the surface. It is also important to decrease the beam size when higher magnifications are used because at some point, the beam becomes larger than

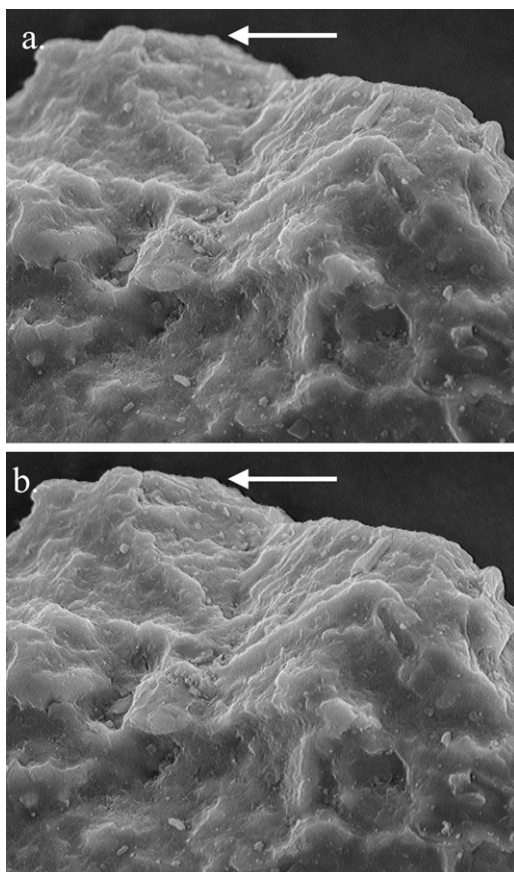


Fig. 10–11. An example of the effects that sample working distance has on the depth of focus of a secondary electron image. Both images were focused at the center of the photograph. (a) Image with a close working distance. (b) Image with a longer working distance. Notice the difference of focus near the particle edge at the top of the images.

the size of a pixel and the signal produced ceases to be a measure of the signal at a single place on the specimen and becomes the weighted average of the signal produced in the area near the place on a specimen. For example, if a beam size of $50 \text{ \AA}$ is assumed, the smallest distance that can be represented with 256 pixels without interactions between adjacent pixels would be $50 \text{ \AA} \times (1 \text{ \mu m}/10,000 \text{ \AA}) \times 256 \text{ pixels} = 1.28 \text{ \mu m}$. Therefore, for observation at high magnifications, a smaller spot size is required. A problem can be the lack of knowledge of the exact size of the beam probe, which is affected by aperture size, working distance, and focus, among other settings on the microscope.

EXAMINATION OF THE SAMPLE

Once the parameters to be used for examination are determined, examination of the sample can commence. First, the prepared sample must be placed in the sample chamber at a working distance optimal for examination, the chamber evacuated for examination, and the microscope operating parameters set. Normally an intermediate beam size is used initially and decreased if needed to obtain optimal micrographs or increased as needed to improve the counting statistics with the EDS. The best working distance is one in which the EDS data can be obtained. At this point, the beam can be saturated (if needed) for use. Other settings that require attention include beam and lens alignment. These and many of

the following steps vary greatly from microscope to microscope and must be learned for individual instruments.

When sample examination begins, the contrast and brightness settings are probably not yet optimized. Most microscopes have both manual and automatic contrast settings. The contrast and brightness screen usually has a series of horizontal lines, the lowest line corresponding to no screen brightness (black) and the highest line corresponding to complete screen saturation (white), with lines between corresponding to shades of gray. Usually, going to this screen slows the beam scan rate to a speed where the degree of brightness for each point in a scanned area can be observed as a constantly changing jagged curve moving on the screen. Any parts of the curve that are above the highest stationary line are oversaturated and will appear as white, while any parts of the curve that are below the lowest stationary line will appear as black. If you wish to observe everything in the view region, then you will set the contrast and brightness to keep the entire curve between the top and bottom stationary line. The contrast and brightness can be set using the automatic contrast and brightness settings in this situation. In some cases, the important information is in one part of the view region, and the remainder of the view area can be allowed to be black or white without loss of information. In these cases, manually setting the contrast and brightness is preferable. The first step in setting the contrast and brightness is to change the brightness setting to the point where the screen brightness is above the line corresponding to black. The contrast setting is then adjusted to keep the brightness below the white line. Often, adjustment of the contrast will affect the brightness and so both will need to be adjusted until optimal.

Once the contrast and brightness are set to allow viewing, the screen is switched to view the sample (some microscopes may allow both the contrast and brightness and visual screens to be observed simultaneously on different monitors or windows). The view can then be focused. As you change the focus setting in a direction, there will be a position in which the focus is optimized. If you continue changing the focus past this point, the sample will become increasingly defocused. In cases where the sample never comes into focus, reverse the focus direction and the sample should come into focus at a point past where you started the focusing operation. If, when focusing the sample, the object being viewed appears to move, there is a problem with lens astigmatism. Usually the viewed area moves, or streaks appear which move in one direction when the focus is adjusted past focus in one direction and change to a direction perpendicular to the original direction when the focus is adjusted past optimal in the other direction. Lens astigmatism arises from the fact that the magnetic lenses may not be perfectly symmetrical. Astigmatism settings allow for electronic correction of the problem. Adjusting for astigmatism is usually performed at magnifications at least one order of magnitude higher than the magnification that will be used for observation. Adjusting astigmatism is best done when viewing a small, round feature at high magnification. Linear features are poor choices for performing the adjustment, as astigmatism may not be easily observed in one direction, whereas it is more obvious when observing a round feature. In most microscopes, there are two directions of astigmatism adjustment, and each is adjusted observing the effects of up- and down-focusing until sample changes are minimized.

Once focus, the brightness and contrast settings, and astigmatism settings are optimized, sample examination can commence. It is very difficult to obtain representative results using SEM examination because the human eye is drawn to objects that are different. The tendency to find the different object can be an advantage in searching for trace minerals or unusual morphologies, but is a disadvantage when the objective is to observe the representative sample. One method to ensure representative results is to examine the

sample using a point-count or line-count method such as is commonly applied to petrographic examination.

When a grain or area of the specimen is chosen for examination, obtaining an EDS pattern is usually the best first step. Once the mineral composition is established, then morphology can be used to refine the mineral identification.

IDENTIFICATION OF MINERALS

Much of the results obtained from the use of an SEM in soil mineralogy studies are the result of experience in the use of the instrument and from studying many materials from different locations. The first step in identifying minerals with SEM is to determine the mineral phases present. Determination of the minerals present in a sample is always aided by size fractionation (Towe, 1974) because most minerals tend to be concentrated in one or more size fractions. The interpretation of SEM data is greatly aided by knowledge of the sample mineralogy and mineral assemblages. The sample mineralogy data can be the result of XRD patterns or from grain counts using an optical microscope. Important information may also include the source material for the sample (e.g., saprolite from a granite). Knowledge about the sample provides an overview, giving clues about the degree of weathering and the mineral population, serving as a guide to the identification of individual particles. Do not assume that a mineral is not present if not identified by XRD, but assume that the minerals that are easily identified by XRD will be found more often when examined with the SEM than those not identified by XRD. Some minerals are common as accessory minerals with a concentration too low to be observed in a bulk XRD pattern. These minerals are often easily identified when examined by SEM because their morphology is distinctly different from the majority minerals. For example, a rod shaped mineral will be easily noticed when it is placed in a group of round grains. Almost all SEM interpretations are based on knowledge of mineralogy obtained from the bulk mineralogy of the sample, the chemical composition of the particle as determined by the EDS spectra, and from the particle morphology. In general, when determining the mineralogy of a grain observed with SEM, the EDS spectrum is employed first and combined with the operator's knowledge of mineral morphologies to identify the mineral.

Morphology will often lead one astray if it is not used in combination with the chemical properties provided by EDS (Fig. 10–12). Morphology may not be diagnostic because the mineral may not occur as crystals, may be altered by weathering or grain fracturing, or may be present with minerals having similar crystallographic characteristics or even pseudomorphs. In fact, morphology alone is a poor criterion to interpret mineralogy unless other diagnostic information is available.

Interpretation of EDS Spectra

The chemical makeup of most minerals is within limits distinctive. The differences in the chemical makeup at a qualitative level can be used to identify a mineral in a sample with knowledge of common substitution limits. It is important to have some knowledge of a sample before an attempt is made to infer the mineralogy from the chemical composition. Saturation of the cation exchange complex with a cation that is not common in octahedral positions is also very helpful in avoiding uncertainties about exchangeable Mg and Al versus structural Mg and Al. Cation saturation places all the exchangeable cations into a single EDS peak, making the peak stronger and therefore more detectable and quantifiable (White et al., 1982). Sodium and calcium are probably the best choices for cation saturation, although the use of an exotic cation prevents interpretation problems from the

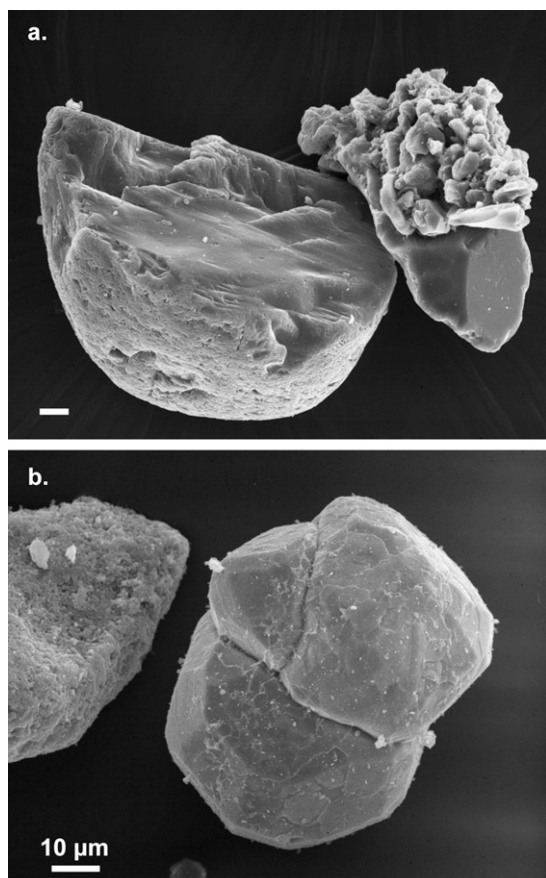


Fig. 10–12. Examples of grain morphologies that might result in errors in identification. (a) An example of a feldspar grain (grain on left) with surface rounding more characteristic of quartz. (b) Fe oxide particle pseudomorph after a pyrite pyritohedron. Data from White and Dixon (1998).

presence of minerals with exchangeable cations present in nonexchangeable locations. It should also be remembered that some minerals do not occur with a fixed formula but have a range in compositions. For example, the amphibole group has a general formula of $(W,X,Y)_{7-8}(Z_4O_{11})_2(OH)_2$, where W are the large cations, Na, Ca, and sometimes trace K; X represents the smaller cations, Mg, Fe^{2+} , and sometimes Mn; Y represents Fe^{3+} , Ti and Al in octahedral coordination; Z represents Si and Al in tetrahedral coordination; and the OH may be replaced partially by F and Cl. There are limits to substitution for some cations, but still a wide range in compositions can result.

Qualitative interpretation of EDS patterns requires some basis for comparison. That basis is usually the ratio of the EDS peaks to each other and to the background. A low peak/background ratio can be the result of a thin sample. If the particle is thick, a low peak/background ratio indicates that the sample is being blocked from the EDS detector by a larger particle between the two, or is organic. Grain morphology may also limit EDS data interpretation by partially absorbing its own spectra if the EDS detector is not in the unobstructed line of sight with the point analyzed. Given there is no physical problem, as mentioned above, and a reasonable peak/background ratio, relative concentrations of the elements within a sample can be estimated using the ratios of the EDS peaks. In general, the relative peak heights of the X-ray fluorescence peaks observed in an EDS pattern are

directly related to the molar concentrations of the elements in the area being scanned. The major factors preventing a direct conversion of peak heights to relative molar concentrations are various atomic factors, detector efficiency, and X-ray absorption (see Guillemette, 2008, this volume for a more detailed discussion). Conversion of EDS spectra to qualitative data is easiest by considering peak ratios, usually Si for silicate minerals.

More detail on conversion of X-ray data to composition is contained in the electron microprobe chapter (Guillemette, 2008). The following discussion briefly describes the issues that arise when using EDS patterns.

Confounding factors prevent the direct conversion of elemental ratios to molar ratios. These factors include the proportion of X-rays produced versus the production of Auger electrons, the detector efficiency, the difference in energy between the electron energy and the X-ray energy (overvoltage), and fluorescence and absorption effects.

The first factor is the production of a certain proportion of Auger electrons rather than characteristic X-rays. Production of Auger electrons is more important in the low atomic number elements and reduces the intensity of the low atomic number peaks. Less than 1% of the collisions capable of producing X-rays result in X-ray production for elements with atomic numbers lower than 11 (i.e., Na). The proportion increases sharply from this point to about 3.3% for Si, 29% for Fe, 51% for As, etc.

Energy dispersive X-ray spectroscopy detectors are produced from Si chips. Materials tend to absorb X-rays from elements with atomic numbers less than the predominant element in the material. As a result, EDS detectors lose efficiency for energies lower than the Si K-peak, therefore resulting in less intense peaks for elements with atomic numbers below 14 (i.e., Si). The combination of detector efficiency with the poor X-ray yield from low atomic number elements make EDS a poor choice for detection of elements with atomic numbers lower than 11 (i.e., Na).

The number of X-rays produced increases logarithmically with the difference in energy between the electron beam and the X-rays produced (overvoltage). This greatly enhances counting statistics but has less effect on the determination of the composition because the overvoltage increases for all elements present in the sample as the beam voltage increases. One effect of overvoltage is that X-rays can be produced deeper in a sample with increased beam voltage. For thick samples, this can result in better data as the number of X-rays increases, but requires more data manipulation because of the effects of fluorescence and absorption described below. For thin samples, the increased depth of beam penetration and X-ray production can result in a mixing of the data for the grain being analyzed with data from the grains below or near the grain because of beam scattering. If, for example, a mineral coated with Fe oxides is analyzed, use of a low beam voltage may only detect the Fe in the Fe oxide, while increasing beam voltage would result in increasing contributions from the coated mineral.

Fluorescence and absorption describes the intensification and reduction of X-ray strength resulting from interactions of the X-rays produced with other atoms in the sample. As the X-rays move toward the surface of the sample to escape and be detected, there is an interaction with the atoms between the point where the X-ray was produced and the surface. Depending on the atomic number of atoms interacting with the X-rays, the total number of counts will be increased by fluorescence or decreased by absorption.

With these factors in mind, a few examples of EDS spectra will be examined. It must be remembered that many minerals, including most of the easily weathered silicates such as amphiboles and pyroxenes, do not have a fixed composition, due to solid solution series between multiple endmembers, or a distinctive crystal morphology that can be used to

identify them in a sample without the knowledge of the bulk mineralogy. Other minerals, by virtue of having only a few elements or a distinctive morphology can be easily identified. The feldspar series, with its limited substitution, and the phyllosilicate minerals, muscovite and kaolinite, are good examples for the determination of mineralogy by EDS spectra.

Feldspars have the general formulas, KAlSi_3O_8 for microcline and orthoclase, and $(\text{Na}_{1-x}\text{Ca}_x)(\text{Si}_{4-x}\text{Al}_{1+x})\text{O}_8$ for the plagioclase series with limited K substitution. The molar ratios of the cations in the K feldspars are 1 K to 1 Al to 3 Si. One would expect an EDS pattern for a K feldspar that has an Al peak about 0.3 as tall as the Si peak and a K peak a little taller than the Al peak. It is impossible to distinguish the difference between the K feldspars with EDS, except for sanidine, which can be distinguished by a small amount of Na substitution into the structure. For the Na end of the plagioclase feldspar series, albite, the EDS pattern has about the same Al/Si peak ratio as the K feldspars but with a smaller Na EDS peak due to the lesser X-ray production and decreased detector efficiency in the Na region of the spectra. As Al content increases with Ca substitution in the Na plagioclase, the Al peak intensity increases relative to the Si peak intensity. As the Ca substitution increases up to the endmember anorthite, $\text{CaAl}_2\text{Si}_2\text{O}_8$, the Al peak is almost as large as the Si peak, and the Ca peak is just more than one-half as tall as the Si peak.

Kaolinite and muscovite both have equal molar contents of Al and Si if negligible Fe substitution is assumed. Therefore, kaolinite cannot be differentiated from muscovite on the basis of the Al/Si peak ratio. As a result of the slightly decreased detector efficiency and lesser X-ray production of Al, the Al EDS peak is slightly smaller than the Si EDS peak. Muscovite, however, has about 1 mol of K to every 3 mol of Al and Si. It follows that muscovite can be differentiated from kaolinite by the occurrence of a K EDS peak with intensity a little over a third as tall as the Al and Si peaks. The spectrum of muscovite can be altered, however, by the addition of Mg and Fe, with a concomitant reduction in the Al peak, and by the substitution of Na and NH_4^+ (the latter has no EDS spectra) for K. The possibility of other phyllosilicates should also be considered.

It is normally possible to differentiate muscovite from K feldspar by morphology alone, but if it were not possible, the much higher Al/Si peak ratio of muscovite should be adequate. Potassium feldspars, as mentioned earlier, have approximately equal K and Al peak intensities and Si peaks about three times as intense as the Al peaks. Muscovite, on the other hand, has almost equally intense Si and Al peaks and a less intense K peak.

It is important when using EDS spectra to identify minerals to be wary of surface coatings. Surface coatings, such as Fe oxides or secondary silica, will cause significant differences in EDS patterns and should be taken into consideration when viewing grains with surfaces that do not appear fresh. See Soukup et al. (2008, this volume) or Shang and Zelazny (2008, this volume) for techniques to remove Fe oxides, carbonates, silica and other coatings during sample pretreatment. It is also important to remember that many minerals do not weather congruently. That is, the EDS spectra obtained may be very much different from the ideal spectrum due to preferential leaching of one or more elements from the mineral surface.

Identification of Minerals Using Particle Morphology

Often it is possible to tentatively identify soil minerals by particle morphology alone. The reason that this is possible is that the outward appearance of minerals is related to their crystal structure. This is not always reliable, because morphology may result from crystal growth into a confined space or from grain abrasion or smoothing from sedimentary transport (Fig. 10–12a), or the grain itself may be a pseudomorph of the mineral from which the pres-

ent mineral formed as a result of weathering (Fig. 10–12b). In addition, some minerals occur in massive form and therefore are not recognizable on the basis of morphology alone.

Some minerals, such as the inosilicates, cyclosilicates, the diaspore group, and some other oxides such as rutile, have structures made up of repeating units of chains. The linear chains in these minerals cause these minerals to have particle morphologies elongated in one direction, forming needles or long prisms. If the mineral has a distinctive EDS spectrum, the morphology aids in the differentiation of these minerals from minerals with a similar chemical makeup. Some other minerals, such as halloysite and chrysotile, may appear similar, but their elongated crystals are the result of a tubular crystal structure. Phyllosilicates, due to their two-dimensional structure and perfect {001} cleavage, have a platy morphology, as do some other minerals, such as gibbsite and lepidocrocite.

The morphology of authigenic phyllosilicates has been the source of intense study by the oil industry because of their effects on oil reservoir properties and their potential as a geothermometer. The most complete study of the SEM observation of authigenic clay is that of Wilson and Pittman (1977), which is a standard reference for almost all later work in this area. In a study of many samples using SEM, EDS, and X-ray diffraction they found that each major authigenic clay group exhibited a limited number of independent morphologies. Smectite, with thin crystals not easily resolvable by SEM, occurs as a very wrinkled or honeycomb-like pore filling. Illite was found as overlapping plates with curled edges and highly elongated lath-shaped flakes. Chlorite exhibited a cardhouse, honeycomb, or rosette arrangement of pseudohexagonal plates lining pores. Kaolinite and dickite tended to fill pores with books of stacked pseudohexagonal plates. Mixed layered phyllosilicates have morphologies mixing the morphologies of the endmembers.

Other common minerals have a three-dimensional structure that does not assist in their identification. Many, such as quartz and some feldspars, potentially have mineral morphologies distinctive enough to allow for their identification, but weathering, transport, or other factors destroy their ideal morphologies. In these cases, there may be other clues that aid in their identification. How weathering or transport breaks up the mineral provide clues to their identification. Some minerals, such as feldspars and many carbonates, have good cleavage in one or more directions. Cleavage produces flat surfaces at set interfacial angles that can help in mineral identification. However, transport abrasion or chemical weathering may obscure cleavage traces. Other minerals such as quartz do not exhibit good cleavage. The lack of cleavage is distinctive as an aid in identification, as are etch pitting and breakage patterns, such as the conchoidal fracture of quartz.

The effects of weathering on a mineral surface may help in the identification of a mineral (Fig. 10–13). Feldspar may exhibit very distinctive crystallographically controlled weathering patterns resulting from weaknesses along cleavage planes and demixing of cationic substitution (Berner and Holdren, 1979). Many K feldspars are perthitic with microscopic intergrowths of Na feldspar. Likewise, the plagioclase series intermediate between albite and anorthite are often demixed into Na-rich (albite) and Ca-rich (anorthite) domains with regions separated by crystallographic boundaries and sizes recognizable by light microscopy. Weathering of these feldspars is almost always incongruent, with one type of the feldspar weathering faster than the other depending on the chemistry of the weathering environment, leaving the feldspar with a “rotten” appearance (Fig. 10–13a and 10–13b) and an EDS pattern tending toward the endmember composition. Weathering and transport may also emphasize crystallographic planes (Fig. 10–13c). Incongruent weathering also is common for many other minerals (see, e.g., Nahon and Colin, 1982), such as inosilicates resulting in a linear appearance similar to rotted wood (Fig. 10–13d).

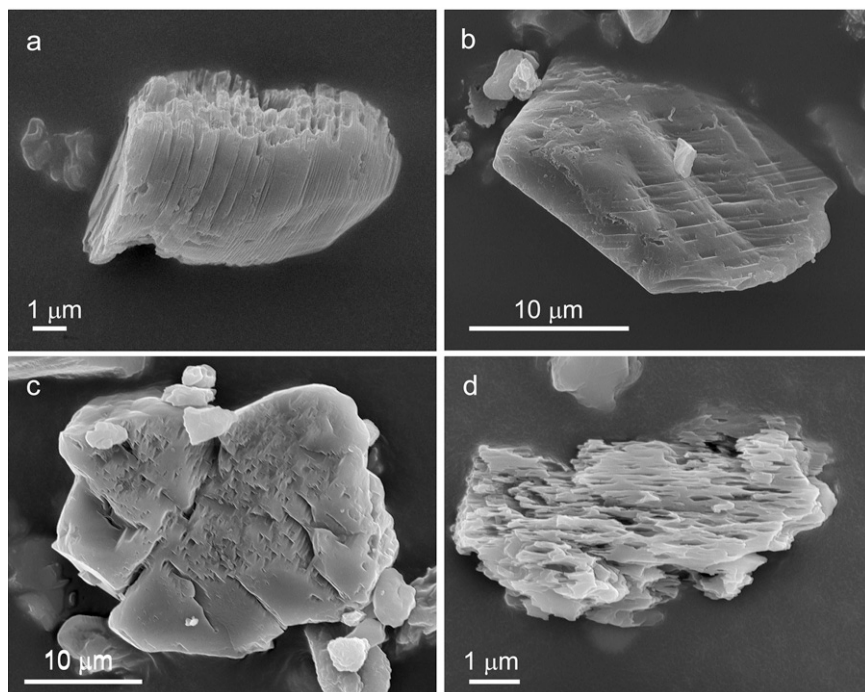


Fig. 10–13. Examples of grain morphologies affected by mineral weathering. (a) Perthitic microcline particle showing the effects of preferred weathering of the Na component of the perthite. (b) Slightly weathered intermediate plagioclase particle with the beginnings of removal of the Ca-rich anorthite phase. (c) Orthoclase or microcline grain (K feldspar) with cleavage planes appearance enhanced by weathering or transport. (d) Hornblende particle with the crystallographically controlled etching, resulting in "rotted" appearance.

Quartz surface morphologies have been extensively studied for clues to the depositional and chemical history of sediments. These studies have taken two directions: one using quartz morphology to determine the depositional history of the sediment and the second using the degree of etching or secondary silica precipitation to determine the chemical history or relative age of a soil. It is important to remember, however, that not all that with an EDS composition of Si is quartz. Very commonly, surface soils have opal phytoliths present as a result of silica uptake by plants, and some soils contain volcanic glass. Opal phytoliths can be differentiated by SEM from quartz by a rotted appearance with many cavities. Often volcanic glass can be differentiated from quartz by a bubble-like morphology, but after transport by wind or water it may greatly resemble quartz. However, usually there are trace amounts of Al and Si present in the glass to differentiate it by chemistry.

An important early reference on use of the surface of quartz to aid in the determination of sedimentary history is the book by Krinsley and Doornkamp (1973). In this work, they concluded that the quartz surface features could be divided into four categories: i) conchoidal fractures, (ii) flat cleavage plates and their expression at grain margins, (iii) upturned plates on cleavage or crystal faces, and (iv) the alteration of the features above. The use of these features to distinguish sedimentary depositional environments is very controversial because most grains may have relict features from a past depositional sequence or have been moved for such a short distance that microfeatures resulting from transport may not

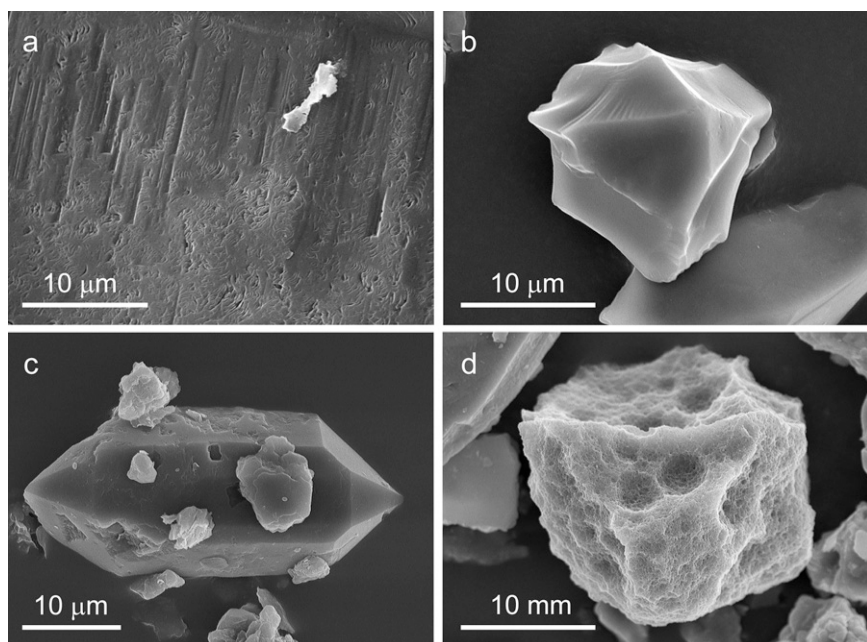


Fig. 10–14. Morphologies of silica from some soils. (a) Surface of a quartz grain showing long linear etch features and random crescent shaped features apparently from grain transport. (b) Very angular quartz grain with faces dominated by conchoidal fractures. (c) Euhedral, doubly terminated, silt-sized quartz crystal showing some etch pits and overgrowths. (d) Opal phytolith.

have become expressed on most grains. The work is more useful if the depositional and chemical features are considered separately. It should also be noted that relative expression of the features are affected by particle size. Krinsley and Doornkamp used only sand grains in a single size fraction. Generally, conchoidal fractures, flat faces, and some other features are much more common in the silt fraction than in the sand fraction, possibly due to differing modes of transport.

Krinsley and Doornkamp observed that quartz grains transported by littoral processes had rounded grain morphologies and mechanically formed V-shaped depressions caused by grain collisions. Use of these V-shaped depressions should be used with caution as chemical etching of quartz results in similar features. The two types of pits can be differentiated—etch pits are oriented along some direction and will change visual appearance slightly on different surfaces of the grain, whereas mechanically produced pits are randomly oriented.

Grains deposited by aeolian processes appeared more angular and less rounded than those deposited by littoral processes. Conchoidal fractures, flat faces resulting from parting or cleavage, and relatively sharp edges are common features. These features are especially evident in the silt particle size fraction.

Quartz, in all but the most recent soils, has been altered by soil forming processes. Most commonly, silica is precipitated on the surface of quartz because of the high Si concentrations resulting from dissolution of more weatherable minerals and phytoliths (Fig. 10–14). The precipitation features may be too thin to be detected by optical microscopy but are apparent when observed by an SEM. Krinsley and Doornkamp (1973) concluded that

if silica precipitation is rapid, an undulating surface topography results. Very slow silica precipitation takes place on projections and may lead to quartz terminations. Quartz particles from highly weathered chemical environments are highly etched and show signs on a macroscopic level of intense surface disintegration. Oriented etch pits are very common (Krinsley and Doornkamp, 1973) but are highly variable in the degree of their expression (Subramanian, 1975).

The features described by Krinsley and Doornkamp (1973) have been used in several soil studies. Glassman and Kling (1980) found the environmental features useful in interpreting the geomorphic history and genesis of soils formed in stratified sediments in the Willamette Valley, Oregon. They found that differences in sediment provenance could be determined and the results used to establish lithologic discontinuities in the soils studied. Rabenhorst and Wilding (1986) also used the quartz grain morphology to aid in the recognition of a discontinuity. Douglas and Platt (1977) found the degree of expression of the alteration of quartz surfaces useful in dating Quaternary soils. They concluded that the amount of chemical surface alteration was proportional to the water-holding capacity of the soil and to the age of the parent material. Residual soils were found to have the most severely altered quartz surfaces, in agreement with Krinsley and Doornkamp (1973). They also found that quartz surfaces were more altered by precipitation in the lower B and upper C horizons than in the A horizons in young soils with the differences decreasing with soil age. They also found that quartz surface morphology was dependent on texture, with more alteration occurring in soils with a higher fine silt and clay content. They hypothesized that the differences were due to the A horizons drying out faster than the B or finer-textured soils.

SUMMARY AND CONCLUSIONS

Scanning electron microscopy, especially when combined with energy dispersive spectroscopy (SEM-EDS), is a very valuable ancillary technique in the study of soils. Minerals that occur only in trace amounts in soils can be detected in soil size separates using X-ray mapping if the minerals contain relatively uncommon elements above atomic number 11. Mineral grain morphologies reveal information about the source of the soil parent materials and weathering processes. Examination of broken ped surfaces allows observation of morphological features of the minerals and their interaction within the soil. Examination of thin sections or polished blocks allows for more quantitative analysis of elemental relationships and may be used to gain additional information from slides previously examined by petrographic microscopy.

REFERENCES

- Berner, R.A., and G.R. Holdren, Jr. 1979. Mechanism of feldspar weathering. II. Observations of feldspars from soils. *Geochim. Cosmochim. Acta* 43:1173–1186.
- Douglas, L.A., and D.W. Platt. 1977. Surface morphology of quartz and the age of soils. *Soil Sci. Soc. Am. J.* 41:641–645.
- Glassman, J.R., and G.F. Kling. 1980. Origin of soil materials in foothill soils of Willamette Valley, Oregon. *Soil Sci. Soc. Am. J.* 44:123–130.
- Goldstein, J.I., D.E. Newbury, P. Echlin, and D.C. Joy. 1992. *Scanning electron microscopy and X-ray microanalysis: A text for biologists, materials scientists, and geologists*. Plenum Pub. Corp., New York.
- Goldstein, J., D. Newbury, D. Joy, C. Lyman, P. Echlin et al. 2003. *Scanning electron microscopy and X-ray microanalysis*. Kluwer, New York.
- Goldstein, J.I., and H. Yakowitz. 1975. *Practical scanning electron microscopy*. Plenum Press, New York, 582 pages.
- Guillemette, R. 2008. Microprobe techniques p. 335–366. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.

- Hillier, S., and T. Clayton. 1992. Cation exchange 'staining' of clay minerals in thin-section for electron microscopy. *Clay Miner.* 27:379–384.
- Krinsley, D.H., and J.C. Doornkamp. 1973. Atlas of quartz sand surface textures. Cambridge Univ. Press, London.
- Nahon, D.B., and F. Colin. 1982. Chemical weathering of orthopyroxenes under lateritic conditions. *Am. J. Sci.* 282:1232–1243.
- Rabenhorst, M.C., and L.P. Wilding. 1986. Pedogenesis on the Edwards Plateau, Texas: I. Nature and continuity of parent material. *Soil Sci. Soc. Am. J.* 50:678–687.
- Shang, C., and L.W. Zelazny. 2008. Selective dissolution techniques for mineral analysis of soils and sediments. p. 33–80. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods.* SSSA Book Ser. 5. SSSA, Madison, WI.
- Soukup, D.A., B.J. Buck, and W. Harris. 2008. Sample pretreatment for mineralogical analyses. p. 13–32. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods.* SSSA Book Ser. 5. SSSA, Madison, WI.
- Subramanian, V. 1975. Origin of surface pits on quartz as revealed by scanning electron microscopy. *J. Sed. Res.* 45:530–534.
- Towe, K.M. 1974. Quantitative clay petrology; the trees but not the forest? *Clays Clay Miner.* 22:275–278.
- White, G.N., V.E. Berkheiser, F.N. Blanchard, and C.T. Hallmark. 1982. Thin-film analysis of clay particles using energy dispersive X-ray analysis. *Clays Clay Miner.* 30:375–382.
- White, G.N., and J.B. Dixon. 1998. Morphology and mineralogy of water dispersible sands and silts from agricultural soils of the southeastern states *In* John Seaman et al (ed.) *Mineralogy & charge properties of readily-dispersible colloidal fractions from selected soils and sediments of the southern region.* Southern Cooperative Series Bulletin 388. Available at <http://www.cals.ncsu.edu/research/s207/cover.htm> (verified 13 Dec. 2007).
- Wilson, M.D., and E.D. Pittman. 1977. Authigenic clays in sandstones: Recognition and influence on reservoir properties and paleoenvironmental analysis. *J. Sediment. Petrol.* 47:3–31.

Use of Atomic Force Microscopy to Study Soil Particle Properties and Interactions

Patricia A. Maurice, University of Notre Dame, Notre Dame, Indiana

Steven K. Lower, The Ohio State University, Columbus

Atomic force microscopy (AFM) was first described in 1986 by Binnig et al. and has since become an important instrument for high resolution imaging of the micromorphology and microtopography of surfaces, a technique to probe fundamental inter- and intramolecular forces between substances, and a tool to manipulate atoms and molecules. The technique is particularly important to various aspects of soil science and biogeochemistry because it allows for high resolution imaging of organic, microbial, and mineral surfaces and characterization of surface properties under environmentally relevant to extreme conditions. Indeed, experiments can be conducted at ambient pressure in gas or in solution at temperatures ranging from 4 to 700 K (Shao et al., 1995, 2000; Sheng et al., 1999).

In this chapter we discuss applications of AFM to soil science, including soil mineralogy, soil organic matter, and soil microbiology. We begin with a discussion of basic operating principles of AFM in air and solution, both for imaging and force measurements. We then provide a number of specific examples of AFM applications in various aspects of soil science, including new applications in soil geomicrobiology.

BASIC OPERATING PRINCIPLES OF ATOMIC FORCE MICROSCOPY

Components—Piezoelectric Scanner, Cantilever, Photodiode Detector

The basic operating principles of AFM have been described previously in the literature (Binnig et al., 1986; Hochella, 1990) and are reviewed in brief here. For most commonly used AFMs (Fig. 11–1), a sample is mounted atop a piezoelectric scanner composed of materials such as lead-zirconium-titanite that permit movement with Angstrom-level precision. The sample is scanned continuously in two dimensions (x – y) beneath a force-sensing probe consisting of a tip that is attached to or part of a cantilever. z -direction (height) movements are also made by the scanner as required to compensate for changes in sample height or forces between the tip and the sample. Attractive or repulsive forces between the tip and the sample cause the cantilever to bend or deflect. This deflection can be monitored in a number of ways. Most commonly, laser light reflects off the back of the cantilever into a photodiode detector. This so-called optical-lever detection system is sensitive to Angstrom-level movements of the cantilever (Clark and Baldeschwieler, 1993; Hansma et al., 1994; Kato et al., 1995). For soil microbiologic purposes, some AFMs keep the sample stationary while the scanner rasters the cantilever above it, so that the AFM can be used concurrently with an inverted optical microscope.

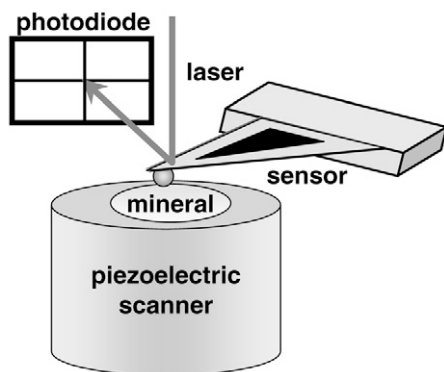


Fig. 11–1. Schematic image of an atomic force microscope. A sample (in this case a mineral) is positioned on a piezoelectric scanner, which moves the sample in three dimensions relative to the sensor (i.e., cantilever). The cantilever deflects due to attractive or repulsive forces between itself and the sample. Deflection of the cantilever is monitored by reflecting a laser off the top and into a photodiode array. The cantilever often terminates in a tip, which can be activated with various substances such as functional groups or cells (shown as a sphere on the end of the sensor). From Lower et al. (2001).

Forces between the Tip and the Sample

A basic understanding of the forces between the AFM tip and the sample is essential for properly utilizing the instrument and analyzing data. A variety of forces that come into play between the tip and the sample are summarized in Table 11–1. The discussion that follows will focus on contact-mode AFM, which is the most commonly used imaging mode.

The total forces (F_t) between the tip and the sample result from the sum of various attractive (F_a) and repulsive (F_r) forces, as described below. One context for understanding AFM tip–sample interactions is provided by considering the Lennard–Jones function, which describes the change in intermolecular potential energy (ϕ) that occurs as two particles, such as atoms or molecules (on tip and sample), are brought closer together (Fig. 11–2). The function is:

$$\phi = 4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6]$$

where σ is approximately the atomic or molecular diameter (distance of closest approach), ϵ is the minimum value of the potential energy or the depth of the potential energy "well," and r is the separation distance (Israelachvili, 1992; Morris et al., 1999). Referring to Fig. 11–2, as the particles are brought closer together from relatively distant separations, the $(1/r)^6$ term (i.e., van der Waals term) describes the slow change in attractive forces. As the particles are brought very close together, the $(1/r)^{12}$ term describes the strong repulsion

Table 11–1. Examples of some fundamental intra- and intermolecular forces that occur between molecules or colloidal particles.

Type of force	Dependence of energy on distance	Energy	Range
	d	kJ mol ^{–1}	nm
Molecular			
Intramolecular (ionic or covalent)	1/d	100s	<1
London dispersion	1/d ⁶	1 to 3	0.5–5
H-bonding	1/d ³	15 to 20	0.5–3
Dipoles	1/d ³	5 to 10	0.5–3
Colloidal			
Electrostatic	e ^{–d}	10 to 100	10s–100s
van der Waals	1/d	1 to 5	<5–10
Solvation	~e ^{–d}	1 to 10	<5
Hydrophobic	~e ^{–d}	1 to 5	10s–100s

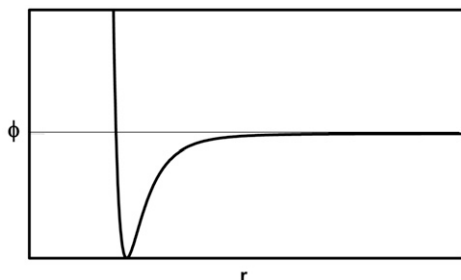


Fig. 11–2. Change in interaction potential energy with distance, graphically representing the Lennard–Jones potential energy function.

that occurs when the electron clouds strongly repel one another due to the Pauli exclusion principle.

The van der Waals (VdW) interaction forces are long-range, relatively weak attractive forces. The origin of the VdW forces is quantum mechanical in nature; they result from a variety of interactions, primarily induced dipole and quadrupole interactions (Israelachvili, 1992). The VdW forces are nonlocalized, meaning that they are spread out over many atoms. Goodman and Garcia (1991) estimated VdW forces for AFM on the order of 10 to 20 nN.

The so-called atomic force, which is a result of the Pauli exclusion principle, is the primary repulsive force at close approach. The magnitude of this force is difficult to predict without a detailed understanding of surface structure.

Several additional forces or interactions must be considered for an AFM tip and sample surface. Capillary adhesion is another important attractive force during imaging in air. This capillary force results from formation of a meniscus made up of water and organic contaminants sorbed on the surface of the tip and the sample (Weisenhorn et al., 1992) (Fig. 11–3). The capillary force has been estimated to be on the order of 100 nN or greater (Weisenhorn et al., 1989). When the tip and the sample are completely immersed in liquid, a meniscus does not form and the capillary forces are absent. Some tips and samples may have hydrophobic properties; in which case, hydrophobic interactions must also be considered.

Water near hydrophilic surfaces is structured (Senden and Ducker, 1994). When the tip and the sample are brought into close contact during force microscopy in solution or humid air, repulsion arises as the structured water molecules on the surfaces of the tip and the sample are pushed away.

In aqueous solutions, electrical double-layer forces, which may be either attractive or repulsive, are present near the surfaces of the tip and the sample. These double-layer forces arise because surfaces in aqueous solution generally are charged. For minerals, the surface charge may come about through permanent structural charge due to defects or substitutions in the crystal lattice, by hydroxylation and ionization of surface functional groups (formation of $>\text{SOH}$ groups), and by adsorption of ions from solution, which may create charge on a previously uncharged surface or alter the direction and magnitude of charge.

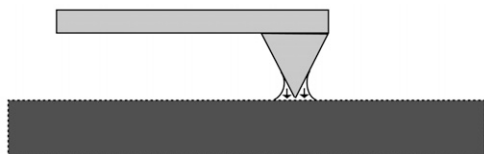


Fig. 11–3. Schematic illustration of the strong capillary force that tends to drive the tip and sample together during imaging in air. This can result in significant damage to the sample surface.

Lateral frictional forces also must be taken into account as the sample is rastered beneath the tip. At low forces, a linear relation should hold between the lateral force, F_L and the force normal (vertical) to the surface, F_v , with a proportionality constant equal to the coefficient of friction, μ (Eggleston, 1994). Warmack et al. (1994) showed this linear relation to be valid to ~ 30 nN repulsive force. Frictional forces vary on an atomic scale, and with temperature, scan velocity, relative humidity, and tip and sample materials.

ATOMIC FORCE MICROSCOPY IMAGING IN AIR AND SOLUTION

Contact-Mode Atomic Force Microscopy

The most traditional and common all-around mode of AFM imaging is the so-called contact mode. Contact-mode imaging can be conducted in air or immersed in a liquid such as an electrolyte solution. In contact-mode AFM, the tip is brought directly into contact with the sample surface, which is scanned beneath it. The tip-sample interaction is primarily repulsive in nature (e.g., due to the short-range forces associated with the Pauli exclusion principle). The interaction force can be as low as 10^{-9} N, which is approximately the interaction force between atoms in solids. Once the tip is brought into contact with the sample surface, the piezoelectric scanner drives the sample upward in the z direction a predefined distance. This causes the cantilever to deflect. The predefined cantilever deflection is preset by the system software and is known as the "set point." A feedback loop maintains a constant set-point level throughout the imaging process. When the tip goes over a feature on the sample surface, the computer feedback loop adjusts the z -piezo to keep the cantilever deflection at the set-point value. The imaging is conducted at a constant force, so that this mode of imaging is often called "constant force" imaging. The greater the cantilever deflection, the higher the imaging force exerted by the tip on the sample. A three-dimensional AFM image is generated as the computer plots the correction to the z piezo as a function of x - y position throughout the scan.

Imaging can also be conducted wherein the cantilever is allowed to deflect freely as the sample is scanned beneath it. This is known as "constant height" imaging or "variable deflection mode." In this mode of imaging, the height data are taken from the measurement of cantilever deflection. Other modes are also possible (see Morris et al., 1999). Contact-mode AFM has a real advantage in ease of use, and typical contact-mode tips (Si_3N_4) are relatively cheap and readily available. However, there is a significant disadvantage for AFM imaging of soft materials of interest in soil science such as adsorbed natural organic matter (NOM) or bacteria. In air, the capillary force between the tip and the sample can effectively drive the tip into the surface and cause significant damage to one or both. Shear or lateral forces that occur during the scan procedure as the sample is dragged underneath the tip can also be highly damaging.

Noncontact-Mode Atomic Force Microscopy

One way to minimize the damage caused by tip-sample interactions is to take advantage of the relatively longer-range attractive VdW force to conduct "noncontact" imaging. In this mode, the tip does not actually come into contact with the sample surface, but rather maintains a distance several nanometers above it. The tip oscillates with an amplitude of about 5 nm. The VdW force causes the amplitude of the oscillation to be decreased, and the amplitude decrease can be used to map the surface microtopography. This method has the advantage of very low forces between the tip and the sample, and hence less possibility of damage. Lateral and shear forces also are decreased. Noncontact-mode AFM can be very useful for obtaining atomic-scale images in solution because the decreased forces prevent

deformation of the surface atomic structure (e.g., Ohnesorge and Binnig 1993). However, in many instances, the resolution is not as good as with contact-mode imaging for similar surfaces. Imaging in air can be quite problematic. If the tip does come into contact with the sample during imaging in air, then the capillary force can drive the tip and the sample together, causing damage to one or both. This can be a major problem, especially for imaging soft microbiologic features.

TappingMode Atomic Force Microscopy

As described above, in conventional contact-mode AFM, the tip essentially drags across the sample, resulting in a three-dimensional topographical map of the sample surface. The lateral shear forces associated with this dragging motion, along with the adhesive forces that occur between the tip and the sample during imaging in air, can result in substantial damage to the tip and/or the sample and can lead to a variety of artifacts. Soft and easily deformable materials of interest in soil science are often particularly susceptible to such scan-induced damage. One way to alleviate this problem is by imaging in fluid (to reduce the capillary adhesion force). Unfortunately, many materials are too soft, easily mobile, or reactive in fluid to be imaged successfully, although new preparation techniques are evolving to facilitate such imaging. As noted above, noncontact-mode AFM probes exhibit relatively weak attractive VdW forces. Hence, the probe is maintained at a small distance above the sample surface, thereby minimizing surface damage. However, noncontact mode often provides lower resolution, unless carefully operated. When forces are very carefully controlled, atomic-scale resolution is possible in solution (e.g., Ohnesorge and Binnig, 1993).

TappingMode AFM (TMAFM) was developed by Digital Instruments (Veeco, Santa Barbara, CA) in an attempt to overcome the problem of elastic deformation and hence damage to soft surfaces, and it is successful for many (although not all) surfaces of interest to soil scientists. In TappingMode, a tip attached to a relatively stiff cantilever is intermittently brought into contact with the surface and lifted off. This helps to avoid the lateral shear forces normally associated with contact-mode AFM and also helps to keep the tip from being trapped by the capillary force that are present in air. Indeed, the effective forces on the sample surface in TappingMode have been shown to be smaller than in contact mode (Wong and Descouts, 1995). In ambient air, a piezoelectric crystal is used to oscillate the cantilever assembly at or near the cantilever's resonant frequency. When the tip is distant from the sample surface, the piezo movement results in a high amplitude oscillation that is usually ≥ 20 nm. The tip is brought closer to the surface until it makes contact with and begins to gently tap the surface at a frequency of 50,000 to 500,000 cycles per second (Prater et al., 2004). As the tip taps the surface, some energy is lost to the surface, resulting in a reduction in amplitude of the oscillation. When the tip goes over a high spot on the sample surface, there is less room for oscillation and the oscillation amplitude decreases. When the tip goes over a low spot, the oscillation amplitude increases. A feedback loop constantly adjusts the separation distance between the tip and the sample to keep the oscillation amplitude constant. In TappingMode, one can vary the tip-sample interaction force by changing the driving amplitude, A_0 , and the set-point amplitude, A_{sp} . The effective tip-sample force increases as A_0 and/or $A_0 - A_{sp}$ increases. TappingMode is often called noncontact-mode AFM, although this can cause some confusion with regard to the true noncontact-mode described above.

TappingMode can also be used to image in fluids, but there are some subtle differences in operation. Because there is no capillary force, the cantilever does not need to be as stiff. The fluid medium damps the cantilever's resonant frequency; however, the fluid

cell itself can be oscillated to drive the cantilever into oscillation. By selecting an appropriate frequency (from 5000 to 40,000 cycles per second; Prater et al. 2004), the cantilever's oscillation amplitude will decrease upon first tapping the surface. As in air, the feedback system adjusts the tip position to keep the oscillation amplitude constant.

Prater et al. (2004) reported that stable imaging forces <200 pN have been measured during operation in TappingMode. Despite the fact that TappingMode reduces frictional and shear forces on the sample and allows relatively low forces to be maintained, sample damage sometimes can occur. Figure 11–4 shows aggregates of NOM (fulvic acid) imaged on the surface of mica by TappingMode in 0.03 M CaCl_2 and $\sim 50\text{ mg C L}^{-1}$, pH 6. Fairly uniform aggregates are observed that are somewhat elongated, possibly due to drift (see below in “Other Common Artifacts”). The aggregates are often aligned into chains with an orientation that may potentially be controlled by muscovite surface structure, although this requires further study. The width of the aggregates measured along the shortest cross section is 64 to 200 nm, with an average of 78 nm. Their heights range from 1.3 to 6.8 nm and average 3.9. Attempts to image at smaller scan sizes (i.e., greater magnification) resulted in removal and smearing of the particles. In the central upper portion of Fig. 11–4, there is an approximately square-shaped area (~ 2 by $2\text{ }\mu\text{m}$) wherein most of the fulvic acid aggregates were removed by imaging before zooming out. We believe that the low adsorption affinity of the fulvic acid for mica at pH 6 allowed the fulvic acid aggregates to be removed from the surface during the small-region scan. For this sample, TappingMode imaging was still preferable to contact mode, which did not result in successful imaging at any scan size (10s of nm to 10s of μm scan size).

Phase Imaging

As the tip interacts with the sample, variations in some surface properties, such as composition, adhesion, friction, and viscoelasticity, can cause the phase of the cantilever oscillation to vary relative to the signal sent to the cantilever's piezo driver (Radmacher et al., 1994; Magonov et al., 1997a) (Fig. 11–5). Tip-sample interaction forces can shift

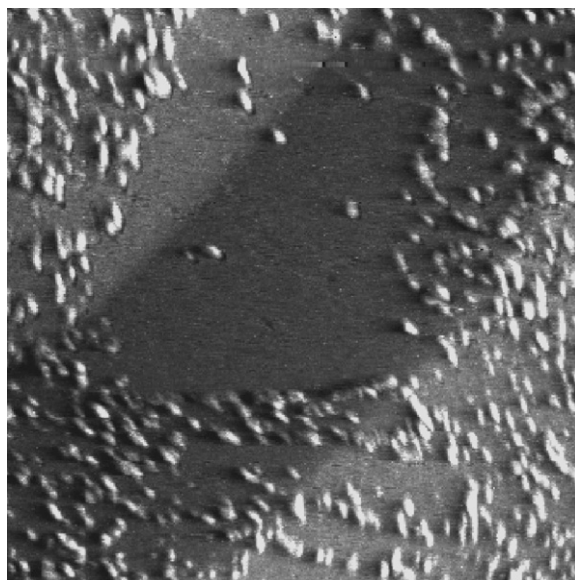


Fig. 11–4. TappingMode AFM image in solution showing aggregates of fulvic acid (rounded globules) adsorbed to muscovite. In the central upper portion of this image some of the aggregates were removed during a previous small-region scan. Hence, although TMAFM is useful for imaging soft and easily deformable surfaces, some damage can occur, particularly during small-region scans. Image is $5\text{ }\mu\text{m}$ on a side. From Namjesnik-Dejanovic and Maurice (2001).

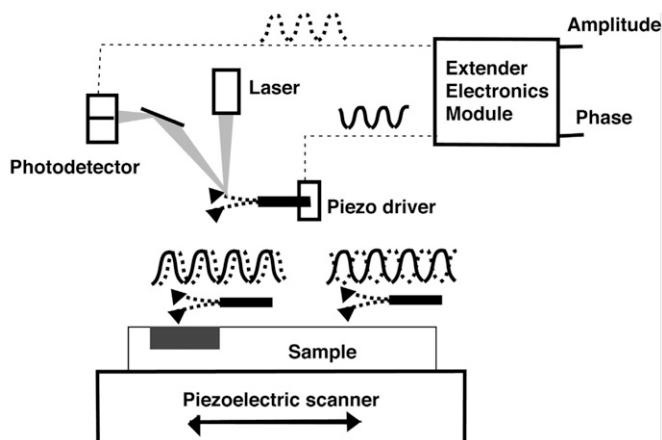


Fig. 11–5. Schematic illustration of phase imaging TMAFM.

the resonant frequency of the oscillating tip to higher or lower frequencies, with related phase changes. The resulting phase changes can be mapped to display a phase image simultaneously with the typical TappingMode image, and with the same potential resolution. Magonov et al. (1997a, 1997b) showed that differences can occur in TappingMode height and phase image quality and reproducibility upon changing the ratio between the A_0 (i.e., the drive signal applied to the cantilever oscillator) and A_{sp} (i.e., the parameter that defines the amplitude of the cantilever oscillation signal to be maintained by the feedback loop). Differences in this ratio can lead to so-called hard ($A_{sp}/A_0 < 0.2$), intermediate ($0.2 < A_{sp}/A_0 < 0.8$), or light ($A_{sp}/A_0 > 0.8$) tapping conditions. Magonov and Reneker (1997) discussed differences in phase shifts at different A_{sp}/A_0 for various materials.

Phase-mode images often are useful for differentiating between materials with different properties, such as bacteria attached to mineral surfaces, or even different portions of a single bacterium. Moreover, phase imaging often provides real-time contrast enhancement. These benefits can be particularly important when dealing with natural field samples of interest in soil science, particularly because such samples often have relatively rough microtopography and because micromorphology alone often is not adequate for interpretation of surface components. For example, Fig. 11–6 compares TMAFM height, phase, and amplitude mode images of a microcline feldspar surface that had been buried in a peat bog by Drs. Jennifer Roberts Rogers (University of Kansas) and Phil Bennett (University of Texas at Austin). The height mode image (Fig. 11–6a) reveals steps, fine particles, and some fibrous structures (e.g., fulvic acid or extracellular polysaccharide) present on the surface. The phase image (Fig. 11–6b) reveals far more detailed structures, including a network of fibrous structures that are likely organic matter attached to the feldspar surface. The network of organic fibers (perhaps humic substances or biofilm material) is only slightly apparent in the TappingMode height or amplitude mode images, but it is clearly highlighted in the phase image. Figure 11–6c is an amplitude mode image. Amplitude mode images are essentially the first derivative of the height mode images, and tend to provide enhanced highlighting of edges. They cannot be used for z -direction (height) measurements. As shown here, the amplitude mode image did not provide all of the intricate detail of fine structures shown in the phase mode image.

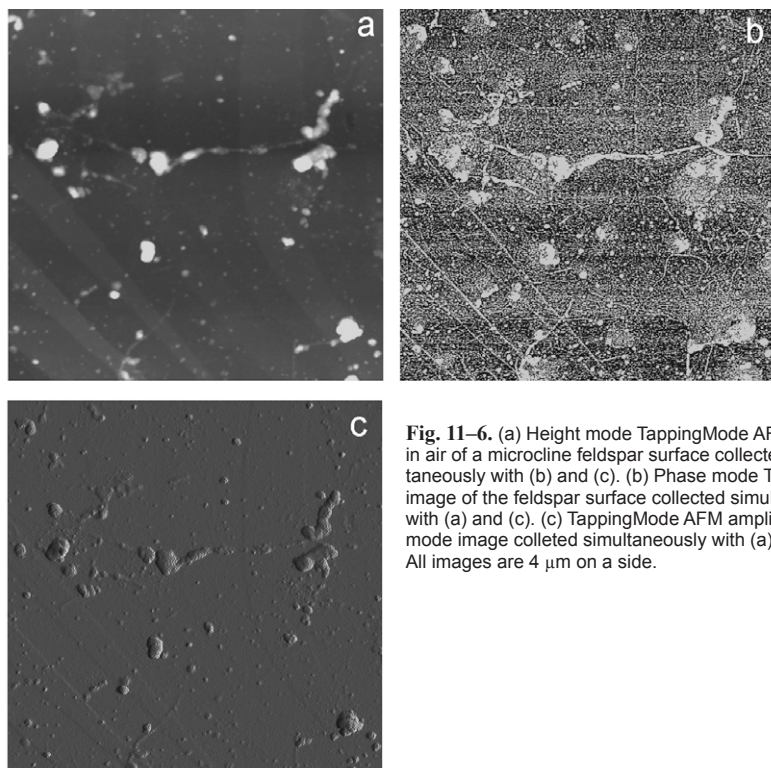


Fig. 11-6. (a) Height mode TappingMode AFM image in air of a microcline feldspar surface collected simultaneously with (b) and (c). (b) Phase mode TMAFM image of the feldspar surface collected simultaneously with (a) and (c). (c) TappingMode AFM amplitude mode image collected simultaneously with (a) and (b). All images are 4 μm on a side.

Some Common Artifacts in Atomic Force Microscopy Imaging

Tip Size and Shape

Many of the most common artifacts of AFM imaging are related to the finite size and shape of the tip. Most commonly used AFM probes, such as the standard Si_3N_4 and TMAFM etched Si, have pyramidal tips (Albrecht et al., 1990; Tortonese, 1997). These tips can have a radius of curvature as small as 1 nm (Sheng et al., 1999), but often the radius of these tips is much larger. For imaging vertical features several tens of nanometers or more in height, the tip half angle limits resolution. This is because when the tip moves over a sharp feature, the sides of the tip rather than just the tip apex contact the edges of the feature (Fig. 11-7). The resulting image is a nonlinear combination of the sample shape and the tip shape. In the worst-case scenario, the tip shape dominates the image and pyra-

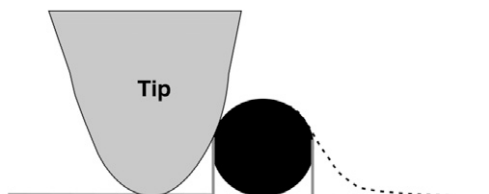


Fig. 11-7. Schematic illustration of tip broadening that tends to occur for biomolecules and other features less than about 30 nm high.

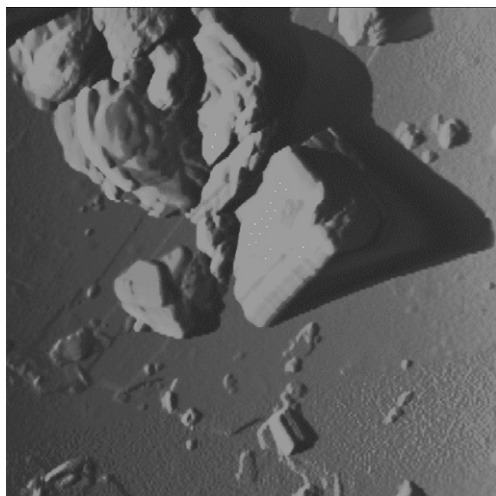


Fig. 11–8. A reverse tip (pyramidal structure just to the right of center) generated on imaging a mica sample that had been buried in an Antarctica Dry Valley (see the section "TappingMode Atomic Force Microscopy Imaging of Biofilm Structure"). The salts or precipitates on the weathered mica surface "convoluted" with the tip shape. This image also has a "shadowing" artifact along the right edges of some features because of the slow response of the feedback loop as the tip moved over a topographic high on the sample. This "shadowing" artifact can be decreased by imaging at a slower scan speed. Image is 2 μm on a side.

midal structures (reverse tip images) appear on the surface (Fig. 11–8). More subtle cases include features such as sloping step edges. Tip shape artifacts tend to be prevalent when imaging nuclei formed during biomineralization reactions. A review of this general phenomenon, including examples of artifactual images, was presented by Maurice and Lower (1998). Various image "deconvolution" software packages are available online, although such software must be used with caution, tempered by the experience of the user.

For features with vertical relief less than about 30 nm, it is the radius of curvature of the tip that limits resolution, resulting in tip broadening of the feature of interest. For tips that are significantly rounded, images of molecular features may be enlarged in x – y . Often, an assumption is made that the features are cylindrical, so that height measurements can be used as somewhat more accurate estimates of width or diameter.

Multiple Tips

An ideal tip ends in a single apex. However, manufacturing anomalies and/or contamination may lead to double or even multiple endings. When this occurs, features on the sample surface are mapped by the tip(s) more than once. For example, a double tip will result in a regularized doubling of features as shown in Fig. 11–9. Such artifacts are commonly termed double- or multiple-tip images. Contaminants on a tip can also interact with a sample surface, leading to repeated patterns of the contaminants scattered across the surface (e.g., Maurice and Lower, 1998). Cleaning of AFM tips and cantilevers is recommended (Lo et al., 1999).

Other Common Artifacts

When the gain parameter of the control loop is set too high, rippling artifacts can occur along the edges of features. These gain ripples (Fig. 11–10) tend to occur along the leading edge of a feature and will generally change position when the scan direction (or forward–reverse data collection) is altered. Shadow artifacts (Fig. 11–8) generally occur along the trailing edge of a feature, when the feedback loop is unable to compensate for a rapid change in topography. Shadow artifacts often can be minimized by reducing the scan speed. Sample damage or deformation during scanning also is an important artifact, par-



Fig. 11-9. Double tip artifact encountered on image of *Pseudomonas mendocina* following reaction with aluminous goethite. Several intertwined bacterial flagella are clearly doubled in this image.

ticularly for soft surfaces and during imaging in air. An example of scan-induced damage was shown above (Fig. 11-4).

Either piezoelectric and/or thermal drift can distort images, particularly at the start of scanning (Fig. 11-4). Imaging near the center of the z -piezo and allowing the AFM instrument and the sample to sit for a period of time to allow equilibration can substantially improve drift-related problems. When an o-ring style fluid cell is used, drag on the o-ring during scanning can also produce drift. This can be alleviated by applying an appropriate “grease” between the o-ring and the substrate surface.

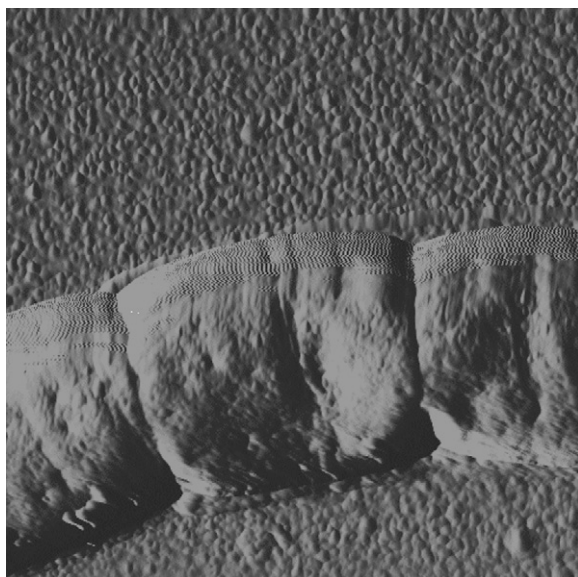


Fig. 11-10. When the gains that control the feedback loop are set too high, gains ripples such as those appearing along the top edge of this *Callithrix* can occur. Image is 8 μm on a side.

USE OF ATOMIC FORCE MICROSCOPY TO MEASURE INTER- AND INTRAMOLECULAR FORCES

As described above, the AFM images a sample by sensing and responding to forces between a tip and the sample. Because the force resolution of the AFM is so sensitive (0.1–1 pN; Aoki et al., 1997; Cappella and Dietler, 1999), it is a powerful tool for probing inter- and intra-molecular forces between two substances. Researchers have taken advantage of this sensitivity to quantify fundamental forces between a sample and some substance linked to the AFM cantilever or tip.

Functionalization of the Tip

Cantilevers or tips can be functionalized with entities of direct interest in soil science such as colloidal beads, chemical functional groups, biomolecules, or even living cells (Table 11–2). Thus functionalized, the AFM can be used to probe the forces between these various entities and the sample surface, whether that is a mineral, cell, or some other material.

There are many ways to functionalize an AFM tip or cantilever. All are constrained by one overriding principle—the bonds between the tip/cantilever and the functionalizing substance (i.e., the forces holding the substance of interest to the tip/cantilever) must be much stronger than those between the functionalizing substance and the sample (i.e., the forces that are actually measured by the AFM). Otherwise, the functionalizing substance would be ripped from the tip/cantilever during force measurements.

Single, colloidal size beads, a few micrometers in diameter, can be routinely attached to a cantilever using an epoxy resin (Ducker et al., 1991, 1992). Such beads may be simple latex or silica spheres or more complex designer beads imprinted with biomolecular recognition sites using molecular imprinting techniques (Caruso et al., 1998; Kriz et al., 1997). Care must be taken to select an epoxy that is inert in the aqueous solution and that will not melt under the laser of the optical-lever detection system (Pincet et al., 1995).

Table 11–2. List of various substances that have been linked to AFM tips or cantilevers.

Substance linked to tip/ cantilever	Linkage chemistry	Reference†
Protein	adsorption, imide, glycol tether, antibody–antigen	1, 6
Nucleic acid	thiol	7, 8
Polysaccharide	non-specific adsorption	9
Glass or latex bead	epoxy	10, 11
Living microbial cell	silane, poly-lysine	12, 13
Nonviable microbe	gluteraldehyde	14, 15
Eukaryotic cell	epoxy, nonspecific adsorption	16, 17
Organic monolayer	self-assembling monolayer, silane	18–21
Nanotube	epoxy	22
Shards of mineral	epoxy	23

† References: (1) Florin et al., 1994; (2) Moy et al., 1994; (3) Hinterdorfer et al., 1996; (4) Willemsen et al., 1999; (5) Dammer et al., 1995; (6) Vansteenkiste et al., 2000; (7) Lee et al., 1994; (8) Boland and Ratner, 1995; (9) Rief et al., 1997; (10) Ducker et al., 1991; (11) Ducker et al., 1992; (12) Lower et al., 2000; (13) Lower et al., 2001; (14) Razatos et al., 1998; (15) Ong et al., 1999; (16) Antonik et al., 1997; (17) Bowen et al., 1998; (18) Noy et al., 1997; (19) Noy et al., 1998; (20) Wong et al., 1998; (21) Tsukruk and Bliznyuk, 1998; (22) Hafner et al., 1999; (23) Eggleston and Jordan, 1998.

Simple carboxylic, methyl, hydroxyl, or amine functional groups can be formed by self-assembling monolayers on gold-coated tips (Frisbie et al., 1994; Noy et al., 1997, 1998) or by creating a silane monolayer directly on the tip (Bliznyuk et al., 1998). Organosilane modification of a tip is slightly more robust because it avoids the use of gold, which forms a relatively weak bond with the underlying silicon or silicon nitride surface of the tip in the case of self-assembling monolayers. Carbon nanotubes that terminate in select functional groups can also be attached to cantilever tips (Dai et al., 1998; Hafner et al., 1999; Wong et al., 1998a, 1998b). Their high aspect ratio and mechanical strength create functionalized cantilevers with unprecedented strength and resolution capabilities. Direct growth of carbon nanotubes onto cantilevers by methods such as chemical vapor deposition (Hafner et al., 1999) will likely make this method more accessible to a large number of researchers.

Biomolecules such as polymers, proteins, and nucleic acids have been linked to AFM tips or deposited directly on the cantilever (Biggs, 1995; Boland and Ratner, 1995; Bowen et al., 1998a; Dammer et al., 1995; Florin et al., 1994, 1995; Lee et al., 1994; Ludwig et al., 1999; Moy et al., 1994; Mueller et al., 1999; Rief et al., 1997a). One of the simplest attachment techniques is by nonspecific adsorption between a protein, for example, and silicon nitride. The adsorbed protein can then serve as a receptor for yet another protein or ligand. In so doing, virtually any biomolecule can be linked to a cantilever either directly or by means of a bridging molecule. Thiol groups on proteins or nucleic acids are also useful because a covalent bond can be formed between sulphydryl groups on the biomolecule and gold coatings on a tip. Such attachment protocols have been very useful, but there are some disadvantages. The linkage procedure may disrupt the native conformation or function of the biomolecule—for example, if the attachment procedure disrupts a catalytic site. It is well known that a protein attached to a solid substrate (e.g., a cantilever or tip) may exhibit a significantly different conformation, function, and/or activity relative to its native state within a membrane or dissolved in solution (Ellen and Burne, 1996; Ingersoll and Bright, 1997; Stotzky, 1986; Turner et al., 1996, 1997). Hence, care must be taken to design control experiments that test the specificity of a particular biomolecule as it occurs in its natural state (e.g., the infusion of a solution of antibody specific for a protein linked to the tip).

The linkage protocol becomes even more challenging when dealing with complex biomolecular networks such as entire cells. For large fungal cells, the linkage is relatively straightforward. Large spores, for example, may be attached to a cantilever in much the same way as colloidal beads (Bowen et al., 1998b). For bacterial cells on the order of ≤ 2 μm , it is much more difficult to manipulate the cell onto the cantilever. Nevertheless, several researchers have succeeded in attaching dead (Ong et al., 1999; Razatos et al., 1998a, 1998b) or even living bacterial cells (Lower et al., 2000, 2001) to cantilevers. The former method relies on the attachment of cells that have been chemically fixed with relatively harsh chemicals (e.g., glutaraldehyde) such that proteins and other biomolecules are denatured, and the bacteria are killed, whereas the latter method allows cells to be attached in their native, fully functional state. For the attachment of live cells, a polycationic linker molecule (e.g., aminopropyltriethoxysilane or poly-lysine) can be used to either link the bacteria to a small glass bead that is then attached to the cantilever (Fig. 11–11), or the bacteria can be linked directly onto the cantilever (Lower et al., 2001). Polycationic linkers work well because most bacteria are negatively charged over a wide range of pH conditions. Hydrophobic molecules (e.g., octadecyltrichlorosilane) are also attractive linkers because many microorganisms have hydrophobic surfaces. Techniques similar to affinity chromatography (e.g., Egger et al., 1992; Pleuddemann, 1991; Rezanian et al., 1999) may be employed to design custom linker molecules (e.g., ligand-receptor or antibody-antigen) that work on virtually any bacterial species. The use of polycationic linkers or similar mol-

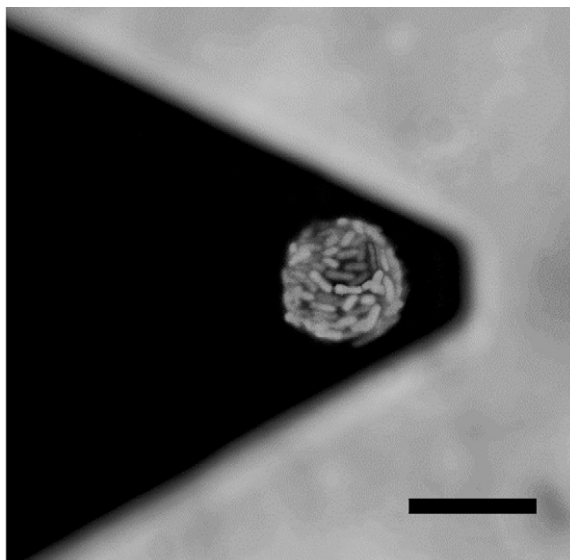


Fig. 11–11. Biologically active force probe fabricated by linking living bacterial cells to a small glass bead on the end of an AFM cantilever. Single cells can also be linked to the AFM tip. Scale bar is 10 μm . From Lower et al. (2000).

ecules preserves the natural conformation, structure, and function of the macromolecules on the microbial surface. When live cells are used, force measurements may be collected under different physiological or environmental conditions in real time (Lower et al., 2001). New nanotechnologies such as optical tweezers and nanotweezers (Kim and Lieber, 1999; Svoboda and Block, 1994) should allow single bacterial cells to be linked to cantilevers with greater ease.

Force Measurements

Force measurement data are collected by monitoring cantilever deflection as the sample approaches, makes contact with, and then retracts from the cantilever. The raw data of cantilever deflection are not in units of force; rather, the cantilever deflection is monitored by a photodiode detector and therefore has units of volts. Further, the distance (or separation) between the tip and the sample is not measured directly. Rather, the AFM measures the displacement of the piezoelectric scanner that supports the sample. A so-called “voltage–displacement curve” plot of cantilever deflection (in diode volts) and corresponding piezo displacement (in meters) (Fig. 11–12a) must be manipulated to produce a force–distance curve (i.e., force of interaction in Newtons vs. separation between the sample and the cantilever in meters; Fig. 11–12b).

Determining the Force Component

To obtain the force part of the force–distance curve, photodiode values must be converted into Newtons of force using Hooke’s Law, $F = k_{\text{sp}} d$, where F is force (Newtons) d is cantilever deflection (meters), and k_{sp} is the cantilever spring constant (N m^{-1}). To use Hooke’s Law, cantilever deflection measured by the photodiode in volts must be converted into meters. A diode–displacement conversion factor (also called “optical lever sensitivity” in V nm^{-1}) is obtained from the region of the force curve where the sample is in contact with the cantilever. For every nanometer that the piezos scanner displaces the sample, the cantilever is pushed upward 1 nm, which registers as a certain voltage output on the photodiode array. Hence, the slope of the force curve in the region where the cantilever is in con-

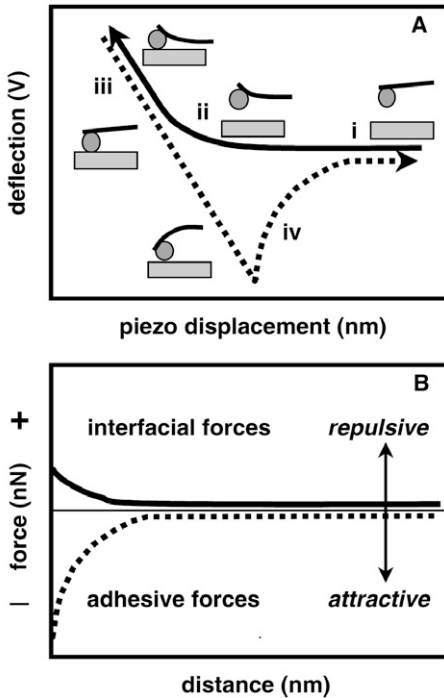


Fig. 11–12. Schematic diagrams of a (A) force curve and (B) force–distance curve (B). When the tip (shown here as a circle on the end of the cantilever) and sample are far apart (i) they exhibit no interaction (region of zero force). As the sample approaches the tip, intermolecular forces between the tip and sample cause the cantilever to deflect upward (ii) due to repulsive forces shown here. Eventually the tip makes contact with the sample (iii) and their movement becomes coupled (region of constant compliance). The sample is then retracted from the tip (iv) until the tip or cantilever and sample return to their original positions, thereby completing one cycle (an entire cycle requires nano- to milliseconds). Hysteresis, shown here, may occur on retraction due to adhesion forces. See text for discussion of converting cantilever deflection and piezo displacement into force and distance, respectively. Interfacial forces are measured on approach, and adhesion forces are measured on retraction. Repulsive forces are positive and attractive forces are negative. From Lower et al. (2001).

tact with the sample defines the optical lever sensitivity (Fig. 11–12A, iii). This part of the force curve is called the region of constant compliance or region of contact. The reciprocal slope can be used to convert measured cantilever deflection from volts to meters.

It is important to note that using the constant compliance region of the force curve to convert photodiode response to deflection will overestimate the force of interaction if the cantilever is not the most compliant component of the system (Fig. 11–13). This is often the case when soft, deformable substances like polymers or cells are used in force measurements (either as a sample or linked to the tip or cantilever). In instances where some other substance (e.g., cells or biomolecules linked to the cantilever or tip) is more compliant than the cantilever, or in cases where one wants to avoid damaging cells or biomolecules with fragile appendages, other methods must be used to accurately convert the measured deflection of the cantilever (in volts) into a force of interaction (in Newtons) (D’Costa and Hoh, 1995; Sader et al., 1999). For example, one could determine the optical lever sensitivity by pressing the tip or cantilever against a hard sample (e.g., mica) before and after it is used on a soft sample. However, this method often does not work because the optical lever sensitivity is strongly dependent on a number of factors such as the position and shape of the laser spot, and it is difficult to precisely align the laser on the same position of the cantilever from experiment to experiment. Further, this method will not work if it is the tip or cantilever that supports the most compliant component of the system (e.g., a cell attached to the cantilever). Another method that relies on the “photodiode shift voltage,” a parameter that is very sensitive to the position and shape of the laser of the photodetector, can be used to convert volts of cantilever deflection into meters of deflection (D’Costa and Hoh, 1995; Lower et al., 2001).

Photodiode shift voltage is measured as the difference in output voltage when the photodiode detector is shifted approximately 318 μm (one full turn of the positioning screw

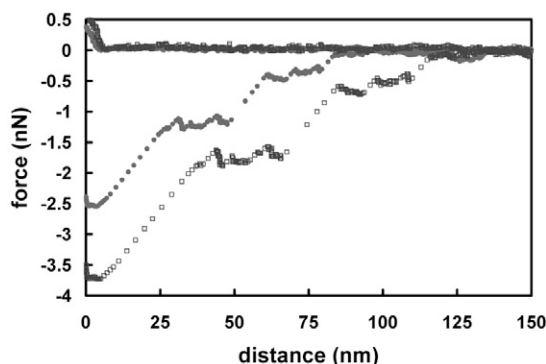


Fig. 11–13. Force–distance curves between *Shewanella oneidensis* and diaspore (AlOOH). Raw data were converted to force and distance values using the optical lever sensitivity determined by either the constant compliance region of the force curve (open squares) or the photodiode shift voltage (solid circles). Notice that both axes are dependent upon which method is used to determine optical lever sensitivity. This is because cantilever deflection is not only used to determine the force of interaction, but it is also used to convert piezo displacement to separation values. The top curves correspond to approach data and the bottom curves to retraction data.

for a DI MutliMode AFM, Veeco Instruments) on either side of the zero setting. Once this correlation between photodiode shift voltage and optical lever sensitivity is established for a given instrument, piezoelectric scanner, fluid cell, and cantilever, the diode–displacement conversion can be accurately determined without pressing the cantilever against any other surface. This method ensures that forces can be determined regardless of the compliance of the cantilever relative to any other component in the AFM, and also ensures the preservation of fragile macromolecules, which may be present of the sample or attached to the cantilever.

The final parameter of Hooke's Law is the cantilever's characteristic spring constant, which is dependent on its composition and dimensions. Nominal values listed by manufacturers may be off by more than an order of magnitude (Bliznyuk et al., 1998). Therefore, it is necessary to determine the spring constant for each cantilever or for each wafer of cantilevers. The variation in spring constant from cantilever to cantilever within the same wafer is minimal (Senden and Ducker, 1994). Parameters such as Young's modulus (related to composition), as well as cantilever length and thickness, can be used in theoretical equations to calculate a spring constant (Sader et al., 1995; Sader, 1995; Neumeister and Ducker, 1994). However, calculated values are often grossly inaccurate because of compositional inconsistencies of the cantilever (e.g. the stoichiometry of silicon nitride can be anything from Si_3N_4 to Si_{15}N_4 (Weisenhorn et al., 1992) and variations in cantilever thickness, which is a dominant parameter in theoretical equations. The spring constant depends on the cantilever thickness raised to the third power, so even small variations in the thickness will result in large variations in the calculated spring constant (Bliznyuk et al., 1998; Hutter and Bechhoefer, 1993).

For these reasons, a cantilever's spring constant should be measured experimentally. The two most common methods are the added-mass method (Cleveland et al., 1993) and the thermal method (Hutter and Bechhoefer, 1993; Sader et al., 1999). In the added-mass method, beads of known mass are attached to the end of the cantilever. The additional mass causes the cantilever's resonant frequency to decrease proportional to the mass. A plot of added mass versus resonant frequency yields a straight line whose slope corresponds to the spring constant. In the thermal-noise method, the root mean square (rms) fluctuations of a freely moving cantilever are measured, and the data are examined as a power spectral density having a Lorentzian line shape (Hutter and Bechhoefer, 1993). The integral of the power spectrum (i.e., area below the peak) can be used to determine the cantilever spring constant.

The hardware available with most commercial AFMs can be used to measure the Brownian motion of a cantilever due to its absolute temperature. Hence, the thermal noise method is an attractive choice for determining a cantilever's spring constant. Further, the thermal-method is nondestructive such that once the spring constant is determined the very same cantilever can be used for force measurements. With the added-mass method, beads are glued to the cantilever such that it can no longer be used for force measurements. Rather, another cantilever from the same wafer is used for force measurements.

The force axis of a force–distance curve is completed by selecting a zero force value. This reference value is determined as the force curve region where cantilever deflection is independent of piezo displacement (Fig. 11–12A, i). That is, the cantilever and the sample are not interacting because they are far apart (often referred to as the region of noncontact). Repulsive forces are then defined as positive values, whereas attractive forces are negative.

Determining the Distance (or Separation) Component

To obtain the distance or separation part of the force–distance curve, a point of contact (i.e., zero separation) must be defined and the recorded piezoelectric scanner position (i.e., displacement) must be corrected by the measured deflection of the cantilever. The latter is achieved by simply adding or subtracting the deflection of the cantilever to the movement of the piezoscaner. For example, if the sample attached to the piezo-scanner moves 10 nm toward the cantilever, and the cantilever is repelled 2 nm due to repulsive forces, then the actual cantilever–sample separation changes by only 8 nm. The origin of the distance axis, the point of contact, is chosen as the beginning of the region of constant compliance; that is, the point on the force curve where cantilever deflection becomes a linear function of piezo-scanner displacement (Fig. 11–12A, iii).

Just as it was difficult to convert photodiode voltage to metric units for soft, deformable materials, it is not always easy to select the point of contact because there is no independent means of determining cantilever–sample separation. For deformable samples, the cantilever indents into the sample such that the region of constant compliance may be non-linear (A-Hassan et al., 1998) and the beginning point cannot be easily defined. Techniques like fluorescence resonance energy transfer (FRET) may provide an independent means of determining separation. With FRET an external energy source is used to excite one of two fluorophores. This fluorophore emits a relatively long wavelength of light, which in turn excites the second fluorophore when the two are in close proximity (i.e., a few Angstroms of each other). By combining AFM with FRET it may be possible to determine the point of contact for soft samples with greater accuracy.

Challenges and Artifacts of Force Measurements

There are a number of artifacts that have been identified in force curves. Many of these problems are a result of interference by the laser, viscosity effects of the solution, or elastic properties of soft samples. When the sample and the cantilever are far apart such that there is no interaction, the force curve data should conform to a horizontal line (i.e., the region of noncontact, Fig. 11–12A, i). However, the laser has a finite spot size that may be larger than the size of the cantilever such that the laser beam reflects off the sample as well as the cantilever. This is particularly troublesome for reflective substrates, often resulting in optical interference (Jaschke and Butt, 1995; Weisenhorn et al., 1992), which manifests itself as a sinusoidal oscillation or as a slight slope in the noncontact region of the force curve (Fig. 11–14). This affects the way in which one defines attractive or repulsive forces. A simple solution is to realign the laser on the cantilever such that the beam

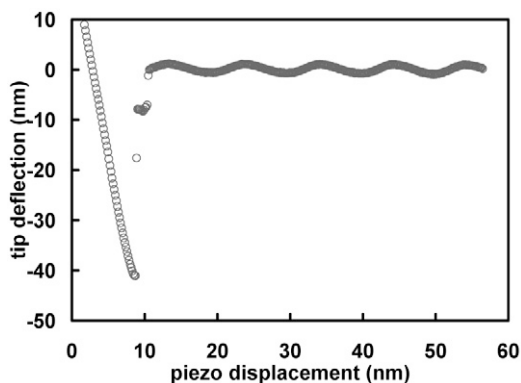


Fig. 11–14. Force microscopy retraction data collected between small glass bead and mica. Optical interference results in the oscillation that is present at displacement values >10 nm.

does not impinge on the underlying sample. Alternatively, the oscillation artifact may be removed from the force curve by knowing the wavelength of the laser (Jaschke and Butt, 1995). Another artifact is hysteresis between the approach and retraction curves in the noncontact area. The approach and retraction curves often do not overlap in high viscosity media (e.g., glycerol = 15 Pa) because of fluid dynamic effects (Hoh and Engel, 1993). Decreasing the scan rate (i.e., the rate at which the piezo-scanner translates the samples toward and away from the cantilever) can help to minimize hysteresis in the noncontact area by decreasing the drag caused by the fluid.

Along the same lines, the approach and retraction curves may not overlap in the region of contact but rather may be offset laterally with respect to one another (Fig. 11–15). Such effects make it difficult to define the point of contact, which is necessary to obtain separation values between the sample and the tip. Such hysteresis is due to frictional effects as the tip (which is mounted in the AFM at an angle of typically 10 to 15° relative to the sample) slides on the sample surface (Hoh and Engel, 1993). This hysteresis is dependent on the scan rate and reaches a minimum below which friction is dominated by stick-slip effects and above which friction is dominated by shear forces (Hoh and Engel, 1993). This artifact may be corrected by mounting the sample perpendicular to the cantilever, thereby eliminating lateral movement of the cantilever on the sample. Alternatively, the average of the approach and retraction curves may be used to define the point of contact.

Viscoelastic properties of soft samples, or soft substrates attached to the cantilever or tip, can also make it difficult to determine the point of contact and to measure accurately

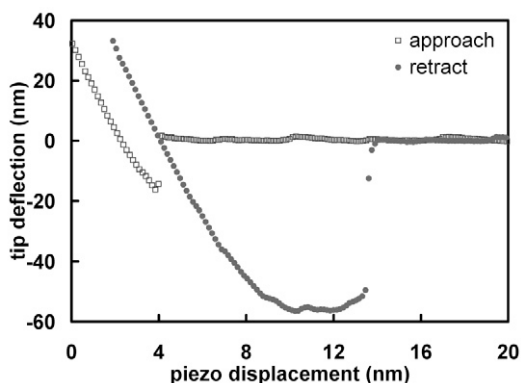


Fig. 11–15. Force curve between mica and small glass bead coated with poly-lysine in 10^{-2} M KCl. The approach and retraction data are offset in region of contact.

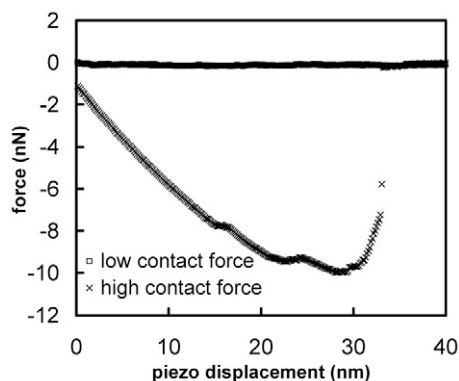


Fig. 11–16. Force curve between *Escherichia coli* and muscovite in 0.1 M NaCl. Only retraction data are shown. The adhesive force, determined by multiplying tip deflection by the cantilever spring constant, is much stronger with increasing "contact force" between the cell and mineral.

the forces of adhesion. When the cantilever makes contact with a soft sample, the sample may be indented by the cantilever such that the region of contact is nonlinear (A-Hassan et al., 1998). Hence, it is hard to determine the point at which contact begins. Furthermore, the scan rate (i.e., rate at which the sample approaches or retracts from the tip) affects the adhesive force measured on soft samples (e.g., membranes or cells). This is because the tip and sample are weakly joined over a large contact area that does not decouple fast enough as the tip is withdrawn at very high scan rates (Hoh and Schoenenberger, 1994). Thus, the cell or membrane deforms upward as the tip withdraws, causing an increased force of adhesion. Contact between a soft sample (e.g., a cell) and the tip also affects the measured adhesion force in other ways. As a tip is driven into a soft sample, the contact area increases as the sample deforms around the tip. Hence, increasing the contact force between the tip and sample increases the contact area, which in turn increases the number of interactions between the tip and sample. Therefore, increasing contact force results in an increased adhesive force between the tip and sample (Fig. 11–16). To compare measured adhesion values, the contact force should be selected such that it does not vary from experiment to experiment. Additionally, slow scan rates should be used to allow the tip and sample to separate during retraction.

ATOMIC FORCE MICROSCOPY APPLICATIONS IN SOIL SCIENCE

TappingMode Atomic Force Microscopy Imaging of Biofilm Structure

Many studies have shown that mineral surfaces in nature tend to be colonized by bacteria (e.g., Zobell 1943; Marshall et al., 1971; Krumbein and Stahl, 1991; Hiebert and Bennett, 1992; Costerton et al., 1995; Barker et al., 1997; Forsythe et al., 1998a, 1998b). The attached bacteria often form biofilms wherein the microorganisms are surrounded by extracellular polymers that form a tangled matrix of fibrous support structures (e.g., Characklis and Marshall, 1990; Forsythe et al., 1998a, 1998b). The environment within the biofilm can be quite distinct from the external environment in terms of pH, dissolved oxygen, inorganic species, and organic components (Little et al., 1997). Recent studies have shown that biofilm architecture tends to be complex and three-dimensional, permitting through flow of water and hence of nutrients and waste products (Little et al., 1997 and references therein).

Atomic force microscopy is not currently the method of choice for characterizing three-dimensional biofilm architecture because typical AFMs require relatively firm attachment of a sample to a substrate surface for imaging. Moreover, one cannot image beneath

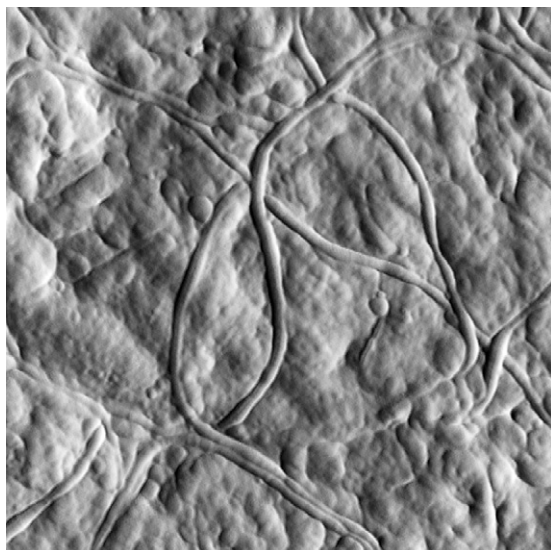


Fig. 11–17. TappingMode AFM amplitude mode image in air of a biofilm formed by *P. mendocina* grown in a succinate medium in the presence of hematite as an Fe source (modified from Forsythe et al., 1998). The biofilm is held together by a network of fibrous attachment features approximately 20 nm in diameter and up to several micrometers in length. The biofilm was deposited on a glass slide, air-dried, and gently rinsed with deionized water to remove salts before imaging. Image is 1.5 μm on a side.

the biofilm surface to probe its interior. Nevertheless, AFM can provide detailed images of nanometer-scale biofilm structures. Figure 11–17 is a TMAFM amplitude-mode image in air of a biofilm formed by aerobic *Pseudomonas mendocina* bacteria in the presence of the Fe oxide mineral hematite (supplied as an Fe source) (Forsythe et al., 1998b). The AFM image shows that the biofilm is bound together by an intertwined network of fibrous attachment features. The average diameter of the fibrous attachment features estimated from their heights is approximately 20 nm, and they extend several micrometers in length.

The detailed features of the biofilm can be highlighted using phase imaging. Figure 11–18 compares simultaneously collected TMAFM amplitude and phase images of a goethite (FeOOH) sample reacted with the *P. mendocina* bacterium for 8 d, deposited onto a glass slide, air dried, and imaged in air. The phase image provides real-time contrast enhancement, allowing the fibrous attachment features to be more readily discernible. Depending on phase imaging parameters, different portions of the surface were highlighted, for example, the bacterial cells, their flagella, the fibrous attachment features, or the goethite (other images not shown here). Hence, phase imaging was shown to differentiate various components of a biofilm based on their disparate surface properties such as stiffness and elastic moduli (Forsythe et al., 1998b). This is important because it provides an additional means of interpreting AFM images beyond microtopography alone.

Phase imaging can be particularly important for interpretation of AFM images of complex field samples. The importance of phase imaging became apparent during AFM imaging of mineral samples that had been buried in the hyporheic zone of an Antarctic Dry Valley stream (Maurice et al., 2002). Although the Dry Valleys receive very little precipitation (as snow), glacial meltwater flows through streams for a period of 6 to 10 wk each summer. Some of this water also flows through the hyporheic zone, which is the region of sediments directly adjacent to and underlying the stream. Bacteriological analysis of the hyporheic zone sediments (Maurice et al., 2002) revealed fairly abundant small (<1 μm long), oblong bacteria, including α -proteobacteria consistent with the oligotrophic conditions. Figure 11–19 compares TMAFM amplitude and phase mode images of the surface of a mica sample that was buried in the hyporheic zone during Austral summer (Dec.

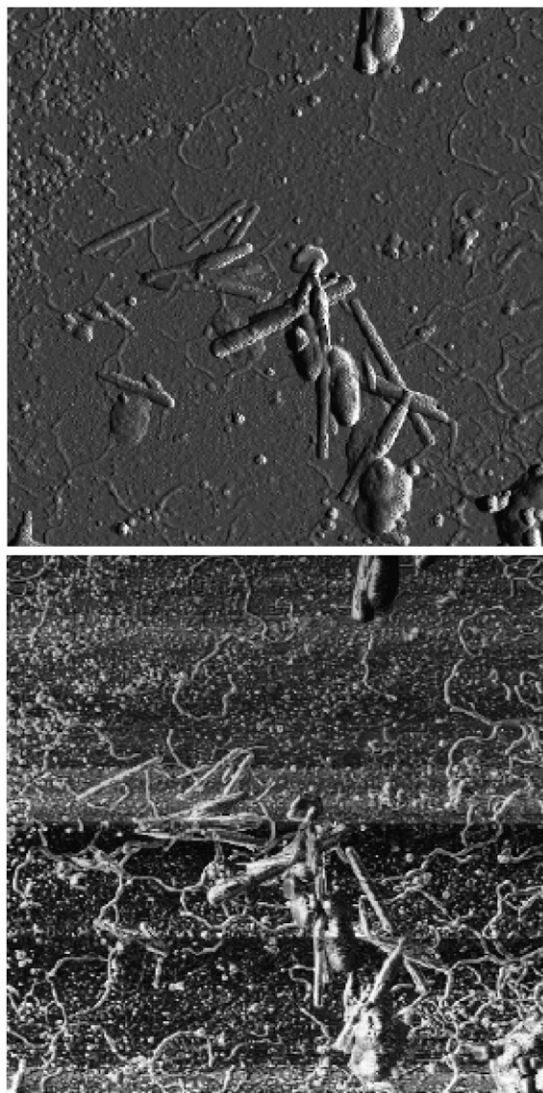


Fig. 11–18. TappingMode AFM (top) amplitude and (bottom) phase mode images in air of a biofilm consisting of bacteria (oblong), goethite (needle-like), and fibrous attachment features deposited on a glass slide. Phase-mode imaging provides real-time contrast enhancement, highlighting the network of intertwined fibrous attachment features. Each image is 10 μm on a side.

1999–Jan. 2000). Both of these simultaneously collected images show one small, oblong bacterium. The size and shape of this bacterium is consistent with epifluorescence microscopy identification of bacteria in the hyporheic zone sediments. In addition, the phase mode image reveals a complex, porous biofilm structure that is largely obscured and difficult to interpret in the amplitude- and height-mode images (latter not shown here). X-ray photoelectron spectroscopy (XPS) analysis of this sample revealed that it was significantly enriched in C and N and depleted in Fe relative to unreacted mica controls. Although C can be from a variety of sources, including adventitious C and carbonates, the great abundance of C, the increase in N and the decrease in Fe are all consistent with microbial activity at the mica surface.

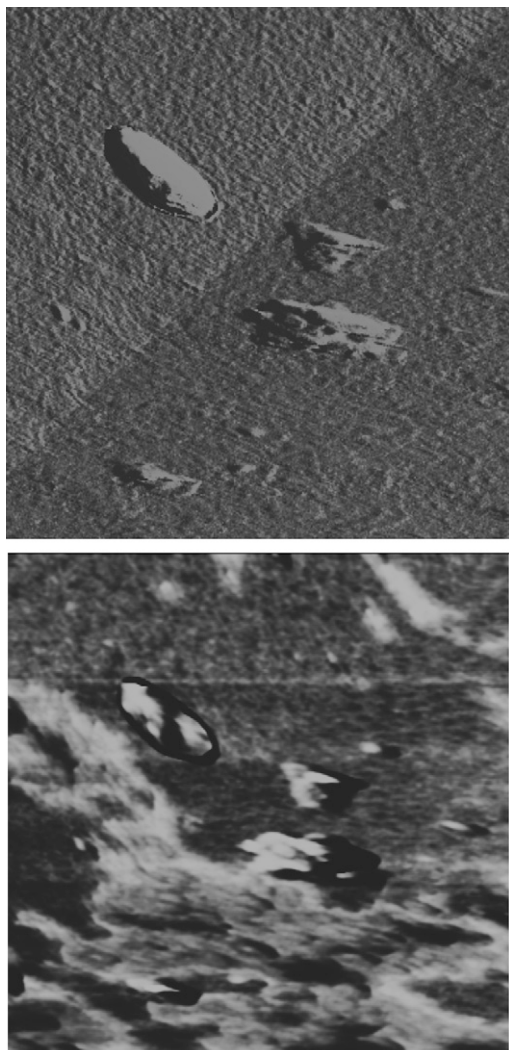


Fig. 11–19. Comparison of TMAFM (top) amplitude and (bottom) phase mode images of a mica surface that had been buried in the hyporheic zone of an Antarctic Dry Valley stream. A single oblong bacterium is apparent. A complex biofilm can be seen clearly in the lower portions of the phase mode image but is largely obscured and not easily identifiable in the amplitude mode image. Each image is 1 μm on a side.

In-Solution Imaging of Sorbed Natural Organic Matter

Atomic force microscopy has also been shown to be of critical importance for understanding the mechanisms of adsorption of NOM to mineral surfaces. Natural organic matter is a ubiquitous component of soils, streams, lakes, aquifers, and estuarine waters (Thurman, 1985), and it exerts a profound influence on many hydrobiogeochemical processes. Natural organic matter helps to control the growth and dissolution of soil and aquifer minerals, the mobilities of pollutants such as trace metals and organic compounds, the aggregation kinetics of colloids, the rates and mechanisms of many photochemical reactions, and the stabilities of soil aggregates. At least some forms of NOM serve as sources of organic C to microorganisms (e.g., Sun et al., 1997; Hunt et al., 2000), and NOM affects microbial attachment to mineral surfaces (Fein et al., 1999). Atomic force microscopy is revolutionizing the study of NOM sorption to mineral surfaces by permitting in-solution imaging

of sorbed NOM at molecular-scale resolution (Maurice and Namjesnik-Dejanovic, 1999; Namjesnik-Dejanovic and Maurice, 2001). Although in-air AFM imaging of sorbed organic matter (e.g., Namjesnik-Dejanovic and Maurice, 1997; Santschi et al., 1998; Mertig et al., 1999) provided valuable insight into the NOM properties, in-solution imaging was shown to be essential to prevent drying-induced artifacts including changes to conformations and aggregate structures (Namjesnik-Dejanovic and Maurice, 1997).

A recent study by Namjesnik-Dejanovic and Maurice (2001) showed that sorbed NOM (i.e., aquatic and soil humic and fulvic acids) conformation and aggregate structure was highly sensitive to solution conditions (pH, ionic strength, NOM concentration, concentration of di- and trivalent ions) and mineral adsorbate surface properties (see Table 11–3). Moreover, electrolyte identity and concentration exerted profound effects on image stability, observed particle size, and sorption density. For example, on mica, the presence of Ca^{2+} led to much more stable images of NOM than did Na^+ . This is in agreement with previous observations that Ca^{2+} tends to promote NOM adsorption relative to Na^+ (Tipping, 1981; Dempsey and O'Melia, 1983) in that stronger sorption should lead to more stable images.

Some of the most intriguing structures were observed at relatively high NOM concentration (soil fulvic acid, approaching 100 mg C L^{-1}) at pH 5, in CaCl_2 on mica. Under these conditions, Maurice and Namjesnik-Dejanovic observed that sorbed fulvic acid formed ring-shaped aggregates with diameters on the scale of several tens of nanometers (Fig. 11–20). Nanometer-scale pores were apparent along the circumferences. These nanopores might represent hydrophobic domains for the partitioning of hydrophobic organic pollut-

Table 11–3. Summary of experimental conditions and results for in-solution AFM imaging of aquatic natural organic matter (NOM). Revised from Namjesnik-Dejanovic and Maurice (2001).

Sample	pH, electrolyte conditions, and NOM concentration	Adsorbed NOM shape	Mean $x - y \pm \text{SD}$	Mean height $\pm \text{SD}$	Substrate
nm					
Stream fulvic acid	pH 3 0.1 M LiCl, 25 mg C L ⁻¹	spheres	3.4 ± 1.0	0.6 ± 0.2	mica
		aggregates of spheres	12.2 ± 3.3	0.6 ± 0.2	
	pH 3, 0.001 M CaCl ₂ , 20–100 mg C L ⁻¹	spheres	1.9 ± 0.8	1.3 ± 0.7	
	pH 5, 0.1 M CaCl ₂ , <100 mg C L ⁻¹	ring-shaped aggregates	$49 \pm 10^\dagger$	$3.5 \pm 1.7^\dagger$	
	pH 6, 0.03 M CaCl ₂ , 75 mg C L ⁻¹	oblate-spherical aggregates	78 ± 13 (shortest cross section)	3.9 ± 1.4	
Muck fulvic acid	pH 8, 0.001 M CaCl ₂ , 100 mg C L ⁻¹	linear aggregates	53 ± 21 width	1.5 ± 0.6	mica
	pH 11 adjusted with NaOH, no additional electrolytes, 50 mg C L ⁻¹	fine linear features	6.4 ± 1.9 width	0.25 ± 0.1	
Ground water NOM	pH 4, no electrolyte added (naturally high Fe and Al), 27 mg C L ⁻¹	globular or toroidal aggregates	300–1000	‡	hematite

† Maurice and Namjesnik-Dejanovic (1999).

‡ Height undetermined.

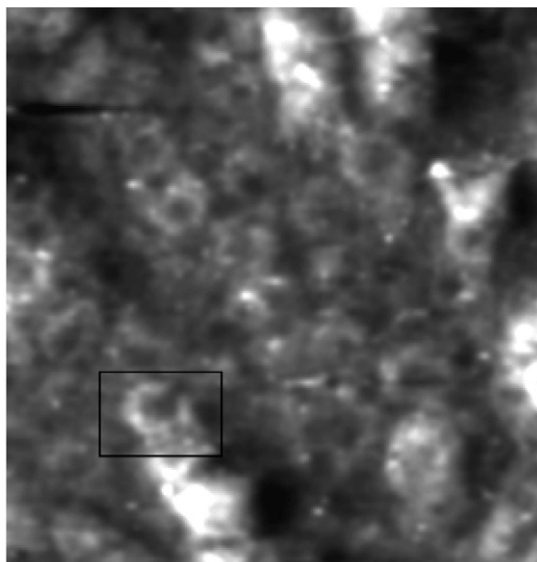


Fig. 11–20. Amplitude mode TMAFM image in solution of surface-water fulvic acid on mica. The surface is covered by a network of ringed-structures tens of nanometers in diameter. Nanometer-scale pores are apparent along the diameters of some of these rings (e.g., see structures highlighted within box). Image is 700 nm on a side. Modified from Maurice and Namjesnik-Dejanovic (1999).

ants. Such pore structures were previously hypothesized from macroscopic observations by researchers such as Chiou et al. (1979). Ring structures were observed when fulvic acid was deposited from solution on mica, allowed to air dry, and imaged in air, but the rings were flatter and the small pores were not apparent.

Although other in situ techniques such as imaging X-ray (synchrotron) microscopy are providing highly detailed images of natural organic matter (Myneni et al., 1999), AFM currently provides the highest resolution for adsorbed structures. Indeed, these techniques are complementary and show that surface NOM conformations are often similar to conformations in solution. Atomic force microscopy is providing direct and quantifiable evidence of NOM surface coverage, demonstrating that NOM does not adsorb as uniform monolayers. The direct, in situ AFM images can be integrated with results of batch adsorption experiments to gain new insight into adsorption phenomena.

Applications to Microbially Mediated Mineral Dissolution

Atomic force microscopy has broad applications to the study of microbially mediated mineral dissolution because of the ability to image mineral surfaces immersed in aqueous solution at nanometer-scale resolution and over the course of reaction. In-air AFM can also be used to image dissolution structures such as etch pits before and/or following reaction in the laboratory or the field. The advantage of AFM for this sort of study is in the high resolution and the ability to image the sample surface without the need for coating as often required by scanning-electron microscopy (SEM).

Grantham and Dove (1996) and Grantham et al. (1997) used in-solution AFM to image microbially mediated dissolution of iron oxyhydroxide surface coatings by the facultative anaerobe, *Shewanella putrefaciens*. Dissolution rates were determined by combining in-solution TMAFM imaging with measurements of dissolved Fe(II) concentration and pH of the reacting medium. Aerobic dissolution resulted in localized pitting directly at the locations of bacterial adhesion to the Fe oxyhydroxide-coated quartz surface. The localized dissolution resulted in circular and vermiform dissolution features (Fig. 11–21). In contrast,

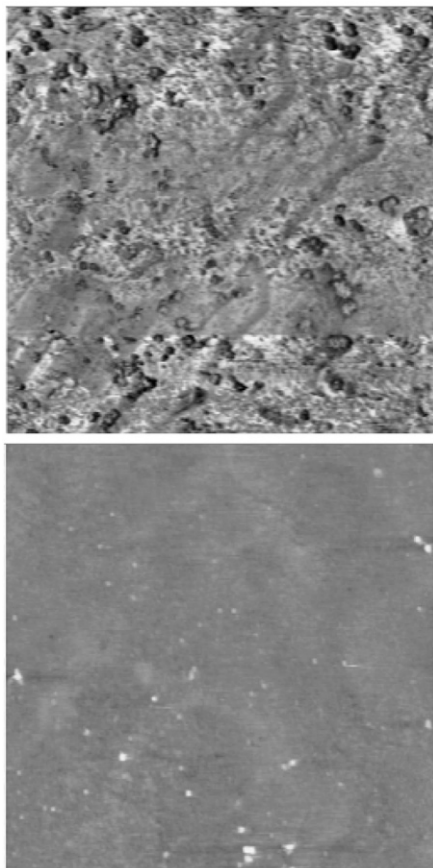


Fig. 11–21. Atomic force microscopy images of an iron oxide sample following exposure to a solution containing (top) dissimilatory-metal reducing bacteria and (bottom) an abiotic control under anaerobic conditions. The bacteria-exposed substrate shows numerous pits. These dissolution features are absent in the abiotic control. Each image is 20 μm on a side. From Grantham and Dove (1996).

anaerobic processes resulted in more delocalized dissolution. Hence, AFM provided important insight into potential reaction “mechanisms.”

Atomic force microscopy has also been shown to be extremely helpful for identifying reaction rates and mechanisms of field-dissolved samples. For example, Nugent et al. (1998) imaged albite feldspar surfaces that had been buried in reaction vessels in a spodosol soil near State College, Pennsylvania, USA for up to 3 yr. X-ray photoelectron spectroscopy and secondary-ion mass spectrometry (SIMS) analysis revealed that the 3-yr-reacted feldspar surfaces were enriched in Al relative to Si. This at first appeared to be contradictory to laboratory experiments, which generally tend to show Al depletion during feldspar dissolution under acidic conditions. Atomic force microscopy imaging revealed a patchy coating consisting of nanometer- to micrometer-scale particles covering approximately one-third of the surface. This coating was largely undetected by SEM. Energy dispersive X-ray analysis (EDX) during transmission electron microscopy (TEM) revealed that the coating contained Al and Si, but little Na and Ca. It was this coating that was most likely responsible for the elevated surface Al/Si ratio. Partial removal by ultrasonification significantly decreased surface Al/Si. The underlying feldspar surface was likely depleted of Al.

The albite feldspar used in this field weathering experiment contained exsolution lamellae (alternating bands of different feldspar composition and structure). Dissolution

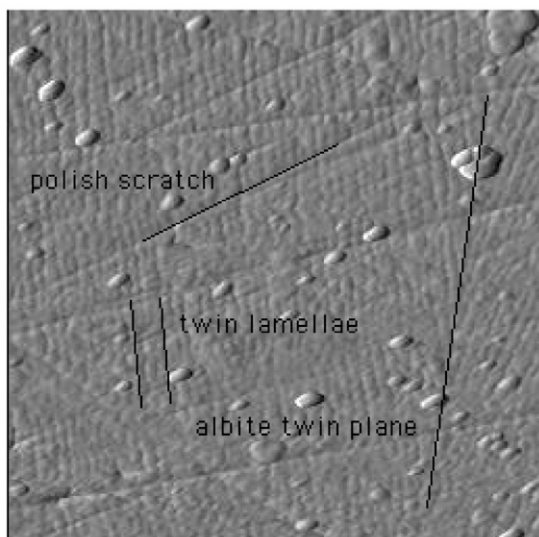


Fig. 11–22. TappingMode AFM amplitude image in air of albite feldspar weathered 3 yr in a spodosol. Samples buried for more than 6 mo contained a surface coating of fine particulates, some of which were removed by ultrasonification of this sample before imaging. Weathering preferentially removed oligoclase feldspar, highlighting albite (010) twins and peristerite lamellae of albite and oligoclase. The grooves created by the peristerite lamellae are 1.5 nm deep, on average. Scratches induced by polishing are present before and after weathering.

highlighted the exsolution lamellae by preferentially dissolving the less stable (oligoclase) component. Figure 11–22 provides an example of the detailed surface structure developed during weathering and revealed by TMAFM imaging. Three-dimensional measurements of changes in microtopography on weathering were used to determine dissolution rates of 10^{-16} to 10^{-18} moles feldspar $\text{cm}^{-2} \text{s}^{-1}$. These rates are consistent with previously reported rates of feldspar dissolution under acidic conditions. Although the Nugent et al. (1998) study was not designed to address microbially mediated dissolution per se, it demonstrated the applicability of the technique to field-scale studies of dissolution rates and mechanisms. This study also emphasized the importance of combining a suite of surface analytical and microscopic techniques to interpret properly the progress of surface (bio)geochemical reactions.

Atomic Force Microscopy Imaging of Biomineralization

Atomic force microscopy has clear potential for studies of biomineralization, for example, in terms of determining microbial–mineral associations, quantifying mineral nucleation and growth rates, helping to identify reaction mechanisms, and relating cell wall characteristics to mineralization processes. However, this area of geomicrobiologic research is still very much in its infancy. Recently, Warren et al. (2001) used AFM as part of a study of microbially mediated CaCO_3 precipitation. In this study, *Bacillus pasteurii* degraded urea, increasing solution pH:



and thus promoting CaCO_3 precipitation. This carbonate precipitation could be used as a mechanism for sequestering contaminants such as Sr.

Scanning electron microscopy imaging of solid reaction products indicated a variety of morphologies reminiscent of those observed in the marine stromatolite literature, including grape-like structures, spherical structures, dumbbells, and rhombohedra. However, because many of the carbonate precipitates were small (tens of micrometers or less) with relatively rough microtopography/micromorphology, AFM imaging was of only limited success.

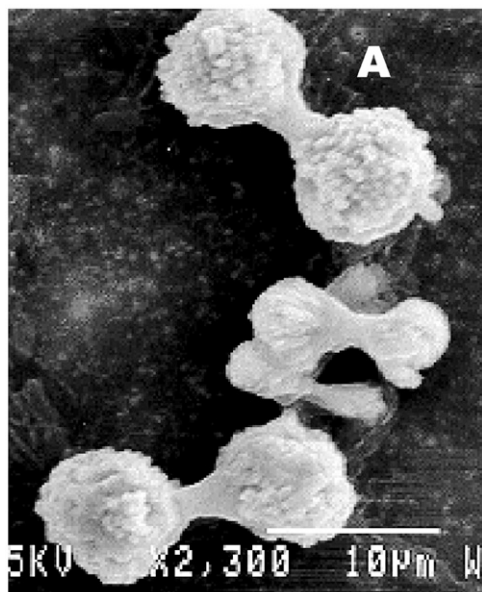


Fig. 11–23. Scanning electron microscopy (SEM) image of carbonate clusters formed during biomineralization by *B. pasteurii*. The spherical shape and rough microtopography make these particles extremely difficult to image by AFM. From Warren et al. (2001).

Spherical, dumbbell, and/or grape-like carbonate precipitates revealed by SEM (Fig. 11–23) were extremely difficult to image by AFM because of limitations caused by the finite size and shape of the TMAFM Si tip (see “Tip Size and Shape” section above). Although the particles could not be imaged in their entirety by AFM, high-resolution AFM images of their surfaces revealed complex microtopography consisting of parallel, drapery-like structures (Fig. 11–24). The AFM images of *B. pasteurii* surfaces sometimes showed small, rounded nuclei, which comparison with scanning electron microscopy with energy dispersive spectrometer (SEM/EDS) images suggested are likely carbonates (Fig. 11–25). The SEM/EDS analysis of reacted bacteria revealed Ca-containing surface coatings, con-

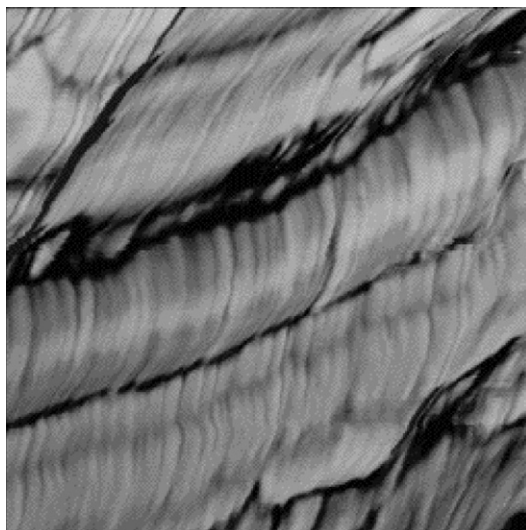


Fig. 11–24. The few images that were obtained on the surfaces of the particles such as those shown in Fig. 11–23 revealed parallel, drapery-like structures. Hence, although it was very difficult to image the small, rounded structures, when AFM was successful it provided a level of detail not accessible by SEM. Image is 250 nm on a side. From Warren et al. (2001).

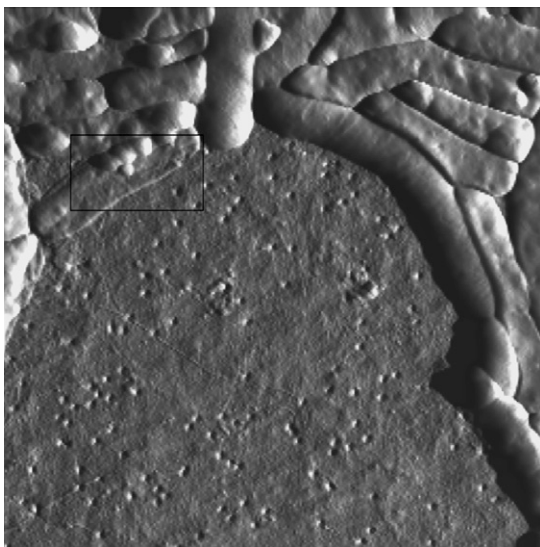


Fig. 11–25. *Bacillus pasteurii* filtered and dried on Nuclepore (Whatman, Brentford, U.K.) polycarbonate membrane (see pores) imaged by TMAFM in air. Some rounded carbonate nuclei are found in association with bacteria, but not on the filter membrane, suggesting that the bacterial surfaces serve as nucleation sites for these structures. Image is 10 μm on a side. From Warren et al. (2001).

sistent with carbonate precipitation at the bacterial surface. However, because the samples were dried before imaging, it is impossible to tell whether the association of the bacteria with the carbonate particles was present in the original reacting solution.

Somewhat larger rhombohedral carbonate particles also were observed by SEM (not shown here). Atomic force microscopy imaging of the particle surfaces revealed an absence of spiral growth structures but islands indicative of birth-and-spread. This observation is consistent with nucleation and growth under highly supersaturated conditions (e.g., Dove and Hochella, 1993). Both straight and jagged-edge steps were apparent. The presence of jagged-edged steps suggests that an inhibiting adsorbate might have been present (e.g., Dove and Hochella, 1993) to effect mineral growth in at least some locations on the carbonate surface (Fig. 11–26). The surfaces of large rhombohedral calcite particles tended

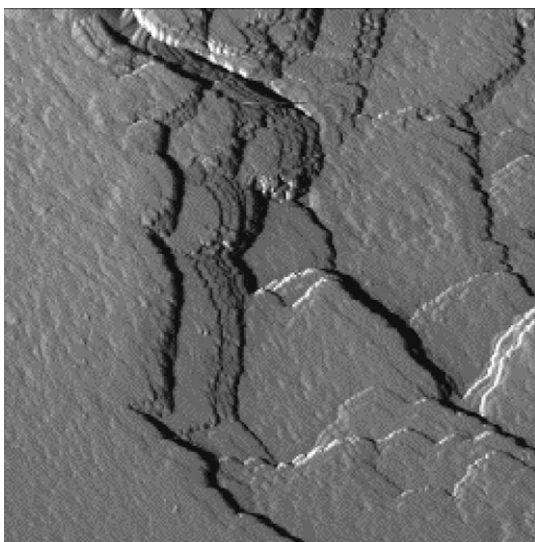


Fig. 11–26. Jagged edged steps present on carbonate precipitates suggest the presence of an adsorbing inhibitor. On other portions of the surface, straight-edged steps were apparent (not shown here). Image is 5 μm on a side. From Warren et al. (2001).

to be relatively free of bacteria, suggesting that their growth was facilitated primarily by passive means (i.e., pH increases). Thus, AFM provided evidence for a reaction “mechanism” that was not available using SEM. However, this study pointed out the limitations of AFM when dealing with small, irregularly surfaced reaction products of biomineralization. Hence, a combination of techniques is advisable.

Chemical and Biological Force Microscopy

Inter- and intramolecular forces affect a variety of phenomena, including membrane structure, molecular recognition, protein folding or unfolding, and adhesion reactions between cells and solid surfaces (e.g., minerals or other cells). Atomic force microscopy is a powerful tool for probing these interactions because it can resolve forces that are several orders of magnitude smaller than the weakest chemical bond, and it has spatial resolution on the order of a few Angstroms. In recent years, researchers have taken advantage of these attributes to create chemical and biological force microscopy (CFM and BFM, respectively) (Frisbie et al., 1994; Lower et al., 2000). Atomic force microscopy probes (i.e., cantilevers or tips) are functionalized with chemical functional groups, biomolecules, or living, fully functional cells to make them sensitive to specific interactions at the molecular to cellular level (see above).

Chemical Force Microscopy

Chemical force microscopy is used to measure the adhesive force (i.e., the force required to pull two surfaces apart) necessary to separate a sample from a chemically modified tip (Noy et al., 1997, 1998; Bliznyuk et al., 1998; Tsukruk and Bliznyuk, 1998). Hundreds of force measurements are collected and histograms are used to compare adhesive forces. For example, Frisbie et al. (1994) used carboxylic acid- and methyl- terminated tips on samples supporting self-assembling monolayers of carboxylic acid and methyl groups. Because hydrophilic groups can form hydrogen bonds, the adhesive force between two carboxylic acid groups was much stronger than the adhesive force between hydrophobic groups (i.e., methyl–methyl interactions), and cross reactions between carboxylic acid and methyl groups (Frisbie et al., 1994). These quantifiable differences in adhesive forces were used to create maps showing the spatial distribution of chemical groups on a surface.

Efforts are now underway to use CFM on biological surfaces. This is challenging because samples need to be relatively flat or topographic data will overshadow chemical information. Nevertheless, antibody-functionalized tips have been used with some success to map the location of specific proteins on mineral or cell surfaces (Gad et al., 1997; Hinterdorfer et al., 1999; Radmacher et al., 1994; Willemsen et al., 1999). Methods being developed to “deconvolute” chemical and topographic data (Heinz and Hoh, 1999) should prove to be very helpful in future studies that use CFM on cells and membranes.

Biological Force Microscopy

Biological force microscopy provides a means of communicating with a living cell as it interrogates the chemical and structural properties of another surface. Biological force microscopy measures interfacial and adhesion forces as a function of the distance between a solid surface and a cantilever activated with a cell. Lower et al. (2000), for example, used this technique to measure nanoscale forces between *Escherichia coli* and muscovite, goethite, and graphite surfaces in aqueous solutions (pH 6, 25°C) of varying ionic strength (10^{-5} to 10^{-1} M). Attractive and repulsive interfacial forces between the bacteria and the mineral surfaces were detected at ranges up to 400 nm separation. The magnitude and sign

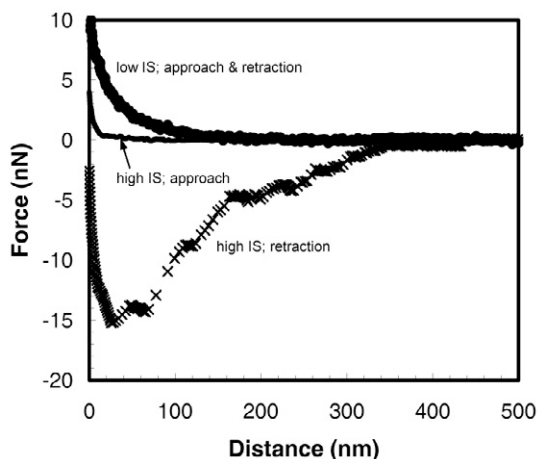


Fig. 11-27. Force-distance curve between *Escherichia coli* and the (001) surface of muscovite in aqueous solution. From Lower et al. (2000).

of these forces depended on the ionic strength of the solution, along with the mineral surface charge and hydrophobicity.

An example of a force-distance curve for *E. coli* and the muscovite (001) surface is shown in Fig. 11-27. Both the *E. coli* and the muscovite surfaces are negatively charged at pH 6. Thus, the repulsive interfacial forces that were observed for the approach curves, are consistent with electrostatic forces. At high ionic strength, compression of the double layer resulted in a decrease in both the magnitude and the distance over which this repulsive electrostatic force operated. The retraction curve at high ionic strength revealed the presence of adhesion force(s). Adhesion at less than about 50 nm was attributed to hydrogen bonds between lipopolysaccharide hydroxyl groups and surface hydroxyl or structural water molecules on muscovite. Rupture of adhesive fibers or fibrils might have resulted in the pull-off events beginning at a separation distance of approximately 150 nm. The results of this experiment demonstrated that BFM can provide detailed data on interfacial interactions between bacteria and mineral surfaces at a scale appropriate for the development and testing of quantitative models. Research is currently underway to use techniques like BFM to study the biomolecules and intermolecular forces associated with microbial adhesion to surfaces (Razatos et al., 1998b; Ong et al., 1999; Sagvolden et al., 1999; Bowen et al., 2000; Camesano and Logan, 2000; Lower et al., 2001).

Other Uses of Force Measurements

Chemical force microscopy and BFM represent only two examples of the use of force microscopy to study phenomena related to the microbiologic and biochemical aspects of soil science. These types of tools have been used to address a number of issues (Table 11-4). Force microscopy has also been used to probe the strength of covalent bonds (Grandbois et al., 1999) and hydrogen bonds between nucleic acids (Boland and Ratner, 1995; Lee et al., 1994), to determine the elasticity of polysaccharides and proteins (Rief et al., 1997a; Rief et al., 1997b), to measure surface charge on membranes (Butt, 1992; Heinz and Hoh, 1999), and to determine pK values of surface groups (Noy et al., 1997) and points of zero charge on minerals (Eggleston and Jordan, 1998).

Table 11–4. Some uses of force microscopy in issues related to geomicrobiology. A through bibliography can be found on Digital Instruments homepage (<http://www.di.com>).

Type of interaction studied	Reference†
covalent bond	1, 2
antibody-antigen	3–6
H-bonding	7, 8
Watson–Crick base pair	7, 8
hydrophobic	9
electrostatic, van der Waals	10–12
bacterial adhesion	13–17
surface charge	18, 19

† References: (1) Grandbois et al., 1999; (2) Lantz et al., 2001; (3) Florin et al., 1994; (4) Moy et al., 1994; (5) Hinterdorfer et al., 1996; (6) Willemsen et al., 1999; (7) Lee et al., 1994; (8) Boland and Ratner, 1995; (9) Yoon et al., 1997; (10) Ducker et al., 1991; (11) Ducker et al., 1992; (12) Senden and Drummond 1995; (13) Razatos et al., 1998; (14) Bowen et al., 1998; (15) Lower et al., 2000; (16) Ong et al., 1999; (17) Razatos et al., 2000; (18) Butt, 1992; (19) Considine et al. (2000).

Acknowledgments

Research conducted by both S. Lower and P. Maurice has been funded by the Department of Energy and the National Science Foundation.

REFERENCES

- A-Hassan, E., W.F. Heinz, M.D. Antonik, N.P. D'Costa, S. Nageswaran, C.A. Schoenenberger, and J.H. Hoh. 1998. Relative microelastic mapping of living cells by atomic force microscopy. *Biophys. J.* 74:1564–1578.
- Albrecht, T.R., S. Akamine, T.E. Carver, and C.F. Quate. 1990. Microfabrication of cantilever styli for the atomic force microscope. *J. Vac. Sci. Technol. A* 8:3386–3396.
- Antonik, M.D., N.P. D'Costa, and J.H. Hoh. 1997. A biosensor based on micromechanical interrogation of living cells. *IEEE Eng. Med. Biol.* 16:66–72.
- Aoki, T., M. Hiroshima, K. Kitamura, M. Tokunaga, and T. Yanagida. 1997. Non-contact scanning probe microscopy with sub-piconewton force sensitivity. *Ultramicroscopy* 70:45–55.
- Barker, W.W., S.A. Welch, and J.F. Banfield. 1997. Biogeochemical weathering of silicate minerals. p. 391–428. *In* J.F. Banfield and K.H. Nealson (ed.) *Geomicrobiology: Interactions between microbes and minerals*. Reviews in Mineralogy 35. Mineralogical Society of America, Washington, DC.
- Biggs, S. 1995. Steric and bridging forces between surfaces bearing adsorbed polymer: An atomic force microscopy study. *Langmuir* 11:156–162.
- Binnig, G., C.F. Quate, and C. Gerber. 1986. Atomic force microscope. *Phys. Rev. Lett.* 56:930–933.
- Bliznyuk, V.N., J.L. Hazel, J. Wu, and V.V. Tsukruk. 1998. Quantitative probing in atomic force microscopy of polymer surfaces. p. 252–265. *In* B.D. Ratner and V.V. Tsukruk (ed.) *Scanning probe microscopy of polymers*. ACS Symposium Series. American Chemical Society, Washington, DC.
- Boland, T., and B.D. Ratner. 1995. Direct measurement of hydrogen bonding in DNA nucleotide bases by atomic force microscopy. *Proc. Natl. Acad. Sci. USA* 92:5297–5301.
- Bowen, R.W., N. Hilal, R.W. Lovitt, and C.W. Wright. 1998a. Direct measurement of interactions between adsorbed protein layers using an atomic force microscope. *J. Colloid Interface Sci.* 197:348–352.
- Bowen, R.W., N. Hilal, R.W. Lovitt, and C.W. Wright. 1998b. Direct measurement of the force of adhesion of a single biological cell using an atomic force microscopy. *Colloids Surf., A* 136:231–234.
- Bowen, W.R., R.W. Lovitt, and C.J. Wright. 2000. Direct quantification of *Aspergillus niger* spore adhesion in liquid using an atomic force microscope. *J. Colloid Interface Sci.* 228:428–433.
- Butt, H.J. 1992. Measuring local surface charge densities in electrolyte solutions with a scanning force microscope. *Biophys. J.* 63:578–582.
- Camesano, T.A., and B.E. Logan. 2000. Probing bacterial electrosteric interactions using atomic force microscopy. *Environ. Sci. Technol.* 34:3354–3362.

- Cappella, B., and G. Dietler. 1999. Force-distance curves by atomic force microscopy. *Surf. Sci. Rep.* 34:1–104.
- Caruso, F., R.A. Caruso, and H. Mohwald. 1998. Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating. *Science* 282:1111–1114.
- Characklis, W.G., and K.C. Marshall. 1990. Biofilms: A basis for an interdisciplinary approach. p. 3–15. *In* W.G. Characklis and K.C. Marshall (ed.) *Biofilms*. John Wiley and Sons, New York.
- Chiou, C.T., L.J. Peters, and V.H. Freed. 1979. Physical concept of soil-water equilibria for non-ionic organic compounds. *Science* 206:831–832.
- Clark, S.M., and J.D. Baldeschwieler. 1993. A high performance scanning force microscope head design. *Rev. Sci. Instrum.* 64:904–907.
- Cleveland, J.P., S. Manne, D. Bocek, and P.K. Hansma. 1993. A nondestructive method for determining the spring constant of cantilevers for scanning force microscopy. *Rev. Sci. Instrum.* 64:403–405.
- Considine, R.F., D.R. Dixon, and C.J. Drummond. 2000. Laterally-resolved force microscopy of biological microspheres-oocysts of *Cryptosporidium parvum*. *Langmuir* 16:1323–1330.
- Costerton, J.W., Z. Lewandowski, D.E. Caldwell, D.R. Korber, and H.M. Lappin-Scott. 1995. Microbial biofilms. *Annu. Rev. Microbiol.* 49:711–745.
- Dai, H., N. Franklin, and J. Han. 1998. Exploiting the properties of carbon nanotubes for nanolithography. *Appl. Phys. Lett.* 73:1508–1510.
- Dammer, U., O. Popescu, P. Wagner, D. Anselmetti, H.J. Guntherodt, and G.N. Misevic. 1995. Binding strength between cell adhesion proteoglycans measured by atomic force microscopy. *Science* 267:1173–1175.
- D'Costa, N.P., and J.H. Hoh. 1995. Calibration of optical lever sensitivity for atomic force microscopy. *Rev. Sci. Instrum.* 66:5096–5097.
- Delawski, E., and B.A. Parkinson. 1992. Layer-by-layer etching of two-dimensional metal chalcogenides with the atomic force microscopy. *J. Am. Chem. Soc.* 114:1661–1667.
- Dempsey, B.A., and C.R. O'Melia. 1983. Proton and calcium complexation of four fulvic acid fractions. p. 239–273. *In* R.F. Christman and E.T. Gjessing (ed.) *Aquatic and terrestrial humic materials*. Ann Arbor Sci., Ann Arbor, MI.
- Dove, P.M., and M.F. Hochella, Jr. 1993. Calcite precipitation mechanisms and inhibition by orthophosphate—In-situ observations by scanning force microscopy. *Geochim. Cosmochim. Acta* 57:705–714.
- Ducker, W.A., T.J. Senden, and R.M. Pashley. 1991. Direct measurement of colloidal forces using an atomic force microscope. *Nature* 353:239–241.
- Ducker, W.A., T.J. Senden, and R.M. Pashley. 1992. Measurements of forces in liquids using a force microscope. *Langmuir* 8:1831–1836.
- Egger, M., S.-P. Heyn, and H.E. Gaub. 1992. Synthetic lipid-anchored receptors based on the binding site of a monoclonal antibody. *Biochim. Biophys. Acta* 1104:45–54.
- Eggleston, C.M. 1994. High resolution scanning probe microscopy: Tip-surface interaction, artifacts, and applications in mineralogy and geochemistry. p. 1–90. *In* A.E. Blum and K. Nagy (ed.) *Scanning force microscopy of clay minerals*. Clay Minerals Society, Boulder, CO.
- Eggleston, C.M., and G. Jordan. 1998. A new approach to pH of point of zero charge measurement: Crystal-face specificity by scanning force microscopy (SFM). *Geochim. Cosmochim. Acta* 62:1919–1923.
- Ellen, R.P., and R.A. Burne. 1996. Conceptual advances in research on the adhesion of bacteria to oral surfaces. p. 201–247. *In* M. Fletcher (ed.) *Bacterial adhesion: Molecular and ecological diversity*. American Society for Microbiology, Washington DC.
- Fein, J.B., J.F. Boily, K. Guclu, and E. Kaulbach. 1999. Experimental study of humic acid adsorption onto bacteria and Al-oxide mineral surfaces. *Chem. Geol.* 162:33–45.
- Florin, E.-L., V.T. Moy, and H.E. Gaub. 1994. Adhesion forces between individual ligand-receptor pairs. *Science* 264:415–417.
- Florin, E.-L., M. Rief, H. Lehmann, M. Ludwig, C. Dornmair, V.T. Moy, and H.E. Gaub. 1995. Sensing specific molecular interactions with the atomic force microscope. *Biosens. Bioelectron.* 10:895–901.
- Forsythe, J., P. Maurice, and L. Hersman. 1998a. Attachment features between an aerobic *Pseudomonas* sp. bacteria and hematite observed with atomic-force microscopy. p. 927–930. *In* Arehart and Hulston (ed.) *Water-Rock Interaction International IX*. Balkema, Rotterdam, The Netherlands.
- Forsythe, J.H., P.A. Maurice, and L.E. Hersman. 1998b. Attachment of a *Pseudomonas* sp. to Fe(III)-(hydr)oxide surfaces. *Geomicrobiology* 15:293–308.
- Frisbie, C.D., L.F. Rozsnyai, A. Noy, M.S. Wrighton, and C.M. Liber. 1994. Functional group imaging by chemical force microscopy. *Science* 265:2071–2074.

- Fritz, J., M.K. Baller, H.P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.-J. Güntherodt, Ch. Gerber, and J.K. Gimzewski. 2000. Translating biomolecular recognition into nanomechanics. *Science* 288:316–318.
- Gad, M., A. Itoh, and A. Ikai. 1997. Mapping cell wall polysaccharides of living microbial cells using atomic force microscopy. *Cell Biol. Int.* 21:697–706.
- Goodman, F.O., and N. Garcia. 1991. Roles of the attractive and repulsive forces in atomic-force microscopy. *Phys. Rev. B* 43:4728–4731.
- Grandbois, M., M. Beyer, M. Rief, H. Clausen-Schaumann, and H.E. Gaub. 1999. How strong is a covalent bond? *Science* 283:1727–1730.
- Grantham, M.C., and P.M. Dove. 1996. Bacterial-mineral surface interactions: Investigations using fluid tapping atomic force microscopy. *Geochim. Cosmochim. Acta* 60:2473–2480.
- Grantham, M.C., P.M. Dove, and T.J. DiChristina. 1997. Microbially catalyzed dissolution of iron and aluminum oxyhydroxide mineral surface coatings. *Geochim. Cosmochim. Acta* 61:4467–4477.
- Hafner, J.H., C.L. Cheung, and C.M. Lieber. 1999. Direct growth of single-walled carbon nanotube scanning probe microscopy tips. *J. Am. Chem. Soc.* 121:9750–9751.
- Hansma, P.K., B. Drake, D. Grigg, C.B. Prater, F. Yashar, G. Gurley, V. Elings, S. Feinstein, and R. Lal. 1994. A new, optical-lever based atomic force microscope. *J. Appl. Phys.* 76:796–799.
- Heinz, W.F., and J.H. Hoh. 1999. Relative surface charge density mapping with the atomic force microscope. *Biophys. J.* 76:528–538.
- Hiebert, F.K., and P.C. Bennett. 1992. Microbial control of silicate weathering in organic-rich ground water. *Science* 258:278–281.
- Hinterdorfer, P., W. Baumgartner, H.J. Gruber, K. Schilcher, and H. Schindler. 1996. Detection and localization of individual antibody-antigen recognition events by atomic force microscopy. *Proc. Natl. Acad. Sci. USA* 93:3477–3481.
- Hinterdorfer, P., A. Raab, and H. Schindler. 1999. Molecular recognition force microscopy/spectroscopy. *PICO* 3:1–2.
- Hochella, M.F., Jr. 1990. Atomic structure, microtopography, composition, and reactivity of mineral surfaces. p. 87–132. *In* M.F. Hochella, Jr. and A.F. White (ed.) *Mineral-water interface geochemistry. Reviews in Mineralogy* 23. Mineralogical Society of America, Washington, DC.
- Hoh, J.H., and A. Engel. 1993. Friction effects on force measurements with an atomic force microscope. *Langmuir* 9:3310–3312.
- Hoh, J.H., and C.A. Schoenenberger. 1994. Surface morphology and mechanical properties of MDCK monolayers by atomic force microscopy. *J. Cell Sci.* 107:1105–1114.
- Hunt, A.P., J.D. Parry, and J. Hamilton-Taylor. 2000. Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. *Limnol. Oceanog.* 45: 237–241.
- Hutter, J.L., and J. Bechhoefer. 1993. Calibration of atomic-force microscope tips. *Rev. Sci. Instrum.* 64:1868–1873.
- Ingersoll, C.M., and F.V. Bright. 1997. Using fluorescence to probe biosensor interfacial dynamics. *Anal. Chem.* 69:403A–408A.
- Israelachvili, J. 1992. *Intermolecular and surface forces*. Academic Press, London.
- Jaschke, M., and H.-J. Butt. 1995. Height calibration of optical lever atomic force microscopes by simple laser interferometry. *Rev. Sci. Instrum.* 66:1258–1259.
- Kato, N., I. Suzuki, H. Kikuta, and K. Iwata. 1995. Force-balancing force sensor with an optical lever. *Rev. Sci. Instrum.* 66:5532–5536.
- Kim, P., and C.M. Lieber. 1999. Nanotube nanotweezers. *Science* 286:2148–2150.
- Kriz, D., O. Ramstrom, and K. Mosbach. 1997. Molecular imprinting: New possibilities for sensor technology. *Anal. Chem.* 69:345A–349A.
- Krumbein, W.E., and L.J. Stahl. 1991. The geophysiology of marine cyanobacterial mats and biofilms. *Kieler Meeresforsch.* 8:158–163.
- Lantz, M.A., H.J. Hug, R. Hoffmann, P.J.A. van Schendel, P. Kappenberger, S. Martin, A. Barattoff, and H.-J. Güntherodt. 2001. Quantitative measurement of short-range chemical bonding forces. *Science* 291:2580–2583.
- Lee, G.U., L.A. Chrisey, and R.J. Colton. 1994. Direct measurement of the forces between complementary strands of DNA. *Science* 266:771–773.
- Lillehei, P.T., and L.A. Bottomley. 2000. Scanning probe microscopy. *Anal. Chem.* 72(12):189R–196R.
- Little, B.J., P.A. Wagner, and Z. Lewandowski. 1997. Spatial relationships between bacteria and mineral surfaces. *Rev. Mineral.* 35:123–159.
- Lo, Y.-S., N.D. Huefner, W.S. Chan, P. Dryden, B. Hagenhoff, and T.P. Beebe, Jr. 1999. Organic and inorganic contamination on commercial AFM cantilevers. *Langmuir* 15:6522–6526.

- Lower, S.K., M.F. Hochella, Jr., and T.J. Beveridge. 2001. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and α -FeOOH. *Science* 292:1360–1363.
- Lower, S.K., C.J. Tadanier, and M.F. Hochella, Jr. 2000. Measuring interfacial and adhesion forces between bacteria and mineral surfaces with biological force microscopy. *Geochim. Cosmochim. Acta* 64:3133–3139.
- Lower, S.K., C.J. Tadanier, and M.F. Hochella, Jr. 2001. Dynamics of the mineral-microbe interface: Use of biological force microscopy in biogeochemistry and geomicrobiology. *Geomicrobiol. J.* 18:63–76.
- Ludwig, M., M. Rief, L. Schmidt, H. Li, F. Oesterhelt, M. Gautel, and H.E. Gaub. 1999. AFM, a tool for single-molecule experiments. *Appl. Phys. Mater. Sci. Process.* 68:173–176.
- Magonov, S.N., J. Cleveland, V. Elings, D. Denley, and M.-H. Whangbo. 1997a. Tapping-mode atomic force microscopy study of the near-surface composition of a styrene-butadiene-styrene triblock copolymer film. *Surf. Sci.* 389:201–211.
- Magonov, S.N., V. Elings, and M.-H. Whangbo. 1997b. Phase imaging and stiffness in tapping-mode atomic force microscopy. *Surf. Sci.* 275:L385–L391.
- Magonov, S.N., and D.H. Reneker. 1997. Characterization of polymer surfaces with atomic force microscopy. *Ann. Rev. Mater. Sci.* 27:175–222.
- Manne, S., and H. Gaub. 1997. Force microscopy: Measurement of local interfacial forces and surface stresses. *Curr. Opin. Colloid Interface Sci.* 2:145–152.
- Marshall, K.C., R. Stout, and R. Mitchell. 1971. Mechanism of the initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.* 68:337–348.
- Maurice, P.A., and S.K. Lower. 1998. Using atomic force microscopy to study soil mineral reactions. *Adv. Agron.* 62:1–43.
- Maurice, P.A., D.M. McKnight, L. Leff, J.E. Fulghum, and M. Gooseff. 2002. Direct observation of aluminosilicate dissolution in the hyporheic zone of an Antarctic Dry Valley Stream. *Geochim. Cosmochim. Acta* 66:1335–1347.
- Maurice, P., and K. Namjesnik-Dejanovic. 1999. Aggregate structures of sorbed humic substances observed in aqueous solution. *Environ. Sci. Technol.* 33:1538–1540.
- Mertig, M., D. Klemm, W. Pompe, H. Zanker, and M. Bottger. 1999. Scanning force microscopy of spin-coated humic acid. *Surf. Interface Anal.* 27:426–432.
- Morris, V.J., A.R. Kirby, and A.P. Gunning. 1999. Atomic force microscopy for biologists. Imperial College Press, London.
- Moy, V.T., E.-L. Florin, and H.E. Gaub. 1994. Adhesion forces between ligand and receptor measured by AFM. *Colloids Surf.*, A 93:343–348.
- Mueller, H., H.-J. Butt, and E. Bamberg. 1999. Force measurements on myelin basic protein adsorbed to mica and lipid bilayer surfaces done with the atomic force microscope. *Biophys. J.* 76:1072–1079.
- Myneni, S.C.B., J.T. Brown, G.A. Martinez, and W. Meyer-Ilse. 1999. Imaging of humic substance macromolecular structures in water and soils. *Science* 286:1335–1337.
- Namjesnik-Dejanovic, K. and Maurice, P.A., 1997. Atomic force microscopy of soil and stream fulvic acids. *Colloids Surf. A* 120:77–86.
- Namjesnik-Dejanovic, K., and P.A. Maurice. 2001. In solution observations of natural organic matter single molecules and aggregates adsorbed to clay mineral surfaces. *Geochim. Cosmochim. Acta* 65:1047–1057.
- Neumeister, J.M., and W.A. Ducker. 1994. Lateral, normal, and longitudinal spring constants of atomic force microscopy cantilevers. *Rev. Sci. Instrum.* 65:2527–2531.
- Noy, A., C.H. Sanders, D.V. Vezennov, S.S. Wong, and C.M. Lieber. 1998. Chemically-sensitive imaging in tapping mode by chemical force microscopy: Relationship between phase lag and adhesion. *Langmuir* 14:1508–1511.
- Noy, A., D.V. Vezennov, and C.M. Lieber. 1997. Chemical force microscopy. *Ann. Rev. Mater. Sci.* 27:381–421.
- Nugent, M., S. Brantley, C. Pantano, and P. Maurice. 1998. The influence of natural mineral coatings on feldspar dissolution. *Nature* 395:588–591.
- Ohnesorge, F., and G. Binnig. 1993. True atomic resolution by atomic force microscopy through repulsive and attractive forces. *Science* 260:1451–1456.
- Ong, Y.-L., A. Razatos, G. Georgiou, and M.M. Sharma. 1999. Adhesion forces between *E. coli* bacteria and biomaterial surfaces. *Langmuir* 15:2719–2725.
- Pincet, F., E. Perez, and J. Wolfe. 1995. Does glue contaminate the surface forces apparatus? *Langmuir* 11:373–374.
- Pleuddemann, E.P. 1991. Silane coupling agents. Plenum Press, New York.
- Prater, C.B., P.G. Malvard, K.J. Kjoller, and m.G. Heaton. 2004 TappingMode imaging applications

- and technology. Digital Instruments/Veeco application note A1V04. Available at www.veeco.com (verified Jan. 2008).
- Radmacher, M., M. Fritz, J.P. Cleveland, D. Walters, and P.K. Hansma. 1994. Imaging adhesion forces and elasticity of lysozyme adsorbed on mica with the atomic force microscope. *Langmuir* 10:3809–3814.
- Razatos, A., Y.L. Ong, M.M. Sharma, and G. Georgiou. 1998a. Evaluating the interaction of bacteria with biomaterials using atomic force microscopy. *J. Biomater. Sci. Polymer Educ.* 9:1361–1373.
- Razatos, A., Y.-L. Ong, M.M. Sharma, and G. Georgiou. 1998b. Molecular determinants of bacterial adhesion monitored by atomic force microscopy. *Proc. Natl. Acad. Sci. USA* 95:11059–11064.
- Razatos, A., Y.L. Ong, F. Boulay, D.L. Elbert, J.A. Hubbell, M.M. Sharma, and G. Georgiou. 2001. Force measurements between bacteria and poly(ethylene glycol)-coated surfaces. *Langmuir* 16:9155–9158. *Langmuir* 16:9155–9158.
- Rezania, A., R. Johnson, A. Lefkow, and K.E. Healy. 1999. Bioactivation of metal oxide surfaces. 1. Surface characterization and cell response. *Langmuir* 15:6931–6939.
- Rief, M., M. Gautel, F. Oesterhelt, J.M. Fernandez, and H.E. Gaub. 1997a. Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* 276:1109–1112.
- Rief, M., F. Oesterhelt, B. Heymann, and H.E. Gaub. 1997b. Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science* 275:1295–1297.
- Sader, J.E. 1995. Parallel beam approximation for L-shaped atomic force microscope cantilevers. *Rev. Sci. Instrum.* 65:4583–4587.
- Sader, J.E., J.W.M. Chon, and P. Mulvaney. 1999. Calibration of rectangular atomic force microscope cantilevers. *Rev. Sci. Instrum.* 70:3967–3969.
- Sader, J.E., I. Larson, P. Mulvaney, and L.R. White. 1995. Method for the calibration of atomic force microscope cantilevers. *Rev. Sci. Instrum.* 66:3789–3798.
- Sagvolden, G., I. Giaever, E.O. Pettersen, and J. Feder. 1999. Cell adhesion force microscopy. *Proc. Natl. Acad. Sci. USA* 96:471–476.
- Santschi, P.H., E. Balnois, K.J. Wilkinson, J.W. Zhang, and J. Buffle. 1998. Fibrillar polysaccharides in marine macromolecular organic matter as imaged by atomic force microscopy and transmission electron microscopy. *Limnol. Oceanogr.* 43:896–908.
- Senden, T.J., and W.A. Ducker. 1994. Experimental determination of spring constants in atomic force microscopy. *Langmuir* 10:1003–1004.
- Senden, T.J., and C.J. Drummond. 1995. Surface chemistry and tip-sample interactions in atomic force microscopy. *Colloids Surf., A* 94:29–51.
- Shao, Z.F., D. Shi, and A.V. Somlyo. 2000. Cryoatomic force microscopy of filamentous actin. *Biophys. J.* 78:950–958.
- Shao, Z.F., J. Yang, and A.P. Somlyo. 1995. Biological atomic force microscopy: From microns to nanometers and beyond. *Annu. Rev. Cell Dev. Biol.* 11:241–265.
- Sheng, S., D.M. Czajkowsky, and Z. Shao. 1999. AFM tips: How sharp are they? *J. Microsc.* 196:1–5.
- Stotzky, G. 1986. Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. p. 305–428. *In* P.M. Huang and M. Schnitzer (ed.) *Interactions of soil minerals with natural organics and microbes*. SSSA Spec. Publ. 17. SSSA, Madison, WI.
- Sun, L., E.M. Perdue, J. Meyer, and J. Weiss. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia River. *Limnol. Oceanogr.* 42:714–721.
- Svoboda, K., and S.M. Block. 1994. Biological applications of optical forces. *Annu. Rev. Biophys. Biomol. Struct.* 23:247–285.
- Thurman, E.M. 1985. *Organic geochemistry of natural waters*. Nijhoff/Junk Publ., Dordrecht, The Netherlands.
- Tipping, E. 1981. The adsorption of aquatic humic substances by iron oxides. *Geochim. Cosmochim. Acta* 45:191–199.
- Tortonesi, M. 1997. Cantilevers and tips for atomic force microscopy. *IEEE Eng. Med. Biol.* 16:28–33.
- Tsukruk, V.V., and V.N. Bliznyuk. 1998. Adhesive and friction forces between chemically modified silicon and silicon nitride surfaces. *Langmuir* 14:446–455.
- Turner, D.C., B.M. Peek, T.E. Wertz, D.D. Archibald, R.E. Geer, and B.P. Gaber. 1996. Enzymatic modification of a chemisorbed lipid monolayer. *Langmuir* 12:4411–4416.
- Turner, D.C., M.A. Testoff, D.W. Conrad, and B.P. Gaber. 1997. Enzymatic hydrolysis of a chemisorbed peptide film using beads activated with covalently bound chymotrypsin. *Langmuir* 13:4855–4860.
- Vansteenkiste, S.O., S.I. Corneillie, E.H. Schacht, X. Chen, M.C. Davies, M. Moens, and L. Van Vaeck. 2000. Direct measurement of protein adhesion at biomaterial surfaces by scanning force microscopy. *Langmuir* 16:3330–3336.

- Warmack, R.J., X.Y. Zheng, T. Thundat, and D.P. Allison. 1994. Friction effects in the deflection of atomic-force microscope cantilevers. *Rev. Scie. Instrum.* 65(2):394–399.
- Warren, L., P. Maurice, N. Parmar, and G. Ferris. 2001. Microbially mediated calcium carbonate precipitation: Implications for interpreting calcite precipitation and for solid-phase capture of inorganic contaminants. *Geomicrobiol. J.* 18:93–115.
- Weisenhorn, A.L., P.K. Hansma, T.R. Albrecht, and C.F. Quate. 1989. Forces in atomic force microscopy in air and water. *Appl. Phys. Lett.* 54:2651–2653.
- Weisenhorn, A.L., P. Maivald, H.-J. Butt, and P.K. Hansma. 1992. Measuring adhesion, attraction, and repulsion between surfaces in liquids with an atomic-force microscope. *Phys. Rev. B* 45:11226–11232.
- Willemsen, O.H., M.M.E. Snel, L. Kuipers, C.G. Figdor, J. Greve, and B.G. De Grooth. 1999. A physical approach to reduce nonspecific adhesion in molecular recognition atomic force microscopy. *Biophys. J.* 76:716–724.
- Wong, T.M.H., and P. Descouts. 1995. Atomic force microscopy under liquid: A comparative study of three different AC-mode operations. *J. Microsc.* 178:7–13.
- Wong, S.S., E. Joselevich, A.T. Woolley, C.L. Cheung, and C.M. Lieber. 1998a. Covalently functionalized nanotubes as nanometre-sized probes in chemistry and biology. *Nature* 394:52–55.
- Wong, S.S., A.T. Woolley, E. Joselevich, C.L. Li Cheung, and C.M. Lieber. 1998b. Covalently-functionalized single-walled carbon nanotube probe tips for chemical force microscopy. *J. Am. Chem. Soc.* 120:8557–8558.
- Yoon, R.H., D.H. Flinn, and Y.I. Rabinovich. 1997. Hydrophobic interactions between dissimilar surfaces. *J. Colloid Interface Sci.* 185:363–370.
- Zobell, C.E. 1943. The effect of solid surfaces on bacterial activity. *J. Bacteriol.* 46:39–56.

Electron Microprobe Techniques

RENALD N. GUILLEMETTE, Texas A&M University, College Station

The electron microprobe, more formally called an “electron probe microanalyzer” (EPMA) is an electron beam instrument similar in design to the scanning electron microscope (SEM), but with several important differences. Whereas the principal purpose of the SEM is to generate images of the material being observed, the primary purpose of the electron microprobe is to obtain chemical analyses, although imaging capabilities have consistently improved over the years to the point where modern electron microprobes can be used as medium-power SEMs. The electron microprobe consists of an electron column with a heated tungsten filament or, less frequently, a lanthanum hexaboride (LaB_6) electron source. The electrons are accelerated by a high voltage down the column and shaped by a series of electromagnetic lenses and apertures to yield a beam with a diameter of about 0.1 to 1 μm . The electron beam impacts the sample material, and the resulting electron and photon signals are measured by several specific detectors. The type of X-ray detection system that distinguishes the electron microprobe from other electron beam instruments is the wavelength-dispersive spectrometer (WDS).

Historically, the birth of the electron microprobe can be traced to Castaing and Guinier (1950), who described the first practical electron microprobe, and to Castaing’s (1951) doctoral thesis, in which he worked out most of the design principles. The first commercial instrument was manufactured by Cameca, Inc. in 1956. By the early 1970s, about a half dozen companies were manufacturing electron probe microanalyzers. Since about 1980, Cameca (Paris) and JEOL (Tokyo) have been the sole manufacturers of dedicated electron microprobes, although several other companies have marketed SEMs with a single inclined wavelength dispersive spectrometer. The design of the electron microprobe has improved as technology has advanced, and performance gains have come as a result of advances in both theory and engineering.

ELECTRON BEAM AND MATTER INTERACTION

Description of Interactions

A high-energy electron beam (5–50 keV) hitting a sample target results in the generation of several products (Fig. 12–1):

- Electrons
 - Secondary
 - Backscattered
 - Absorbed
 - Auger

- X-rays
 - Characteristic
 - Continuous
 - Fluorescent
- Visible light
 - Cathodoluminescence
- Heat

Electrons

Secondary electrons (SE) are low-energy electrons (generally <50 eV) produced as a result of inelastic collisions in the sample. They primarily provide topographic information in surface imaging. Their low energy means that a detector placed at a low angle off to one side of the specimen will see substantial variations in secondary electron intensity depending on the tilt of the sample relative to the detector and on the surface roughness.

Backscattered electrons (BSE) are elastically rebounding electrons leaving the surface with a substantial proportion of their incoming energy (typically about 80%). The production of backscattered electrons is strongly correlated with the mean atomic number (Z) of the target. Thus, the backscattered electron signal finds its primary application in atomic number imaging on polished horizontal surfaces. In addition, the BSE multidetector arrays present in many microprobes permit directional examination of rough surfaces, resulting in a topographically controlled signal similar to SE, but with different surface details often visible.

Absorbed electrons are those that have been decelerated by multiple inelastic collisions and remain within the sample. To prevent a charge buildup, the sample is connected to ground through a metering circuit, and the output can be used for imaging purposes. The result is a signal that is essentially the inverse of the BSE signal. Because of the generally slower response time of this measurement, signal quality is not as good as with BSE, and therefore absorbed electron imaging is seldom used in microprobes.

Auger electrons are photoelectrons generated when an X-ray interacts with a bound electron in the sample, resulting in the ejection of that electron. Auger electrons have characteristic energies equal to the difference between the interacting X-ray and the binding energy of the ejected electron. Auger electrons can be used in the chemical analysis of the upper several tens of angstroms of the sample, the maximum depth from which these elec-

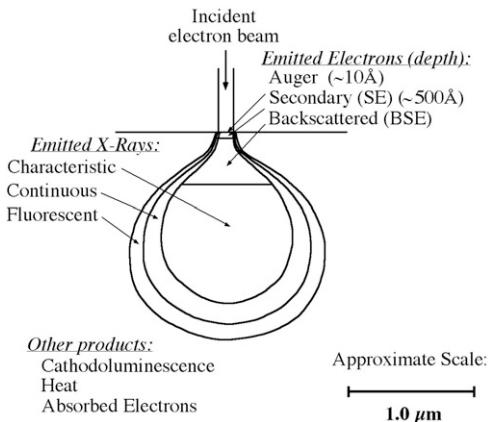


Fig. 12-1. A high-energy electron beam (5–50 keV) hitting a sample target results in the generation of several products.

trons can exit the sample. Microprobes, however, are generally not equipped with Auger spectrometers due to design constraints.

X-rays

Characteristic X-rays are those generated as the result of a specific transition within the electron shell of an atom, and whose energy (or wavelength) is therefore going to be indicative of the chemical element that produced it.

Continuous X-rays (or white or “Bremstrahlung radiation”) are generated by inelastic processes and span a wide range of energies. Typically, these contribute to the background noise and serve no useful function.

Fluorescent X-rays (also called secondary X-rays) are produced by X-ray and matter interaction rather than by electron beam and matter interaction. A “primary” X-ray (produced by electron beam and matter interaction) interacts with an atom, resulting in the formation of a characteristic X-ray from that atom.

Visible Light

Visible light can be produced in some materials by a variety of mechanisms. The most significant is usually cathodoluminescence, where a visible or near visible photon is produced in the target area by the recombination of an electron with a hole produced by electron beam matter interaction. In addition, visible light may also be produced briefly by thermoluminescence. Only a small percentage of compounds are intrinsically cathodoluminescent, with another group exhibiting cathodoluminescence when an activating impurity is present in the absence of quenching impurities. Cathodoluminescence can be a valuable tool to study different generations, and therefore different conditions of growth of natural and synthetic materials (Marshall, 1988; Pagel et al., 2000). Cathodoluminescent detectors, with or without spectrometers, can usually be installed as an option on electron microprobes.

Heat

Heat is an undesirable byproduct of the electron beam and matter interaction, and is the principal mechanism by which the electron beam energy is dissipated within the sample. Because the electron microprobe typically operates at beam currents two to three orders of magnitude higher than a SEM, heat damage to the specimen can be a serious concern. At a typical operating condition of 15 kV accelerating voltage, a beam current of 10 nA, and a beam diameter of 1 μm , the power density per square centimeter would be approximately 20 kW if all of the beam energy were converted to heat. Even if as little as 70% were converted, this would result in heating the immediate target area of a poor thermally conducting soil mineral like quartz to several hundred degrees Celsius. If the sample material cannot tolerate this much heat, the beam must be defocused to a maximum diameter of 10 to 20 μm , thus reducing the power density (and the related heating effect) by up to two orders of magnitude.

Generation and Nomenclature of X-rays

X-rays are produced in the sample by two different mechanisms. A beam electron may be decelerated when interacting with the nucleus or tightly bound inner electrons, resulting in the emission of X-ray photons having a continuous range of energies up to the accelerating potential of the beam. A beam electron may also transfer enough of its energy to an inner shell electron to result in the ejection of that electron from the atom (i.e., to at least

the first vacant position in an outer shell). This second process then results in an outer shell electron dropping into the vacant inner shell position, with two possible consequences: (i) the emission of an X-ray photon having an energy equal to the difference between the starting and ending shell energies or (ii) the ejection of an electron also having an energy related to the outer to inner shell transition. In the electron microprobe, the emitted characteristic X-ray photons allow all elements heavier than Li to be identified, and the concentration of each element in an unknown sample to be calculated by measuring the number of X-ray photons per unit time.

Figure 12-2 shows an electron shell diagram of a hypothetical atom and some of the statistically more important X-ray lines that could be emitted as a result of allowed transitions if a K shell electron were to be initially ejected by a primary beam electron. Which transitions are allowed and which forbidden are determined by quantum theory selection rules. A good source for a detailed discussion of these rules as well as the naming conventions for the different lines is Bertin (1978). The X-ray spectral lines with the K, L, or M series nomenclature result respectively from the filling of a K, L, or M vacancy by an electron from a higher shell. The shell designation is followed by a lowercase Greek letter, and then usually by a subscripted numeral or lowercase italic letter. The alpha line X-rays are the most important in a series, being the statistically most likely to be generated, and resulting from an electron transition from the next higher shell. Whether a K, L, or M series X-ray will be chosen for analytical work will be determined primarily by the atomic number of the element to be analyzed. For practical reasons involving the choice of beam accelerating voltage, the absorption of X-rays within the sample, and the abilities of different X-ray detectors, $K\alpha$ X-rays are most often selected for elements in the atomic number range of 4 (Be) to about 33 (As), $L\alpha$ from about 34 (Se) to about 74 (W), and $M\alpha$ from about 75 (Re) to 92 (U).

The energy of an X-ray photon is related to the wavelength by:

$$\lambda = \frac{hc}{eE} = \frac{hc}{e} \frac{1}{E} = \frac{12.40}{E}$$

where h is Planck's constant, c is the speed of light, E is the energy (keV), e is the electron charge, and λ is the wavelength in angstroms (Å). Thus, a Cu $K\alpha$ X-ray with an energy of 8.04 keV will have a wavelength of 1.54 Å and a C $K\alpha$ X-ray with an energy of 0.279 keV

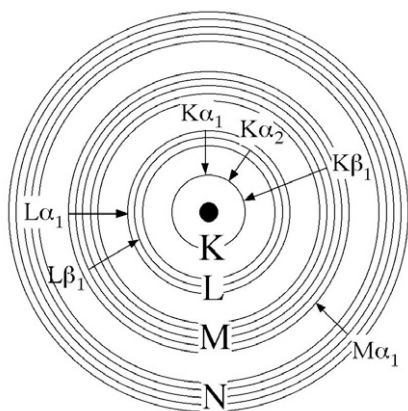


Fig. 12-2. An electron shell diagram of a hypothetical atom and some of the statistically more important X-ray lines that could be emitted as a result of allowed transitions if a K shell electron were to be initially ejected by a primary beam electron.

will have a wavelength of $44.4 \text{ \AA}$. The use of both energy-dispersive and wavelength-dispersive spectrometers in the electron microprobe will require both methods of describing X-rays.

Description of the Electron Microprobe

Figure 12-3 shows a schematic cross-sectional view of an electron microprobe. The following major components and subsystems are present:

- Electron optics system
 - Electron gun
 - Gun alignment coils
 - Electromagnetic lenses
 - Scanning coils
 - Associated power supplies and other electronics
- Optical microscope
- Sample stage
- Electron detectors
 - Secondary electrons
 - Backscattered electrons
 - Absorbed (sample current)
- X-ray spectrometers
 - Energy-dispersive spectrometry
 - Wavelength-dispersive spectrometry
- Vacuum system

Electron Optics System

The electron gun in almost all electron microprobes utilizes a conventional triode design consisting of a tungsten hairpin filament, a Wehnelt cylinder (grid cap), and an anode. Electrons are emitted thermionically from near the tip of the filament (heated to about 2700°C), focused and controlled by the surrounding Wehnelt cylinder, and accelerated toward and through a hole in the anode plate that is set at a positive potential difference of from about 1 to 40 kV relative to the filament. A crossover point occurs above the

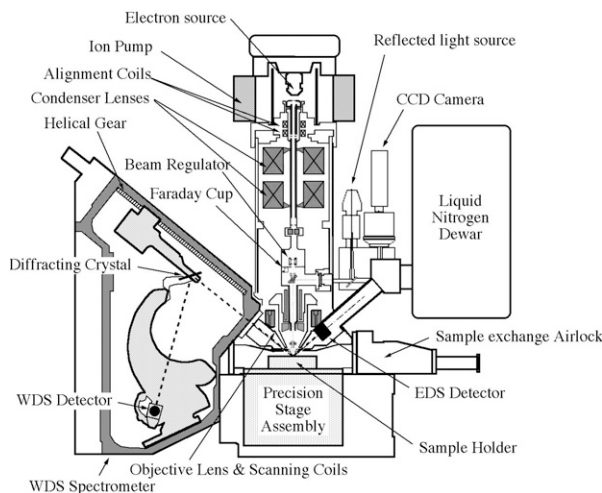


Fig. 12-3. A schematic cross-sectional view of an electron microprobe.

anode. The beam then continues down the column through the magnetic lens system. The condenser lens assembly (usually consisting of two lenses) demagnifies the gun crossover image, directing it through one or more apertures into the objective ("probe-forming" or "final") lens, which then focuses the image onto the sample. The condenser lenses effectively control the beam current, while the objective lens controls the beam diameter. A stigmator lens assembly is also located near the objective lens to adjust the circularity of the beam cross section. In some designs, a set of beam alignment coils may be located between the anode and the condenser lens. Scanning coils (located just above or within the objective lens assembly) permit rastering of the beam so that the electron microprobe can also be used as a SEM. However, the larger beam current requirements relative to a traditional SEM and the geometric constraints imposed by the presence of WDS spectrometers generally result in a lower image resolution; the maximum practical magnification achieved is usually less than 20,000 $\times$ for SE images and <5000 $\times$ for BSE.

Quantitative analysis requires that excellent accelerating voltage and beam current stability be maintained for periods of a day or more, with maximum drift of 1% or less being desirable. This requirement must be taken into account in the design of electron sources, lenses, and power supplies. In addition, an insertable beam current monitoring device (Faraday cage) is located just below the objective aperture. This can be inserted into the beam path between each quantitative analysis to measure and, if necessary, adjust the current to the desired value. In addition, beam current can be dynamically measured and controlled by a feedback mechanism involving the objective aperture or other associated apertures.

In cases where a higher beam current or a smaller beam source size is desirable, the tungsten hairpin filament can be replaced by a lanthanum hexaboride (LaB_6) source in most modern electron microprobes. This would provide the capability to image samples at higher magnifications, while also permitting high current applications if desired. The LaB_6 source also has a longer lifetime, up to about 1000 h of operation compared with several hundred for tungsten. The main disadvantages of LaB_6 sources are substantially higher operating costs and the need for a better vacuum in the gun area. Presently, most electron microprobes use tungsten hairpin filaments, but it is expected that the use of LaB_6 will increase in the future. Field emission electron guns are not suitable for use in electron microprobes. Although they provide an excellent very small bright source for high magnification SEM imaging, their relatively poor time-dependent stability makes them unsuitable for quantitative analysis. Field emission electron guns also cannot, at this time, provide the high beam currents required for specialized WDS quantitative applications such as trace element analysis.

Optical Microscope

Unlike a SEM, all microprobes also have an optical microscopy system as an integral part of their design. This is not just a luxury, but also a necessity dictated by the most common WDS spectrometer design. The optical microscope system must have a minimum capability of providing a high quality, reflected light image, with a field of view of several hundred micrometers, and must have a deliberately designed poor depth of field. This is because the optical microscope is designed to be parfocal with the focusing WDS spectrometers, so that when the optical image of the sample surface is in focus, the WDS X-ray spectrometers are also in focus.

Although many designs have historically been used in electron microprobes, current models all use a reflecting microscope, which permits simultaneous viewing of both optical and electron images along the beam path and normal to the sample surface. A hole in

the center of each of the optical mirrors permits the beam to pass down the column. The light source can be located above the lens system for reflected light operation, or below the sample stage for transmitted light uses. The latter is generally available as an option for those laboratories where this is needed. In addition, polarizers can usually be installed in the reflected and/or transmitted light paths for optical crystallography applications. Another option that may be available is a lower power or zoom magnification capability. Current microprobes usually use a video camera to acquire the optical image, with the output being directed to a digital display and image capture system.

Sample Stage

Sample stage requirements are similar but not identical to those of a SEM. The stage must be reproducibly moved in increments of a micrometer or less over the entire sample area in the x , y , and z directions and must also be fully controlled under computer automation for long unattended quantitative analysis or sequential stage-scanning image acquisition sessions. The x and y ranges will have to be at least 10 cm to accommodate the types of flat polished samples usually analyzed in a microprobe, but the range of z motion may be restricted by geometrical constraints imposed by the large numbers of detectors and spectrometers and their designs. In many microprobes, z motion may be restricted to only several millimeters with stock sample stages. Rotation and tilting of specimens may not be available as standard features, but special stage designs may be implemented as an option. It is also desirable to be able to raster the stage under the fixed electron beam to image large sample areas.

Electron Detectors

Three types of electron signals are usually detected in electron microprobes: secondary (SE), backscattered (BSE), and absorbed (ABS). The most commonly used SE detector is of the Everhart–Thornley type, typically consisting of a thallium-doped sodium iodide scintillator optically connected to a photomultiplier tube (Fig. 12–4). The most commonly used BSE detector consists of an array of four to six solid-state plates arranged symmetrically around the beam path just above the sample and between the WDS ports (Fig. 12–5). The selection of different geometrical combinations of these plates permits the emphasis to be placed on either compositional contrast or topographic information. The strong dependence of the BSE signal from a horizontal polished surface on the mean atomic number of each region of the sample will usually result in the selection of the signal contrast mode. The ABS signal consists of the electrons being conducted to ground from the sample, and this is usually measured by a digital nanoammeter in the signal path.

X-ray Spectrometers

Two types of X-ray detection systems are present in electron microprobes: energy-dispersive spectrometry (EDS) and wavelength-dispersive spectrometry (WDS). An EDS sys-

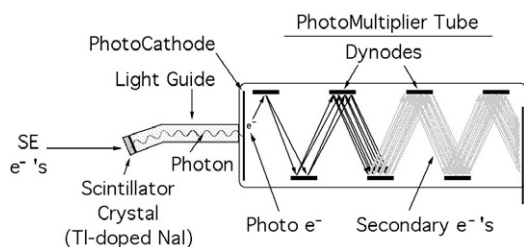


Fig. 12–4. The most commonly used secondary electron detector is of the Everhart–Thornley type, typically consisting of a thallium-doped sodium iodide scintillator optically connected to a photomultiplier tube.

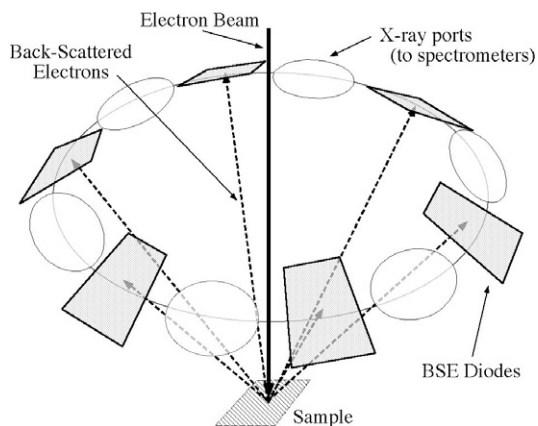


Fig. 12-5. The most commonly used backscattered electron detector consists of an array of four to six solid-state plates arranged symmetrically around the beam path just above the sample and between the wavelength-dispersive spectrometer ports.

tem has the ability to simultaneously acquire the entire X-ray spectrum and to accumulate and display that data set within tens to hundreds of seconds. This system is thus ideal for rapid preliminary examination of an unknown sample. A WDS spectrometer must sequentially acquire only a portion of the X-ray wavelength range, with up to five spectrometers usually being used in tandem to cover the entire range. However, WDS spectrometers can typically resolve closely spaced peaks about an order of magnitude better than EDS, and can also display about an order of magnitude better peak/background ratio, resulting in the ability to detect much smaller concentrations of an element. This makes them ideal for use in quantitative elemental analysis, complementing the rapid qualitative performance of the EDS system.

Energy-dispersive spectrometry systems are generally the same as those used in SEMs, with only minor differences. Because higher beam currents are typically used in electron microprobes, it may be necessary to insert a limiting aperture in front of the detector to prevent dead-time problems due to excessively high X-ray count rates at high beam currents. Most systems use either a Li-drifted silicon [Si(Li)] or a high-purity germanium (HpGe) detector. Atmospheric thin organic polymer windows are more commonly used than Be windows or windowless operation. Most systems use liquid nitrogen (LN₂) cooling of the detector and associated field-effect transistor preamplifier to reduce thermal noise and prevent diffusion of Li out of the active front surface of the detector. However, silicon drift detectors cooled only by a Peltier element are also becoming more widely used.

Figure 12-6 is a simplified cross-sectional diagram of the commonly used Si(Li) EDS detector. The arrival of an X-ray photon generates electron-hole pairs, and the number of pairs produced is proportional to the energy of the X-ray photon. The output of the detector is amplified and fed to a multichannel pulse-height analyzer system where the signal is measured and divided into 1024 or 2048 channels, each having a width of 10 or 20 eV. The X-ray spectrum is then displayed as a histogram plot with computer superimposed marker lines showing the energies of the characteristic X-rays for the elements of interest.

As its name implies, a WDS spectrometer discriminates one X-ray from another using their wavelengths. It consists of a rotatable, oriented diffraction crystal coupled with a proportional gas-filled ionization detector in a geometric relationship following Bragg's Law:

$$n\lambda = 2d\sin\theta$$

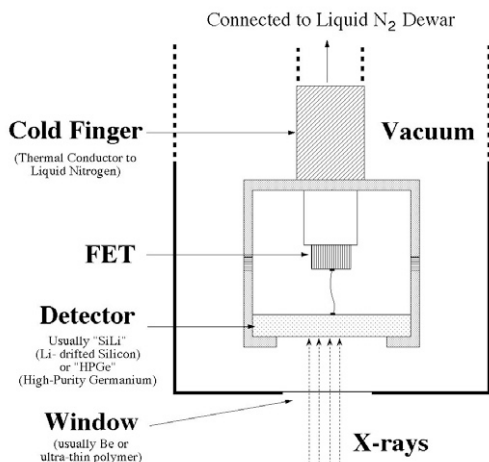


Fig. 12-6. A simplified cross-sectional diagram of the commonly used Si(Li) energy-dispersive spectrometry detector.

where n is the order of diffraction (an integer), λ is the wavelength of the X-ray ($\text{\AA}$), d is the spacing of the oriented set of planes in the diffracting crystal ($\text{\AA}$), and θ is the angle of incidence of the X-ray to the diffracting crystal.

Constructive interference of two X-rays of the same wavelength arriving at the crystal in phase will only occur at certain angles, such that the path length difference between the two “reflected” X-rays will be an integer multiple of their wavelength, allowing them to exit in phase (Fig. 12-7). For example, for Fe $K\alpha$ X-rays with a wavelength of $1.937 \text{ \AA}$ incident on the 200 (Miller indices or hkl) planes of LiF with a d -spacing of $2.0135 \text{ \AA}$, first-order ($n = 1$) constructive interference will only occur at an incident angle of 28.751° .

Each diffracting crystal must subtend a large enough angle to collect enough X-rays for practical quantitative work—in most spectrometer designs this corresponds to a crystal size of 2 to 4 cm. If a single flat crystal of this size were used, the X-rays arriving at the edges of the crystal would no longer be incident at the Bragg angle for a given wavelength. Therefore, it is necessary to use a diffracting surface that actually consists of a “curved” crystal, where the surface is also ground to maintain Bragg’s Law conditions. This results in the Johansson spectrometer design (Fig. 12-8). The crystal is bent to the diameter of the Rowland circle, and its front surface is ground to the radius of the circle. The X-rays coming from a point source on the sample are focused into the detector after being diffracted over a large area of the crystal, dramatically improving count rates and therefore sensitiv-

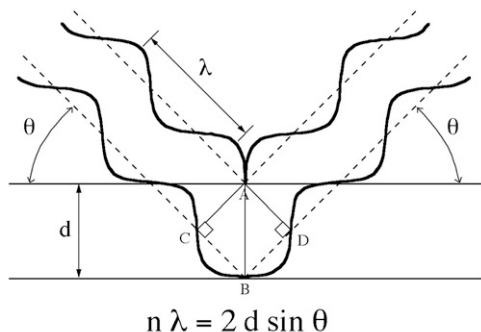


Fig. 12-7. Constructive interference of two X-rays of the same wavelength arriving at the crystal in phase will only occur at certain angles, such that the path length difference between the two “reflected” X-rays will be an integer multiple of their wavelength, allowing them to exit in phase.

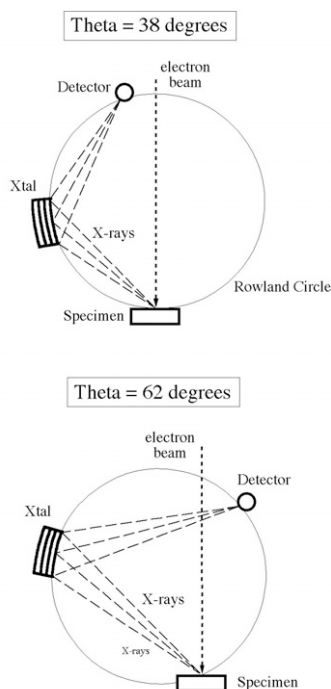


Fig. 12–8. The Johansson spectrometer design maintains Bragg's Law conditions allows for the X-rays arriving at the edges of the crystal. The diffracting surface consists of a "curved" crystal. The crystal is bent to the diameter of the Rowland circle, and its front surface is ground to the radius of the circle.

ity compared with a flat crystal. The disadvantage of this design is that it requires that the surface of the sample be positioned at a very particular height to fulfill Johansson focusing conditions. In the EPMA, this is accomplished by adjusting the height of the sample until it is in optical focus under the parfocal reflected light microscope. If the location of the specimen surface is less well known, as in an SEM fitted with a WDS spectrometer, then an inclined rather than vertical spectrometer design can be employed, substantially reducing the effect of vertical mispositioning. An inclined spectrometer would also allow samples with rough surfaces or with a large range of vertical relief to be better analyzed, although this would not eliminate all analytical problems associated with these types of samples. Figure 12–9 shows the count rates for both vertical and inclined spectrometers as a function of vertical distance away from the ideal Johansson focal point. Inclined spectrometers are not commonly used in microprobes because they occupy more space, reducing the maximum number of spectrometers that can be installed.

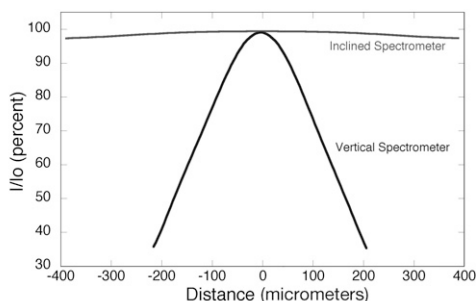


Fig. 12–9. The count rates for both vertical and inclined spectrometers as a function of vertical distance away from the ideal Johansson focal point

Table 12-1. Commonly used diffracting materials in wavelength-dispersive spectrometers.

Diffracting material	Type	2d Å	xtal planes
Currently used WDS analyzer crystals:			
Lithium fluoride (LIF)	XI	4.028	(200)
Pentaerythritol (PET)	XI	8.742	(002)
Thallium acid phthalate (TAP)	XI	25.90	(10-10)
Pseudocrystals	(high and low Z alternating layers) (e.g., W/Si or Mo/B ₄ C)	~50-200	—
Older types of diffraction crystals:			
Quartz	XI	8.52	(100)
Ammonium dihydrogen phosphate (ADP)	XI	10.64	(101)
Mica	XI	19.8	(100)
Potassium acid phthalate (KAP)	XI	26.63	(100)
Rubidium acid phthalate (RAP)	XI	26.12	(100)
Pb-stearate	Pseudocrystal	200	—

Diffraction is most efficient when the wavelength of the X-ray closely matches the d-spacing of the oriented set of planes in the crystal. Therefore, to be able to efficiently diffract the range of X-rays from 1 to 100 Å, it will be necessary to use several diffracting crystals. In addition to their d-spacing, several other parameters figure in the choice of crystals that can be used in a WDS spectrometer. Some desirable properties are high efficiency of diffraction, long-term stability under both vacuum and atmospheric conditions, low fluorescence, low intensity of higher order diffraction, and small coefficients of thermal expansion. Table 12-1 lists the most commonly used diffracting materials as well as some that have been used historically. Figure 12-10 shows the ranges of X-ray lines and elements by atomic number that are covered by some of these crystals. Because no crystal has a suitable set of planes that can be used to diffract very long-wavelength X-rays (i.e., > ~20 Å), regularly interlayered synthetic structures have been constructed for this purpose. These materials, are often referred to as “pseudocrystals,” consist of alternating layers of heavy and light atomic elements, such as W and Si, deposited by evaporation or other suitable process. Several different multilayer materials are required to cover the entire range of long-wavelength X-rays.

The detector type used in a WDS is usually a flow-through proportional ionization detector (Fig. 12-11). A high purity gas consisting of 90% argon and 10% methane (“P10”)

Crystal	2d (nm)	Atomic Number
LIF	0.403	Sc 21 K _α Rb 37 Te 52 L _α U 92
PET	0.874	Si 14 K _α Mn 25 Sr 38 L _α Yb 65 W 74 M _α U 92
TAP	2.58	F 9 K _α P 15 Mn 25 L _α Zr 40 La 57 M _α Au 79
Pseudo 1	~ 4.5	N 7 Na 11
Pseudo 2	~ 6.0	C 6 Ne 10
Pseudo 3	~ 9.5	B 5 O 8
Pseudo 4	~ 20.0	Be 4 C 6

Fig. 12-10. The ranges of X-ray lines and elements by atomic number that are covered by some of these crystals.

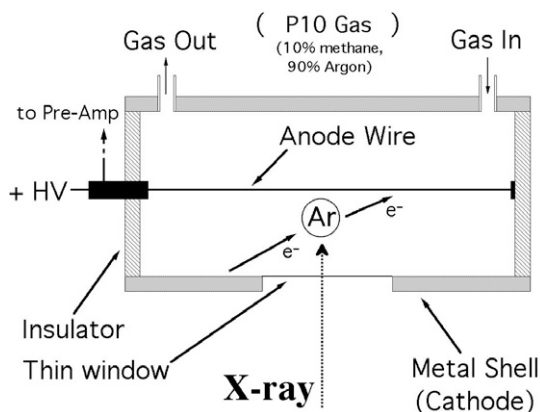


Fig. 12-11. A flow-through proportional ionization detector.

is pumped through a cylindrical tube containing a thin window that permits X-rays to enter from the diffracting crystal. A central wire is kept at a positive potential of 1000 to 2000 V relative to the outer tube. An incoming X-ray triggers an ionization “avalanche” in the gas, resulting in electrons flowing to the central wire; the methane acts as a quench gas to stop the ionization until another X-ray enters the detector. The number of electrons produced is proportional to the energy of the X-ray. The output is then fed to a preamplifier, pulse-height analyzer and counting circuitry. The pulse-height analyzer can be used to eliminate the higher order (and therefore higher energy) coincident diffracted X-rays from elements other than the one of interest.

Vacuum System

Electron microprobes typically maintain column vacuum conditions in the 1×10^{-4} to 10^{-5} Pa range ($\sim 1 \times 10^{-6}$ to 10^{-7} torr). Depending on the types of samples being analyzed, the pressure in the sample chamber area may be higher by one or two orders of magnitude. The spectrometers may be either at the same conditions as the sample chamber or only at about 1×10^0 to 10^{-1} Pa. Two or three levels of vacuum pumping are employed to achieve these conditions. Oil rotary mechanical pumps (or, more recently, “dry” pumps) are used to “rough” pump the system from atmospheric pressure down to about 1×10^{-1} Pa, and to “back up” the next higher level of pumps. Once this first level of vacuum has been reached, an oil diffusion pump or a turbomolecular pump can then lower the pressure to about 1×10^{-5} Pa. Finally, in microprobes that use a LaB_6 gun or when a longer W filament lifetime is desired, an ion pump is used in the immediate gun area to improve the vacuum by about another order of magnitude. Ion pumps can also be helpful in more rapidly reestablishing operating conditions when accelerating voltages in excess of 30 kV are used.

For the best results when analyzing light elements (e.g., Be, B, C, N, O), it is important to minimize the buildup of carbon contamination on the surface of the sample during analysis. Carbon buildup at the point of impact of the beam on the sample results from the breakdown of hydrocarbons. One of the most important sources of these hydrocarbons is the oil from the mechanical and diffusion pumps. The amount of oil can be greatly reduced by using dry roughing pumps and a turbomolecular pump. Other methods that will reduce carbon contamination include a LN_2 baffle above the diffusion pump, a “cold finger” (LN_2 cooled plate) just above the specimen, or a minuscule nitrogen gas jet directed across the sample at the target area (Scott and Love, 1983).

QUALITATIVE ANALYSIS

Qualitative analyses can be defined as those that provide information about which elements may be present, but provide little data about absolute or even relative concentrations. A trained analyst who is familiar with a particular instrument under a given set of conditions may be able to make some crude estimate of possible concentrations by examining typical qualitative results, but this approach is likely to be inaccurate.

The most common type of qualitative analysis in a microprobe is the acquisition of an EDS spectrum on a spot or area of a sample. Figure 12–12 shows an EDS spectrum acquired with a 15-keV electron beam on biotite mica. The EDS detector is capable of receiving X-rays from the entire area of sample that can be scanned at the lowest magnification; whatever area is being viewed as a SE or BSE image will contribute to the total spectrum. Only X-rays generated in the upper one or two micrometers will be seen on average, and in the case of the easily absorbed low-energy, long-wavelength X-rays, the observed X-rays will come from even shallower depths in the sample. The smallest area that can be sampled without interference from surrounding phases will depend on the beam accelerating voltage, the mean atomic number of the sample, and the energies of the emitted X-rays. In general, spectra obtained on the center of grains smaller than about 5 μm in diameter should be suspected of showing a contribution from the surrounding material.

Care should be taken in interpreting an EDS spectrum. A Si(Li) detector fitted with an ultra-thin atmospheric window can usually observe $K\alpha$ X-rays from elements of as low an atomic number as carbon (6), and under ideal conditions, perhaps boron (5). However, some polymer ultra-thin windows may have a strong absorption edge for nitrogen, and may not permit the detection of this element even in nitrates. Researchers trying to detect light elements with an EDS system should first obtain spectra on known compounds containing those elements to acquaint themselves with the characteristics of the system in use.

The smallest concentration of an element whose major X-ray lines can be seen on a spectrum acquired during a scan of several minutes is about several tenths of a percent by weight. This qualitative “lower limit of detection” will be lowest for intermediate atomic number elements ($Z \sim 15\text{--}27$) present in relatively low atomic number matrices. Limits of detection will be highest for light elements accompanied by heavy elements, where several percent by weight may need to be present before a peak can be seen for the light element. Care should always be taken in considering overlapping interfering peaks from other elements in the sample.

Researchers should be aware of several artifacts that can result from basic detector characteristics and geometry and can appear in the spectrum as apparent small peaks of elements that may not actually be present in the sample. Because the EDS detector “sees” such a large area of the sample and even the walls of the sample chamber, backscattered

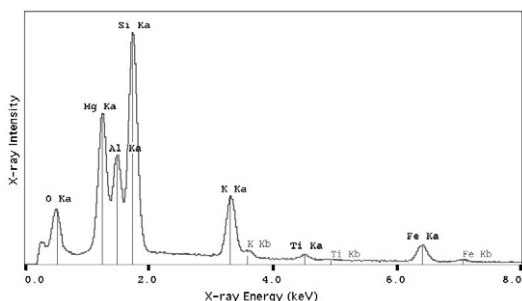


Fig. 12–12. An energy-dispersive spectrometry spectrum acquired with a 15-keV electron beam on biotite mica

electrons may generate X-rays from areas away from the primary target and contribute a minor peak to the spectrum. Also, a very small Si peak is often seen in spectra generated using a Si(Li) detector, even when no Si is present in the sample itself. Sometimes very small peaks that usually do not correspond to any X-ray lines can be seen in spectra as a result of the coincident arrival of two X-ray photons within such a short time period that the detector was not able to distinguish them as two separate events, but instead recorded them as a single event having an energy equal to the sum of the two energies ("sum" or "coincidence" peaks).

Since most modern electron microprobes are equipped with an EDS spectrometer, the WDS spectrometers are usually not used for routine full-spectrum scans as a means of identifying all elements in the sample. However, there are several circumstances where limited WDS qualitative scans may be very valuable. If the correct diffracting pseudocrystals are used, the presence of elements as light as Be ($Z = 4$) can be detected, including an element such as N, whose characteristic X-rays may have been absorbed by the EDS detector window. Wavelength-dispersive spectrometers can also be used to resolve any uncertainty in an EDS spectrum when severe peak overlaps prevent an unambiguous determination of which one of two elements may be present. The much better resolution of the WDS spectrometers will usually resolve even closely spaced peaks, permitting a clear identification to be made. Wavelength-dispersive spectrometer qualitative scans over the immediate neighborhood of peaks selected for WDS quantitative analysis will also provide detailed information about the best locations for measuring off-peak backgrounds.

QUANTITATIVE ANALYSIS

The goal of quantitative analysis is to determine the concentration of one or more elements present in a sample with an acceptably small error. Using WDS analysis on an electron microprobe, the concentrations of major elements (those present at $\geq 10\%$ by weight) can routinely be determined to within $\pm 2\%$ of the amount present, and if great care is taken, $\pm 1\%$ may be achieved.

Optimizing Analytical Conditions

Analytical conditions must be optimized to yield the best possible results. These include:

- X-ray analytical line to be used for each element
- beam accelerating voltage and current
- beam size
- location of off-peak positions for background measurements
- counting times
- choice of analytical standards

Analytical Line

The best analytical line to use for quantitative analysis will usually be the highest energy, most intense one that can be generated by the electron beam and detected by the wavelength-dispersive spectrometers. The shortest wavelength X-ray that can be efficiently diffracted by a crystal (LiF) has a wavelength of about $0.9 \text{ \AA}$. This means that $K\alpha$ lines can only be used to analyze elements whose atomic number, Z , is less than about 38 (Sr). For higher Z elements, the $L\alpha$ line would be the one of choice, followed by $M\alpha$ for the heaviest elements if a beam accelerating voltage of 20 kV or less is used. Before making a final choice of analytical lines based on these criteria, it is important to confirm that there will be no spectral interferences by any other X-ray line on each chosen analytical line.

Although WDS spectrometers have excellent resolution, interferences do still occur, especially in complex multi-element compounds. The interferences are particularly bothersome if they are the result of a strong major element line overlapping the analytical line of a trace or minor element. In certain cases it may be necessary to select an alternate, less sensitive line to avoid a strong interference or to apply a correction to compensate for the interference. Higher order interferences ($n = 2$ or higher) can usually be successfully filtered out with a judicious setting of each spectrometer's pulse-height analyzer.

The accelerating voltage is selected to be high enough to excite the desired characteristic X-ray line for every element to be analyzed. This usually corresponds to a voltage that will be 1.5 to 2 times above the critical excitation energy required to eject an electron from one of the inner shells. For example, to analyze Fe using the Fe $K\alpha$ X-ray with an energy of 6.40 keV, the critical excitation energy required to eject a K shell electron would be 7.11 keV, and the optimum accelerating voltage should be at least 11 to 14 kV. Most WDS analyses are performed at beam voltages of 10 to 25 kV, with 15 or 20 being the most popular. This yields good peak/background ratios and allows the entire periodic table to be analyzed using the appropriate K, L, or M lines.

Beam current is selected to yield sufficiently high X-ray count rates for good counting statistics, while not exceeding about 20,000 cps, above which detector "dead-time" errors start to become significant. When both major and trace elements are to be analyzed, a dual approach may be used: the major elements can be analyzed first at a lower beam current, followed by high beam current analysis of the trace elements. The choice of beam current will also be dictated by the ability of the sample to withstand elevated temperatures in the area of the target without damage. In the worst case, damage may take the form of sample decomposition, such as converting calcium carbonate to calcium oxide or releasing H_2O from gypsum or zeolites. In other cases, it may be manifested by the diffusion of an element across a thermal or electrical gradient. The latter is more likely to occur with small univalent ions in a relatively open or hydrous crystal lattice or glass. Examples include the diffusion of Na away from the hot beam area in feldspars and of F away from the high electron region in apatite (Stormer et al., 1993). Typical beam current values used in an EPMA would be 10 to 30 nA. When analyzing trace elements in refractory minerals or in metals, beam currents in excess of several hundred nanoamperes may be used. It should be noted that most SEMs operate at beam currents of <1 nA.

The beam diameter can be defocused between about 1 and 20 μm for quantitative WDS analysis. The lower limit is determined by the size of the X-ray emission volume, while the upper limit is dictated by the defocusing of the WDS. The smaller beam is used when it is desirable to obtain higher spatial compositional resolution. Use of the larger beam can minimize sample damage. A good policy is to use a beam size of around 10 μm for routine analyses where high spatial resolution is not critical.

Background counts on a WDS spectrum are usually measured at equal distances on either side of each X-ray peak and averaged under the peak. The larger the d-spacing of the diffracting crystal, the wider the peak and the further away that background must be measured. Typical values for each crystal can be used as a starting point, but for optimal results a qualitative scan should be performed over each analytical peak in a sample, especially for trace and minor elements where the peak/background ratio will be low. This is important to avoid interfering peaks at proposed background positions. The lower the concentration of the element to be analyzed, the more important it becomes to carefully select background positions. In some cases where an unavoidable interference is present on one side of the peak, the background may be measured only on the other side, and a slope calculated to extrapolate the measured background to the peak center.

Counting times will be determined either by the precision required by the analyst or by the lower limit of detection to be achieved (i.e., what is the percentage of error that can be tolerated, or what is the smallest concentration that must be detected). Typical major and minor element analyses would use counting times of 10 to 40 s on peak (and less than or equal to that on background) to typically achieve lower limits of detection of several hundred parts per million by weight ($\sim 0.05\%$ [w/w]). Counting times of 2000 s or more are occasionally used for trace element analyses, but most researchers find that a typical analysis of eight to ten elements can be satisfactorily performed in about 180 to 480 s (3–8 min) of total counting time. Refer to Williams (1987) for a detailed summary of optimized counting strategies to fit different analytical goals.

One of the most critical requirements for accurate quantitative analysis is a good set of elemental reference standards. For a material to be a good standard, it must meet a number of important criteria. The composition must be accurately known, the material must be compositionally homogeneous, and it must be stable under exposure to the electron beam in a vacuum and under exposure to ambient atmospheric conditions. Pure elements, oxides, silicates, sulfides, and others may all be good standards if they meet these criteria. It is preferable if the composition of the standard is close to that of the unknown, especially for major element analysis, but this is often not possible.

Quantitative analysis is a comparative method, relating the X-ray intensity for an element obtained on an unknown sample to the intensity on a standard of known composition. In its simplest form,

$$C_{\text{unk}} = C_{\text{std}} (I_{\text{unk}}/I_{\text{std}})$$

where C_{unk} and C_{std} are the concentrations of an element in an unknown (unk) sample and in a standard (std), and I_{unk} and I_{std} are the background subtracted X-ray intensities for the unknown sample and the standard. This simple relationship does not work well because it does not take into account how the compositional differences between the unknown and standard will affect the electron beam's interaction with the two materials in generating X-rays, and the X-rays' interaction with the materials in exiting. Thus, a correction factor is required for this equation. Since the three major components of this matrix or composition correction are the mean atomic number of the sample, the absorption of generated X-rays as they exit the sample, and the generation of secondary fluorescent X-rays, the letters "ZAF" are used to represent it, and three sets of equations are usually used to calculate it:

$$C_{\text{unk}} = (\text{ZAF})C_{\text{std}} (I_{\text{unk}}/I_{\text{std}})$$

The "Z" or atomic number correction (also called the generation factor) can be considered in two parts: the BSE and the stopping power (also called ionization-penetration) terms. Incoming beam electrons that are elastically backscattered will not contribute to the generation of X-rays, and this effect will be most pronounced for high atomic number targets. However, the electrons that do enter the sample and interact inelastically are more likely to produce X-rays in a higher atomic number material. These two factors have opposite effects and will usually largely cancel each other out, resulting in a relatively small combined Z correction in most materials.

The "A" or absorption correction occurs because the X-rays that are generated down to a depth of one or two micrometers must pass through the material on their way to the detectors and will interact with all other elements in the sample along the exit path (typically at an angle of 40 to 50° from the horizontal, the "takeoff angle" to the detectors, fixed in each microprobe instrument). Absorption will of course always decrease the intensity of

Table 12–2. An analysis of a magnesium and iron silicate (olivine), with Z, A, and F correction factors.

Element and X-ray line				Intensities		Backgrounds	
	Oxide	Element	Atomic %	Unknown	Std.	Unknown	Std.
	—— % (w/w) ——						
Mg K α	50.77	30.62	26.17	75702	75556	237	240
Si K α	41.02	19.17	14.19	13768	13809	110	115
Fe K α	7.73	6.01	2.24	5781	5702	157	162
O K α †	43.72	57.40					
Total	99.52						
Corrections							
	Z		A		F		
	Backscattered electrons	Ion penetration	Absorption	Fluorescence	Total		
Mg K α	0.996	1.020	1.420	0.997	1.439		
Si K α	0.982	1.038	1.419	0.999	1.446		
Fe K α	0.932	1.251	0.998	1.000	1.164		

† O was determined by stoichiometry; iterations required: 6.

X-rays, and this correction is usually the most significant of the three ZAF terms. Lower energy, longer wavelength X-rays will generally be more severely absorbed. However, even a higher energy X-ray could be severely attenuated if, by coincidence in a particular sample, there exists a major element that is a particularly efficient absorber of that X-ray. This efficient absorption would occur if the energy required to eject one of the absorbing atom's inner shell electrons should happen to be just below the energy of the X-ray (i.e., absorption edge effect).

The “F” or fluorescence correction occurs because of the possibility of emitting a secondary X-ray when a primary X-ray is absorbed. The primary X-ray can be either a characteristic X-ray emitted from another element in the sample or part of the continuous or “white” spectrum. In almost all cases, the former is the dominant contributor to the fluorescence correction.

To apply these corrections for any one element, it is necessary to know the identity and concentration of all other major and minor elements in the material being analyzed, an apparent “Catch 22” situation. This is resolved in practice by employing an iterative correction program, where a first estimate is made of the concentration of each element from the uncorrected equation, and progressively better estimates of the concentration are then obtained in each computational cycle using the ZAF correction factors, until the concentrations converge; generally four to eight iterations are necessary. Table 12–2 shows an analysis of a magnesium and iron silicate (olivine), with the magnitude of the Z, A, and F correction factors listed. Note the large absorption corrections for magnesium and silicon. If this had been an analysis of a low concentration of Mg in a Fe-silicate, absorption corrections for Mg K α X-rays in excess of 200% might have been observed. For a detailed discussion of the ZAF terms and correction procedures, see Reed (1993).

Quantitative Analysis: Energy-Dispersive vs. Wavelength-Dispersive Spectrometry

Quantitative analyses can be performed using either WDS or EDS systems. In general, EDS quantitative analyses tend to have poorer accuracy and precision than WDS analyses. The main reason for this is the relative inability of the EDS detector to resolve adjacent

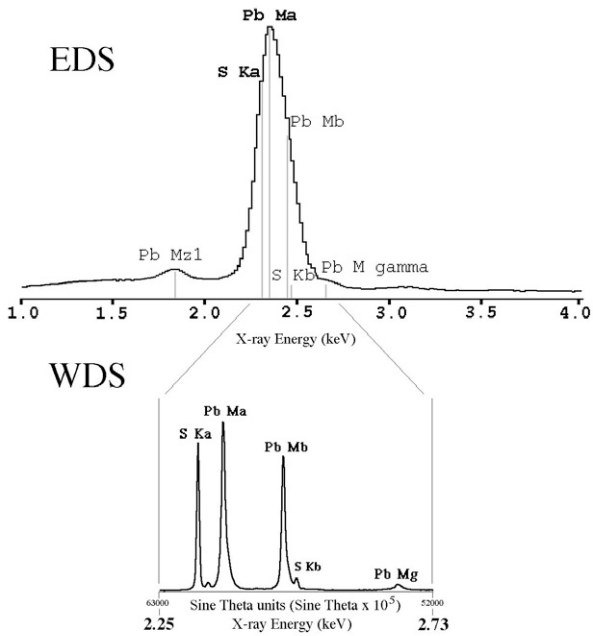


Fig. 12–13. Two scans over the S K α and Pb M α peak regions in PbS, one from a Si(Li) energy-dispersive spectrometry (EDS) system and the other from a wavelength-dispersive spectrometer (WDS) with a pentaerythritol (PET) diffracting crystal.

peaks. An example of this can be seen in Fig. 12–13, which shows two scans over the S K α and Pb M α peak regions in PbS, one from a Si(Li) EDS system (top) and the other from a WDS spectrometer using a pentaerythritol (PET) diffracting crystal (bottom). The inability to resolve closely spaced peaks means that the EDS system must rely on peak deconvolution algorithms to carry out most analyses. This problem with overlapping peaks also results in additional uncertainty with background values. Coupled with the poorer peak/background ratios inherent in EDS systems, these deconvolution errors result in detection limits that are typically an order of magnitude higher than with WDS systems. Table 12–3 shows a comparison between EDS and WDS systems for a number of important parameters related to quantitative analysis. Figure 12–14 shows the distribution of relative errors for a large number of analyses performed using both WDS and EDS systems. The WDS analyses exhibit much better reproducibility.

The best EDS analyses can be performed on elements ranging from about Cl ($Z = 17$) through about Zn (30). The background curve for this range is relatively flat, and overlaps

	WDS	EDS
Elements analyzed	Be (4) to U (92)	Na (11) to U (92)
Resolution	10–15 eV	~150 eV
Diameter of area analyzed	1–20 μ m	1 μ m to >1 mm
Peak/background ratio	100 to >300	20–50
Detection limit	50–500 ppm	>1000–2000 ppm
Background measurement method	Off-peak	Curve-fitting and off-peak
Accuracy (major elements)	± 1 –2%	± 2 –5%

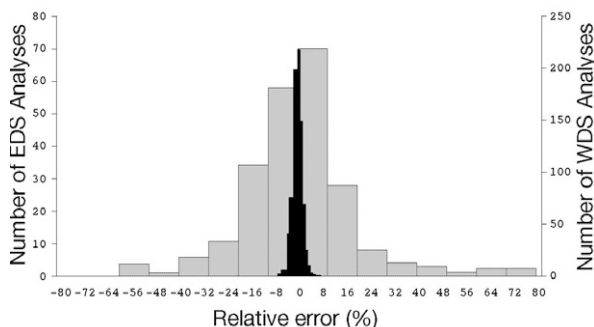


Fig. 12-14. The distribution of relative errors for a large number of analyses performed using both wavelength-dispersive and energy-dispersive spectrometry systems. The wavelength-dispersive analyses exhibit much better reproducibility.

from adjacent peaks are generally not as severe as in the Na (11) through S (16) region. If elements above atomic number 30 must be analyzed, then the accelerating voltage can be increased to use a higher energy line that will be subject to less interference and to a lower, linear background curve.

The EDS analyses are typically performed in one of two modes: with reference standards and standardless. The former is the preferred method, with counts being taken on reference standards under the same analytical conditions as are used on the unknowns. After deconvolution and background stripping computations have been performed on both the standard and unknown spectra, one of the ZAF matrix correction programs is used to obtain the composition. The standardless method is used either to save time or when suitable reference standards are not available. In this case, measured peak intensities from the unknown sample are compared with calculated pure element peak intensities. These analyses are typically less accurate than those using reference standards and have sometimes been referred to as “semi-quantitative.” A semi-quantitative analysis is really just a quantitative analysis with a large uncertainty or error bar.

Most modern EPMA software packages have the option of performing integrated WDS and EDS analyses. Major elements with no interferences and acceptable peak/background ratios can be analyzed from a concurrently acquired EDS spectrum, reducing the total time required for analysis and using the best properties of both types of X-ray systems.

SAMPLE REQUIREMENTS AND PREPARATION

The ideal sample for EPMA analysis would have a perfectly flat horizontal surface, would be polished to better than a 0.25- μm finish, and would consist of easily discernible grains larger than 10 μm in diameter. Successively finer grades (from 8 to 0.25 μm) of diamond pastes or alumina can be used to polish samples (Sawhney, 1986). In many cases, especially in the study of soil materials, samples will deviate from this ideal, and it is important to understand the consequences of dealing with these types of samples.

A tilted surface will introduce errors in the calculation of the ZAF matrix corrections, primarily the absorption correction. X-rays exiting from beneath the surface of a tilted sample toward a given detector will have either a shorter or longer path length to travel within the material, depending on whether the sample is tilting toward or away from the detector. Since most microprobes have three to five WDS spectrometers arranged around the sample, the intensities and therefore the apparent concentrations of different elements in a tilted sample will be affected. A general rule is that each degree of tilt may introduce about a 1% error in calculated concentration; this effect will be dependent on wavelength and will be most severe for longer wavelength X-rays (e.g., C K α vs. Cu K α X-rays). Tilt

may also manifest itself locally in an otherwise horizontal sample. This would be the case where harder grains are surrounded by and protrude out of a softer matrix such as clay, carbonate, or a mounting material such as epoxy resin. The polishing process may generate "relief," with the harder grains being a few microns above the softer grains, accompanied by rounded edges on the harder grains. If this type of rounding is observed optically or with SE imaging, then care must be taken to avoid analyzing these edges; the center, horizontal area of each grain should be selected instead. Areas of softer matrix must also be selected and analyzed with care.

A poorly polished rough surface will result in the scattering of incoming electrons and especially of outgoing X-rays. This effect will be wavelength dependent, attenuating the longer wavelength X-rays relative to the short. A very rough surface (relief of several micrometers) will result in topographic shadowing, where X-rays emitted from some points will encounter surface obstacles they have to penetrate on their way to the spectrometers, adding additional absorption effects that are not corrected by the ZAF program.

A porous material can result in low analytical totals (e.g., not adding up to 100%) if the pores are filled with a mounting material such as epoxy. However, relative element concentrations may not be severely affected, and the analysis could be normalized to 100%.

Many poorly aggregated and porous materials such as soils must be impregnated with a mounting material, often under vacuum or pressure, to fill void spaces, to secure the grains during the grinding and polishing process, and to preserve original texture. The type of sample mount used will depend on the types of studies to be done on the samples, and the design of the sample stage in a given instrument. The two most commonly used sample mounts would be polished thin or thick sections on 25 by 50 mm glass slides, or embedded round 25- or 32-mm cylindrical mounts. Epoxy is usually the preferred mounting material because of its ease of use and acceptable behavior in vacuum under the beam. There are dozens of different epoxy formulations with different properties and compositions. Because some of them contain elements such as Cl, S, and Si, the researcher must be careful to select an epoxy that will produce the fewest problems for the elements to be analyzed. The use of a colored epoxy (usually blue) can make it possible to differentiate between primary porosity and "artificial" porosity created during the process of grinding and polishing the sample mount.

In some cases, the analysis of fine-grained material can only be performed on a pressed powder pellet. Although this is not an ideal surface for quantitative EPMA work, acceptable results can often be achieved if great care and uniformity are used in sample preparation and if reference materials are carefully analyzed to determine the magnitude of any systematic errors. The pellet should be very thin (several hundred micrometers) and prepared using highly polished ($<0.3\ \mu\text{m}$) stainless-steel dies. Frequent repolishing of the dies may be required to maintain a smooth surface during the preparation of a large suite of samples. A defocused beam about 10 to 20 μm in diameter should be used to average out any remaining surface irregularities. The largest errors may occur for those elements that must be analyzed using longer wavelength X-rays ($\lambda > \sim 10\ \text{\AA}$). This was shown in an analytical test performed by Sutter (2000) on the same fluorapatite prepared both as several millimeter highly polished grains and as a pressed powder pellet (unpublished data, Sutter, 1999). He found that the analyzed total was about 5% lower for the pellet than for the polished grain. The following lines were used to analyze each element: Ca ($K\alpha = 3.4\ \text{\AA}$), P ($K\alpha = 6.2\ \text{\AA}$), and F ($K\alpha = 18.3\ \text{\AA}$). The losses for each element measured on the powder sample relative to the polished crystal were approximately 3% for Ca, 5% for P, and 10% for F.

The best pressed powder results are also obtained if the material being analyzed is monomineralic and has a grain size of $<1\ \mu\text{m}$. The analysis of heterogeneous mixtures with grain sizes $>1\ \mu\text{m}$ may result in the generation of matrix correction errors because X-ray photons exiting the sample surface will pass through different composition materials on their way to the different spectrometers and will not all see the same “average” sample composition.

Another method of preparing a poorly aggregated, inhomogeneous sample for bulk electron microprobe analysis is by fusion using a low voltage, high amperage strip heater (Brown, 1977). This method has the advantage of producing a glass that can then be mounted, ground, and polished to a nearly ideal surface, but care must be exercised to ensure homogeneity and to minimize the loss of volatile alkalis. This technique can be used to evaluate mass fluxes within a soil profile or to obtain quantitative analyses of clay or silt size fractionated soil materials.

Before being analyzed on the electron microprobe, all samples must be coated with a very thin layer of an electrically conductive material to remove electrons from the target area and prevent the buildup of a static charge. Such a static charge would distort imaging of the sample and introduce large errors into the quantitative results by deflecting the incoming electron beam away from the intended target. A 50- to 150-Å layer of Au or Au-Pd deposited in a sputter coater is the preferred coating for SE imaging of the surface topography of a rough sample coupled with rapid qualitative EDS analysis. However, an Au or Au-Pd coating will interfere severely with BSE imaging or the quantitative analysis of a polished surface. For this type of work the sample must be coated with an approximately 150-Å layer of high purity C in a carbon evaporator. The very low atomic number of C relative to Au (6 vs. 79) results in minimal absorption effects by the coating on the incoming beam electrons and outgoing BSE electrons and emitted X-rays, while still preventing the buildup of a static charge. Both unknown samples and quantitative reference standards must be coated as identically as possible for best results.

TYPES OF DATA SETS

The most common use for the EPMA is to perform quantitative point analyses using the WDS spectrometers. However, other important modes of data acquisition can also be used, often as preliminary steps in characterizing the sample before quantitative analysis. These include one-dimensional line scans or traverses across a grain or portion of a sample to examine variations in the concentration of one or more elements using uncorrected raw counts and two-dimensional area scans or grids using a wide range of electron or photon signals.

The following is a recommended sequence for carrying out a complete chemical characterization of a typical material with the electron microprobe:

1. Examine the prepared polished surface of the material to be analyzed by reflected and, if appropriate, transmitted light microscopy. If the original material was not consolidated before sample preparation, then it may also be appropriate to conduct a standard SEM examination of the surfaces of the grains on a separately prepared grain mount sample.
2. Examine the prepared sample by BSE imaging to determine grain size and degree of homogeneity.
3. Conduct EDS qualitative determination of the elements present in each of the observable phases of interest in the material.

4. If optical and/or BSE imaging suggests the material is systematically variable across the sample area, then a quick elemental line scan should be performed.
5. If a more extensive examination of spatial elemental distribution patterns is desired, then automated two-dimensional X-ray "maps" can be acquired. These are generated by either scanning the beam over a small region (typically tens of microns), or by moving the stage in a grid pattern beneath a fixed beam to image regions as large as several centimeters across. The result in either case is a set of images showing the areal distribution of elemental X-ray intensities, one element per WDS spectrometer. X-ray maps can also be acquired using an EDS spectrometer, albeit with a much higher background noise level. One caveat when deciding whether to acquire X-ray maps is that X-ray counting statistics often require that a set may take as long as 1 h.
6. Specific points can then be selected for complete quantitative WDS analyses, either manually or by using a software program to automatically pick points at prespecified distances along a traverse.

EXAMPLES OF ELECTRON PROBE MICROANALYZER STUDIES OF SOIL MATERIALS

There have been many publications in the area of soil materials incorporating electron microprobe data. The examples presented below are a small representative sampling. Additional examples can be found in Sawhney (1986).

Quantitative Analysis of Primary and Secondary Soil Minerals: Compositional Changes due to Weathering

The analysis of compositional changes in individual mineral grains or rock fragments due to chemical and biological weathering has been one of the main uses of the electron microprobe in soil science. Ezzaim et al. (1999) studied the formation of weathered rinds in tuff stones in an acid brown soil in Beaujolais, France. They cross-sectioned and prepared polished thin sections of both the weathered rims and unweathered cores of 1- to 40-cm diameter tuffaceous stones found on the surface and in soil pits. Phenocrysts were analyzed by electron microprobe using a 1- μ m beam diameter, and mean phenocryst compositions determined for each identified mineral typically using 25 analyses. Secondary minerals were analyzed in thin sections of the coarse sand fractions. The chemical composition of both fresh and weathered groundmass was determined by averaging one hundred 4- μ m diameter analytical spots for each. Normative composition was calculated for bulk rock (groundmass + phenocrysts) using data from microprobe, powder X-ray diffraction, inductively coupled plasma spectroscopy, scanning electron microscopy (SEM), and optical microscopy. A weathering chemical mass balance was then calculated based on Gressens (1967) method of composition-volume relationships. The authors concluded that the tuff weathering that they documented differed markedly from previous descriptions of granitic weathering in a temperate climate. Whereas granitic weathering results in granular disintegration, which liberates relatively fresh particles, the observed tuff weathering released particles that have experienced strong chemical alteration in the weathered rind.

Many other studies of weathering and soil formation have employed the electron microprobe. Martin-Garcia et al. (1999) studied the weathering of coarse fragments in Mediterranean red soils. They analyzed the composition of the illuvial infillings of fissures in coarse gravel fragments that were selected for their high degree of weathering. Murphy et al. (1998) examined the rate and mechanism of biotite weathering in a tropical water-

shed in the Luquillo Mountains, Puerto Rico. They used the electron microprobe to obtain WDS analyses of the bedrock biotite and chlorite while using a scanning transmission electron microscope (STEM) to obtain EDS analyses of the saprolite biotite and kaolinite. Abreu and Vairinho (1990) used the electron microprobe as one of their tools in studying the alteration of amphibole to vermiculite in ferromagnesian sandy grains of saprolite and C horizon of a Typic Rhodoxeralf formed from gabbro-diorite in southern Portugal.

Determination of In Situ Cation Exchange Capacity of Alteration Minerals

The electron microprobe can be used to measure in situ cation exchange capacities (CEC) using the “Cs-staining” method outlined by Hillier and Clayton (1992). Schiffman and Southard (1996) compared bulk extraction and in situ techniques in measuring the CEC of layer silicates and palagonitized glass in mafic volcanic rocks. They found that the sums of cations extracted by NH_4 -acetate on bulk samples were generally similar to the CEC determined by both the excess salt and in situ methods. Schiffman et al. (2003) also used this technique to help distinguish palagonitized from pedogenically altered basaltic Hawaiian tephra.

Analysis of Experimental Products

Electron microprobe analysis can be used to characterize materials produced or modified during laboratory experiments. Nobles (2001) used the microprobe to obtain the distribution profiles of bromine used as a tracer to study the impact of structural interfaces on the transport of chemicals in Vertisols and Alfisols. Sutter (2000) analyzed synthetic Fe, Mn, and Cu containing hydroxyapatites for those elements in addition to Ca and P. Because of the very small grain size of these hydroxyapatites, all quantitative analyses had to be done on pressed powder pellets. The potential problem of wavelength-dependent scatter was addressed by analyzing a well-characterized North Carolina phosphate rock pellet prepared under the same conditions as the experimental materials (at a pressure of 103.5 MPa). The results showed that the use of pressed powder pellets did not significantly alter elemental ratios, and good agreement was achieved with literature analyses of the North Carolina phosphate rock.

Geochemical Analysis of Minor Elements in Fine-Grained Geologic and Soil Materials

Quantitative analyses of elements present in the hundreds to thousands of parts per million concentration range can be achieved if the correct sample preparation and subsampling procedures are followed. Rao and Berner (1995) developed an electron microprobe method for the determination of P and associated elements in sediments. They used two preparation methods: clay-rich samples were prepared as pressed powder pellets between two polished metal disks, while polished epoxy impregnated grain mounts were used for silt- and sand-rich material. The most difficult materials to work with were those where the clay and the silt or sand were subequal. They achieved detection limits for P of about 50 to 100 ppm. They experienced problems with lower than expected Na with both methods; this could be the result of Na migration under the beam or wavelength-dependent scatter.

Composition of Iron–Manganese Minerals in Soils and Associated Adsorbed Metals

Manganese and Fe–Mn mineral grains can occur in soils as concretionary nodules, as coatings on silicates and carbonates, and as finely disseminated grains. The study of their

abundance and composition is important because of their tendency to adsorb heavy metals. They can be difficult to characterize by X-ray diffraction techniques because of their poor crystallinity and low detection sensitivity due to Fe fluorescence if Cu K α X-rays are used.

Lidiard et al. (1993) used a combination of analytical techniques to identify and characterize Mn oxide minerals in soil-float crusts and mottles in some soils from Devon, UK and to study the uptake of Co and Cu by the minerals using a simple sorption experiment. They employed WDS electron microprobe analysis to analyze for the trace elements Co, Cu, and Ba while employing EDS analysis to determine the major elements Mn, Fe, Ca, K, Na, and Al. They first prepared polished thin sections of Mn-rich soil mottles and soil-float crusts. They then acquired major element X-ray distribution images to map out areas of Fe and Mn oxides. The WDS and EDS analyses were then performed on 15 or 50 (depending on grain size) randomly selected points on each selected Fe–Mn oxide grain. The results of these analyses were then combined with X-ray diffraction, SEM, and bulk chemical analyses of acid-digested samples by inductively coupled plasma emission spectroscopy along with the results of the sorption experiments to characterize the Fe–Mn minerals and determine their distribution over the study site.

This author (Guillemette, 2002) presented an example of the types of data sets that could be acquired with the electron microprobe to chemically characterize soil materials such as a Fe and Mn soil concretion. An approximately 1.5-cm outer section of an Fe and Mn soil concretion from Brazos County, Texas (courtesy of Dr. Ronald Parker) was embedded in epoxy in a 2.5-cm-diameter phenolic ring form. The surface was ground and polished with silicon carbide and alumina abrasives until a 0.3- μ m final surface polish was achieved. The sample was then coated with a 150-Å conductive carbon coating for electron beam work. The same procedure used here for a concrectionary material could also be adapted for use with epoxy-impregnated poorly indurated soil materials. Five types of data sets were collected to assess the compositional and textural variability of the fine-grained Fe–Mn concrectionary aggregate and to obtain quantitative micrometer-scale point analyses:

- Reflective optical scan of the polished (but not yet C-coated) surface of the sample using a 1200-dpi flatbed optical scanner in 24-bit color mode
- EDS spectra acquisition at each optically or BSE different zone observed
- Two-dimensional stage raster imaging by BSE and X-ray signals (i.e., acquisition of X-ray elemental and BSE distribution “maps”)
- One-dimensional line scan using X-ray signals (if two-dimensional imaging is not possible)
- Fully quantitative point analyses along a traverse or for individual points selected as a result of the compositional information provided by the optical, BSE, and X-ray “maps”

Reflective optical scans of polished specimen surfaces with a standard high resolution (>1200 dpi) color flatbed scanner provide a fast and inexpensive method of observing subtle textural variations and selecting areas for more detailed electron beam imaging and analysis. Transmitted light scans with a 35-mm scanner would also be appropriate for polished thin sections of coarser grained material. Figure 12–15 is a grayscale version of a strongly color-enhanced optical scan of the polished surface of the epoxy-embedded concretion with analyzed areas marked. Zoning that could be seen only subtly with the naked eye becomes clearly observable after contrast enhancement with an image enhancement program such as Adobe PhotoShop or NIH Image (Rasband and Bright, 1995).

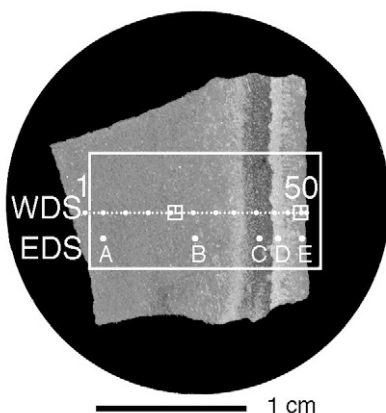


Fig. 12–15. A grayscale version of a strongly color-enhanced optical scan of the polished surface of the epoxy-embedded concrete with analyzed areas marked.

Figure 12–16 shows EDS spectra for five regions from the inner to outer Fe–Mn concretion. These provide qualitative information about the major and minor elements present in texturally different regions of the sample, allowing the researcher to make decisions about which elements should be examined in more detail in the imaging and quantitative analysis portions of the study.

Figure 12–17 contains a BSE image; Fe, Mn, Ca, and Ba two-dimensional elemental distribution maps; averaged and simple line profiles; and WDS quantitative line plots. The 15.36 by 7.68 mm area of the sample was rastered beneath a 15-keV, 20-nA, 1- μ m-diameter stationary beam to generate a 1024 by 512 point grid, with an average beam dwell time of 15 ms at each 15- μ m grid square. The total time needed to simultaneously acquire the BSE and four elemental maps was approximately 2 h. This time is dictated by the maximum scan rate of the stage assembly and the need to obtain statistically reasonable X-ray counts at each pixel. This time might be shortened by using higher beam currents and/or faster stage scan rates. The mean X-ray columnar value line profiles were generated from the BSE and X-ray maps using the “plot profile” function in NIH Image. The simple line profile was acquired by moving the sample beneath the beam for 1024 15- μ m-long, 1-s duration steps; total acquisition time was about 25 min. Limiting the number of steps and/or the time spent at each point could shorten this. The WDS oxide concentration percentage (w/w) plots were generated from the WDS quantitative analysis data set. This data set consisted of 50 ten-element analyses spaced 30 μ m apart, acquired with a 15-keV, 20-nA, 1- μ m-diameter beam. All elements were calibrated with well-characterized mineral standards, with oxygen calculated by stoichiometry. The calibration time was 90 min, and each analysis took about 3 min. The raw data were corrected using the PAP (Pouchou and Pichoir, 1985) ZAF matrix correction program. Table 12–4 contains 14 complete representative analyses.

The large area X-ray maps in Fig. 12–17 suggest a change in elemental distribution texture between the center and the edge of the rind, but with a grid spacing of 15 μ m, they do not have sufficient resolution to show any details. Two additional higher resolution X-ray maps were therefore required. Each covers a square millimeter at a grid spacing of only 2 μ m. Figure 12–18 shows the upper right 256 by 256 pixel quadrant of these two detailed, higher magnification Mn and Ca two-dimensional elemental distribution maps (locations shown in Fig. 12–15). The greater resolution allows the differences in particle size to be clearly observed.

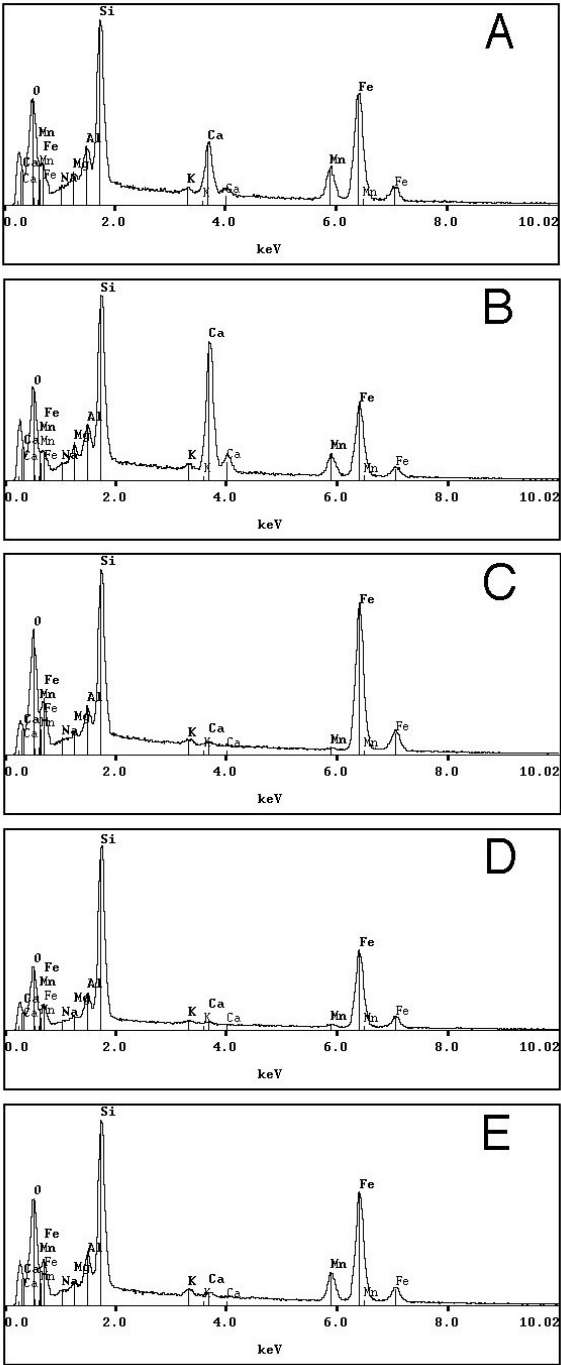


Fig. 12–16. Energy-dispersive spectra for five regions from the inner to outer Fe–Mn concretion.

Fig. 12–17. A backscattered electron image; Fe, Mn, Ca, and Ba two-dimensional elemental distribution maps; averaged and simple line profiles; and wavelength-dispersive spectrometer quantitative line plots.

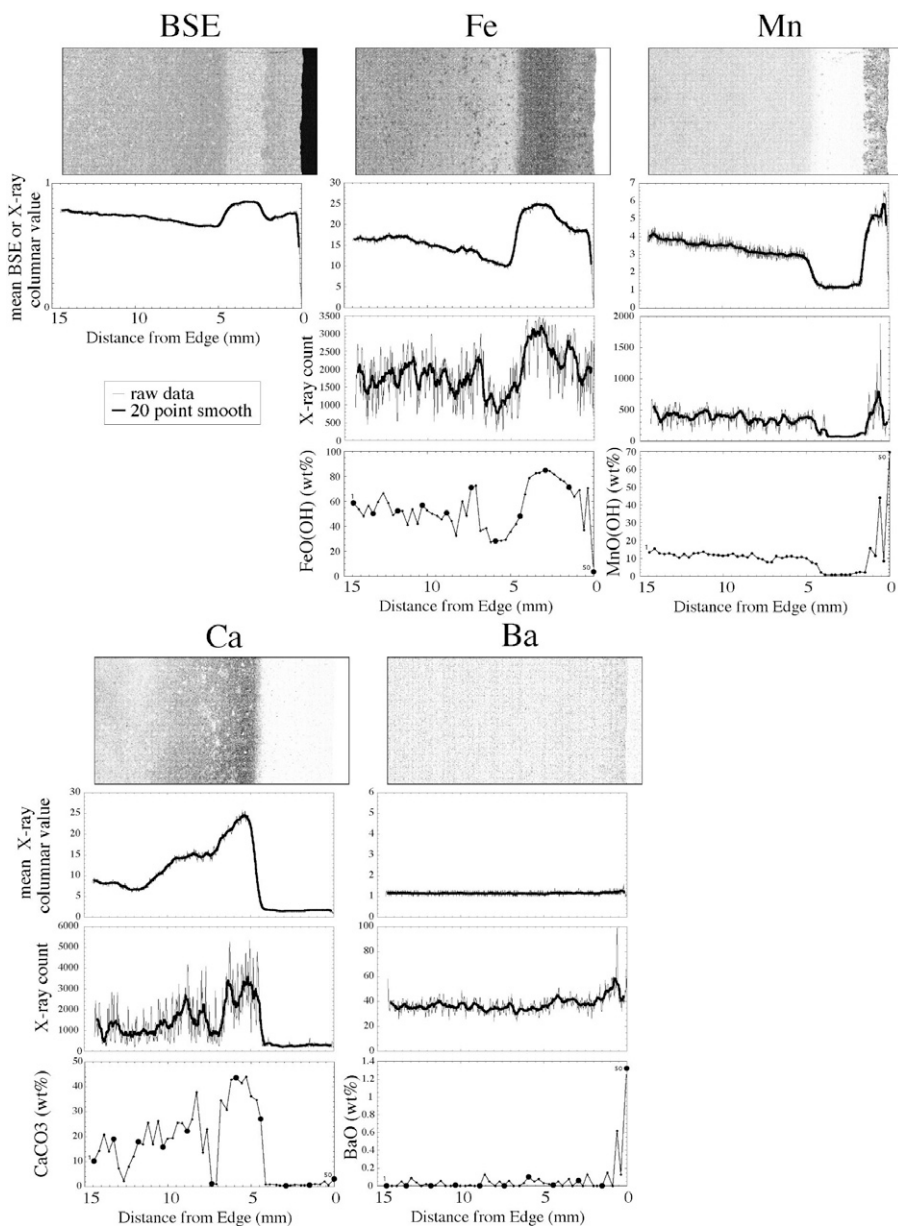


Table 12–4. An example of complete representative analyses acquired with the electron microprobe to chemically characterize soil materials.

Analysis no.	1	5	10	15	20	25	30	34	39	43	46	49	50
Distance from edge, mm	14.4	13.2	11.8	10.3	8.8	7.4	5.9	4.7	3.3	2	1.2	0.3	0
FeO(OH), % (w/w)	58.8	50.2	52.2	56.7	50.5	70.8	28.2	41.6	82.8	77.5	63.6	70.6	3.4
MnO(OH)	13.2	12.6	12.7	11.6	11.5	7.9	11.1	7.6	0.6	1.7	15.7	8.4	69.5
CaCO ₃	10.2	19.1	18.0	15.9	22.2	1.1	43.6	34.8	0.4	0.6	1.0	0.6	3.1
BaO	0	0	0	0	0	0	0.1	0	0	0	0.2	0.1	1.3
Na ₂ O	0	0	0	0	0	0	0.1	0	0	0	0.2	0	0.9
Al ₂ O ₃	0.5	1.1	0.6	0.4	0.7	1.1	0.7	0.4	0.6	1.4	2.0	1.3	0.9
MgCO ₃	2.9	3.2	3.0	3.0	3.8	3.8	4.4	3.5	0.9	1.4	1.8	1.1	3.6
SiO ₂	5.2	4.5	3.5	2.1	2.6	4.8	2.4	2.7	7.2	7.8	7.3	7.1	3.0
K ₂ O	0	0.1	0	0	0.1	0.3	0.1	0	0.1	0.1	0.2	0.2	0.5
TiO ₂	0.1	0.1	0	0	0	0.3	0	0	0.1	0.1	0	0.1	0.1
Total	90.9	90.8	89.9	89.7	91.4	90.1	90.6	90.7	92.7	90.6	91.8	89.6	86.1

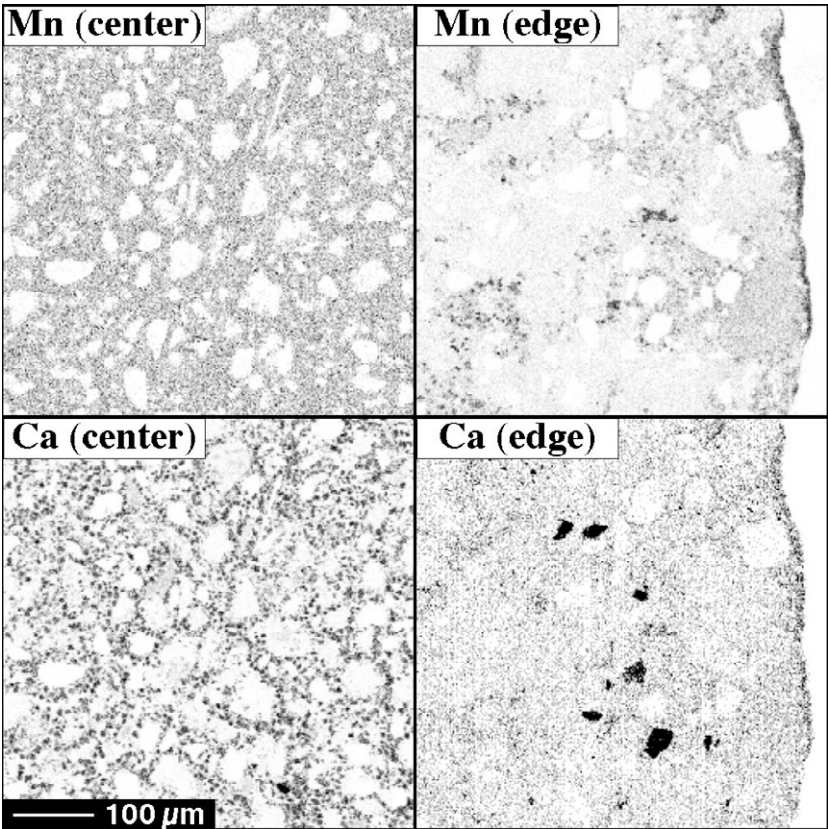


Fig. 12–18. The upper right 256 by 256 pixel quadrant of detailed, higher magnification Mn and Ca two-dimensional elemental distribution maps. Locations are shown in Fig. 12–15.

This study of a Fe–Mn soil concretion leads us to the following conclusions regarding the use of the different types of data sets, which can be acquired with an electron microprobe.

1. Backscattered electron and X-ray elemental distribution “maps” can be very useful in characterizing fine, inhomogeneous aggregate materials. They provide a preliminary overview of the material to determine the degree of inhomogeneity and to initially recognize any obvious systematic compositional or textural trends. If the area to be examined is sufficiently small (generally $< \sim 1$ mm in diameter), BSE images alone can be acquired much more quickly in beam-scanning mode rather than in the slower stage-scanning mode. Backscattered electron intensities are usually so high that low noise images can be acquired in tens of seconds. X-ray images, however, will still take at least tens of minutes because of relatively low X-ray detection rates, even for major elements. Also, because of the defocusing behavior of the Johansson spectrometers, beam scanning WDS X-ray maps can only be obtained over areas less than about 50 μm . Larger area WDS X-ray maps would still have to be acquired by rastering the stage beneath a fixed beam. Lower quality X-ray maps could also be acquired with the EDS spectrometer; these would exhibit much higher noise and poorer spectral resolution than WDS maps. Once acquired, these images are amenable to image analysis techniques that can yield quantitative or relative information about chemical zonation and grain morphology and size distributions. For coarser grained materials, modal mineral information can be obtained in lieu of manual point counts with an optical microscope. Averaged line profiles such as those obtained in the images above can be created for any orientation in the image, and using any selected number of pixel rows or columns. The freely available image analysis programs NIH Image for the Macintosh operating system (Rasband, 2006), the related Scion Image for the Windows operating system (Scion Corporation, 2006), and ImageJ for Macintosh, Windows, and Linux (Rasband, 2006) provide most functions needed for this type of analysis. These images provide critical information needed to choose the minimum number of points required for full quantitative WDS analyses. An examination of the images and extracted plot profiles in the case of the Fe–Mn concretion, for example, would suggest that substantially fewer than 50 points could have been chosen to adequately describe this material.
2. Simple single line profiles can be useful in some materials, but may not adequately sample an inhomogeneous material such as a fine-grained aggregate. These simpler profiles should generally be used only when the equipment or the time and resources available preclude the acquisition of two-dimensional area images.
3. Fully quantitative analyses have an important role as a final step in characterizing most materials. The best results are obtained when analyses can be performed on discrete mineral grains that are at least 5 to 10 μm in diameter. When a finer grained aggregate is analyzed, analytical totals may not reach 100% due to intergranular void spaces or due to X-ray scatter from a poorly polished surface. In the worst case, such scatter may alter elemental ratios by several percent as a result of the X-ray wavelength dependence of the scatter.

REFERENCES

- Abreu, M.M., and M. Vairinho. 1990. Amphibole alteration to vermiculite in a weathering profile of gabbro-diorite. p. 493–500. *In* Soil micromorphology. Proc. 8th meeting of soil micromorphology, San Antonio, TX. 1988. Developments in Soil Science 19. Elsevier, New York.
- Bertin, E.P. 1978. Introduction to X-ray spectrometric analysis. Plenum Press, New York.
- Brown, R.W. 1977. A sample fusion technique for whole rock analysis with the electron microprobe. *Geochim. Cosmochim. Acta* 41:435–438.
- Castaing, R. 1951. Ph.D. diss. Univ. of Paris.
- Castaing, R., and A. Guinier. 1950. Proc. Int. Conf. on Electron Microscopy, 1st. Delft, The Netherlands. 1949.

- Ezzaim, A., M.P. Turpault, and J. Ranger. 1999. Quantification of weathering processes in an acid brown soil developed from tuff (Beaujolais, France) Part I. Formation of weathered rind. *Geoderma* 87(3-4):137-154.
- Gresens, R.L. 1967. Composition-volume relationships of metasomatism. *Chem. Geol.* 2:47-65.
- Guillemette, R.N. 2002. Electron microprobe quantitative and X-ray imaging applications in soil analysis. p. 376. *In* Annual Meetings Abstracts. ASA, CSSA, and SSSA, Madison, WI.
- Hillier, S., and T. Clayton. 1992. Cation exchange "staining" of clay minerals in thin-section for electron microscopy. *Clay Miner.* 27:379-384.
- Lidiard, H.M., J.E. Rae, and A. Parker. 1993. Identification of Mn-oxide minerals in some soils from Devon, UK, and their varying capacity to adsorb Co and Cu. *Environ. Geochem. Health* 15(2-3):93-104.
- Marshall, D.J. 1988. Cathodoluminescence of geological materials. Unwin Hyman, London.
- Martin-Garcia, J.M., G. Delgado, J.F. Parraga, E. Gamiz, and R. Delgado. 1999. Chemical, mineralogical and (micro)morphological study of coarse fragments in Mediterranean red soils. *Geoderma* 90(1-2):23-47.
- Murphy, S.F., S.L. Brantley, A.E. Blum, A.F. White, and D. Hailiang. 1998. Chemical weathering in a tropical watershed, Luquillo Mountains, Puerto Rico: II Rate and mechanism of biotite weathering. *Geochim. Cosmochim. Acta* 62:227-243.
- Nobles, M.M. 2001. Impact of structural interfaces on the transport of chemicals in Vertisols and Alfisols of Texas. Ph.D. diss. Texas A&M Univ., College Station.
- Pagel, M., V. Barbin, P. Blanc, and D. Ohnenstetter (ed.) 2000. Cathodoluminescence in Geosciences. Springer Verlag, New York.
- Pouchou, J.L., and R. Pichoir. 1985. "PAP" (phi-rho-z) procedure for improved quantitative microanalysis. p. 104-106. *In* J.T. Armstrong (ed.) Microbeam analysis. San Francisco Press, San Francisco.
- Rao, J.L., and R.A. Berner. 1995. Development of an electron microprobe method for the determination of phosphorus and associated elements in sediments. *Chem. Geol.* 125(3-4):169-183.
- Rasband, W.S., and D.S. Bright. 1995. NIH Image: A public domain image processing program for the Macintosh. *Microbeam Anal. Soc. J.* 4:137-149.
- Rasband, W.S. 2006. ImageJ. Image processing and analysis in JAVA. Available at <http://rsb.info.nih.gov/ij/> (verified 20 Mar. 2007).
- Rasband, W.S. 2006. NIH Image. Available at <http://rsb.info.nih.gov/nih-image/> (verified 20 Mar. 2007).
- Reed, S.J.B. 1993. Electron microprobe analysis. 2nd ed. Cambridge Univ. Press, Cambridge, UK.
- Sawhney, B.L. 1986. Electron microprobe analysis. *In* A. Klute (ed.) Methods of soil analysis, Part 1. Physical and mineralogical methods. Agron. Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.
- Schiffman, P., and R.J. Southard. 1996. Cation exchange capacity of layer silicates and palagonitized glass in mafic volcanic rocks: A comparative study of bulk extraction and in situ techniques. *Clays Clay Miner.* 44:624-634.
- Schiffman, P., R.J. Southard, D.D. Eberl, and J.L. Bishop. 2003. Distinguishing palagonitized from pedogenically-altered basaltic Hawaiian tephra: Mineralogic and geochemical criteria. p. 393-405. *In* J.L. Smellie and M.G. Chapman (ed.) Volcano-ice interactions on earth and mars. Spec. Publ. 202. Geological Society of London.
- Scion Corporation. 2006. Scion Image for Windows. Port of NIH Image for Mac. Available at <http://www.scioncorp.com> (verified 20 Mar. 2007). Scion Corp., Frederick, MD.
- Scott, V.D., and G. Love. 1983. Quantitative electron-probe microanalysis. Ellis Horwood Ltd., Chichester, UK.
- Stormer, J.C., Jr., M.L. Pierson, and R.C. Tacker. 1993. Variation of F and Cl X-ray intensity due to anisotropic diffusion in apatite during electron microprobe analysis. *Am. Mineral.* 78(5-6):641-648.
- Sutter, B. 2000. Structural properties and dissolution of iron, manganese and copper containing synthetic hydroxyapatite. Ph.D. diss. Texas A&M Univ., College Station.
- Williams, K.L. 1987. An introduction to X-ray spectrometry: X-ray fluorescence and electron microprobe analysis. Allen and Unwin, London.

ADDITIONAL READING

- Goldstein, J., D. Newbury, P. Kchlin, D. Joy, C. Lyman, E. Lifshin, L. Sawyer, J. Michael, and M. Staniforth (ed.) 2003. Scanning electron microscopy and X-ray microanalysis. 3rd ed. Plenum, New York.
- Heinrich, K.F.J. 1981. Electron beam X-ray microanalysis. Van Nostrand Reinhold Company, New York.
- Heinrich, K.F.J., and D.E. Newbury. 1991. Electron probe quantitation. Plenum Press, New York.

- Krinsley, D.H., K. Pye, and S. Boggs (ed.) 1998. Backscattered scanning electron microscopy and image analysis of sediments and sedimentary rocks. Cambridge Univ. Press, Cambridge, UK.
- Lee, R.E. 1993. Scanning electron microscopy and X-ray microanalysis. Prentice Hall, New York.
- Lyman, C.E. 1990. Scanning electron microscopy, x-ray microanalysis, and analytical electron microscopy: A laboratory workbook. Plenum, New York.
- Newbury, D.E., D.C. Joy, P.E. Echlin, and C.E. Fiori. 1986. Advanced scanning electron microscopy and X-ray microanalysis. Plenum, New York.
- Postek, M.T., K.S. Howard, A.H. Johnson, and K.L. McMichael. 1980. Scanning electron microscopy—A student's handbook. Ladd Research Industries, Williston, VT.
- Potts, P.J., J.F.W. Bowles, and S.J.B. Reed (ed.) 1995. Microprobe techniques in the earth sciences. Mineralogical Society Series 6. Chapman and Hall, New York.
- Reed, S.J.B. 1996. Electron microprobe analysis and scanning electron microscopy in geology. Cambridge Univ. Press, Cambridge, UK.
- Russ, J.C. 1990. Computer-assisted microscopy—The measurement and analysis of images. Plenum Press, New York.
- Russ, J.C. 2006. The image processing handbook. 5th ed. CRC Press, Boca Raton, FL.

Diffuse Reflectance Spectroscopy

JOSÉ TORRENT and VIDAL BARRÓN, University of Córdoba, Spain

The appearance of a soil results from the interaction of its different constituents with incident light. Color and various other attributes of the appearance of soil are highly sensitive to the nature, proportion, particle size and morphology, and spatial association of its mineral and organic components. In fact, color has been used for more than 75 yr to obtain information about these soil properties with a goal of characterizing and distinguishing soil types.

The demand for a standardized method to describe soil color was met by the adoption of the Munsell notation by the USA Soil Survey Program in 1949 and, about 10 yr later, by the International Society of Soil Science (Simonson, 1993). Since then, Munsell Soil Color Charts (Munsell Color, 1975) have been systematically used by pedologists. Visual estimation of soil color, however, is subject to substantial error due to various psychophysical and physical factors. For this reason, the use of colorimeters and spectrophotometers has gained widespread acceptance among soil scientists as a means to measure color accurately and precisely. Moreover, different types of spectrophotometers afford the elucidation of the spectrum of light reflected by a soil illuminated in various ways.

Reflectance, which is the base quantity that characterizes the process of reflection, is defined as the ratio of the reflected radiant flux (or power) to the incident radiant flux (or power) (Wyszecki and Stiles, 1982). Generally, the reflectance of a soil at any wavelength λ can be considered to be the sum of two components: *regular* (or specular, or mirror) reflectance and *diffuse* (or volume or nondirectional) reflectance (defined in more detail below). Reflectance measurements in the field are usually made on relatively large areas ($>10 \text{ cm}^2$). Under these conditions, both specular and diffuse reflectance usually contribute to the total reflectance of a soil surface, the magnitude of which depends on particle size, structure, microrelief, and other properties that define the “surface state” (Escadafal, 1989). In contrast, laboratory measurements of soil reflectance are usually made on small areas ($<10 \text{ cm}^2$) of disturbed soil materials that are usually sieved or ground to a small size. In this case diffuse reflectance predominates, which depends mainly on soil composition.

This chapter describes available laboratory methods for recording diffuse reflectance spectra for soil materials and ways to handle the information to identify and characterize soil minerals. Only the visible and narrow vicinal ultraviolet (UV) and infrared (IR) regions of the spectrum are considered here because reflectance in the IR region is the subject of another chapter (Johnston and Aochi, 1996)).

PRINCIPLES

A brief introduction to the theoretical principles of diffuse reflection is provided here. For an in-depth description of this phenomenon, interested readers are referred to textbooks such as those of Wendlandt and Hecht (1966), Kortüm (1969), Wyszecki and Stiles (1982), and Hapke (1993).

A beam of light impinging on a flat polished surface of a crystal larger than the beam cross section is partly specularly reflected and partly refracted following the laws of geometric optics (contained in the Fresnel equations). In absorbing materials, the radiant flux is absorbed according to the well-known Lambert Absorption Law:

$$I = I_0 e^{-K_T x} \quad [1]$$

where I is the radiation flux transmitted from an initial flux I_0 following passage through a layer of thickness x of a medium with an absorption (or extinction) coefficient K_T measured in transmission.

When the dimensions of the particle are small compared with the beam cross section but large relative to the light wavelength, diffraction phenomena also occur because rays striking the crystal and passing by it result in interferences among elementary waves. In powders of randomly oriented particles of such size, part of the incident light goes back at all angles into the hemisphere of provenance of the light. The phenomenon resulting from the reflection, refraction, diffraction, and absorption by particles oriented in all directions is called *diffuse* (or *volume*) *reflection*, in contrast with *regular* (or *directional*) reflection from a plane phase boundary. For ideal diffuse reflection, the angular distribution of reflected light is independent of the angle of incidence and obeys the Lambert Cosine Law. This law states that the remitted radiation per unit surface and unit solid angle is proportional to the cosine of the angle i of incident light and the cosine of the angle of observation, e . There is no such thing as an ideal diffuse reflector, but near-Lambertian behavior is normally observed in tightly pressed powder samples.

If the dimensions of the particle are similar to, or smaller than, the wavelength, then the contributions of reflection, refraction, and diffraction to the intensity and angular distribution of the remitted radiation flux are comparable and impossible to separate. The phenomenon is then designated as *scattering*. Various theories have provided a reasonably solid basis to interpret single scattering by isolated molecules of absorbing or nonabsorbing isotropic particles. However, as the distance between particles decreases, single scattering gives way to *multiple scattering*, which logically predominates in densely packed crystal powders and pigment mixtures.

The angular distribution of light scattered by a single particle is far from isotropic. On the other hand, simple semiquantitative investigations have shown that, with sufficiently large number of particles and layer thickness, an isotropic distribution eventually arises within the sample (Kortüm, 1969). Essentially, this corresponds to diffusely reflected radiation for a layer of densely packed coarse particles, for which, as stated above, the laws of reflection, refraction, diffraction, and absorption still hold. Support for this assumption is provided by the experimentally confirmed validity of the Lambert Cosine Law for multiple scattering by fine particles.

There is no general quantitative solution to the problem of multiple scattering. Purely phenomenological theories have thus been developed to describe the system properties. Several theories are based on two constants that characterize the absorption and scatter-

ing per unit layer thickness of the medium. These so-called *coefficients of absorption and scattering* are generally taken to be properties of the irradiated layer, assumed to be a continuum, and are experimentally accessible.

The Kubelka–Munk Theory

The Kubelka and Munk (1931) theory assumes that a plane-parallel layer of thickness X capable of both scattering and absorbing radiation is irradiated in the $-x$ direction with a diffuse monochromatic radiation flux I (Fig. 13–1). The layer is very extensive relative to X and can be split into infinitesimal layers of thickness dx . The diffuse radiation flux in the negative and positive x directions are designated I and J , respectively. If, in passing through dx , the downward flux I is decreased by an amount K/dx by absorption, and increased by an amount S/dx by scattering, and a similar reasoning is made for the upward flux J , then the following differential equations can be derived:

$$\frac{-dI}{dx} = -(K + S)I + SJ \quad [2]$$

$$\frac{dJ}{dx} = -(K + S)J + SI \quad [3]$$

where K and S are the absorption and scattering coefficient of the sample, respectively.

Kubelka (1948) obtained explicit hyperbolic solutions to this equation that were discussed in detail by Wyzecki and Stiles (1982). The most general solution is

$$R = \frac{1 - R_g(a - b \coth bSX)}{a - R_g + b \coth bSX} \quad [4]$$

where R is the reflectance of the layer over a background of reflectance R_g , $\coth bSX$ the hyperbolic cotangent of bSX , X the layer thickness, $a = 1 + K/S$, and $b = (a^2 - 1)^{0.5}$.

Routinely, many measurements are made on layers thick enough to ensure that a further increase in thickness will fail to change the reflectance. Under these conditions, the reflectance is given by R_∞ and Eq. [4] yields

$$\frac{K}{S} = \frac{(1 - R_\infty)^2}{2R_\infty} = F(R_\infty) \quad [5]$$

where $F(R_\infty)$ is usually termed the *remission* or *Kubelka–Munk (K–M) function*.

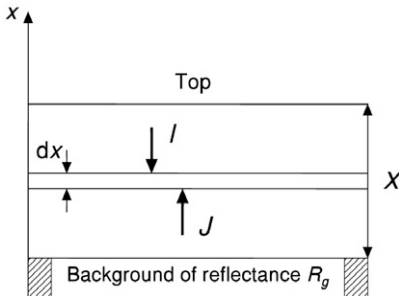


Fig. 13–1. Cross-sectional diagram of a powder layer.

It must be noted that the reflectance at any wavelength is a function of the K/S ratio rather than of the absolute values of K and S . As a result, these two absolute values cannot be obtained from Eq. [5].

The validity of Eq. [5] has been tested through carefully designed measurements on samples of colored glass for which the scattering coefficient was shown to be independent of the wavelength, and both K (Eq. [5]) and K_T (the absorption coefficient in transmission, Eq. [1]) were measured. K was found to be proportional to K_T by a factor similar at all wavelengths (Kortüm, 1969). Hence, the logarithmically plotted reflectance spectrum could be vertically offset to make it coincide with the logarithmically plotted transmission spectrum. For this reason, either $F(R_\infty)$ or the apparent absorbance $[\log(1/R_\infty)]$ are proxies for the actual absorption spectrum. Such a significant finding has been used to identify the characteristic absorption bands of minerals (Sherman and Waite, 1985; Burns, 1993).

As noted, the “typical” absorption spectrum constructed from reflectance measurements [i.e., the $F(R_\infty)$ curves] should reflect the true absorption spectrum only if the scattering coefficient is independent of the wavelength. This is only the case when the average grain size is large relative to the wavelength. Otherwise, the scattering coefficient usually decreases with increasing wavelength. Exceedingly coarse grains pose other problems (heterogeneity of the mixture and significant regular reflection) that greatly reduce differences between maxima and minima in the absorbance curves. For this reason, fine-grained (0.1–1 μm) samples are preferred, even though the decrease in scattering coefficient with increasing wavelength results in a flattening of the typical color curves toward the UV region and a “red-shift” of maxima in the color curve.

The dependence of the K – M function or the apparent absorbance $[\log(1/R_\infty)]$ on particle size is rather complex, particularly in heterogeneous mixtures such as ground soil materials, where both particle size range and differences in absorption coefficient among minerals are wide. The absorption coefficient (either K or K_T) invariably decreases with increasing particle size throughout the size range of interest, and the spectrum flattens. However, differences between weak and strongly absorbing materials continue to exist as relates to the size-dependence of absorbance. In strongly absorbing materials, absorbance increases with decreasing particle size for sizes smaller than the wavelength through an increased absorption coefficient. With particle sizes larger than the wavelength, the absorbance also usually decreases with increasing particle size because (i) the fraction of regular reflection increases with increasing particle size and (ii), as predicted by the Fresnel equations, regular reflection has much weight in strongly absorbing materials. In weakly absorbing materials, on the other hand, an increase in particle size above the wavelength results in an increase in absorbance because the decrease in K is offset by that in S (which is proportional to the reciprocal of particle size). Because K is small, light can penetrate deeper into the sample and increase the absorbance.

In summary, the K – M theory allows one to obtain the typical absorption spectrum for an absorbing mineral or mineral mixture, but one must consider those factors affecting the curve. In practice, significant deviations from the theory occur at $R_\infty < 0.6$. For this reason, dilution of the sample to be measured with a “white” standard possessing a known scattering coefficient at the different wavelengths has usually been recommended. K values can then be calculated from the K – M function on the proven assumption that the scattering coefficient of the diluted sample can be approximated by that of the diluent. Absolute values of S can be determined by measuring the reflectance of several mounts consisting of thin layers of standard against a background of reflectance $R_g = 0$. S can then be obtained from the slope of the plot against thickness, X , of the following function:

$$SX = \frac{1}{b} \coth^{-1} \frac{1 - aR_0}{bR_0} \quad [6]$$

where a and b are defined as for Eq. [4], and R_0 is the reflectance in front of the black background.

The K – M theory has been criticized on several fronts, namely: misinterpretation of the K and S parameters because they are mutually dependent, lack of physical meaning of the reflectance because it can be measured in different ways, and poor definition of parameter S . In addition, the practice of diluting the sample with a white standard to prevent saturation of the remission function may not be effective (Hapke, 1993).

Recent Theories

In the early 1980s new theories were developed that provided solutions to the problem of radiative transfer in a complex particulate medium. The simplest of such theories was that of Hapke (1981, 1993), in which the primary quantity in reflectance is not the remission function, but the *particle single-scattering albedo*, w . This represents the probability of a photon surviving an interaction with a single particle (i.e., the ratio of the total amount of power scattered/total power removed from the wave). For particulate media, the *volume single-scattering albedo*, $\bar{w}$, is the average of the single-scattering albedo for the individual particles. The theory starts with the formalism of the equation of radiative transfer, which is used to determine how the intensity of an electromagnetic wave is changed by the processes of emission, absorption, and scattering as the wave propagates through a complex medium. Hapke coined the term *diffusive reflectance* to designate the reflectance derived from the simplest two-stream solution to the radiative transfer equation. This diffusive reflectance has no physical meaning (K – M is in fact a form of diffusive reflectance) and is defined on the assumption that the radiation impinges on a plane corresponding to $z = 0$ that separates an empty half-space from an infinitely thick lower half-space filled with particles that scatter and absorb light. The assumptions in the diffusive reflectance formalism are that the incident radiance does not penetrate into the medium, but rather acts as if the upper boundary were covered by an invisible membrane scattering incident light uniformly in all downward directions, and that no sources of radiation exist within this medium. The problem is then to determine the radiance in the medium and the scattered radiance emerging from the $z = 0$ plane in the upward direction into the half-space. In the two-stream approximation, the solid angle is entirely occupied by the upward- and the downward-going hemispheres.

Hapke (1981) derived a relation between the particle single-scattering albedo, the complex index of refraction, the grain size, and a scattering parameter to describe scattering centers within imperfect grains. For the case of volume single-scattering albedo of isotropic scatterers, he developed analytical approximations for the different cases of reflectance (measured relative to a white standard). For the diffusive reflectance, the expression was

$$\bar{w} = \frac{4R}{(1 + R)^2} \quad [7]$$

Because diffusive reflectance lacks physical meaning, this equation is only of use for rapid semiquantative estimation of $\bar{w}$. A variety reflectance types exists in terms of spectrophotometric measurements that depend on the degree of collimation of the light

source and detector, the usual attributes used being *directional*, *conical* and *hemispherical*. As signaled by Hapke (1993), all measured reflectances are biconical because neither perfect collimation nor perfect diffuseness can be achieved in practice. In the particular case of directional-hemispherical reflectance (where the object is illuminated by a highly collimated source and the total amount diffuse light scattered into the upward hemisphere is measured, $\bar{w}$ is given by

$$\bar{w} = \frac{R^2(4\cos^2 i - 1) + R(4\cos i + 2)}{(1 + 2R\cos i)^2} \quad [8]$$

in which i is the incidence angle. When $i = 0$, Eq. [8] reduces to

$$\bar{w} = \frac{3R^2 + 6R}{(1 + 2R)^2} \quad [9]$$

Equations [8] and [9] have been successfully used to estimate the absorption coefficients of minerals and for quantitative analysis, as discussed later in this chapter.

Analysis of Mixed Powders

Duncan (1940, 1949) showed the absorption and scattering coefficients of a mixture to be simple additive functions of the respective coefficients of the mixture components weighed according to their proportions. Hence, the K - M function for the mixture can be written as

$$F(R_M) = \frac{(1 - R_M)^2}{2R_M} = \left(\frac{K}{S}\right)_M = \frac{\sum C_i K_i}{\sum C_i S_i} \quad [10]$$

where subscript M refers to the mixture, R_M is R_∞ for the mixture, and C_i is the mass fraction of component i with absorption coefficient K_i and scattering coefficient S_i .

Equation [10], which is frequently used in quantitative analysis, can be further simplified for mixtures containing a large excess of a diluent such that the scattering coefficient is completely dictated by the diluent:

$$F(R_M) = \frac{1}{S} \sum C_i K_i \quad [11]$$

Equation [10] can also be used to determine the K and S values for a single- or multi-mineral sample, K_p and S_p . For this purpose, the sample is mixed with variable amounts of an appropriate white standard, ST, whose reflectance is set to 1 (i.e., $K_{st} = 0$, according to Eq. [5]). Equation [10] for any mixture thus becomes

$$F(R_M) = \frac{(1 - R_M)^2}{2R_M} = \frac{C_p K_p + C_{st} K_{st}}{C_p S_p + C_{st} S_{st}} = \frac{C_p K_p}{C_p S_p + C_{st} S_{st}} \quad [12]$$

which can be expressed as

$$\frac{2R_M}{(1 - R_M)^2} = \frac{S_p}{K_p} + \frac{C_{st}}{C_p} \frac{S_{st}}{K_p} \quad [13]$$

By plotting the left-hand side of Eq. [10] against C_{st}/C_p for different mixtures, the slope of the regression line gives S_{st}/K_p and the intercept S_p/K_p . For simplicity, a value of $S_{st} = 1$ is usually adopted whereby relative rather than absolute values of S_p are obtained. In this way, Eq. [13] yields K_p and S_p at any wavelength.

One alternative analysis not suffering from some of the drawbacks of the K – M approach is based on the volume single-scattering albedo. According to Hapke (1981), the value of this variable for a multiminer surface, $\bar{w}_M$, is given by

$$\bar{w}_M = \frac{\sum (C_i w_i / \rho_i d_i)}{\sum (C_i / \rho_i d_i)} \quad [14]$$

where i denotes the i th component, C_i the mass fraction, ρ_i the density of the mineral, d_i the mean grain diameter, and w_i the single scattering albedo of the i th component.

Color Notation and Calculations

Torrent and Barrón (1993) provided a brief introduction to soil color and its laboratory measurement, and Wyszecki and Stiles (1982) supplied a comprehensive treatment of colorimetric concepts. Here, it suffices to say that the diffuse reflectance spectrum in the visible region allows one to numerically specify the color of a soil or mineral mixture.

Briefly, color notation is based on an empirical trichromatic generalization. In psychophysical terms, any color can be identified by only three color stimuli, a *color stimulus* being radiant energy of a given intensity and spectral composition entering the eye and producing a sensation of color. Therefore, any spectrum reaching the observer's eye can be converted into the so-called *tristimulus* values, designated X , Y , and Z . For this purpose, the so-called *standard observer*, that is, the "ideal" observer established by the Commission Internationale de l'Eclairage (CIE) in 1931, is considered. Tristimulus values result from the combination of the reflectance spectrum of the observed object with the spectrum of the illuminating light. X , Y , and Z must thus be defined with reference to a standard illuminant.

Color can be specified by using X , Y , and Z , or the *chromaticity coordinates*, $x = X/(X + Y + Z)$, $y = Y/(X + Y + Z)$, which, together with Y , define a region of well-defined limits or *color solid* in a three-dimensional space. Generally, other color spaces that are more uniform in psychophysical terms are used, the coordinates of which are calculated from X , Y , and Z (which are calculated, in turn, from the reflectance spectrum). Soil and other earth scientists use the part of the Munsell Color Space represented in Munsell Soil Color Charts (Munsell Color, 1975). The coordinates of this system are designated with the familiar terms *hue*, *value*, and *chroma*. One useful alternative system is the CIE 1976 ($L^*a^*b^*$) space (Commission Internationale de L'Eclairage, 1978), which is based on the coordinates lightness (L^*), redness–greenness ($+a^* \rightarrow -a^*$) and yellowness–blueness ($+b^* \rightarrow -b^*$).

METHODS

Apparatus

Diffuse reflectance measurements are usually made by using a UV-visible spectrophotometer equipped with a diffuse reflectance accessory (integrating sphere) capable of collecting the reflected flux. Currently, many high-performance, research spectrophotometers can be fitted with an integrating sphere/detector module, which usually replaces the cell

holders used for transmission measurements in the measuring compartment. Commercial sources¹ of these spectrophotometers include PerkinElmer Analytical Instruments (Norwalk, Connecticut), Shimadzu Scientific Instruments, Inc. (Columbia, Maryland), and Varian, Inc. (Palo Alto, California). Integrating spheres adaptable to different spectrophotometers are supplied by Labsphere (North Sutton, New Hampshire) and others.

An integrating sphere is essentially a hollow sphere internally coated with a white material of diffuse reflectance close to 1. The sphere has apertures through which a beam of radiant energy can penetrate and ports for mounting samples and standards and placing the appropriate detectors. Commercially available spheres range from 50 to 250 mm in diameter and are internally coated with highly diffusing polytetrafluoroethylene (PTFE) or barium sulfate (BaSO_4). Their performance increases with increasing diameter and decreasing port fraction. As a general rule, no more than 5% of the sphere surface area should be occupied by port openings.

As discussed above, the K - M and other forms of diffusive reflectance are strictly mathematical artifices with no direct physical meaning. In practice, however, reflectance measurements are performed under specific geometric conditions. For instance, under the specific hemispherical-directional reflectance conditions illustrated in Fig. 13-2a, reflectance is measured by illuminating the sample with diffuse light (generated by a beam of incident light impinging on a spot of the sphere surface), the angle between the normal to the sample surface and the axis of the viewing beam not exceeding 10° . Most commercial spectrophotometers measure directional-hemispherical reflectance, whereby the sample is illuminated by a beam whose axis does not depart by more than 10° from the normal to the sample and the reflected radiation collected by the sphere goes to the detector (Fig. 13-2b). Some instruments possess cylindrical (ring) integrating surfaces where measurements are made under the 45/0 or the 0/45 conditions, which are analogous to the diff/0 or 0/diff conditions illustrated in Fig. 13-2 but with the illuminating or the viewing beam at $45 \pm 5^\circ$ to the normal to the sample. Integrating spheres usually contain small baffles placed between the sample and the area of the sphere that is illuminated or viewed. These baffles prevent directly reflected light from superimposing on the illuminated sample or on the area of the surface that is being viewed.

Standards

The perfect reflecting diffuser, defined as the ideal uniform diffuser with reflectance equal to 1, does not exist in nature, as discussed above, so the white standards used in

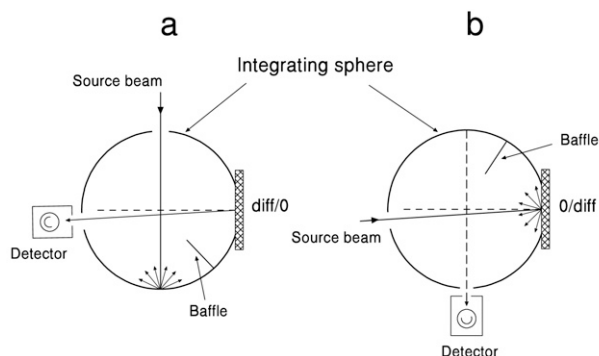


Fig. 13-2. Schematic diagrams showing (a) the diff/0, and (b) the 0/diff standard illuminating and viewing geometries in an integrating sphere.

¹ Reference to trade and company names is purely for the convenience of the reader and does not imply endorsement by the Universidad de Córdoba, nor that similar products may not be satisfactory.

diffuse reflectance measurements are secondary standards from which the reflectance is known by means of special calibration procedures (Wyszecki and Stiles, 1982).

Smoked magnesium oxide (MgO) was used as a standard for many years because its absolute reflectance in the visible range is close to 0.98. At present, barium sulfate and halon (PTFE) are considered suitable standards by the CIE. The Merck DIN 5033 barium sulfate powder standard (EM Industries Inc., Hawthorne, NY) has an absolute reflectance of 0.973 to 0.988 in the 380- to 750-nm wavelength range, and >0.95 in the 750- to 1500-nm wavelength range. When used as a spectrophotometric standard, it must be pressed to 2 g cm^{-3} (ASTM, 1973). Halon has a microcrystalline structure formed under pressure and excellent reflectance properties. Pressed tablets of halon, available under the trade name of Spectralon (Labsphere, North Sutton, NH) are washable and exhibit reflectance values >0.99 in the 400- to 1500-nm wavelength range, and >0.95 in the 250- to 2500-nm wavelength range.

Materials suitable for working standards include some opal glasses, ceramic plates, and various powder mixtures. However, they require periodic recalibration since dust, moisture, and exposure to radiation affect their optical properties. In practice, the use of barium sulfate as described below is convenient for most applications.

Sample Preparation

Diffuse reflectance spectra are significantly sensitive to the manner in which the soil or mineral mixture sample is prepared. There is no universal best procedure for this purpose. The operator must thus carefully consider the factors potentially influencing those features of the spectrum from which useful information is to be derived.

Particle size is the factor most strongly affecting reflectance, as shown by the occasionally dramatic changes in soil color upon grinding. For the reasons discussed above (mainly sample heterogeneity and regular reflection), the best results are obtained with small particle sizes, so it is generally advisable to grind the sample to a fine silt ($<10 \mu\text{m}$) size. For sandy samples, this may result in a dramatic increase in lightness and fading of colors due to coloring minerals, which are diluted in the mass of fine particles produced by grinding of sand. Despite this apparent drawback, most spectral features of interest are usually preserved after grinding.

The grinding procedure and intensity influence the spectrum for the end product. The fast and effective grinding provided by ball mills is not always advisable because, above a certain grinding energy, some minerals are transformed into others (e.g., goethite becomes hematite). Also, amorphous coatings may be formed on the surface of some minerals. Consistent results and preservation of the spectral features of interest can be achieved in most instances simply by grinding the sample in an agate mortar, so that minerals are in the volume-scattering region. Barrón and Torrent (1986) used a grinding time of 10 min in the agate mortar for samples of about 0.5 g; further grinding caused only minor changes in the spectral features of the samples.

Preferential orientation of particles of layer silicates, and occasionally other minerals (e.g., feldspars), must be avoided because it results in regular reflection, thus breaking the laws of diffuse reflection. For this reason, the soil powder is usually pressed against a rough surface such as frosted glass or unglazed paper. Dilution of the sample with a barium sulfate standard usually reduces preferential orientation to an acceptable minimum. On the other hand, preferential orientation can be advantageously used to detect low proportions of minerals with platy morphology (as is the case with oriented mounts in X-ray diffraction). This has scarcely been used in practice, however.

Moist soil samples have occasionally been used, particularly by pedologists interested in observing color changes upon moistening. Moist pastes can be prepared by adding the desired proportion of water to the soil sample according to the previously determined moisture content at different suction pressures. Moisture loss during measurement, which can be a major source of error, must be evaluated by measuring the sample spectrum at variable intervals. Bedidi et al. (1992) showed the diffuse reflectance spectrum for a soil to undergo nonlinear changes with increasing moisture content. Therefore, the spectral signatures of moist soils usually cannot be derived from those of their dry counterparts.

Sample Holders

Instrument manufacturers have provided a variety of sample holders that can be adapted to the sample ports of different integrating spheres or ring collectors. Many holders possess a cover glass to prevent loose powder from falling into, and damaging, the integrating sphere when sample ports are vertical or horizontally positioned on top of the sphere. Because cover glasses cause substantial qualitative deviations from the $K-M$ theory (Kortüm, 1969), only special reasons justify their use. Therefore, the only choice available with vertical ports is to use self-supporting pressed powder mounts. Preparation of these mounts requires that the powder be fine and that the area of the holder hole be relatively small. Rectangular or ovalated holes with a maximum size of 8 to 10 by 12 to 16 mm are usually suitable. The minimum size is logically the viewing area of the spectrophotometer, which is in the region of several tens of a square millimeter in most instruments. Fernandez and Schulze (1987) used Plexiglas 44 by 44 by 13 mm holders with a 20 by 8 mm hole with rounded ends to prevent samples from sticking in the corners and to facilitate cleaning. Sample holders with a hole of appropriate size and external dimensions for placement in the allocated space on the port area can be cut out from boards of various opaque plastic materials. Preferably, the surface of these boards should lack gloss, which can be ensured by abrading the surface with corundum powder.

Portable spectrophotometers allow the rapid measurement of the reflectance of unaltered soil surfaces or ground soil samples without the need for special preparation. However, they generally measure reflectance at relatively large wavelength steps. This restricts detailed band analysis and quantitative calculations.

Acquisition of Reflectance Spectra

Materials

- Agate mortar (60–70 mm diameter).
- Reference standards (disks of Spectralon or pressed barium sulfate).
- Standard barium sulfate powder (Merck DIN 5033 or similar).
- Two sample holders and a plunger adapted to the size of the holder hole.
- 5. 2 g of the soil or mineral sample ground to <2 mm or finer.

Procedure

Position the diffuse reflectance accessory in the sample compartment of the spectrophotometer and plug it in. Align the optical system according to the manufacture's operating instructions and let the instrument warm up for a few minutes. Set up the measuring program: wavelength range(s) of interest, mode (reflectance or absorbance), double beam, and scan speed.

When the instrument is ready, place one of the reference disks over the sample port and the other over the reference port. Alternatively, prepare two standards from barium sulfate powder. To do this, place the holder over a frosted plate glass or a flat surface covered with unglazed paper. Add the required amount of barium sulfate powder in several layers and distribute it uniformly in the hole. Press it firmly with the plunger to obtain the required packing density ($>2 \text{ g cm}^{-3}$) and a thickness $>3 \text{ mm}$. Remove the plunger and check that the barium sulfate surface is perfectly flat—this is required to minimize phase angle effects. Remove loose particles remaining on the surface with the aid of a gentle jet of filtered dry air before placing the holder over the instrument port.

Collect the baseline scan with the standard in place and replace the standard on the sample port with the soil sample. This must be prepared previously by grinding 2 g of the $<2\text{-mm}$ sample in the agate mortar for 3 min. Then, take a subsample of 200 to 500 mg (depending on the size of the holder hole) and grind it for 8 to 10 min, or until any apparent grittiness disappears and further grinding causes no apparent color change. Press the resulting powder into the holder hole, as for the barium sulfate powder, to a minimum thickness of 3 mm. Place the holder on the sample port, and record the spectrum.

Unless time for spectrum acquisition is limited, it is recommended for most purposes to obtain reflectance measurements in 0.5- to 1-nm steps for instruments with a wavelength bandwidth of 2 nm. This can take 10 to 20 min for the visible (380–750 nm) range, or 20 to 30 min for the visible-near IR range, in most spectrophotometers.

Comments

If barium sulfate is used as standard, it is advisable to prepare “fresh” standards before each set of measurements. The use of permanent disks saves time, but they tend to lose their reflectance properties with time and necessitate replacing (or washing and polishing).

Special care must be exercised to ensure stability of the powder mount in vertically placed sample holders with no cover glass.

Analysis and Parameterization of Reflectance Spectra

Data Storage

Modern spectrophotometers are computerized, so they allow reflectance data (and the results of various mathematical calculations on them) to be saved in suitable formats. ASCII files are convenient for most applications and can be easily transformed into the file types utilized by common software.

Color Calculations

The visible portion of the spectrum is used to calculate the tristimulus values by multiplying the reflectance values by the values of the so-called *color-matching functions*, which have been tabulated for different illuminants by Wyszecki and Stiles (1982). In the past this was done manually, now software provided by spectrophotometer manufacturers allows much faster calculation. Many software packages also provide conversion of the X , Y , and Z values to the Munsell and CIE 1976 ($L^*a^*b^*$) notations. These conversions can also be made with free software that can be downloaded from the Gretagmacbeth & Munsell Color's site (New Windsor, NY; www.gretagmacbeth.com/index/products/products_color-standards.htm [verified 26 Mar. 2007]).

Analysis of Absorption Bands

As stated above, the typical absorption spectrum for a soil sample can be approximated by the absorption coefficient, K , in the K - M function. To calculate K , Eq. [13] is used once the reflectances for several mixtures of the sample with a white standard have been obtained. One can use either the absorbance ($\log 1/R_\infty$) or the K - M function [i.e., $F(R_\infty) = (1 - R_\infty)^2/2R_\infty$] as a proxy for the typical absorption spectrum. Although both functions have been used to identify and characterize soil minerals, the latter is preferred for theoretical reasons.

K , absorbance, or K - M spectra can be deconvoluted by using Gaussian functions of the form:

$$\varphi(\lambda) = \sum_{i=1}^n h_i \exp \left[4 \ln(2) \left(\frac{\lambda - \lambda_i}{FWHH_i} \right)^2 \right] \quad [15]$$

where $\varphi(\lambda)$ is the spectral function; λ is wavelength (alternatively, wave number can be used); and h_i , λ_i , and $FWHH_i$ are the height, position and full width at half height of the i band, respectively. Scheinost et al. (1999b) successfully applied the Marquardt nonlinear regression method (SAS Institute, 1988) to spectra of absorbance against wavelength. Sunshine et al. (1990) found a modified Gaussian model better than the frequently used Gaussian model to deconvolute absorption bands in complex mixtures of pyroxenes and olivine.

Clark and Roush (1984) successfully used the concept of *continuum* to isolate particular absorption features for the analysis of spectra. The continuum is the “background absorption” onto which other absorption features are superimposed and represents either the absorption due to a different process in a specific mineral or absorption from a different mineral in a mixture. Continua can be modeled in terms of the mean optical path length through different minerals (or separated into different absorption processes) using the equation

$$\exp[-k\bar{l}] = \exp \left(- \sum_{i=1}^i k_i \bar{l}_i \right) \quad [16]$$

where k and $\bar{l}$ are the equivalent absorption coefficients and mean optical path lengths, respectively, for the surface as a whole, k_i is the absorption coefficient of the i th mineral (or particular absorption process) having a mean optical path length $\bar{l}_i$ for a multiminerale surface in an intimate mixture. Because of its definition, the continuum should be removed by subtraction from apparent absorbance and remission spectra but by division in reflectance spectra.

Direct resolution of the absorption spectra is hardly ever straightforward, so a number of ad hoc assumptions are frequently needed. In practice, different mathematical transformations of the spectra (or their proxies) make band resolution easier. The most widely used transformations are the first and, especially, second derivative. The first derivative of a Gaussian band has a maximum and a minimum to the left and right, respectively, of the maximum in the original band. The difference in ordinate between the maximum and minimum is proportional to the band amplitude. Unfortunately, when bands overlap, the wavelength at which the derivative is zero may be shifted relative to the true band position, or the derivative may not reach a zero value.

The second-derivative curve exhibits a minimum at the point of maximum curvature (i.e., the peak) in the original absorption band. The second-derivative curve provides more information than does the first-derivative curve because the original band, even when overlapped with other bands and not yielding a true absorption maximum (reflectance minimum), always yields a minimum in that curve. Figure 13–3 illustrates this important feature for a soil containing significant amounts of goethite and hematite as main pigments (and yielding bands in the visible region). Second-derivative diffuse reflectance spectroscopy has thus become a powerful tool for identifying and characterizing colored soil minerals (Kosmas et al., 1984, 1986; Scheinost et al., 1998).

Because spectra are acquired stepwise, they must usually be smoothed for calculation of their successive derivatives. Various algorithms are available for this purpose. The cubic spline fitting procedure (Press et al., 1992) involves joining end-to-end a number of cubic polynomial segments. Each segment is based on a certain number of adjacent data points, with the restriction that continuity in the first and second derivative must exist at the joints. The method of Savitzky and Golay (1964) uses least-squares methodology to fit a polynomial curve to a set of contiguous data points (usually from 13 to 31) and calculates the ordinate at the central value of the abscissa (wavelength). A new polynomial curve is then obtained by moving the set one point and calculating the new ordinate. For each polynomial, the slope at the central value of the abscissa gives the derivative. The number of

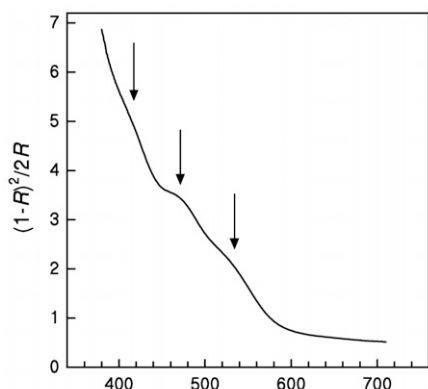
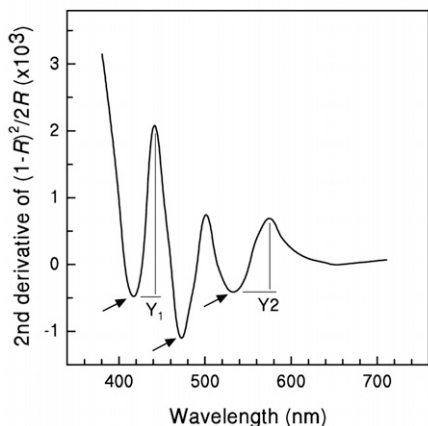


Fig. 13–3. (upper) Kubelka–Munk function spectrum and (lower) its second derivative for a Brazilian Oxisol containing hematite and goethite. The relatively inconspicuous absorption bands (marked with arrows) in the original spectra appear as strong minima in the second-derivative spectrum. The amplitudes of the bands between the minimum at ~415 nm and the maximum at ~445 nm (Y_1) and between the minimum at ~535 nm and the maximum ~580 nm (Y_2) were used in the quantitative analysis of these two minerals.



points in each set is a crucial input parameter in both procedures. Goodness of fit increases, but the degree of smoothing decreases (and resolution coarsens) with decreasing number of points, so a compromise must be adopted. Gálvez et al. (1999) found 22 points to provide the best results when applying a cubic spline procedure to spectra recorded in 0.5-nm steps. Software for smoothing and derivative calculations can usually be obtained from instrument manufacturers and used with little proficiency in mathematics.

APPLICATIONS

Identification of Mineral Species

Some common soil minerals can be identified by their characteristic bands in the absorbance or $K-M$ function spectra. Spectral libraries, such as that of the USGS (2003) provide reflectance spectra of the different minerals usually found in soils.

By far, iron oxides have been the most intensively examined soil minerals to date. This is not surprising because together with organic compounds, they constitute the most important soil pigments. They exhibit absorption bands in the UV to near IR wavelength range that are due to the following electronic transitions within the $3d^5$ shell of Fe^{3+} ion: (i) Fe^{3+} ligand field transitions, (ii) transitions due to magnetically coupled Fe^{3+} cations at adjacent sites, and (iii) ligand-to-metal charge transfer. On the basis of the ligand field theory, Sherman and Waite (1985) assigned three bands to the following ligand field transitions: ${}^6A_1({}^6S) \rightarrow {}^4T_1({}^4G)$, ${}^6A_1({}^6S) \rightarrow {}^4T_2({}^4G)$, and ${}^6A_1({}^6S) \rightarrow {}^4E, {}^4A_1({}^4G)$. A fourth band was assigned to the electron pair transition or ${}^6A_1({}^6S)$ double exciton $2({}^6A_1) \rightarrow 2[{}^4T_1({}^4G)]$. This double exciton process usually yields the strongest band, so it exerts a decisive influence on hue. Thus, goethite is yellow and hematite is red because the corresponding band lies at a shorter wavelength in the former (~480 nm) than in the latter (~535 nm).

Scheinost et al. (1998) used the second derivative of the $K-M$ function to examine the intensity of these four bands for 176 synthetic and natural Fe oxide samples. They found significant differences in band position and intensity among the eight minerals studied and also among different samples of each mineral. These differences are the result of (i) $Fe(O, OH)_6$ octahedra being linked in different ways, (ii) distortion of the octahedra decreasing symmetry and hence altering the ligand field and band positions, and (iii) differences in the Fe-to-Fe distance and in the degree of occupancy of octahedra likely affecting magnetic coupling of neighboring Fe^{3+} ions and hence the position and intensity of the double exciton band. The combined effect of these factors results in substantial overlap of the bands for different oxides, as shown in Fig. 13–4. Thus, Scheinost et al. (1998) found that (i) pure samples of goethite, maghemite, and schwertmannite could not be discriminated; (ii) at least 80% of pure akagenéite, ferroxhyte, ferrihydrite, hematite, and lepidocrocite samples were correctly classed by using discriminant functions; and (iii) in soil mixtures containing other Fe oxides, only hematite and magnetite could be discriminated from other Fe oxides. Band overlap can thus hinder Fe oxide identification by diffuse reflectance spectroscopy. For instance, this problem, together with poor spectral resolution, has precluded positive identification of Fe oxides in Martian soils and dust (Bell et al., 2000).

Detection limits in second-derivative diffuse reflectance spectroscopy are typically lower than in alternative identification techniques for Fe oxides. Thus, <0.1% goethite and hematite can be detected in mixtures with other soil minerals (Scheinost et al., 1998), and the same applies to Ti oxides (Malengreau et al., 1995). This proportion is more than one order of magnitude smaller than the detection limit of ordinary X-ray diffraction. Low detection limits are also provided by first-derivative spectroscopy (Deaton and Balsam, 1991).

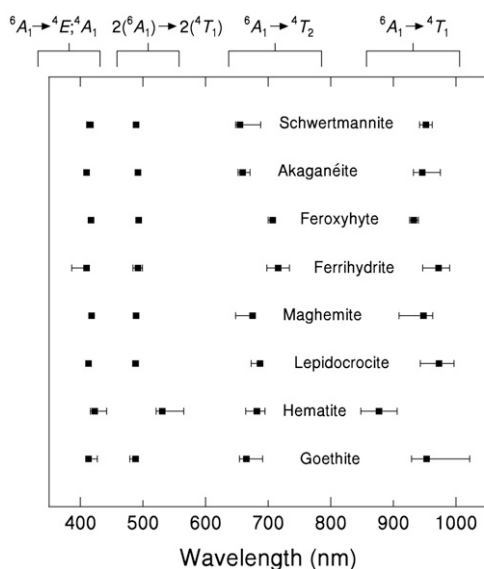


Fig. 13-4. Median and range of the crystal field band positions as determined from second-derivative minima. Adapted from Scheinost et al. (1998).

Elucidation of Crystal Properties

The decisive influence of crystal parameters on the position and intensity of the absorption bands of many minerals is well documented (Burns, 1993). Comparatively little research has been conducted on soil minerals in this respect, however. As a result, much available information has been derived from either natural or synthetic pure mineral specimens.

The spectral features of the different minerals have usually been interpreted in light of the crystal field and ligand field theories. Specifically, differences among spectra for Fe minerals have been ascribed to distortions in $\text{Fe}(\text{O}, \text{OH})_6$ octahedra or to differences in octahedral linkages, which alter the ligand field. Substitution of Al for Fe is a common source of such differences. For instance, the ${}^6A_1 \rightarrow {}^4T_1$ transition of synthetic goethite at ~950 nm shifts to a lower energy (longer wavelength) when Al enters its structure (Buckingham and Sommer, 1983; Scheinost et al., 1999b). This was ascribed to the decrease in Fe–(O, OH) distances caused by Al substitution, as also observed in synthetic hematite by Morris et al. (1992). Scheinost et al. (1999b) found Fe–(O, OH) distances to change by 0.2 pm, which, as noted by these authors, is one order of magnitude smaller than the sensitivity of extended X-ray absorption fine structure (EXAFS) spectroscopy. On the other hand, band positions and the degree of Al substitution in populations of natural minerals are not always related (Malengreau et al., 1997).

One other example of the use of diffuse reflectance spectroscopy to elucidate the structural properties of Fe oxides was provided by Gálvez et al. (1999), who worked with synthetic hematite prepared in the presence of phosphate. The presence of structural P in these hematites had been inferred from X-ray diffraction, IR spectroscopy, and acid dissolution studies. Diffuse reflectance spectra exhibited an absorption band typical of goethite (~488 nm) in addition to the typical hematite band (~538 nm). The “goethitic” component in hematite was proportional to the P content in hematite and was not observed in pure hematite with surface adsorbed phosphate. The previous evidence supports the hypothesis

that P is structural and probably occupies tetrahedral sites, which results in deficiency in the cationic substructure.

Isomorphous substitution by transition element cations (Co, Cr, Ni, Mn, V) in Fe oxides and other minerals markedly alters diffuse reflectance spectra, which provides valuable geochemical information. Thus, Scheinost (2000) reported the presence of several additional bands in the spectrum for goethite substituted by these elements due to M^{3+} crystal field transitions and M-to-Fe charge transfer. However, such effects are unlikely to be observed in soils unless they contain a high content in Fe oxides.

Diffuse reflectance spectroscopy has also been used to characterize nanometric surface precipitates. Thus, Scheinost et al. (1999a) studied secondary Ni precipitates on various silicates and gibbsite, and found the band for the $^3A_{2g} \rightarrow ^3T_{1g}$ transition to be shifted to a shorter wavelength when the host mineral contained Al. This suggested that Al was dissolved from the sorbent and substituted for Ni in the brucite-like hydroxide layers of the precipitating phase, thus decreasing Ni–O distances and raising the crystal-field splitting energy.

Quantitative Analysis

Equation [11] is generally useful for quantitative analysis. Based on it, at any wavelength, the K – M function for a mixture of a small amount of a colored mineral in a matrix of white or colorless soil minerals (i.e., with very low K values) is proportional to, and depends almost exclusively on, the concentration of that mineral provided S is constant. Usually, S differs little among matrices of white or colorless soil minerals with a low percentage of the colored mineral. With higher concentrations, constancy of S can reasonably be approached in practice by diluting the sample with a “white” mineral or a white standard. If several colored minerals are present, then the K – M function is proportional to their K values as weighed according to their concentrations.

Quantitative analysis by Eq. [11] of even very small amounts of colored minerals can be accurate provided the K and S values for the colored minerals in the samples concerned are known. This is rarely the case, as it calls for an effective method to separate each mineral from the matrix and examine it separately. K and S are obtained by applying Eq. [13] to mineral–white standard mixtures. More often, the K and S values for external standards must be adopted. One is thus confronted with the fact that K and S (and color) for most minerals range widely as a function of their size, shape, and surface and crystal properties. This has been observed, for instance, in synthetic hematites and goethites (Barrón and Torrent, 1986), and in various natural and synthetic Fe oxides (Scheinost et al., 1998; Scheinost and Schwertmann, 1999). For mere comparison of samples from soils not differing markedly in properties, one can adopt average K and S values. Thus, Barrón and Torrent (1986) used a wide population of soils to obtain such average values for “soil” goethite and hematite.

Quantitative analysis can be performed by “inverting” Eq. [14] if the values of ρ_p , d_p , and w_i for each i th mineral are known (Clark, 1999). Quantitative analyses are also performed by measuring the intensity of the characteristic bands for each mineral in the K – M spectral curve or in the second-derivative curve. Thus, Scheinost et al. (1998) found the amplitudes of the typical goethite and hematite bands in the second-derivative curve to be proportional to the contents of these minerals when mixed with a deferrated matrix in a proportion less than about 2%. Increasing the proportion of any of these minerals beyond this level resulted in a less-than-proportional increase in band amplitude. As noted above, this saturation problem can be mitigated by diluting the sample with a white standard.

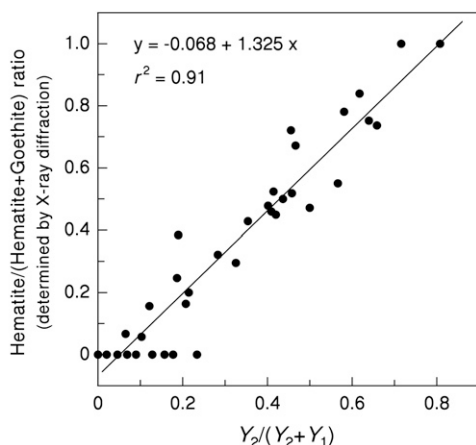


Fig. 13-5. Relationship between the hematite/(hematite + goethite) ratio as determined by differential X-ray diffraction and the ratio $Y_2/(Y_2 + Y_1)$ (Fig. 3) as determined by diffuse reflectance spectroscopy. Y_1 is the amplitude of the band between the minimum at ~415 nm and the maximum at ~445 nm, and Y_2 is the amplitude of the band between the minimum at ~535 nm and the maximum ~580 nm. Adapted from Scheinost et al. (1998).

Matrix effects on quantitative analyses make it advisable to estimate relative rather than absolute amounts of colored minerals in soils and mineral mixtures, provided the minerals exhibit characteristic, essentially nonoverlapping bands. For instance, many soils and sediments contain significant amounts of goethite and hematite as the only Fe oxides. On the basis of this, Scheinost et al. (1998) used the intensity of the second-derivative curve band between the ~415-nm minimum and the 445-nm maximum for goethite (denoted by Y_1 in Fig. 13-3), and that between the ~535-nm minimum and the 580-nm maximum for hematite (denoted by Y_2 in Fig. 13-3) to estimate the hematite/goethite ratio. Significant correlation was thus found between the hematite/(hematite + goethite) ratio and the $Y_2/(Y_1 + Y_2)$ ratio for a population of 40 soils with a wide range of properties (Fig. 13-5). Indeed, one can obtain the absolute proportions of two minerals for which the content ratio is known provided a method for determining the sum of the two contents exists. With goethite and hematite, the difference between the amounts of Fe extracted by the dithionite/citrate/bicarbonate mixture (Mehra and Jackson, 1960) and acid oxalate (Schwertmann, 1964) can be assigned to total Fe in both minerals.

Simple indices based on the various color notation systems have been used for more than 20 yr to estimate hematite content in soils, starting with the “redness rating” proposed by Torrent et al. (1980). The reader is referred to Torrent and Barrón (1993) for a description of such indices and their use. The indices have the advantage that color notation can be obtained either visually or with the aid of a tristimulus colorimeter, with no need to use a spectrophotometer to record the diffuse reflectance spectrum.

Acknowledgments

The authors acknowledge the financial support from Spain’s CICYT within the framework of Project PB98-1015. Dr. A. Scheinost [European Synchrotron Radiation Facility (ESRF)] kindly revised an earlier draft of the manuscript.

REFERENCES

- ASTM. 1973. Preparation of reflectance white standards. ASTM Standard E 259-66 (reapproved 1973). American Society for Testing and Materials, Philadelphia, PA.
- Barrón, V., and J. Torrent. 1986. Use of the Kubelka–Munk theory to study the influence of iron oxides on soil colour. *J. Soil Sci.* 37:499–510.
- Bedidi, A., B. Cervelle, J. Madeira, and M. Pouget. 1992. Moisture effects on visible spectral characteristics of lateritic soils. *Soil Sci.* 153:129–141.

- Bell, J.F.I., H.Y.J. McSween, J.A. Crisp, R.V. Morris, S.L. Murchie, N.T. Bridges, J.R. Johnson, D.T. Britt, M.P. Golombek, H.J. Moore, A. Ghosh, J.L. Bishop, R.C. Anderson, J. Brückner, T. Economou, J.P. Greenwood, H.P. Gunnlaugsson, R.M. Hargraves, S. Hviid, J.M. Knudsen, M.B. Madsen, R. Reid, R. Rieder, and L. Soderblom. 2000. Mineralogical and compositional properties of Martian soil and dust: Results from Mars Pathfinder. *J. Geophys. Res.* 105:1721–1755.
- Buckingham, W.F., and S.E. Sommer. 1983. Mineralogical characterization of rock surfaces formed by hydrothermal alteration and weathering—Application to remote sensing. *Econ. Geol.* 78:664–674.
- Burns, R.G. 1993. Mineralogical applications of crystal field theory. 2nd ed. Cambridge Univ. Press, Cambridge, UK.
- Commission Internationale de L'Eclairage. 1978. Recommendations on uniform color spaces, color-difference equations, psychometric color terms. Suppl. 2 to Publ. 15, Colorimetry. Bureau Central de la CIE, Paris.
- Clark, R.N. 1999. Spectroscopy of rocks and minerals and principles of spectroscopy. p. 3–58. *In* A.N. Renz (ed.) Manual of remote sensing. John Wiley & Sons, New York.
- Clark, R.N., and T. Roush. 1984. Reflectance spectroscopy: Quantitative analysis techniques for remote sensing applications. *J. Geophys. Res.* 89:6329–6340.
- Deaton, B.C., and W.L. Balsam. 1991. Visible spectroscopy—A rapid method for determining hematite and goethite concentration in geological materials. *J. Sed. Pet.* 61:628–632.
- Duncan, D.R. 1940. The colour of pigment mixtures. *Proc. Phys. Soc.* 52:390–401.
- Duncan, D.R. 1949. The colour of pigment mixtures. *J. Oil Colour Chem. Assoc.* 32:296–391.
- Escadafal, R. 1989. Caractérisation de la surface des sols arides par observations de terrain et par télédétection. Editions de l'ORSTOM, Collections Études et Thèses, Paris.
- Fernandez, R.N., and D.G. Schulze. 1987. Calculation of soil color from reflectance spectra. *Soil Sci. Soc. Am. J.* 51:1277–1282.
- Gálvez, N., V. Barrón, and J. Torrent. 1999. Preparation and properties of hematite with structural phosphorus. *Clays Clay Miner.* 47:375–385.
- Hapke, B. 1981. Bidirectional reflectance spectroscopy. 1. Theory. *J. Geophys. Res.* 86:3039–3054.
- Hapke, B. 1993. Introduction to the theory of reflectance and emittance spectroscopy. Cambridge Univ. Press, New York.
- Johnston, C.T., and Y.O. Aochi. 1996. Fourier transform infrared and Raman spectroscopy. p. 269–322. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. ASA and SSSA, Madison, WI.
- Kortüm, G. 1969. Reflectance spectroscopy. Springer-Verlag, Berlin.
- Kosmas, C.S., N. Curi, R.B. Bryant, and D.P. Franzmeier. 1984. Characterization of iron oxide minerals by second-derivative visible spectroscopy. *Soil Sci. Soc. Am. J.* 48:401–405.
- Kosmas, C.S., D.P. Franzmeier, and D.G. Schulze. 1986. Relationships among derivative spectroscopy, color, crystallite dimensions, and Al substitution of synthetic goethites and hematites. *Clays Clay Miner.* 34:625–634.
- Kubelka, P. 1948. New contributions to the optics of intensely light-scattering materials. Part I. *J. Opt. Soc. Am.* 38:448–460.
- Kubelka, P., and F. Munk. 1931. Ein Beitrag zur Optik der Farbanstriche. *Z. Technol. Phys.* 12:593–620.
- Malengreau, N., J.-P. Muller, and G. Calas. 1995. Spectroscopic approach for investigating the status and mobility of Ti in kaolinitic minerals. *Clays Clay Miner.* 43:615–621.
- Malengreau, N., P.G. Weidler, and A.U. Gehring. 1997. Iron oxides in laterites—A combined mineralogical, magnetic, and diffuse reflectance study. *Schweiz. Mineral. Petrogr. Mitt.* 77:13–20.
- Mehra, P.O., and M.L. Jackson. 1960. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. *Clays Clay Miner.* 7:317–327.
- Morris, R.V., D.G. Schulze, H.V. Lauer, D.G. Agresti, and T.D. Shaffer. 1992. Reflectivity (visible and near IR), Mössbauer, static magnetic, and X-ray diffraction properties of Al-substituted hematites. *J. Geophys. Res.* 97:10257–10266.
- Munsell Color. 1975. Munsell soil color charts. Munsell Color, Baltimore, MD.
- Press, W.H., S.A. Teukolsky, W.T. Vetterling, and B.P. Flannery. 1992. Numerical recipes in Fortran. 2nd ed. Cambridge Univ. Press, Cambridge, UK.
- SAS Institute. 1988. SAS/STAT user's guide. SAS Inst., Cary, NC.
- Savitzky, A., and M.J.E. Golay. 1964. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* 36:1627–1640.
- Scheinost, A.C. 2000. Color. p. 27–42. *In* U. Schwertmann and R.M. Cornell (ed.) Iron oxides in the laboratory. Wiley-VCH, Weinheim, Germany.
- Scheinost, A.C., A. Chavernas, V. Barrón, and J. Torrent. 1998. Use and limitations of second-derivative diffuse reflectance spectroscopy in the visible to near-infrared range to identify and quantify Fe oxides in soils. *Clays Clay Miner.* 46:528–536.

- Scheinost, A.C., R.G. Ford, and D.L. Sparks. 1999a. The role of Al in the formation of secondary Ni precipitates on pyrophyllite, gibbsite, talc, and amorphous silica—A DRS study. *Geochim. Cosmochim. Acta* 63:3193–3203.
- Scheinost, A.C., D.G. Schulze, and U. Schwertmann. 1999b. Diffuse reflectance spectra of Al substituted goethite: A ligand field approach. *Clays Clay Miner.* 47:156–164.
- Scheinost, A.C., and U. Schwertmann. 1999. Color identification of iron oxides and hydroxysulfates: Use and limitations. *Soil Sci. Soc. Am. J.* 63:1463–1471.
- Schwertmann, U. 1964. Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat-Lösung. *Z. Pflanzenernähr. Düng. Bodenkd.* 105:194–202.
- Sherman, D.M., and T.D. Waite. 1985. Electronic spectra of Fe^{3+} oxides and oxide hydroxides in the near IR to near UV. *Am. Mineral.* 70:1262–1269.
- Simonson, R.W. 1993. Soil color standards and terms for field use—History of their development. p. 1–20. *In* J.M. Bigham and E.J. Ciolkosz (ed.) *Soil color*. SSSA Spec. Publ. 31. SSSA, Madison, WI.
- Sunshine, J.M., C.M. Pieters, and S.F. Pratt. 1990. Deconvolution of mineral absorption bands: An improved approach. *J. Geophys. Res.* 95:6955–6966.
- Torrent, J., and V. Barrón. 1993. Laboratory measurement of soil color: Theory and practice. p. 21–33. *In* J.M. Bigham and E.J. Ciolkosz (ed.) *Soil color*. SSSA Spec. Publ. 31. SSSA, Madison, WI.
- Torrent, J., U. Schwertmann, and D.G. Schulze. 1980. Iron oxide mineralogy of some soils of two river terrace sequences in Spain. *Geoderma* 23:191–208.
- USGS. 2003. Digital spectral library. Version 4: 0.2 to 3.0 microns. Available at <http://speclab.cr.usgs.gov/spectral.lib04/spectral-lib04.html> (accessed 12 Sept. 2003, verified 23 Mar. 2006).
- Wendlandt, W.W., and H.G. Hecht. 1966. *Reflectance spectroscopy*. John Wiley & Sons, New York.
- Wyszecki, G., and W.S. Stiles. 1982. *Color science: Concepts and methods, quantitative data and formulae*. 2nd ed. John Wiley & Sons, New York.

Analysis of Soils and Minerals Using X-ray Absorption Spectroscopy

S. D. KELLY, Argonne National Laboratory, Argonne, Illinois

D. HESTERBERG, North Carolina State University, Raleigh, North Carolina

B. RAVEL, Argonne National Laboratory, Argonne, Illinois, now at National
Institute of Standards and Technology, Gaithersburg, Maryland

X-ray absorption spectroscopy (XAS) has been applied to numerous problems in soil science, mineralogy, and geochemistry. X-ray absorption spectroscopy was developed in the early 1970s (Sayers et al., 1971) and is widely used at synchrotron radiation facilities. Regardless of the complexity of the sample, the XAS signal comes from all of the atoms of a single element as selected by the X-ray energy. High-quality XAS spectra can be collected on heterogeneous mixtures of gases, liquids, and/or solids with little or no sample pretreatments, making it ideally suited for soils and many other systems. The structural information obtained from XAS is useful for identifying the chemical speciation of an element, including mineral, noncrystalline solid, or adsorbed phases. With the addition of X-ray focusing, samples can be interrogated on length scales comparable to or smaller than some level of natural heterogeneity, thus making it possible to study differences in the atomic environment of an element within or between individual particles and grain boundaries.

The acronym XAS covers both X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopies. X-ray absorption near edge structure can be used to determine the valence state and coordination geometry, while EXAFS can be used to determine the local molecular structure of a particular element within a sample. Micrometer-length scale X-ray measurements are designated μ -EXAFS and μ -XANES. Another variation to the XAS technique utilizes the natural linear polarization of synchrotron X-rays. The use of polarized X-rays allows the atomic environment of the absorber to be probed in the polarization direction and is particularly suited for minerals with layer-type structures such as phyllosilicates and manganese oxides (Manceau et al., 1999).

The nature of a complex sample is best revealed by the application of several different experimental techniques, with each individual measurement providing both unique and complementary information. The most common technique for characterizing abundant soil minerals is X-ray diffraction (XRD), which relies on long range ordering of atomic planes to probe crystalline structure at a length scale of approximately 50 Å or more. X-ray absorption spectroscopy probes the immediate environment of the selected element, within about 6 Å, and its theory and interpretation does not rely on any assumption of symmetry or periodicity. While both XRD and XAS can be used to determine distances between atoms, the information is derived from two very different X-ray interactions with the sample. For most systems the application of XRD and XAS is complementary.

Another complementary technique to both XAS and XRD is X-ray fluorescence (XRF). Since each element fluoresces at a unique energy, the XRF spectrum can be used to determine the elemental distribution within a sample. The results are useful for interpreting XANES and EXAFS data by identifying the elements that are associated with (and possibly coordinated to) the element of interest. A combination of μ -XRF and μ -XAS has been used to characterize geochemical matrices, the association of minerals with plant roots, and to study mineral formation and transformation processes in complex matrices (e.g., Bertsch et al., 1994; Schulze et al., 1995; Tokunaga et al., 1998; Duff et al., 1999; Osán et al., 1997; Niemeyer and Thieme, 1999; Myneni, 2002; Kelly et al., 2006). A combination of spatially resolved μ -XRF, μ -XRD, and μ -EXAFS has been used to determine the coordination environment of Ni within Mn nodules (Manceau et al., 2002).

There are several sources for additional information on XAS and its application to soils, minerals, and other geochemical matrices. Volume 49 of *Reviews in Mineralogy and Geochemistry* (Fenter et al., 2002) contains an excellent set of comprehensive review chapters on XAS applications, synchrotron facilities, and specialized techniques involving synchrotron X-rays. Also, a number of earlier review papers and book sections describe techniques and applications of XAS in geochemistry and soil science (Brown, 1990; Manceau et al., 1992; Fendorf et al., 1994; Schulze and Bertsch, 1995; Brown and Parks, 2001; Bertsch and Hunter, 2001; Brown and Sturchio, 2002). The principles of X-ray absorption fine structure spectroscopy and data analysis were described by Stern (1978), Sayers and Bunker (1988), Fendorf and Sparks (1996), and Fendorf (1999), while more details on the physics of XAS appear in several books (Stern and Heald, 1983; Koningsberger and Prins, 1988; Teo, 1986; Stöhr, 1992). This chapter focuses mainly on the basic principles and methods of XANES and EXAFS spectroscopy of soils, minerals, and mineral-associated (e.g., adsorbed or coprecipitated) chemical species. Emphasis is placed on sample preparation, data collection, and data analysis.

PRINCIPLES OF X-RAY ABSORPTION SPECTROSCOPY

The principles and terminology used in XAS are based on the interactions of X-rays with matter. This section begins with a description of some of the properties of atoms, X-rays, X-ray scattering, X-ray absorption, and X-ray absorption spectra.

Atoms

An atom contains a dense nucleus of positively charged protons and uncharged neutrons (Fig. 14–1) surrounded by negatively charged electrons. The atomic number Z associated with an element in the periodic table corresponds to the number of protons. A neutral atom has an equal number of protons and electrons. Atoms have sets of orbitals that

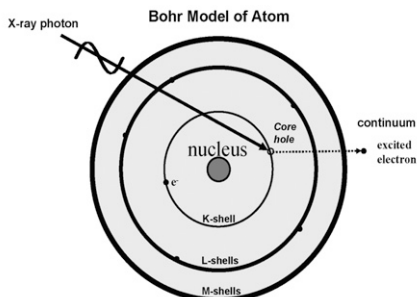


Fig. 14–1. Bohr model of an atom.

can be populated with electrons. The electrons occupy the orbital closest to the nucleus first because this orbital has the lowest energy. Then the other orbitals are populated in order of increasing energy. The energy required to remove an electron from an atom is the *electron binding energy*. The binding energy of an electron in a lower-energy orbital that is closer to the nucleus is greater than the binding energy of an electron in a higher-energy orbital that is farther from the nucleus because the inner electrons screen the positively charged nucleus from the outer electrons.

There are generally two groups of electrons associated with an atom. The loosely bound “valence” electrons occupy the outermost orbitals and participate in chemical bonding, and the tightly bound “core” electrons occupy the innermost orbitals that are an integral part of the atom. Different oxidation states of an atom are determined by charge imbalances caused by the removal of the outermost electrons. For example, Cr(0), Cr(III), and Cr(VI) have lost zero, three, and six electrons, respectively, and Cr(VI) is in a higher oxidation state than Cr(III) or Cr(0).

X-rays

X-rays belong to a class of particles termed *photons*, which are electromagnetic radiation. Photons in different energy ranges are given different names, such as radio and television waves, microwaves, infrared light, visible light, ultraviolet light, X-rays, and gamma rays, as illustrated in Fig. 14–2. Photons are packets of energy that can be described as having both wave-like and particle-like properties. The X-ray is characterized by its energy, expressed in electronvolts (eV). The energy is inversely proportional to wavelength: $E = hc/\lambda$, where h is Planck’s constant ($\sim 4.14 \times 10^{-18}$ keV Hz⁻¹) and c is the speed of light ($\sim 3 \times 10^8$ m s⁻¹), and the wavelength is inversely proportional to the frequency (f): $\lambda = c/f$.

X-ray Scattering

Compton and Rayleigh scattering are due to X-rays interacting with electrons. Compton scattering is an inelastic process because the X-ray loses energy, causing its trajectory to change. Rayleigh scattering is an elastic process in which the oscillating electric field associated with the X-rays induces oscillations in the electron. Because the electrons of an atom are accelerated at the same frequency as the incoming X-ray wave, X-rays of the same wavelength are emitted. Atoms with more electrons have stronger elastic X-rays scattering (Cullity, 1978).

X-ray Absorption

X-ray absorption occurs when an atom acquires all the energy of an X-ray, which is used to excite electrons into higher energy electron orbitals that are unoccupied, or into the continuum where the electron is no longer associated with the atom (Fig. 14–1). The Fermi energy is defined as the energy of the lowest accessible electron orbital of the atom. The excited electrons are termed *photoelectrons* because the absorbed X-ray is a photon. The empty electron orbital is called a hole and the excitation of core electrons creates core

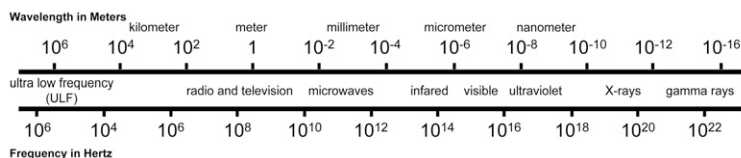


Fig. 14–2. Spectrum of photon energy in wavelength and in frequency with the common names.

holes. A relaxation process occurs with the release of energy as an electron transitions from a higher-energy electron orbital to fill the core hole. In the next subsections, we discuss the properties of these electronic transitions in more detail as a prelude to subsequent sections outlining the measurement and interpretation of X-ray absorption spectra.

Electron Transitions

The energy state of an electron is defined by quantum numbers: the principal ($n = 1, 2, 3, \dots$), the azimuthal ($\ell = 0, 1, 2, \dots, n - 1$), and the total angular momentum ($j = \ell + s$), which depends on spin ($s = +1/2$ or $-1/2$). Electrons populate these states by filling the lowest energy states first, starting with principle quantum number $n = 1$. The principal quantum number defines the electronic shell designated by the letters K-shell ($n = 1$), L-shell ($n = 2$), M-shell ($n = 3$), and so forth, as shown in Fig. 14–1. The azimuthal quantum numbers $\ell = 0, 1, 2, 3$, up to $n - 1$ correspond to the letters s, p, d, f and are used to define the subshell of the electron. An electronic subshell is designated by $n(\text{number})/(\text{letter})$. For example, the K shell ($n = 1$) has a single subshell denoted 1s ($n = 1, \ell = 0$). The L shell ($n = 2$) can contain the 2s ($n = 2, \ell = 0$), 2p_{1/2} ($n = 2, \ell = 1, s = -1/2, j = 1/2$), and 2p_{3/2} ($n = 2, \ell = 1, s = 1/2, j = 3/2$) subshells corresponding to the L_I, L_{II}, and L_{III} subshells, respectively. The M shell ($n = 3$) can contain the 3s, 3p_{1/2}, 3p_{3/2}, and 3d_{3/2} and 3d_{5/2} subshells corresponding to the M_I, M_{II}, M_{III}, M_{IV}, and M_V subshells, respectively.

Electronic transitions due to X-ray absorption are restricted by the dipole selection rule such that angular momentum is conserved. This rule states that transitions can occur only between energy states that differ in the azimuthal quantum number (ℓ) by ± 1 . For example, transitions from a p orbital ($\ell = 1$) to an s orbital ($\ell = 0$) and vice-versa are allowed, but a transition from a 2s orbital ($n = 2, \ell = 0$) to a 1s orbital ($n = 1, \ell = 0$) is not allowed.

Relaxation Processes

The promotion of an electron into a higher energy state by X-ray absorption is short lived. Within approximately a femto-second (10^{-15} s), the core hole is filled by an electron that transitions from a higher energy orbital. The transition is accompanied by a release of energy. The energy released can be in the form of fluorescence radiation, Auger electron production, or secondary electron or photon production (Teo, 1986).

X-ray fluorescence occurs when an electron from a higher-energy orbital fills the core hole by releasing an X-ray. The energy of the fluorescent X-ray is equal to the difference in the energy of the two orbitals. Because the electron orbital energies of each element are unique, the fluorescence X-ray energies for a given electronic transition will be unique for each element.

Auger electron production occurs when an electron from a higher-energy orbital fills a core hole, losing its energy by the emission of another electron from the same or a different atomic shell. Other relaxation processes include secondary electron or photon production, resulting from multiple steps of electrons cascading downward in energy as successive core holes are filled (L shell $\rightarrow$ K shell, M-shell $\rightarrow$ L-shell, etc.), with one of these multi-step processes emitting an electron or photon.

X-ray Absorption Coefficient

The number of X-rays transmitted (I_t) through a sample is given by the intensity of X-rays impinging on the sample (I_0) decreased exponentially by the thickness of the sample (x) and the absorption coefficient of the sample (μ)

$$I_t = I_0 e^{-\mu x} \quad [1]$$

The absorption of X-rays by a material is designated by the percentage decrease in the incident X-ray intensity (I_0) or by the energy-dependent absorption length of the material that is the exponential factor, μx . From Eq. [1] it is apparent that as twice as many X-rays are shined on a sample, twice as many X-rays will go through the sample. This affect is linear. Also, a thicker sample will transmit fewer X-rays than a thin sample. This effect is exponential, such that increasing the thickness of the sample by one absorption length decreases the transmitted X-ray intensity by ~63% ($100\% \times 1 - e^{-1}$). The absorption coefficient is a property of all elements within the sample. Elements such as Pb have greater X-ray absorption coefficients than lighter elements such as O and are therefore used for efficient X-ray shielding. In radiography, an X-ray beam is impinged on the human body. The more-dense bones containing higher-Z elements like Ca absorb more X-rays than the less-dense flesh containing mainly lower-Z elements like C, H, and O. The result is a contrast image on X-ray sensitive film placed behind the subject.

The X-ray absorption coefficient (μ) is the probability for an X-ray to be absorbed by a sample. X-ray absorption spectroscopy involves measuring μ as a function of X-ray energy. A typical experimental setup for XAS is shown in Fig. 14-3A. The X-rays go through an ionization chamber to measure the number of incident X-rays (I_0), then through the sample, and then through another ionization chamber to measure the number of transmitted X-rays (I_t). The X-ray absorption coefficient is determined by rearranging Eq. [1]:

$$\mu x = \ln \left(\frac{I_0}{I_t} \right) \quad [2a]$$

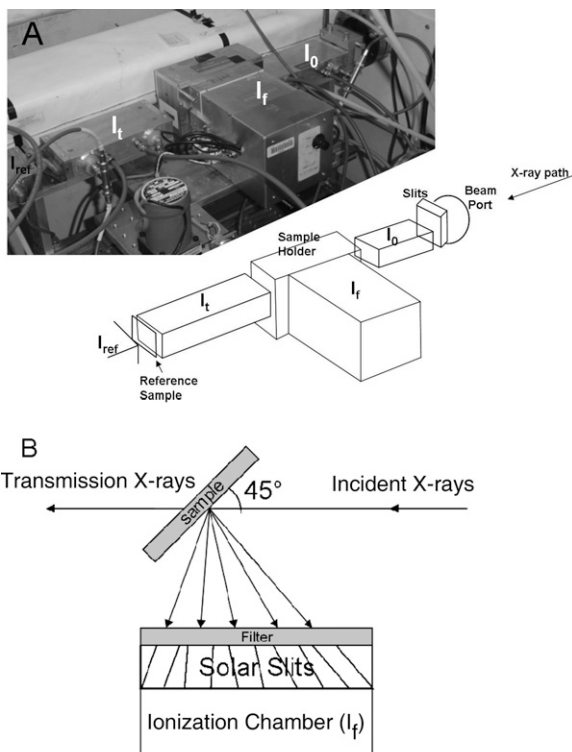


Fig. 14-3. (A) Picture and schematic drawing of an EXAFS experimental setup. (B) Fluorescence sample geometry and detector.

Because core hole production and consequent relaxation processes are proportional to absorption, the absorption coefficient can also be derived from the number of fluorescence X-rays (I_f):

$$\mu x \propto \frac{I_f}{I_0} \quad [2b]$$

The absorption coefficient (μ) is a function of the incident X-ray energy. The probability for absorption increases sharply when the incident X-ray energy equals the energy required to excite an electron to an unoccupied electron orbital. These steps in the absorption coefficient are termed *absorption edges*.

Absorption Edges

Figure 14-4 gives an example of an X-ray absorption edge (for NiO). Each element has a specific set of absorption edges at the binding energies of its electrons. The absorption edge shown in Fig. 14-4 is commonly described as the Ni K-edge XAS spectrum from NiO.

Figure 14-5 shows energies of K-edges or L_{III} -edges for the elements given by their atomic number (Z). Some elements commonly found in the soil matrices or as contaminants have been explicitly noted. The energy of a specific edge increases as the atomic

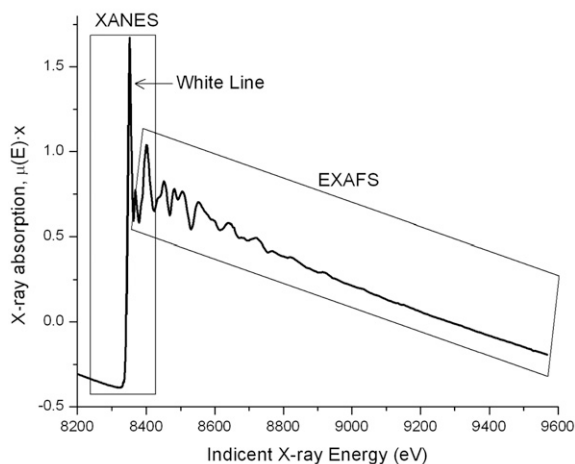


Fig. 14-4. The X-ray absorption spectrum of NiO with the XANES region, EXAFS region, and white line noted.

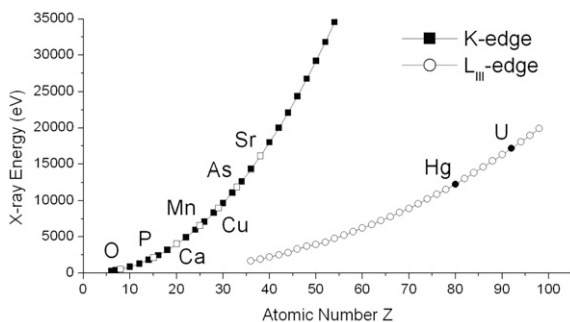


Fig. 14-5. X-ray energy for the K-edge and/or L_{III} -edge for the elements designated by atomic number Z . The K-edge starts at C and ends at Xe and the L_{III} -edge starts at Kr and ends at Cf. Many soft X-ray beamlines are capable of X-ray energies from 100 to 5000 eV and many hard X-ray beamlines are capable of X-ray energies from 5000 to 35,000 eV or higher. Several elements of interest to mineralogist have been explicitly noted. The transition metals like Mn, Fe, Co, Ni, Cu, and Zn with atomic number (Z) from 25 to 30 are accessible at most hard X-ray beamlines.

number of the element increases. This is because elements with greater atomic numbers have more positively charged nuclei from a greater number of protons, and therefore, they have a greater binding energy of an electron in a given atomic orbital. The K-shell electrons are closest to the atom's nucleus (Fig. 14-1), and therefore they have greater binding energy than the L-shell electrons, as shown in Fig. 14-5 for the elements with atomic numbers 35 to 50.

Comprehensive tables of X-ray edge energies are available (e.g., McMaster et al., 1969; Shaltout et al., 2005; Elam et al., 2002). A simple graphical interface based on these tables is accessible through the program HEPHAESTUS by Ravel and Newville (2005).

X-ray Absorption Spectra

A typical X-ray absorption spectrum is shown in Fig. 14-4. A full spectrum is collected from approximately 200 eV below an absorption edge of interest to approximately 1000 eV above the edge.

XANES Spectra

The X-ray absorption near edge structure (XANES) is the part of the absorption spectrum near an absorption edge, ranging from approximately -50 to +200 eV relative to the edge energy (Fig. 14-4). The shape of the absorption edge is related to the density of states available for the excitation of the photoelectron. Therefore, the binding geometry and the oxidation state of the atom affect the XANES part of the absorption spectrum.

For most elements the absorption edge looks mostly like a step, as shown in Fig. 14-4. This absorption edge has the properties that it is linear and smooth below the absorption edge, increases sharply at the edge (like a step), and then oscillates above the edge. The main "step-like" feature of the absorption edge is due to the excitation of the photoelectron into the continuum (Fig. 14-1). The absorption edge for some elements includes decorations in the region of the step. These decorations may be isolated peak(s), shoulder(s), or a strong peak at the top of the step, called a "white" line. The Ni K-edge spectrum in Fig. 14-4 shows an example of a white line. Different features are caused by differences in the density of unoccupied electron orbitals that can be occupied by the excited photoelectron.

The *absorption edge energy* is defined as a specific energy on the step-like part of the absorption edge spectrum. The edge energy for an element in a higher oxidation state is usually shifted by up to several electronvolts to a higher X-ray energy. In a neutral atom, the positive charge of the nucleus is screened by the negative charge of the electrons. In an atom of higher oxidation state with fewer electrons than protons, the energy states of the remaining electrons are lowered slightly, which causes the absorption edge energy to increase. That is, an X-ray with slightly greater energy is required to excite the core electron.

The change in element oxidation state is usually accompanied by a change in centrosymmetry, which will change the features of the absorption edge. A well-known example of a geochemical species exhibiting an isolated peak in the absorption spectrum before the step is hexavalent Cr [Cr(VI)], which exhibits a sharp peak at approximately 5994 eV (Fig. 14-6). For Cr(VI), the Fermi energy is just below the peak at 5994 eV and the continuum is the energy of the step at ~6005 eV. The peak before the edge is sometimes called a "pre-edge" peak, but we contend that this terminology is misleading because the peak is part of the absorption process, and hence it is part of the absorption edge of Cr(VI).

The origin of the Cr(VI) peak at 5994 eV can be understood in terms of its molecular orbital configuration. A neutral Cr atom in the ground state has an electronic configuration denoted as [Ar]4s²3d⁴. The Cr atom also has 4p orbitals that are empty. Symmetry of the

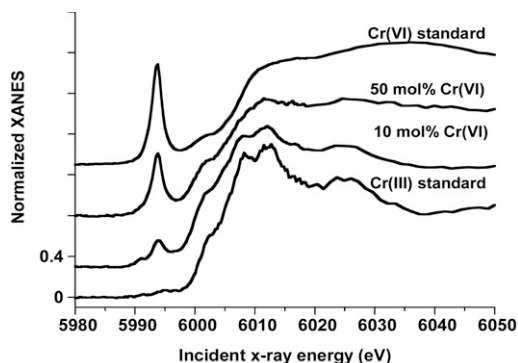


Fig. 14-6. Stacked Cr K-edge spectra for mixtures of Cr(VI) from CrO_3 and Cr(III) from Cr_2O_3 showing systematic changes in the edge region in proportion to the composition of each Cr oxidation state.

orbitals is important for building molecules. The d and s orbitals are threefold symmetric, while the p orbitals have directionality; the latter are dumbbell shaped. To make the tetrahedral configuration of Cr(VI)O_3 stable, the empty 3d and 4p orbitals of Cr(VI) hybridize and overlap with the O 2p orbitals. The Cr K-edge X-ray absorption probes electron transitions of the absorber from the 1s orbital ($\ell = 0$) to empty 4p orbitals ($\ell = 1$) because $\Delta \ell = \pm 1$ as discussed above in “Electron Transitions.” In general, transitions from the 1s ($\ell = 0$) to 3d ($\ell = 2$) are not allowed, but tetrahedral Cr(VI) has hybridized 3d and 4p orbitals, which makes the transition to the 3d states allowable, resulting in the peak at 5994 eV. Octahedral Cr(III) with threefold symmetry does not have hybridized 3d orbitals; hence, this transition is not allowed for Cr(III).

The intensity of the Cr(VI) peak at 5994 eV has been used to quantify the relative proportions of Cr(VI) and Cr(III) in geochemical samples (Zachara et al., 2004). The intensity of the peak depends not only on the concentration of Cr(VI) relative to Cr(III), but also on the symmetry of the tetrahedral coordinated Cr(VI) atom.

EXAFS Spectra

The extended X-ray absorption fine structure (EXAFS) part of the spectrum is the normalized oscillatory part of the absorption coefficient above the absorption edge to approximately 1000 eV or higher (Fig. 14-7). The EXAFS spectral region is used to determine local molecular bonding environments of elements in soils and mineral samples. The EXAFS spectrum contains information on the types and numbers of atoms in coordination with absorber atoms, their interatomic distances, and the degree of local molecular bonding disorder.

Figure 14-7 shows a conceptualization of the X-ray absorption process. The X-ray absorption spectrum of NiO is shown in Fig. 14-7a. At an energy below the absorption edge at approximately 8333 eV, no photoelectron is produced (Fig. 14-7b). At and above the absorption edge, a photoelectron is created (Fig. 14-7c and 14-7d). Above the absorption edge, the energy of the X-ray is used to excite an electron into the continuum, and any remaining energy is given to the photoelectron in the form of kinetic energy (KE). The KE of the photoelectron is equal to the difference between the incident X-ray energy (E) and the electron binding energy of the photoelectron (E_0):

$$\text{KE} = E - E_0 \quad [3]$$

The photoelectrons can be described as spherical waves propagating outward from the absorber atoms (Fig. 14-7c and 14-7d). These photoelectron waves are scattered from the atoms surrounding the absorber. The relative phase of the outgoing photoelectron wave

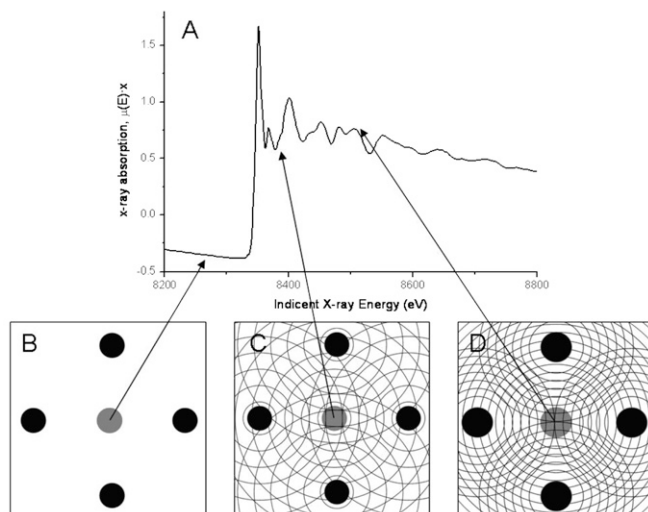


Fig. 14-7. Illustration of the X-ray absorption process. (A) Nickel K-edge X-ray absorption spectrum of NiO. (B–D) Nickel absorber atom (gray filled circle) and four of six neighboring oxygen atoms (black filled circles). The crests of photoelectron waves produced by X-ray absorption by the absorber atom (Ni) and by scattering from neighboring oxygen atoms are shown as concentric circles about Ni and O atoms, respectively. (B) At X-ray energies below the absorption edge no photoelectron is produced. (C) At energies close to but above the absorption edge, the photoelectron wavelength is longer than at higher energies. This particular wave produces a minimum in the oscillatory part of the absorption coefficient because the outgoing and scattered parts of the photoelectron meet at a minimum at the absorbing atom; that is, the peak crests are completely out of phase at the center of the absorber atom. (D) At higher X-ray energies the wavelength of the photoelectron is shorter than shown in C. This particular wave produces a maximum in the oscillatory part of the absorption coefficient because the outgoing and scattered parts of the photoelectron are in phase and meet at a maximum at the absorber atom.

and the scattered wave at the absorbing atom affects the probability for X-ray absorption by the absorber atom. The relative phase is determined by the photoelectron wavelength and the interatomic distances between the absorber and scattering atoms. When the waves are out of phase (Fig. 14-7c), a minimum in absorption occurs, causing a drop in the oscillatory part of the absorption coefficient. As the incident X-ray energy is increased above the edge, the KE increases (Eq. [3]) and the photoelectron wavelength becomes shorter (Fig. 14-7d). Because the average distance between the absorber and the neighboring atoms does not change, the waves will progressively become in phase (Fig. 14-7d), causing a maximum in the oscillatory part of the absorption coefficient. As the incident X-ray energy is increased incrementally over a range of energies, the corresponding photoelectron wavelength progressively decreases, and the sum of the outgoing photoelectron wave and the scattered waves at the absorber atom oscillate with a periodicity that is related to the average distances between the absorber and coordinating atoms. Such EXAFS oscillations only occur for elements with neighboring atoms. In the absence of coordinated atoms the EXAFS oscillations are absent.

Each atom at the same radial distance from the absorber contributes to the same component of the EXAFS signal. This group of atoms is called a shell. The number of atoms in the shell is called the coordination number. Figure 14-8A shows two shells of atoms, each with a coordination number of 4. The phase of the EXAFS signal from each shell is determined by the distance that the photoelectron travels. All photoelectron scattering configurations that start at the absorber atom, go to one or more neighboring atoms, and then return

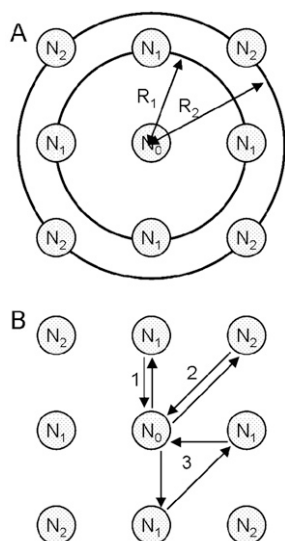


Fig. 14-8. Schematic of the absorber atom (sphere labeled N_0) surrounded by two shells of atoms. (A) Circles with radius R_1 and R_2 denote the first nearest neighbors (spheres labeled N_1) at a distance R_1 , and the second nearest neighbors (spheres labeled N_2) at distance R_2 , respectively. (B) A single scattering path to the first nearest neighbor labeled 1, $N_0-N_1-N_0$; a single scattering path to the second nearest neighbor labeled 2, $N_0-N_2-N_0$; and a triangular multiple scattering path labeled 3, $N_0-N_2-N_1-N_0$.

to the absorber atom contribute to the EXAFS signal (Fig. 14-8B). These configurations are called scattering paths. Single-scattering paths are from one shell of atoms. The degeneracy of a single-scattering path is the coordination number of that shell. The degeneracy of a multiple scattering path is the number of equivalent paths. Figure 14-8B illustrates two single scattering paths with a degeneracy of 4 each, and one triangular multiple scattering path with a degeneracy of 8.

The EXAFS spectrum can be understood in terms of the EXAFS equation. The EXAFS equation can be written in terms of a sum of the contribution from all scattering paths of the photoelectron (Stern, 1978; Stern and Heald, 1983):

$$\chi(k) = \sum_i \chi_i(k) \quad [4]$$

Each path can be written in the form:

$$\chi_i(k) \equiv \frac{(N_i S_0^2) F_{\text{eff}_i}(k)}{k R_i^2} \sin[2k R_i + \varphi_i(k)] e^{-2\sigma_i^2 k^2} e^{\frac{-2R_i}{\lambda(k)}} \quad [5]$$

with

$$R_i = R_{0i} + \Delta R_i \quad [6]$$

and

$$k^2 = \frac{2m_e(E - E_0 + \Delta E_0)}{\hbar} \quad [7]$$

The terms $F_{\text{eff}}(k)$, $\varphi_i(k)$, and $\lambda(k)$ are the effective scattering amplitude of the photoelectron, the phase shift of the photoelectron, and the mean free path of the photoelectron,

respectively, all of which can be calculated by a computer program such as FEFF (Rehr and Albers 2000). The term R_i is the half path length of the photoelectron (i.e., the distance between the absorber and a coordinating atom for a single-scattering event). The value of R_{0i} is the half path length used in the theoretical calculation which can be modified by ΔR_i . The remaining variables, described below, are usually determined by modeling the EXAFS spectrum. Equation [7] is used to express the excess KE of the photoelectron in wavenumber, k , by using the mass of the electron m_e and Plank's constant $\hbar$. It is helpful to note that

$$(E - E_0) \approx 3.81k^2 \quad [8]$$

where $(E - E_0)$ is in units of electronvolts, and k is in units of $\text{\AA}^{-1}$.

The EXAFS equation is easiest to understand for a single scattering path. Each of its terms is described below.

- **$(N_i S_0^2)$:** These terms modify the amplitude of the EXAFS signal and do not have a k -dependence. The subscript i indicates that this value can be different for each path of the photoelectron. For single scattering, N_i represents the number of coordinating atoms within a particular shell. For multiple scattering, N_i represents the number of identical paths. The passive electron reduction factor (S_0^2) usually has a value between 0.7 and 1.0 (Li et al., 1995). S_0^2 accounts for the slight relaxation of the remaining electrons in the presence of the core hole vacated by the photoelectron. S_0^2 is different for different elements, but the value is generally transferable between different species from the same element and the same edge.
- **$F_{\text{eff}}(k)$:** This term is the effective scattering amplitude. For a single scattering path it is the atomic scattering factor used in X-ray diffraction. For a multiple scattering path it is an effective scattering amplitude written in terms of the single scattering formalism (Rehr and Albers, 1990). This term accounts for the element sensitivity of EXAFS. In general, atoms with more electrons scatter photoelectrons more strongly at higher wavenumbers. For example, Fig. 14–9 shows the unique scattering amplitudes for O, P, Fe, and U. Because $F_{\text{eff}}(k)$ depends on the number of electrons, it is similar between elements with nearly the same number of electrons. The EXAFS signal from elements separated by only one atomic number on the periodic table such as O, N, and C can rarely be distinguished on the basis of their scattering amplitude alone.
- **$1/R_i^2$:** The contribution from a shell of atoms at a distance R_i diminishes with increasing distance from the absorber.
- **$\sin[2kR_i + \varphi_i(k)]$:** This term accounts for the oscillations in the EXAFS signal with a phase given by $2kR_i + \varphi_i(k)$. The path of the photoelectron is described by $2R_i$ (the distance to the neighboring atom and then back to the absorber atom), which is multiplied by its wavenumber (k) to determine the phase. $\varphi_i(k)$ is a phase shift of the photoelectron

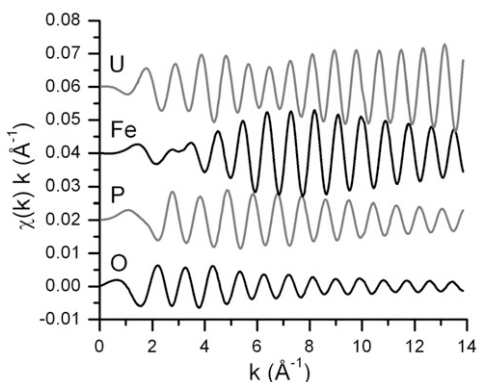


Fig. 14–9. Stacked, theoretical calculations of the EXAFS signals of O, P, Fe, or U atoms at a distance of 4.0 Å from a U atom, showing difference in amplitude and phase as a function of k . For this calculation $N_i S_0^2 = 1$ and $\sigma^2 = 0 \text{ Å}^2$.

caused by the interaction of the photoelectron with the nuclei of the absorber atom and the interaction with the nuclei of the coordinating atoms of the photoelectron path. Because the photoelectron has a negative charge and the nucleus is positively charged, the photoelectron loses energy and its wavelength lengthens as it interacts with the coordinating atoms and the absorber atom. It is this sine term in the EXAFS equation that makes the Fourier transform (FT) of the XAFS signal such a powerful tool, because a FT results in peaks at distances related to R_i , the interatomic distances between the absorber and coordinating atoms. The peak is not precisely at R_i due to the phase shift $\varphi(k)$, which causes a shift in distance of approximately $-0.5 \text{ \AA}$.

- $e^{-2\sigma_i^2 k^2}$: Because all of the coordinating atoms in a shell are not fixed at positions of exactly a distance R_i from the central absorber atom, σ^2 accounts for the disorder in the interatomic distances. σ^2 is the mean-square displacement of the bond length between the absorber atom and the coordination atoms in a shell. This term has contributions from dynamic (thermal) disorder as well as static disorder (structural heterogeneity). A distribution of distances within a single shell decreases the amplitude of the EXAFS signal because the phase differences between outgoing and scattered photoelectrons are shifted slightly for each atom in the coordination shell. The EXAFS process occurs on the femto-second (10^{-15} s) time scale, while thermal vibrations occur on a much longer time scale of 10^{-10} to 10^{-12} s . Because the atoms are essentially “frozen” at one position about their thermodynamic minima during the excitation process, EXAFS spectra measure the distribution of the distances between the absorber atom and each of the coordinating atoms within a shell in terms of a σ^2 value. The static disorder component of σ^2 is due to differences in the position of the minima themselves. Thus, for example, if two interatomic distances are separated by only $0.010 \text{ \AA}$, with one atom at $2.00 \text{ \AA}$ and another atom at $2.10 \text{ \AA}$, the contributing EXAFS signal could be modeled with one scattering path at $2.05 \text{ \AA}$ with a mean disorder of $0.0025 \text{ \AA}^2$.
- $e^{(-2R_i)/\lambda(k)}$: This exponential term depends on $\lambda(k)$, the mean free path of the photoelectron, which is the mean distance that a photoelectron travels after excitation. The mean free path of a photoelectron (Fig. 14–10) decreases from approximately $15 \text{ \AA}$ for short wavenumbers of $1 \text{ \AA}^{-1}$, goes through a minimum of $5 \text{ \AA}$ for wavenumber of $2.5 \text{ \AA}^{-1}$, and then increases to $30 \text{ \AA}$ for large wavenumbers of $15 \text{ \AA}^{-1}$. It is this term that causes the EXAFS signal to be dominated by the scattering contributions from atoms within approximately $10 \text{ \AA}$ of the absorber atom and makes EXAFS a local structural probe.
- ΔR_i : This term represents a change to the interatomic distance relative to the initial path length R_i . The path length can be changed slightly ($<0.1 \text{ \AA}$) to optimize the model to the data.
- ΔE_i : This term relates to a change in the photoelectron energy. This value is used to align the energy scale of the theoretical spectrum to match the measured spectrum.

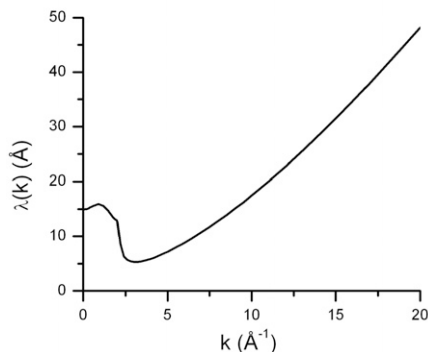


Fig. 14–10. The k dependence of the mean free path, $\lambda(k)$, of the photoelectron. Data are taken from a FEFF calculation of Cu-foil. The general trend is similar for all materials.

The value of ΔE_i can differ between coordination shells, but is more often a constant for all paths used in the model.

X-RAY ABSORPTION MEASUREMENTS

This section is aimed at facilitating the design and implementation of successful X-ray absorption measurements. There are many general practices given in the first section "Design of an X-ray Absorption Project." Typical components of a synchrotron and X-ray beamline are described in "Synchrotron Facilities" and "X-ray Beamlines," respectively. The final section describes several detailed methods for sample preparation and sample measurements. There are many requirements for a successful X-ray absorption project; therefore, it is often desirable to collaborate with an expert that can be found through the synchrotron facility or at www.xafs.org (verified 17 Dec. 2007).

Design of an X-ray Absorption Project

X-ray absorption is potentially useful for a broad array of specialized problems in soil mineralogy and soil chemistry with varying objectives. The need for an X-ray absorption experiment usually begins with the hypothesis that there is an element(s) with a particular local atomic configuration and/or valence state that is affecting the macroscopic behavior of the system. Key factors to consider in the design of a project are the identification of absorber atom(s), their concentration, the absorption edge and data collection method, and the data analysis method. In general, there are two different methods of X-ray absorption analysis. The first method is based on comparing unknown spectra with those of well-characterized, physical "standards." The second method involves comparing or modeling the spectrum for an unknown sample with theoretically calculated spectra. Usually a combination of these two techniques is used for EXAFS analysis, while XANES analysis relies heavily on comparisons with standards. For EXAFS analysis, spectra for physical standards can be used to make comparisons, optimize the theoretical models, and assess the performance of the beamline.

Often projects involve multiple elements that are affected by some physical, chemical, or biological process. Analyzing two or more elements is particularly useful if it is suspected that these elements are coordinated to each other. Consider, for example, a project that involves understanding coprecipitation of Zn with Fe oxides in a contaminated soil or subsurface undergoing redox fluctuations. If 15 mol% or more of the Fe(III) within a poorly crystalline Fe oxide is substituted by Zn(II), then Fe K-edge and Zn K-edge EXAFS measurements on the same sample would potentially produce a well-constrained data set. The Zn EXAFS spectra will contain a signal from the Fe neighbors, and the Fe EXAFS spectra will contain a corresponding signal from the Zn neighbors.

The concentration of the absorber atom may limit data collection to the XANES part of the absorption spectrum. The XANES spectrum contains the signal of the absorption edge itself, while the EXAFS represents only about 10% or less of that (Fig. 14-4). As discussed in a later section, approximately 100 to 1000 times more atoms of the absorber element are required for EXAFS than for XANES. Hence, the number of absorber atoms per unit volume is needed to determine the feasibility of an X-ray absorption experiment. In the example given above, if the concentration of Zn substituted into Fe oxide is <0.01 mol%, then the Zn K-edge measurement becomes difficult, as it requires long measurement times to obtain the required measurement statistics. In addition, if the concentration of Zn is <10 mol%, then the Fe K-edge EXAFS signal will contain very little signal from the Zn atoms because an insufficient number of the Fe

neighboring atoms will be Zn. Even so, the Fe K-edge EXAFS signal may still be useful in quantifying the affect of Zn on the Fe oxide.

X-ray absorption spectra can be collected at more than one edge of one particular element. Many hard X-ray energy edges are well separated by 1000 eV or more, which is essential for EXAFS spectroscopy. Edges at low X-ray energies are typically separated by several hundred electronvolts or less. These edges are suitable for XANES analysis. If an element's oxidation state is of primary interest, then the spectrum at a lower-energy edge might show a larger shift in the edge position with changing oxidation state or centrosymmetry than the spectra at a higher-energy edge. The elements O, N, and C have low-energy XANES spectra with characteristic features that can be used to differentiate the number and types of bonding environments.

Standards chosen for an XAS project usually include well-characterized minerals, adsorbed phases, or aqueous species that contain the element of interest in chemical forms that are considered relevant to the system studied with respect to mineralogy, chemical composition, and pH. The ideal XAS standards exactly match all aspects of the chemical species in the sample. However, it is not possible to synthesize or purchase standards, such as minerals or noncrystalline solids, that match the crystallinity, impurities, and defect structures of similar phases that were formed or degraded under the unique weathering conditions of a soil. In fact, it is often the project objective to determine these aspects of soil minerals. Therefore, a reasonable set of chemically meaningful standards should be included. Through the modeling of standards, the EXAFS signal can be understood and used to help constrain the structural model for the unknown sample. In the example of Zn substituted into an Fe oxide, a set of standards might include various synthesized Fe oxide minerals with different concentrations of coprecipitated Zn, and one or more Zn oxide or Zn hydroxide and Zn-free Fe oxide minerals. In addition, a metallic Zn-foil and Fe-foil are usually included to assess the quality of the beamline settings.

Synchrotron Radiation Facilities

X-ray absorption spectroscopy measurements are made at synchrotron radiation facilities. As of 2002, there were approximately 50 synchrotron radiation facilities in operation or under construction around the world (Sham and Rivers, 2002). Synchrotron X-ray facilities are grouped in terms of generations (currently first, second, and third generation) based on the technologies that result in a general range of capabilities. In the United States, there are four second- or third-generation synchrotron user DOE-funded facilities, including the Advanced Photon Source (APS) at Argonne National Laboratory (Fig. 14–11), the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory, the Stanford Synchrotron Radiation Laboratory (SSRL) at Stanford Linear Accelerator Center, and the Advanced Light Source (ALS) at Berkeley Laboratory. A third-generation facility at Brookhaven National Laboratory is being planned. Additional information, including details on gaining access to these facilities through peer review of proposals, is available online at www.lightsources.org (verified 17 Dec. 2007).

A schematic overview of a synchrotron facility is depicted in Fig. 14–11B. Bunches of charged particles are initially accelerated by a linear accelerator (LINAC) and then accelerated further in a booster ring that injects the particles traveling near the speed of light into a storage ring (Fig. 14–11B). The particles within the storage ring are accelerated toward the center of the ring each time their trajectory is changed so that they travel in a closed loop. This causes X-rays with a broad spectrum of energies (white light) to be emitted tangential to the storage ring. Therefore, a synchrotron storage ring is an N -sided polygon, where N



Argonne
NATIONAL LABORATORY

B

THE ADVANCED PHOTON SOURCE

Sector Allocations & Disciplines

Source Configuration

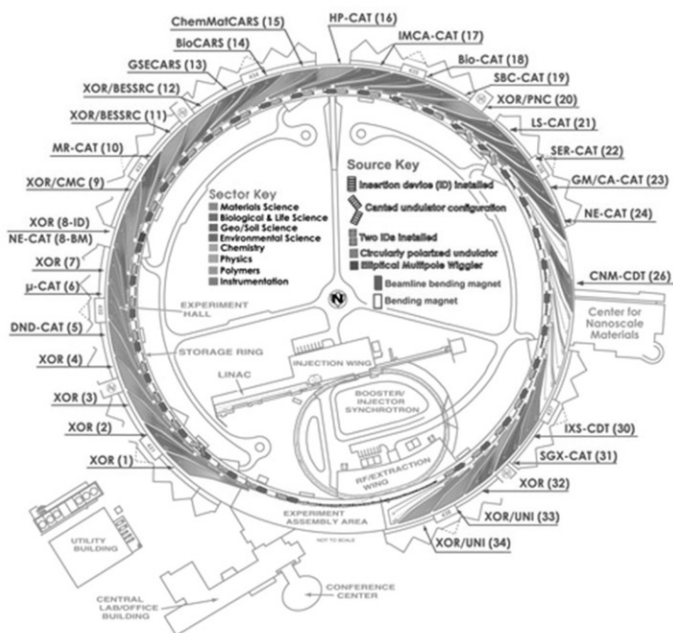


Fig. 14–11. (A) Photograph and (B) Schematic of the Advanced Photon Source at Argonne National Laboratory (www.aps.anl.gov).

is the number of bends. The particle trajectory is bent by magnets. Wigglers and undulators are two types of specialized insertion devices that are placed in the straight sections of the storage ring. These devices are an integral part of third-generation facilities. A wiggler consists of several closely spaced bending magnets that increase the intensity of the X-ray pulse. An undulator oscillates the charged particles using carefully spaced magnets such that the interference between their poles affects the emitted X-ray spectrum. This interference is additive at particular wavelengths, producing an intense X-ray beam at a wavelength that can be selected by varying the gap between the poles of the magnets. Beamlines are placed tangential to the storage ring to use the X-rays emitted by bending the charged particles. Bending-magnet and wiggler beamlines are well suited for XAS measurements because the X-ray energies produced span 1000 eV or more as needed for an XAS spectrum. To preserve the high brilliance of an undulator beamline, the spacing between the magnets is changed as the EXAFS scan is collected. With some loss of intensity, the undulator spacing can be tapered to produce a wider range of the X-ray energies for XAS measurements.

Beamline Setup

Setting up a synchrotron beamline for XAS data collection is typically done by or with the assistance of beamline scientists. Nevertheless, it is useful for the experimentalist to have a basic level of understanding of the optical system. Typical XAS beamlines contain a number of slits to define the X-ray profile, a monochromator to choose the X-ray energy, a harmonic rejection mirror to reduce the harmonic content of the X-ray beam, ionization or other detectors to measure the X-ray intensity, and a sample positioning stage. The absorption of X-rays by air can be significant for measurements made with X-ray energies below 10 keV, so evacuated or He-filled enclosures called “flight paths” are placed in the beam path to reduce the loss of X-ray intensity. The monochromator is often in a first optical enclosure, while the sample and detectors are in a second enclosure. This separation allows the experimenter to access the sample while keeping a constant heat load on the monochromator from the X-ray beam. Mirrors, slits, and flight paths are usually found in both enclosures. A schematic of the first optical enclosure is shown in Fig. 14–12A. A picture and a schematic of a second enclosure and detectors are shown in Fig. 14–3A.

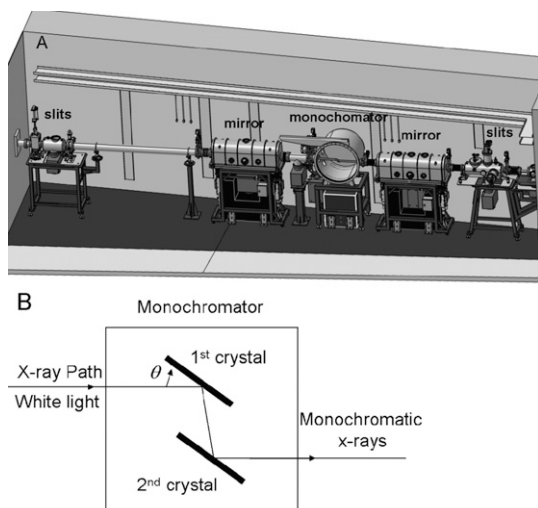


Fig. 14–12. (A) Drawing of the first optical enclosure of a beamline. X-rays enter the optical enclosure from the left. All EXAFS beamlines have a monochromator and slits. Some EXAFS beamlines have one or more mirrors. (B) Schematic of basic monochromator components.

The purpose of optimizing the beamline optical system is primarily to maximize the intensity of monochromatic X-rays of appropriate resolution on the sample. Also, during the XAS scan, the X-ray intensity should change smoothly and the position of the beam on the sample should remain stable. Beamline optics can move out of optimal alignment during the course of an experiment. Monitoring the incident X-ray intensity (I_0) with respect to ring current can help identify misalignment problems.

Slits

Slits are used to define the X-ray beam profile and to block unwanted X-rays. Two common types are fixed and adjustable slits. Fixed slits have a pre-cut opening of fixed heights between 0.2 and 1.0 mm and a width of several centimeters. These slits can be moved into or out of the X-ray beam. Adjustable slits use metal plates that move independently to define each edge of the X-ray beam. Many beamlines have slits located upstream and downstream of the monochromator (Fig. 14–12A) and before the I_0 detector (Fig. 14–3A).

The monochromator slits may need to be adjusted at the start of an experiment or after a large (several kiloelectronvolts) change in X-ray energy. Optimization is done by maximizing the X-ray intensity while changing the position of the slit opening. Vertical slits placed downstream or upstream of the monochromator can be used to increase the energy resolution of the X-ray incident on the sample at the expense of some loss in X-ray intensity.

Monochromator

The monochromator is used to select the X-ray energy incident on the sample. Typically the monochromator is stepped through the XAS scan range or it can be run continuously. The latter scanning mode is called quick-scanning of the monochromator.

An X-ray monochromator usually consists of two parallel crystals (double-crystal monochromator) or a single crystal with a slot cut nearly through it (channel-cut monochromator). Typical monochromator crystals are made of silicon or germanium and are cut and polished such that a particular atomic plane of the crystal, described by the (hkl) indices, is parallel to the surface of the crystal. Common monochromator crystals are Si(111), Si(311), and Ge(111). The energy of X-rays diffracted by the crystal is controlled by rotating the crystals in the white beam. A simplified schematic of a monochromator is shown in Fig. 14–12B. Only the X-rays with energies that satisfy Bragg's Law are diffracted by the crystal. Bragg's Law is:

$$n\lambda = 2d\sin(\theta) \quad [9]$$

where d is the spacing of the atomic planes of the crystal parallel to its surface, θ is the angle of the crystal with respect to the impinging white beam, λ is the wavelength of the diffracted X-ray, and n is an integer. The fundamental X-ray energy corresponds to $n = 1$, and X-rays of higher harmonic energies correspond to $n > 1$.

Harmonic Rejection.

The harmonic X-ray intensity needs to be reduced, as these X-rays will adversely affect the XAS measurement. Harmonic X-rays that are diffracted from the crystal depend on the crystal lattice and the cut of the crystal. It is useful to use a crystal that does not diffract the second harmonic ($n = 2$) because the intensity of the second harmonic is usually much greater than the intensity of the higher harmonics. Silicon crystals with the diamond structure will not allow the harmonics that satisfy the equation $h + k + l = n$, where n is twice an odd number. Hence, the Si(111) crystals do not diffract the second (for an odd number of 1) or sixth (for an odd number of 3) harmonic. For example, by using a Si(111) crystal at 8 keV, the first allowed harmonic

is at 24 keV ($n = 3$), rather than at 16 keV ($n = 2$). When working at high X-ray energies, it is possible that the energy of higher-order harmonics exceeds the maximum energy produced by the synchrotron storage ring. In that case, no harmonic rejection is needed.

Common methods for reducing the harmonic X-ray content include detuning the second crystal of the monochromator or using a harmonic rejection mirror. To detune the monochromator, the angle of the second crystal is slightly offset with respect to the angle of the first crystal, causing the X-ray intensity to be decreased slightly for the fundamental energy while dramatically decreasing the higher harmonic intensity. Figure 14–13 shows the reflectivity of a Si(111) double crystal monochromator with and without detuning, based on calculations from Heald (1988b).

The degree of detuning is determined by the change in the incident X-ray intensity (I_0) relative to its maximum value obtained for parallel crystals. For example, 20% detuning means that I_0 has been decreased by 20% of its maximum value. Detuning is usually done at approximately 200 eV above the absorption edge. The beamline scientist should know the appropriate amount of detuning needed for a particular measurement. The method used at the APS to determine the harmonic content of the X-ray beam can be found at www.xafs.org. At some beamlines, the degree of detuning is not stable and needs to be monitored and regularly adjusted.

The harmonic content can be determined by monitoring the transmission intensity of a foil of one absorption length with an absorption edge just above the energy of the first allowed harmonic. This foil will completely block the fundamental X-rays and transmit 66% of the harmonic X-rays. The detuning angle on the second monochromator crystal can be increased until the transmitted X-ray intensity (I_t) is reduced appropriately.

Another common method for removing harmonic X-rays is to use a harmonic rejection mirror. This mirror is usually made of Si for low energies, Rh for X-ray energies below the Rh absorption edge at 23 keV, or Pt for higher X-ray energies. The mirror is placed at a grazing angle in the beam such that the X-rays with fundamental energy are reflected toward the sample, while the harmonic X-rays are not. This is because the critical angle for reflectance depends on energy. Slits placed downstream of the mirror are used to block the direct beam containing the harmonic X-rays. The mirror angle is optimized by measuring the reflected beam intensity as a function of mirror angle. This measurement is usually performed at several different energies, one in the scan region and another a few kiloelectronvolts higher. The optimum angle of the mirror allows maximum reflection in the X-ray energy region of interest while reflecting insignificant X-rays of higher harmonics.

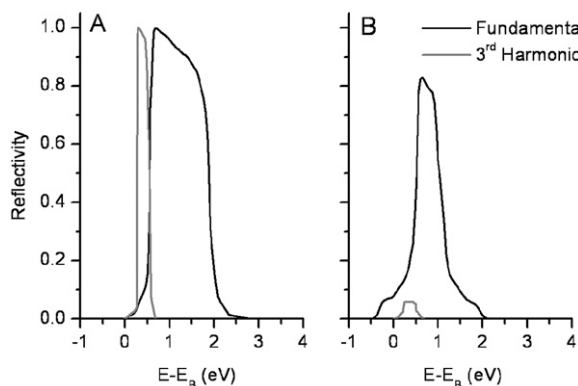


Fig. 14–13. Reflectivity of fundamental energy of 10 KeV and the third harmonic of 30 KeV from a Si(111) monochromator crystal as a change in relative energy from the Bragg energy (E_B) (A) without detuning and (B) with a detuning angle of 3.5 arcsec. The intensity of the third harmonic in B has been multiplied by 100.

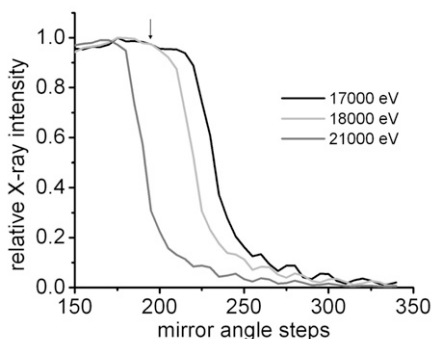


Fig. 14-14. Reflection from a Rh harmonic rejection mirror at three different X-ray energies. The cut-off angle for a U scan from 17,000 to 18,000 eV is indicated by the arrow.

For example, Fig. 14-14 shows the relative intensities of X-rays at three different energies that are reflected from a Rh mirror as a function of mirror angle. Shallow mirror angles corresponding to mirror steps <175 reflect the X-rays at all three energies shown. X-rays with energy of 17,000 eV are reflected strongly until the mirror reaches a critical angle corresponding to mirror steps of approximately 230, then reflectivity drops sharply. Critical angles for X-rays of 18,000 and 21,000 eV correspond to approximately 220 and 180 steps, respectively, showing that the critical angle decreases with increasing energy. To reject higher harmonics during an EXAFS scan from 17,000 to 18,000 eV, the mirror angle is set to approximately 220 steps so that the mirror reflects the 17,000- and 18,000-eV x-rays, but not higher-energy X-rays.

Monochromator Calibration.

The X-ray energy is determined by the angle of the monochromator crystal (Fig. 14-12B) relative to the incident X-ray beam through Bragg's Law (Eq. [9]). Motors that control the monochromator angle can slip, causing the X-ray energy to be miscalibrated. Also, the temperature of the monochromator affects the mean crystal spacing. Therefore, the monochromator energy is typically calibrated at the start of data collection and monitored throughout an experiment. Usually, the energy of the X-rays is determined from the absorption edge of a reference standard. Alternatively, a sample composed of a higher-Z element with an edge falling within the energy scan is suitable for elements that are not easily obtained. For example, a Y foil (K-edge = 17,038 eV) can be used when collecting data for U (L_{III} -edge = 17,166 eV) with a scan region from 16,938 to 18,000 eV.

It is good practice to simultaneously monitor the monochromator X-ray energy for each XAS scan by collecting the absorption spectrum from a reference standard. The reference standard is usually collected in transmission mode by using the X-rays that pass through the sample as shown in Fig. 14-3A, or by using scattered X-rays (Cross and Frenkel, 1998). By aligning the reference spectra collected with each sample spectrum, relative changes in the absorption edge energy from different chemical species can be determined accurately. For comparison of edge energy shifts of XANES spectra, there is a convention used to define an absolute energy scale relative to some feature of the absorption edge. This convention is necessary for comparing spectra from different studies and is discussed in the "Data Analysis" section below.

Detectors

Ionization Detectors. Typical X-ray absorption measurements use ionization detectors. Ionization detectors are gas-filled devices of typical lengths between 2 and 60 cm. Figure 14-3A shows an example of incident (I_0), transmission (I_t), and fluorescence (I_f) ionization

chambers. Alignment is done by using a fixed-position laser or by using X-ray fluorescence material or light-sensitive (“burn”) paper placed across a detector window. After the other beamline optics are positioned, it is good practice to recheck the alignment of the detectors.

The ionization detectors contain two parallel plates separated by a gas-filled space that the X-rays travel through. Some of the X-rays ionize the gas particles. A voltage bias applied to the parallel plates separates the gas ions, creating a current. The applied voltage should give a linear detector response for a given change in the incident X-ray intensity. Linearity tests can be used to determine the response of the detectors (Kemner et al., 1994).

The ionization detectors are filled with one or two inert gases to optimize X-ray absorption for a given edge energy. Absorption increases with increasing atomic number of the gas ($\text{He} < \text{N}_2 < \text{Ar} < \text{Kr}$) and increasing detector length. In general, suitable attenuation of the X-ray intensity by the detectors is 10 to 20% for the I_0 detector and 70 to 90% for the I_i and I_{ref} detectors.

The gas mixture needed to achieve a given absorption is similar to that described below in the “Sample Preparation” section. The open source software program Hephaestus contains a calculator for determining the optimum gas mixture. Typically the ionization detector is purged with the appropriate gas mixture, and then while measurements are made the gas is slowly flowed ($50\text{--}100 \text{ mL min}^{-1}$) through the detector, or the gas mixture is sealed within an air tight detector.

The voltage reading (V) from the ionization chamber is proportional to the number of X-ray photons per second ($N_{\text{X-ray}}/\text{s}$) passing through the detector as given by

$$V = eA \frac{N_{\text{X-ray}}}{s} \frac{E_{\text{X-ray}}}{E_{\text{ion}}} \quad [10]$$

where e is the charge of an electron, A is the ionization detector amplifier gain, and $E_{\text{X-ray}}$ is the X-ray energy in electronvolts. Typical gases have an ionization energy E_{ion} of 30 eV, so the number of X-rays per second becomes

$$\frac{N_{\text{X-ray}}}{s} = \frac{2 \times 10^{20} V}{AE_{\text{X-ray}}} \quad [11]$$

The Hephaestus software program contains a utility for calculating the number of X-rays per second.

Fluorescence Detectors. Fluorescence X-rays are usually measured at a 90° angle relative to the incident beam direction, with the sample being positioned in between at a 45° angle (Fig. 14–3B). The 90° angle between the detector and the incident beam direction is used because the fluorescence X-rays are emitted from the sample isotropically, while the intensity of the scattered X-rays is a minimum at 90° (Compton, 1923). Therefore, the greatest signal/background ratio is obtained for a 90° angle between the incident beam and the detector. Both Stern–Heald (Stern and Heald, 1979) and solid-state detectors are commonly used in XAS analysis of soil samples. The Stern–Heald detector consists of an X-ray filter, solar slits, and a large-window ionization detector (Fig. 14–3B). The X-ray filter is used to preferentially absorb scattered X-rays from the sample and is described in more detail below. The solar slits consist of a fan of thin pieces of metal, the point of the fan going in the X-ray beam direction and the fingers of the fan directed from the sample position to the detector. The solar slits block X-rays that do not originate at the sample. Proper align-

ment of the solar slits is critical. A commercially available Stern–Heald type of detector is shown in Fig. 14–3A. It comprises a sample chamber, solar slits, and ionization detector that are connected for automatic alignment of the solar slits.

The fluorescence filter (Heald, 1988a) for the transition metals usually consists of the element of one atomic number lower than the absorber element, and is called a “ $Z - 1$ filter.” This is because the absorption edge of the filter has an energy between energies of the incident X-rays and the fluorescence X-rays. For example, XAS measurements of Fe may use a Mn filter of one absorption length ($3\ \mu\text{m}$). The incident X-ray energy of 7200 eV is above the absorption edge of Mn at 6539 eV, such that 55% of the scattered 7200 eV X-rays are absorbed, but the fluorescent X-ray energy from the Fe at 6400 eV is below the Mn absorption edge, such that only 13% of these X-rays are absorbed. Therefore, a greater ratio of fluorescent X-rays relative to the scattered X-rays reach the detector, and the measurement is improved. For elements with atomic number greater than 37 a “ $Z - 2$ ” filter can be used.

A Stern–Heald detector is useful for samples with low to moderate absorber concentrations in a matrix that has low background signal because the fluorescence ionization chamber does not discriminate X-rays on the basis of their energy. For example, a Cu (K-edge = 8979 eV) measurement as a minor element in an Fe-rich matrix (Fe K-edge = 7112 eV) will have a large background signal from the Fe. In these situations a solid-state detector that can select the fluorescence X-rays from the Cu atoms is usually a better choice.

Solid-state detectors use a semiconducting material as a sensor for fluorescence X-rays. The most common solid-state XAS detector is a germanium detector, although silicon drift detectors are becoming increasingly popular. The solid-state sensor on a germanium detector is usually protected from the external environment by a thin beryllium window and is operated at liquid nitrogen temperature. Multi-element detectors (MED) have multiple detectors within the sensing unit, and the fluorescence signal from each detector is collected during each scan. Because the area of the X-ray transparent window from each element subtends a different solid angle relative to the position of the incident beam on the sample, the fluorescence signal varies between elements. These differences can be used to properly align the MED to the sample.

The electronics associated with the solid-state detector allow for the X-ray energy to be determined and only those X-rays within a specified energy range to be recorded. For the example of trace amounts of Cu in an Fe matrix, the fluorescence peak from Cu at 8046 eV can be distinguished to a large degree from the fluorescence peak from Fe at 6405 eV. The biggest drawback to the MED detector is that the electronics can be overloaded by too much signal, creating “dead time” when the detector is spending time processing signal rather than collecting signal. It is best to keep the count rate as high as possible and the dead time as low as possible. A maximum dead time of 10% is usually acceptable. The signal can be internally corrected for dead time, so depending on the configuration of the particular solid-state detector, different maximum dead times may be acceptable. Common approaches to limit the dead time of an MED are to reduce the incident X-ray intensity, add low- Z filters (Teflon or Al) to preferentially absorb fluorescence from lower Z elements, or increase the distance between the sample and detector. The “ $Z - 1$ ” filter is not recommended for the MED detector because fluorescence from the absorber element creates additional X-rays for the MED to process, and the signal from the filter can overlap with the signal from the absorber atom.

Electron Yield Detectors. An electron yield detector is particularly useful for collecting data on concentrated standards in the soft X-ray region, where Auger electron production is greater than fluorescence. Electron-yield detection is surface sensitive, and it does not

suffer from self-absorption effects, described in the section “Fluorescence Mode Samples” below. Electron-yield spectra can be collected simultaneously with fluorescence spectra to compare surface and bulk structures, respectively. Additional details can be found in a published handbook by Davis (1976).

XAS Scan Parameters

The main XAS scan parameters are the energy range, energy step size, the counting time, and the monochromator settling time. The energy step size determines the energy resolution of a scan, with the lower limit given by the energy resolution of the monochromator. The counting time is the duration that the detector signal is measured at each energy point. The monochromator settling time is the wait time after each monochromator movement (usually 0.2–0.5 s) to allow settling of mechanical vibrations before collecting the detector signal.

The duration of an XAS scan increases with increasing energy range, decreasing step size, and increasing counting or settling time. To increase the efficiency of data collection, the spectrum is divided into regions that have different energy resolution requirements. These regions are (i) the pre-edge region, (ii) the edge region, and (iii) the post-edge region (Fig. 14–4). The region before the edge defines the baseline, so large steps of 5 to 10 eV are taken. Smaller step sizes of 0.05 to 0.5 eV are used to resolve the rapidly changing features in the edge region. In the EXAFS part of the spectrum (Fig. 14–4), the oscillations broaden with increasing energy. Hence, smaller step sizes are needed to resolve EXAFS oscillations at lower energies than at higher energies. For this reason, the step size is determined in wave number (k) (Eq. [8]). A data point spacing of $0.05 \text{ \AA}^{-1}$ is sufficient to detect signals from atoms about $31 \text{ \AA}$ away from the absorbing atom, as given by the relationship $R = \pi/(2\Delta k)$. Because most EXAFS spectra contain information from atoms 5 to $10 \text{ \AA}$ away from the absorber atom, a typical data point spacing of $0.05 \text{ \AA}^{-1}$ is more than adequate. Collecting an EXAFS spectrum at just the minimum spacing in k is not recommended because one or more aberrant points may need to be removed.

Detector counting times at each step typically vary from 2 to 4 s, with longer times used to measure the weaker EXAFS oscillations at high k values. It is good practice to collect and average data from multiple scans on a sample using parameters that yield collection times between 25 and 45 min, rather than collecting fewer, long (>1 h) scans. It is also generally desirable to use the same data-collection parameters for all samples and standards within a set at a given absorption edge, while the number of scans collected depends on the signal from each sample.

Sample Preparation for XAS

X-ray absorption spectroscopy measurements can be performed on solids, gasses, or liquids, including moist or dry soils, mineral suspensions or pastes, and aqueous solutions. The quality of an XAS spectrum is often limited by the quality of the sample. X-ray absorption spectroscopy samples should preserve the speciation of the absorber atom, have a reasonably uniform distribution of the absorber atom, and have the correct absorption for the measurement.

The X-ray beam typically probes a millimeter-size portion of the sample. Ideally, this small volume is representative of the entire sample. μ -XAS analysis uses a focused X-ray beam that probes the sample on a micrometer scale. On this scale, one can capitalize on the natural heterogeneity of a soil sample to isolate sample regions that are enriched in a

particular chemical species (Manceau et al., 2002; Kelly et al., 2006). Soil samples can be sieved and diluted to homogenize samples for measurements on a millimeter-sized portion of the sample. For oxygen-sensitive samples these procedures should be done in an inert (He, N₂ or Ar) atmosphere (Hesterberg et al., 1997, 2001).

The method used to prepare the XAS sample can depend on the method of data collection. Transmission mode is most suitable for samples containing the element at concentrations greater than 1%. Fluorescence mode is clearly advantageous for samples containing the element of interest at concentrations of tens to hundreds of milligrams per kilogram. When the concentration of the element being analyzed is in between these ranges and the edge energy is in the hard X-ray range, it is advisable to prepare samples suitable for both fluorescence and transmission mode (Fig. 14–3). Both spectra can be collected simultaneously and compared to determine the best measurement. In the soft X-ray region, fluorescence mode is usually most suitable for XAS measurements.

Sample Preservation

Synchrotron X-ray measurements are scheduled for a specific time period for accessing a beamline. Thus, samples collected in the field or prepared ahead of time in the laboratory should be preserved to maintain the chemical state(s) and molecular structure of the element being analyzed for a given objective. The preservation of samples related to soils and minerals can be susceptible to changes in element oxidation state, changes in hydration, and biodegradation. Bartlett and James (1993) discussed methods for preserving the redox status of moist soil samples during storage. Oxygen-sensitive samples can be sealed under an inert gas in gas-impermeable containers such as crimp-capped, borosilicate-glass septum vials and stored at <3°C. Although air-drying of soil samples is a common practice, this step is not necessary for XAS measurements and should be avoided whenever possible. Drying would presumably have less effect on minerals than on adsorbed chemical forms of an element. If drying removes interfacial water, then it alters the local molecular environment probed by XAS for an adsorbed species. Treatments like oxidation of organic matter or removal of Fe oxide minerals are not only unnecessary, but undesirable. Such matrix alterations could change the molecular-level bonding environments of chemical elements, causing changes in the information that XAS measurements provide. It is good scientific procedure to test sample preservation methods to ensure that no detectable changes in the chemical speciation occur.

Transmission Mode Samples

A transmission mode sample needs to absorb as many X-rays as possible to produce a high-quality absorption signal. However, the signal is measured in the transmission ionization chamber (I_t); therefore, the sample also needs to transmit enough X-rays to make this measurement accurate. In general for a uniform sample, transmission mode measurements are optimized when the total absorption from all atoms in the sample is less than 2.5 absorption lengths ($\mu x = 2.5$) while the partial absorption due to the absorber atoms is approximately one absorption length ($\Delta\mu x = 1$) (Heald, 1988a). The upper limit for the total absorption by a sample is most critical. The partial absorption should be maximized within the total absorption limit and is easily measured, as it corresponds to the step height of the absorption edge in transmission mode. The total absorption can be determined by measuring the change in absorption as the sample is moved into and out of the X-ray beam. For inhomogeneous samples the total absorption should be minimized to the extent possible, such that a fluorescence mode measurement may be preferable.

The total absorption limit is affected by the number of X-rays incident on the sample. More intense X-ray beamlines can measure samples with a greater total absorption. As discussed in “Random Noise in EXAFS Spectra” below, the transmission signal (I_t) should measure 10^6 X-rays per data point for a high-quality XAS measurement. Samples with a total absorption length of 2.5 transmit 8% of the incident X-rays. Therefore, an incident X-ray intensity of 10^7 , which is common for most second-generation synchrotrons, is needed to measure a sample with a total absorption length of 2.5. An example absorption calculation for preparing a transmission sample is given in the later section “X-ray Absorption Calculation.”

Fluorescence Mode Samples

The most common problem of fluorescence-mode measurements is from samples that are too concentrated in the absorber atom. For a highly concentrated sample, the fluorescence X-rays are reabsorbed by the absorber atoms in the sample, causing an attenuation of the fluorescence signal. The effect is termed *self-absorption*. In the hard X-ray region, data can be collected in transmission mode and fluorescence mode simultaneously. The amplitude of the oscillations in the XANES region of the spectra where the signal is strong can be compared to determine if the fluorescence signal is attenuated due to self-absorption.

Tröger et al. (1992) mathematically analyzed self-absorption and provided equations for correcting this effect. Some XAS data analysis programs like Athena (Ravel and Newville, 2005) have incorporated algorithms for making this correction. These corrections rely on precise knowledge of the density of all atoms in the path of the X-rays. Because it is difficult to measure these parameters, it is preferable to avoid or at least minimize self-absorption effects, rather than making mathematical corrections to the data.

Concentrated samples and standards can be prepared for fluorescence measurements, particularly in the soft X-ray energy range, by reducing the particle size and diluting the particles in a weakly absorbing matrix. This sample preparation technique allows a greater proportion of the incoming and fluorescence X-rays to pass through each absorbing particle, thereby diminishing self-absorption effects.

Thickness Effects

Of particular concern are samples that contain regions that are nearly X-ray opaque (large particles or dense areas) interspersed with gaps between particles of high X-ray transparency. The resulting spectral distortions are termed *thickness effects* or *pin-hole effects* (Heald, 1988a). These distortions introduce systematic noise in the measured spectrum. Pin-hole and thickness effects are more pronounced in transmission mode than in fluorescence mode. Reducing the particle size of the sample is a remedy for thickness effects because it makes the sample more uniform and diminishes the size of interstices between particles.

Particle Size Reduction

In transmission mode, substantial distortions in the XAS spectra result from inhomogeneous samples. Particle size is less critical for dilute samples measured in fluorescence mode, although uniform samples help avoid spurious diffraction peaks that can occur from coarse-grained samples containing highly crystalline minerals like quartz.

It is generally beneficial, although not always essential, to reduce the particle size of a soil sample to $<50\text{ }\mu\text{m}$ by crushing or physically separating larger grains. Soil samples should at least be sieved by using a 2-mm screen made of noncontaminating material to remove gravel and roots. Ideally, particles will have diameters less than one absorption

length to ensure that a significant portion of the incident X-ray beam is transmitted through the particles. With a particle size of one absorption length and a total sample thickness of 2.5 absorption lengths, the sample is on average 2.5 particles thick. The overlap of several particles helps to minimize thickness effects.

Sample Dilution

Sample dilution can be required for fluorescence measurements to decrease self-absorption effects or for transmission measurements to optimize the amount of absorption. The diluent should have a low absorption coefficient (μ) at the energy range being used. It should be chemically pure, chemically stable and not reactive with the sample, and physically compatible with the sample for proper mixing. Boron nitride (BN) is often used as a diluent in XAS because this compound consists of low-Z elements (B and N) having low absorption coefficients, is chemically stable and inert, and can be purchased as a finely divided powder. Petroleum jelly (USP grade) is a suitable alternative diluent for powdered mineral standards. An important control experiment is to measure the absorption signal from the diluent alone to ensure that it does not produce a signal that interferes with the measured spectrum. An example of how to calculate the amount of diluent and sample to mix together is given in the next section.

X-ray Absorption Calculation

For samples of known composition, the total and step absorptions can be calculated. It is convenient to write the absorption cross-section (μ) in terms of the fractional density of each element. The mass absorption coefficient (μ_p) by a sample is the sum of absorptions by each constituent element:

$$\mu_p = \sum_i f_i \mu_{pi} \quad [12]$$

where f_i is the mass fraction of element i having mass absorption coefficient μ_{pi} . Energy-dependent mass absorption coefficients for the elements are tabulated in handbooks (Elam et al., 2002) and in XAS utility programs such as Hephaestus. The mass absorption coefficient μ_p can be either the total mass absorption coefficient (μ_{pt}) or the step mass absorption coefficient $\Delta\mu_p$, across an absorption edge.

Table 14–1 shows an example calculation for determining the amount of a franklinite standard (ZnFe_2O_4) to dilute in BN to obtain an edge step of approximately 1 at the Zn edge. The particular sample holder is made from a 1-mm-thick sheet of acrylate polymer (i.e., Plexiglas), with a rectangular window of 2.5 by 0.3 cm (0.75-cm² area) cut out (Fig. 14–15A). The capacity of such a sample holder on a mass basis can be estimated by weighing how much BN can be firmly packed into the window. Such calculations can be set up in a computer spreadsheet using the equations given in the footnotes of Table 14–1, or calculated within Hephaestus.

The mass fraction of each element in the standard (e.g., franklinite, Table 14–1) is calculated from their mole fractions and atomic weights. Because the tabulated mass absorption coefficients do not include EXAFS structure, it is reasonable to choose energies several electronvolts above and below the absorption edge of the absorber element (Zn in this example). Note in Table 14–1 that Zn shows a sharp increase in the mass absorption coefficient across the edge energy at 9659 eV, whereas the cross sections of Fe and O remain essentially unchanged. The Zn concentration (ρx , g cm^{–2}) yielding a given edge step (S) (unitless absorption) is calculated as (Table 14–1, second footnote) follows:

Table 14–1. Example calculation to estimate the dilution of franklinite (ZnFe_2O_4) in boron nitride (BN) yielding an edge step of 1.0 across the Zn K-edge (9659 eV) for a sample holder having a rectangular sample window with a cross-sectional area of 0.75 cm^2 ($2.5 \times 0.3 \times 0.1 \text{ cm}$ thick) and a sample capacity of approximately 70 mg of BN, the dominant sample component. In this example, the dilution yielding unit edge step would give a total post-edge absorption (2.65) exceeding the desired maximum of 2.5 for a transmission sample.

Element	Mass fraction in sample	Mass absorption coefficient (μ/ρ) [†]			Element		Mineral amount [§]	Mineral conc. [¶]	Post-edge absorption (per element) [#]
		Pre-edge (9658 eV)	Post-edge (9660 eV)	Δ (μ/ρ)	Conc. for unit edge step [‡]	Amount in sample holder [§]			
		$\text{cm}^2 \text{ g}^{-1}$			g cm^{-2}	mg			
Zn	0.271	35.0	254.0	219	0.00457	3.42	12.64	153	1.16
Fe	0.463	187.0	187.0						1.46
O	0.266	6.6	6.6						0.03
Total post-edge absorption:									2.65

[†] Mass absorption coefficient (μ/ρ) below and above the Zn K-edge at 9659 eV obtained from X-ray absorption tables in Hephæstus for each element, and the change in μ/ρ for Zn across the edge.

[‡] Calculated for Zn as $1.0/\Delta$ (μ/ρ), where 1.0 = the target edge step.

[§] Zinc amount (column 7) in sample (mg) = Zn concentration (g cm^{-2}) $\times$ sample cross-sectional area (0.75 cm^2) $\times$ 1000 mg g^{-1} ; mineral amount (column 8) = Zn amount (column 7)/mass fraction of Zn (column 2).

[¶] Concentration of mineral (mg/g sample) after diluting in BN = mass of mineral (12.64 mg)/mass sample ($0.08264 = 0.01264 \text{ g mineral} + 0.07 \text{ g BN}$).

[#] Total post-edge absorption contribution per element = element mass fraction (column 2) $\times$ mineral amount in grams ($0.001 \times$ column 8) $\times$ post-edge mass absorption coefficient (column 4)/sample cross-sectional area (0.75 cm^2). Total absorption = sum of all element contributions, where the contribution of BN to the total absorption (0.16) was not included here.

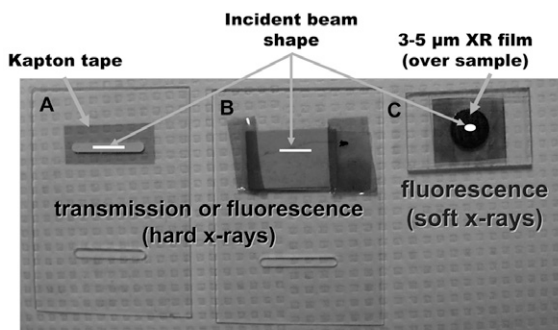


Fig. 14–15. Examples of sample holders used for collection XAS data in either transmission or fluorescence mode.

$$\rho x = \frac{S}{\Delta\mu_p} \quad [13]$$

Multiplying $S/\Delta\mu_p$ by the cross-sectional area of the sample holder (0.75 cm^2 in this example) gives the mass of Zn in the diluted sample (converted to milligrams), which is then converted to the mass of franklinite through its mass fraction of Zn. For the final Zn concentration in this example, the total sample mass includes the masses of franklinite and BN.

Note that in this example, diluting franklinite for an optimal edge step of 1 is expected to give a total absorption that slightly exceeds 2.5 (Table 14–1, column 10). Iron contrib-

utes the most to the total absorption. The dilution that gives a maximum total absorption of 2.5 can be calculated from equations given in Table 14-1. Because these calculations are theoretical estimates and the BN also contributes to the total absorption ($\mu_{\text{BN}} = 0.16$), it is advisable to use a more conservative dilution to ensure that total absorption is < 2.5 . Following the example in Table 14-1, diluting franklinite in BN to obtain a calculated edge step of 0.8 requires 0.0037 g cm^{-2} or 126 mg franklinite g^{-1} sample, giving a suitable total absorption of 2.3 (including the contribution of BN).

Sample Mounting

The system used for sample mounting depends in part on the data collection mode and the beamline end station. Samples to be collected in fluorescence mode are placed at a 45° angle with respect to the incident X-ray beam direction (Fig. 14-3B). Samples for transmission measurements can be placed perpendicular to the beam, or at a 45° angle. The amount of sample that the X-ray beam passes through will be 1.4 times greater for a sample placed at 45° angle (Fig. 14-3B) as compared with a sample placed perpendicular to the beam.

Most beamlines have a mounting bracket placed on a movable stage. The bracket is aligned in the X-ray beam so that sample holders, designed to easily attach to the mounting bracket, are automatically aligned in the X-ray beam. Figure 14-15 shows examples of modular sample holders. Materials such as acrylate polymer or acid-washed Teflon sheeting with low levels of metal impurities are suitable materials for sample holders. A window is cut completely through holders used for transmission mode data collection (Fig. 14-15A and 14-15B). If it is not possible to transmit X-rays through the sample (low-energy edges), a shallow sample well can be cut partially through the sample holder to decrease the quantity of sample needed (Fig. 14-15C). Standards diluted with a low-Z material are packed into a sample holder as shown in Fig. 14-15A. The back and front of the slot is covered with a high-purity, X-ray transparent Kapton (DuPont, Wilmington, DE) tape. Concentrated powder samples are often spread uniformly across the sticky side piece of Kapton tape (Fig. 14-15B). Care must be taken when mounting such samples to avoid pin holes. Sample thickness can be adjusted by folding the sample over to form two or more layers. As shown in Fig. 14-15B, the folded tape can be mounted directly across the window in the sample holder to assure rigidity and alignment. When working in the soft X-ray region, Kapton tape causes too much absorption. For these purposes, various types of X-ray films (e.g., mylar, polypropylene, ultralene) are available in thicknesses of 3 to $6 \mu\text{m}$. The film is taped using Kapton across the face of the sample in a well (Fig. 14-15C).

Another option for mounting soil samples or aqueous pastes measured in transmission or fluorescence mode is to place the sample in a window cut through Teflon sheeting of various thicknesses. The size of the Teflon sheeting and the location of its window are made to match the acrylate sample holder, so the Teflon mount can be taped to the holder for placement in a mounting bracket. It is often necessary to keep moist samples separated from the adhesive on the Kapton tape by first covering the sample with Kapton film.

All incident X-rays should be on a uniform region of the sample. The size of the sample window or well must be larger than the dimensions of the X-ray beam and the sample must be well aligned in the X-ray beam. The beam size can be adjusted with slits with the loss of X-ray intensity. For some small, dilute samples the X-ray beam will need to be focused. Because not all beamlines have focusing capabilities, these details should be discussed with the beamline scientist well before an experiment. If the X-ray beam is partially off of the sample, data quality will be poor, as described under "Thickness Effects" above.

To ensure that the entire beam is incident on a uniform sample, the sample can be moved slightly (~ 1 mm) in each direction while monitoring the signal from the sample (I_t or I_f). If the X-ray beam is located fully on the sample, detector signal will remain constant; and if the sample is clipped, then the intensity will change dramatically as the sample is moved.

Materials used for mounting brackets, and particularly for sample holders, should not contain detectable amounts of the elements being analyzed. Control measurements should be made during each beamtime on a blank holder, including tape or film coverings, to ensure that there is insignificant absorption or fluorescence that would interfere with the sample spectrum. The impact of absorber element impurities on the sample spectrum depends on the impurity concentration relative to the sample concentration. For example, mylar X-ray film contains enough P to significantly alter the XANES spectrum from samples containing 20 to 100 mmol kg⁻¹ of P.

XANES DATA ANALYSIS

To compare and model XANES spectra, the measured absorption spectra are normalized. Qualitative comparisons of normalized XANES spectra are made by overlaying or stacking spectra. Visual comparison of the spectra of samples and relevant standards reveals unique spectral features and gives an idea of possible chemical species represented in the sample spectra.

Linear combination fitting (LCF) is presently the most common approach for quantitative XANES analysis. Linear combination fitting models the spectrum from a sample of unknown speciation with a linear combination of spectra from standards of known structure and composition that are candidate species within the sample. When a set of XAS spectra are collected from a group of samples that are meaningfully related, the LCF can be augmented with a statistical technique called principal component analysis (PCA). The PCA approach provides insight on the number of unique species in the set of samples, and the significance of different standards to represent species in the unknown. Another XANES fitting approach is to model the spectra from an unknown sample in terms of curve fitting of different line shapes. This technique is particularly powerful for soft X-ray spectra for which different features of the absorption edge can be used to quantify a particular binding environment. Theoretical calculations of the XANES spectra can be used to connect a feature in the XANES spectra to a structure and/or cluster size. These approaches are discussed in the following sections.

Normalization

Normalization standardizes XANES spectra to remove variations between samples due to effects of absorber element concentration, sample preparation and thickness, and detector response. Differences in features between normalized spectra are attributed to differences in local molecular coordination environment. The XANES Fe K-edge spectra from a soil sample collected simultaneously in transmission and fluorescence modes (Fig. 14–16A) shows the characteristic downward-sloping spectrum for transmission and the characteristic upward-sloping spectrum for fluorescence. The downward slope in a transmission spectrum occurs because the total absorption of the sample decreases with increasing energy of the incident X-rays, so that more X-rays are transmitted through the sample at higher X-ray energies and $\ln(I_0/I_t)$ (Eq. [2a]) decreases. Samples with a larger total absorption will have a steeper slope than samples with a smaller total absorption. In contrast, in fluorescence mode the incident X-ray detector becomes less absorbing (i.e., the I_0 signal decreases) as the incident X-ray energy increases, while the fluorescent

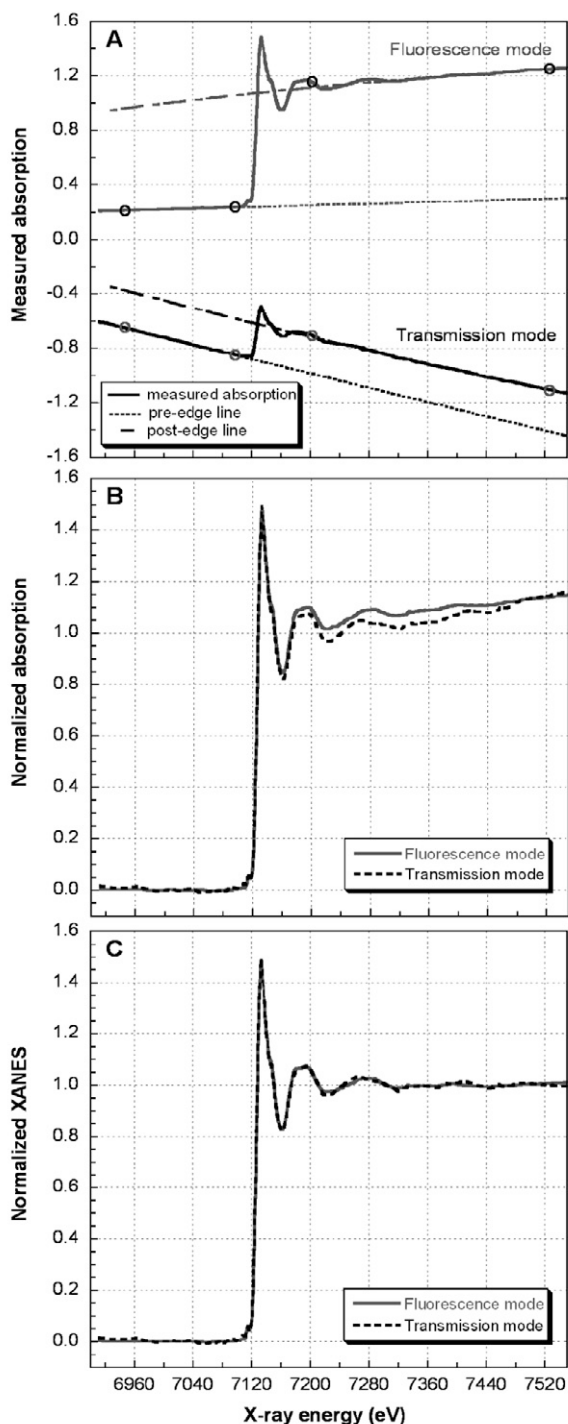


Fig. 14-16. Iron K-edge X-ray absorption spectra for a soil redoximorphic feature. (A) Measured experimental spectra collected simultaneously in fluorescence and transmission modes, showing the pre-edge and post-edge regressions used for normalization as calculated between the regions indicated by circles. (B) Normalized absorption spectra with pre-edge region along zero and an edge-step of one. (C) Normalized XANES spectra showing the pre-edge region along zero, an edge-step of one, and the post-edge region oscillating around one.

X-rays are at constant energy such that the fluorescence X-ray detector has the same relative absorbance. As a result, the slope of I_f/I_0 (Eq. [2b]) increases with increasing energy. Normalization removes detector (and other) effects such that normalized spectra from the same sample are identical as illustrated in Fig. 14–16C. Thus, XANES spectra for samples or standards collected in either fluorescence and transmission mode can be directly compared.

The normalization process involves a regression to the XANES spectrum in the pre-edge region (–200 to –30 eV before the edge energy) and the post-edge region (50–1000 eV above the edge energy) as delimited by the small circles in Fig. 14–16A. Usually, a linear function is regressed to the pre-edge region, and a linear or quadratic function is regressed to the post-edge region. These lines are extrapolated to the absorption edge, E_0 , and the difference in absorption between these projected lines at E_0 is called the edge step. Normalized $\mu(E)$ spectra are produced by subtracting the pre-edge line from the entire data spectrum and then dividing the spectrum by the step height. In the normalized spectrum, the pre-edge region lies along zero absorption and the edge step is 1 (Fig. 14–16B).

The post-edge region of the spectra often has a different slope or curvature than the pre-edge, such that in the normalization procedure described above, the post-edge region of the spectra will not oscillate about 1. Such spectra can be further processed by “flattening,” so the post-edge region oscillates about unit absorption. A flattening function subtracts the difference in slope between the pre- and post-edge regions and is applied only to the post-edge region above E_0 . The spectra in Fig. 14–16B have a non-zero slope and/or curvature that would not be removed without flattening the spectra. This flattening process does not affect the EXAFS spectra discussed below in “Edge Energy and Normalizing $\mu(E)$.” The normalized and flattened spectra are called normalized XANES spectra and consist of a pre-edge region along zero absorption, a step height of one, and a post-edge region that oscillates about one as shown in Fig. 14–16C.

Sometimes, particularly in the soft X-ray energy range, XANES spectra may have inconsistent baseline and background trends, and normalization is difficult. Normalization procedures can affect the intensity of a white line and other spectral features, which consequently affects modeling results. A first, critical step in making an appropriate background correction for all samples and standards is the acquisition of high-quality XAS data. It is a good practice to collect data for normalizing XAS spectra across a broad energy range extending well above the absorption edge (>200 eV) where EXAFS oscillations are weaker. Large (5–10 eV) steps can be taken across this region if only XANES data are being collected. It is also important that a normalization procedure be applied consistently between samples and standards (Sayers and Bunker, 1988).

Linear Combination Fitting

The underlying principle of linear combination fitting (LCF) is the additive nature of the absorption from each species in the sample. It can be applied to XANES, derivative XANES, or EXAFS spectra. As shown in Eq. [12] the total absorption coefficient can be written as a sum of coefficients for all the elements in the sample. It can also be written as a sum of chemical forms or species. For example, if a sample contains 50% goethite and 50% ferrihydrite, the absorption coefficient can be written as a weighted sum of the absorption coefficients for goethite and ferrihydrite, where each of these species has a contribution from Fe, O, and H atoms. As such, LCF involves modeling the spectrum for a sample of unknown chemical speciation using spectra from representative standards.

Linear combination fitting subroutines are available in XAS data analysis programs like Athena (Ravel and Newville, 2005) and Sixpack (Webb, 2007). These programs

compute the scaling factors applied to different standard spectra that provide the best representation of the measured spectrum over a defined energy range. Mathematically,

$$\text{Model} = \sum_i f_i (\text{STD}_i) \quad [14]$$

where Model represents the least-squares fit to the sample spectrum over a selected energy range, and f_i represents the scaling factors applied to each spectrum of the corresponding standard (STD_i) across that energy range of the fit. It is good practice for all spectra included in the LCF to have a similar energy resolution. Consistent normalization is important for LCF. To test for and possibly account for normalization problems, a line can be added into the fit as a standard. The statistical goodness-of-fit parameters for LCF are similar to those described for EXAFS analysis in the section “Comparing Models Using Statistical Tools” below.

Number of Standards

The number of standards included in a XANES LCF depends on the complexity of the chemical system. For example, only two standards would be used to characterize the XANES spectrum for a well-defined chemical system that, based on the known chemical composition, is likely to contain only two chemical species. For complex systems like soils that might contain many species, the number of standards would be limited to the number of species to which the XANES spectrum is sensitive, and caution should be used to avoid overfitting the spectrum with too many standards. For example, Beauchemin et al. (2003) identified the main forms of inorganic phosphate in soil samples. However, each of several possible forms of organic P present in most soils could not be differentiated because each form would likely occur at <10 mol% in the sample, and some of these species have XANES spectra with broad nondistinct features. Statistical methods, such as PCA, are helpful in determining the number of possible species within an unknown sample, although this method has many of the same limitations as LCF.

Fitting Range

The energy range for fitting an XAS spectrum using LCF is flexible, provided that data over that range are available for both samples and standards. Because the dominant feature in a XANES spectrum is the white line, this feature will have the most influence on a XANES fitting result. Therefore, LCF is particularly sensitive to various chemical species when each species gives unique features in the white-line region. However, for a particular problem, it might be desirable to isolate a specific, unique feature that can be used to differentiate between two or more different bonding environments of the absorber element. For example, Khare et al. (2004, 2005) used LCF fitting of a feature unique to Fe(III)-bonded phosphate to quantify the relative amounts of phosphate bound to Fe(III) oxide vs. Al(III) oxide in mineral mixtures. Murray and Hesterberg (2006) performed the same analysis to quantify the proportion of Fe(III)-bonded phosphate in mineral mixtures that underwent abiotic reduction, but they additionally performed LCF across the white line to establish whether the Al(III)-bonded phosphate was more likely adsorbed or precipitated as Al phosphate.

When analyzing soil samples that likely contain multiple forms of unknown chemical species, it is best to visually compare the spectra for samples and standards to determine the energy range that captures unique features of each standard. Then, begin the fitting over this energy range, and refine the energy range as insights are gained about the dominant chemical species present.

Energy Shift Parameter

Most LCF programs provide an option for allowing the energy scale of the sample spectra and/or the standard spectra to be shifted to improve the fit. This option can be used to correct the energy scale of the spectra caused by a miscalibrated monochromator. However, it is best to use a reference standard to align all scans before LCF because energy shifts themselves are useful for differentiating chemical species.

If there is evidence that the energy of a XANES spectral feature for a particular type of chemical species is shifted, perhaps due to minor structural differences, then allowing an energy shift can be justifiable. For example, a spectrum could shift depending on the crystallinity of a mineral. If so, then including an energy-shift parameter in the LCF analysis could serve as a measure of the crystallinity of that mineral as compared with the standard. These types of energy shifts are generally <1 eV (Beauchemin et al., 2003), and a justification for using an energy shift should be provided.

Constraints on Scaling Factors

The scaling factors (Eq. [14]) can be constrained so that they sum to one and are not negative. Negative scaling factors rarely have physical meaning. Using a negative scaling factor might be useful, for example, in isolating spectra for an individual chemical species from a spectrum containing a mixture that cannot be physically separated. With appropriate standards, the sum of scaling factors equals 1, so that the sum of the fractions of all chemical species of an element equals the relative amount of the element in the sample. Physically, the spectra from a standard might contain more atomic scale order than the representation of that component in a soil sample. Structural disorder within the soil sample could be represented in the XANES spectra by a broadening of the spectral line shape relative to the standard spectrum. This mismatch between the standard spectrum and the analogous species in the soil spectrum can be partially accounted for by relaxing the summation constraint. In this case, the fitted scaling factors for the appropriate standards are renormalized to one, so they are intended to represent the approximate fraction of each species in the sample.

Linear Combination Fitting Examples

Linear combination fitting involves a set of XAS spectra, possibly containing a number of spectra from related samples to be characterized and a number of standard spectra. For example, a set of spectra, which is typical of soils, sediments, and other geochemical systems, might contain a sample spectrum that is largely unknown and a wide range of standards for comparison. A particularly powerful implementation of the LCF technique involves using spectra from a series of related samples that are modeled using the end-member spectra as the standards for quantifying intermediate species. In this case, the endmember species closely match the species in the intermediate samples, and the LCF results are more quantitative and definitive. In practice, LCF can contain some aspects of both. These two different LCF projects are illustrated through the examples below.

Figure 14–17 shows LCF fitting results for the Fe K-edge XANES spectrum from a redoximorphic Fe depletion isolated from a subsoil. The standard spectra are from several synthetic Fe oxide minerals common to soils and a laboratory sample of Fe(III) bound to soil organic matter (peat). This example is typical in soil science, where the chemical species of a given element are not known. The difficulty in this problem is to know what standards are most representative of the chemical species in the sample. Fitting with a large number of standards representing a variety of possible chemical species in the sample is a start, but fitting results must be scrutinized by evaluating goodness-of-fit parameters in conjunction with

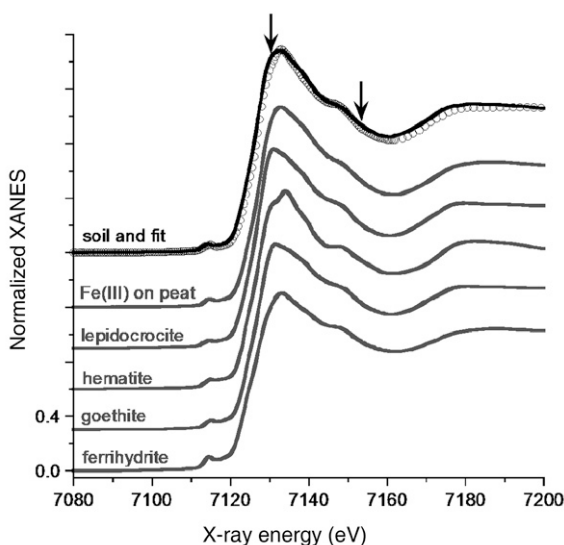


Fig. 14–17. Stacked, normalized Fe K-edge XANES spectra for a sample of a soil redoximorphic feature (data points), selected standards used in fitting, and a linear combination fit to the sample (solid). The fitting result is the sum of the following scaled spectra for standards: 60% Fe(III) on peat, 20% hematite, and 20% lepidocrocite as determined by the range of acceptable models (Table 14–2). The arrows indicate the fitted region of the spectrum.

the geochemistry of the system. For the example, in Fig. 14–17, lepidocrocite and goethite have very similar XANES spectra and gave equally good fits to the data. The part of the spectrum that is unique to the soil sample is from the edge energy to about 25 eV above the edge (arrows in Fig. 14–17). By fitting just this region of the spectra, the five combinations of standard spectra that gave the best representation of the measured spectra are listed in Table 14–2. The goodness-of-fit parameter, χ_v^2 value, scaled to the best fit is also listed. The four best-fit models indicate that an Fe–Peat component is needed because the change in the χ_v^2 value is 3.1 times greater for the best model without an Fe–Peat component. From this analysis we cannot uniquely identify the Fe oxide component, so supplementary analysis, like XRD, is needed to identify Fe oxide mineral(s) and constrain the LCF model. This example illustrates that LCF analysis of XANES spectra can help identify chemical species, like organic-matter bound metal ions, but that supplementary information from other mineralogical or geochemical properties of the soil are essential.

An example of using endmember standards for fitting XANES spectra for intermediate samples is shown in Fig. 14–18. Aqueous solutions of 0.2 M H_3PO_4 and 0.2 M KH_2PO_4 were mixed in varying proportions and pH adjusted between 1.6 and 4.5 to yield different proportions of total P as $\text{H}_3\text{PO}_4^0(\text{aq})$ and $\text{H}_2\text{PO}_4^-(\text{aq})$. Based on speciation calculated from thermodynamics (Visual Minteq version 2.31; Felmy et al., 1984; Gustafsson, 2007), aque-

Table 14–2. Five best models determined from linear combination fitting of Fe in a soil redoximorphic feature (Fig. 14–17).

Model	Fe–peat	Hematite	Lepidocrocite	Goethite	$\chi_{\text{vif}}^2/\chi_{\text{vi}}^2$
	%				
1	60 ± 6	20 ± 6	20 ± 9		1
2	70 ± 5	30 ± 5			1.2
3	66 ± 7		34 ± 7		1.3
4	85 ± 4			15 ± 4	1.5
5		35 ± 10	65 ± 10		3.1

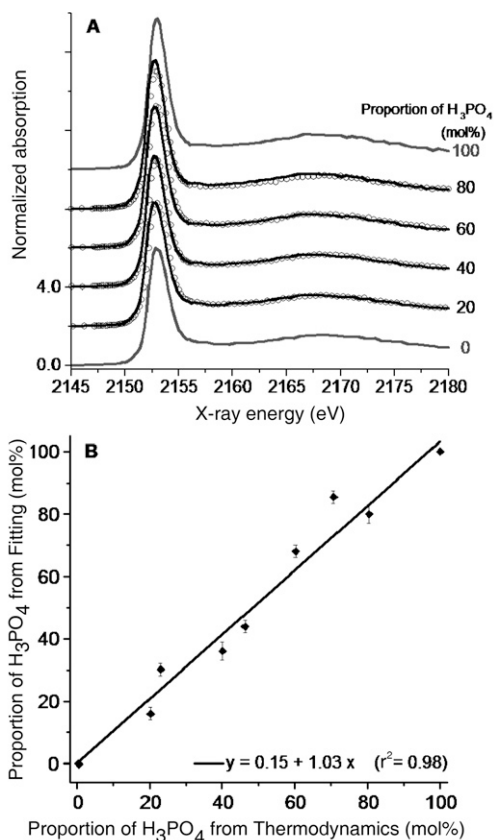


Fig. 14–18. (A) Stacked, normalized P K-edge XANES spectra for selected aqueous solutions containing H_3PO_4 and H_2PO_4^- in different proportions. Intermediate data (points) are fit (overlaid lines) with the endmember solutions containing 0 and 100 mol% H_3PO_4 . (B) Overall relationship between XANES fit and thermodynamic-predicted aqueous speciation for all samples analyzed.

ous complexes of $\text{KH}_2\text{PO}_4^0(\text{aq})$ accounted for ≤ 15 mol% of total dissolved PO_4 and were neglected in the LCF. The XANES spectra for intermediate mixtures (shown as symbols in Fig. 14–18A) were modeled with the endmember species of $\text{H}_3\text{PO}_4^0(\text{aq})$ and $\text{H}_2\text{PO}_4^-(\text{aq})$, which are known in this example to be the dominant species in all solutions. The model fits (solid lines) are overlaid on the measured spectra. XANES fitting results showed agreement within 8% of aqueous speciation based on thermodynamics for all mixtures evaluated (Fig. 14–18B). These data illustrate the accuracy of quantitative fitting using LCF, when standards are highly representative of the chemical species in the samples.

Interpretation of Linear Combination Fitting

Ideally, the scaling factor obtained from LCF represents the fractions of each standard species within the unknown sample. The accuracy of fitting results is limited by the accuracy of the standards in representing the chemical species present in the sample and the uniqueness of XAS spectral features between standards.

The spectra from the standard species are rarely so unique that no other spectrum or combination of spectra could account for the same sample signal, so results should be interpreted with caution. For example, if the total Ca concentration in an alkaline soil is similar to total P concentration, then using several Ca phosphate minerals as standards for LCF is reasonable based on stoichiometry and mass balance. Conversely, if the total molar Ca concentration is only 10% of the total P concentration, then a Ca phosphate mineral

like CaH_2PO_4 having a 1:1 Ca/P ratio can be included in the LCF only up to 10 mol%. If the model assigns more than 10% of total P as CaH_2PO_4 , the result is chemically impossible, indicating that there must be another species within the soil that has a spectrum similar to CaH_2PO_4 . Often more than one combination of standards gives similar quality of fit (Beauchemin et al., 2003), which is attributable to an insensitivity of LCF analysis to different chemical species. Additional geochemical information is needed to evaluate the degree to which the standards represent species within a sample.

It cannot be overemphasized that minerals and other solid-phase chemical species in soils and other natural systems have compositions along a continuum (e.g., different levels of impurities or a range of crystallinities), making it unlikely that the exact distribution of unknown species in a natural sample can be simulated using pure standards. A reasonable model based on LCF does not prove that these standards are the exact chemical species in the soil. Rather, LCF results identify the proportions of standards that yield the best fit to the data, recognizing that if a more representative set of standards are identified, then the accuracy of the results improves.

Principal Component Analysis

The principal component analysis (PCA) approach provides insight on the number of unique components present in a set of samples, and the likelihood that a standard represents a species in the samples via target transformations. This approach is commonly combined with LCF to help determine the number of standard spectra and to define the standard spectra. Principal component analysis is used with a set of spectra that are meaningfully related, such as soils exposed to different chemical treatments or soil samples along a geochemical gradient that naturally vary in the speciation of the absorber element. Research has shown that the number of components obtained statistically by applying PCA to XANES datasets for P in soils, S in humic acid, and Mn in airborne particulates ranged from two to three (Ressler et al., 2000; Beauchemin et al., 2002; 2003). Additional examples of PCA applied to XANES or EXAFS spectra can be found in Fernández-García et al. (1995); Wasserman (1997), and Wasserman et al. (1999). Details of the theory and practice of PCA in chemical systems are given in Malinowski (2002), and the PCA approach for S XANES spectra is described in some detail by Beauchemin et al. (2002).

Principal component analysis uses matrix operations to determine eigenvectors that represent the variation in the set of spectra. The maximum number of eigenvectors is equal to the number of sample spectra. The first eigenvector is defined as a vector along the greatest variation in some aspect of the data set. The second vector is extracted orthogonally to the first one to account for the highest remaining variation. Additional eigenvectors are orthogonally extracted until all the variation in the dataset is captured. There are several criteria that can be used to statistically separate the significant eigenvectors from those representing noise in the data set. The significant eigenvectors are called principle components and are statistical abstractions with no physical relationship to the chemical components or species in the samples.

Ideally, the number of significant components would be equal to the number of chemical species in the sample, thereby constraining the maximum number of standards that should be combined to model a given sample spectrum. In practice, the number of significant components is not necessarily equal to the number of different chemical species contained within the set of samples because the chemical species are not necessarily unique. For example, if two different species are always present in the same fraction within each spectrum of the data set, then these two species will be represented by a single prin-

principal component. The number of principle components more often indicates the minimum number of species needed to characterize a data set.

The next step in PCA is target transformation to relate the principle components to physical standards. Target transformations test the likelihood of a given standard spectrum to explain the variance in the set of spectra, and thereby identify the standards that most likely represent the data set. Because PCA relies on standard spectra, the same precautions used to interpret LCF apply.

Peak Fitting

When the absorption edges and white lines in XANES spectra for a mixture of different species of an element are well separated in energy, curve fitting can be used to quantify the chemical species present. This approach has been commonly used for determining the oxidation states of S in natural samples because the white-line peak position shifts linearly with the oxidation state (Morra et al., 1997; Xia et al., 1998; Prietzel et al., 2003). The spectra are deconvoluted into a series of Gaussian peaks at the energies representing each of the S oxidation states, added to an arctangent function that accounts for the step-like absorption edge. The peak areas of the Gaussian fits to the spectra are related to the proportion of each possible species in the sample (e.g., oxidation state in the sulfur example). Consistent normalization of spectra is critical for quantitative analysis. When using curve fitting, it should be recognized that the intensity of the white line in a normalized XANES spectrum often varies between different chemical species. In contrast to curve fitting, LCF accounts for variations in intensity associated with different species by using the spectra from the different physical standards.

Ab Initio Simulations

Simulation of XANES spectra based on ab initio computations of the underlying physical phenomena giving rise to a spectrum represents the most quantitative approach for relating XANES to the molecular bonding environment of a chemical species. There are several theoretical XANES programs, such as FEFF8.5 (Ankudinov et al., 1998; Rehr et al., 1998; Rehr and Ankudinov, 2005), TT-MULTIPLETS (de Groot, 2005), MXAN (Benfatto et al., 2002), and FDMNES (Joly, 2001). XANES calculations have been applied, for example, to refine the structure of Zn(II) ions adsorbed to ferrihydrite (Waychunas et al., 2003).

The FEFF8.05 theory predicts the XANES spectra based on the production of a core hole during electron excitation, the density of states of the molecular orbitals of the coordinated atoms in this excited state, and multiple scattering of the photoelectron at energies near the absorption edge. The use of a real-space multiple scattering approach for ab initio calculations of XAS spectra unifies the description of both the XANES and EXAFS regions of the spectra. The theoretical calculation of the XANES spectrum is based on a cluster of atoms surrounding the absorber. The theoretical spectrum is then compared with the experimental spectrum, and the molecular structure is refined as needed to provide the best match between theory and experiment. Due to the long mean free path of the photoelectron at the absorption edge (Fig. 14–10), the cluster of atoms needed for an accurate XANES calculation can be greater than 10 Å. Usually a small cluster is used first, and then the size is incremented to determine the effect on the XANES spectra. An exact match implies that the local molecular structure has been solved. To develop the original molecular structure, additional information such as interatomic distances and coordination numbers provided by EXAFS analysis or molecular dynamics simulations are useful. The use of ab initio simulations of XANES data are presently useful for characterizing the fine details of

a structure of a given mineral or adsorbed species, and it could potentially become useful for characterizing multicomponent systems like soils.

X-RAY ABSORPTION FINE STRUCTURE DATA ANALYSIS

The objective of EXAFS data analysis is to develop a structural model of atoms coordinated to the absorber atoms, as a means of identifying the chemical form(s) of the absorber. Interpreting the structural information contained in EXAFS spectra requires knowledge of spectroscopy, a basic understanding of signal processing and Fourier transforms, and knowledge of the fundamental processes that generate the EXAFS signal.

In the following sections, we present the details of how EXAFS spectra are processed and modeled in terms of local molecular coordination. We describe EXAFS spectral processing, building theoretical models, and examples of theoretical modeling.

The “EXAFS Data Processing” section includes preprocessing of XAS spectra, operations performed on the spectra as measured in energy, the basics of Fourier transformations, and background removal procedures for isolating the oscillatory part of the absorption coefficient.

The description of building theoretical models includes an overview of the basic components, the basics of constructing the theoretical EXAFS spectra using FEFF, common methods for constraining EXAFS parameters, the fundamentals of building an EXAFS model, and comparing models using statistical tools.

The EXAFS modeling examples include aligning the data and theory in energy, modeling a metal-oxide (SnO_2), determining a second shell atom type, and modeling a data series.

This section is concluded with tips for reporting EXAFS analysis in published manuscripts and some final concluding remarks. In our descriptions, reference is made to the Athena and Artemis software packages for EXAFS data analysis (Ravel and Newville, 2005). A number of other software packages are available for EXAFS data analysis, and information about many of these programs can be found at www.xafs.org/Software (verified 20 Dec. 2007).

EXAFS DATA Processing

There are several data processing steps that may be needed to refine the final spectrum for analysis. These preprocessing procedures include rebinning measured data points, removal of data points that are clearly in error (deglitching), trimming the absorption spectra to the usable energy region (truncation), changing the energy scale to align several scans, and averaging several scans to produce a high quality spectrum for analysis.

Rebinning

Depending on the energy spacing of XAS data points, the signal/noise ratio may be increased by averaging the signal from closely spaced energy points to obtain a single value at an average energy. This procedure is called rebinning. The above section “XAS Scan Parameters” gives the detailed energy resolution requirements for an XAS scan. Rebinning is particularly useful for spectra collected in quick-scanning mode of the monochromator to produce evenly spaced energy points.

Glitch Removal

At certain energies for a given monochromator crystal, the crystal geometry is such that the primary diffracted beam undergoes a second diffraction from a different set of

atomic planes, resulting in the loss of X-ray intensity going to the sample (Heald, 1988b). The sharp drop in the incident X-ray intensity (I_0) can cause a spike or dip in the XAS spectrum. These points are called monochromator glitches. With very good linear response of the detectors, glitches are cancelled out in the ratio of the detector signals (Eq. [2]), such that the absorption spectra, $\mu(E)$, are unaffected. In some cases, glitches can be avoided by adjusting the beamline optics (e.g., changing the detuning or rotating the crystals laterally or normally to change the region of the crystals reflecting the X-rays). When a glitch persists in the EXAFS spectra, these points can be removed. If glitches contribute to the data structure, then they will distort the background or cause artifacts in the EXAFS spectrum that can affect the final fitting results. These problems can be easily avoided by careful analysis procedures.

One method for determining the significance of a glitch is to remove the background with and without including the data points from the glitch to determine the effect on the EXAFS signal. As with rebinning, often an EXAFS spectrum is oversampled in energy so that the loss of a few data points to remove a glitch will not significantly decrease the information content in the measured spectrum. Caution should be used when omitting glitch points when it results in a loss in energy points corresponding to a radial distance (R) $< 10 \text{ \AA}$. This requires that the remaining data points are not separated by more than $0.16 \text{ \AA}^{-1}$ in k , determined by the relation $R = \pi/(2\Delta k)$. For example, if the data are collected with a $0.05 \text{ \AA}^{-1}$ spacing in k , then three consecutive data points can be removed with tolerable loss of information.

Truncating

Dramatic changes in the X-ray absorption coefficient before or after the absorption edge should not be included in the final spectrum for analysis. These features can produce errors when determining the step height or in the background removal procedures. Possible causes for such a dramatic change in the absorption coefficient include an absorption edge from another element or beamline-specific energy-dependent structure. For example, Fig. 14–19A shows the U L_{III} -edge at 17,166 eV with a pre-edge region that has been obscured by the yttrium K-edge at 17,038 eV. The region of the data that contains the smooth, step-like absorption edge should be isolated from the interfering region by removing data points affected by the Y K-edge, or by adjusting the ranges used to process the data so that the unwanted feature is excluded. The pre-edge region from 16,900 to 17,100 eV (–200 to –50 eV relative to the edge energy) in Fig. 14–19A results in a steep, positively sloped pre-edge line that is clearly not along the baseline (gray line). A more correct pre-edge line would be the line with a negative slope obtained by using the very short data range just before the U edge, from 17,090 to 17,120 eV. Effects of each pre-edge line on the XAS spectrum are shown in Fig. 14–19 B–D and are discussed in more detail below

Aligning and Averaging Spectra

The edge energy of the spectrum from the reference standard can be used to define the absolute energy scale for the measured sample spectra. Shifts in energy during data collection can be caused by mechanical instabilities in the monochromator, such as a missed step of the monochromator motor used to change the energy or changes in the monochromator temperature due to heat load differences between synchrotron ring fills.

Because multiple scans are usually collected from a single sample, the resulting $\mu(E)$ spectra should be aligned to a common energy scale to avoid loss of energy resolution, then averaged to increase the signal/noise ratio in the final spectrum. Alignment of spectra to an absolute energy scale is important for XANES data analysis, whereas it is sufficient for EXAFS analysis to align spectra to a common, relative energy scale.

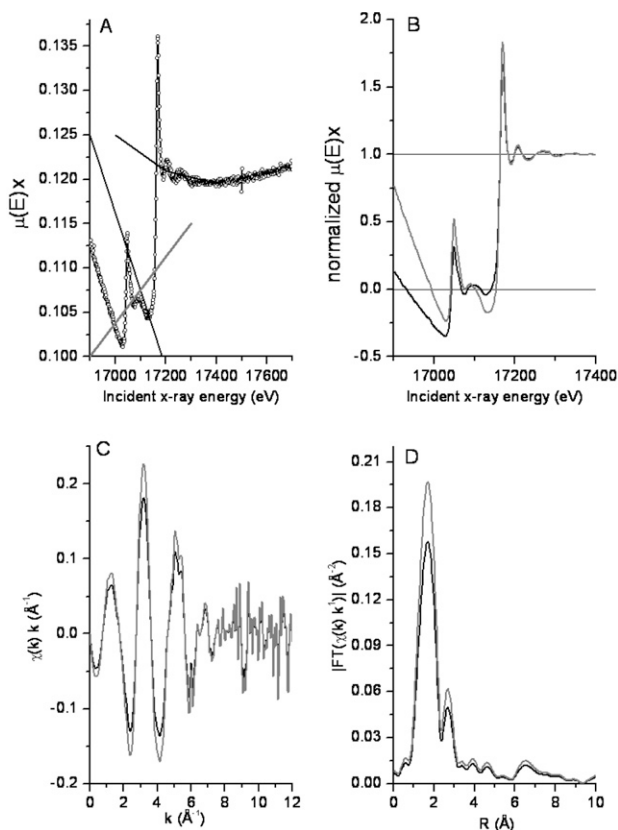


Fig. 14–19. (A) X-ray absorption data from a sediment sample containing both Y and U. The Y K-edge near 17,000 eV obscures the pre-edge region of the U L_{III} -edge at 17,200 eV. The thick gray line with positive slope shows a poor choice for the pre-edge line obtained by using the data from 16,900 to 17,100 eV. The thin black line with negative slope shows a better choice for the pre-edge line. The difference between the post-edge and pre-edge lines at the edge energy determines the absorption step height. The data are processed using both the gray and black pre-edge lines and are shown as gray and black spectra, respectively, in the following panels: (B) normalized X-ray absorption data, (C) EXAFS $\chi(k)k$ data, (D) magnitude of Fourier transform of EXAFS $\chi(k)k$ data.

To align multiple XAS spectra, the reference spectrum corresponding to each sample spectrum is calibrated to its known reference energy, and the resulting energy shifts are applied to each corresponding sample spectrum. For XANES analysis, a particular feature in the absorption $\mu(E)$ spectrum is assigned to a specific absolute energy value. For EXAFS analysis, an absolute energy value is not needed because the process of choosing the edge energy (E_0) on the absorption edge does not depend on an absolute energy reference. Hence, for EXAFS analysis, it is sufficient to align all reference spectra to a common feature on the first reference spectrum. Once the sample spectra are known to be on the same energy grid, they can be averaged without loss of energy resolution.

Before merging aligned sample spectra, it is useful to overlay the absorption $\mu(E)$ spectra and inspect the data in the edge region to check for changes in the spectra as a function of X-ray exposure time. Changes to the speciation of the absorber atom, due to radiation damage or oxidation changes, are often readily visible in the edge region where the absorption coefficient changes rapidly with energy. If the spectra are consistent within the measurement uncertainty, then the absorption spectra can be merged in $\mu(E)$.

The merging of several scans is often performed on the absorption data $\mu(E)$ with a weighting factor of one for each measured spectrum. The weighting factor can be adjusted based on additional information. For example, data sets measured with longer integration times that produce higher quality spectra should be more heavily weighted in the averaging than lower quality spectra.

Edge Energy and Normalizing $\mu(E)$

Definition of E_0

The edge energy represents the energy required to excite a core electron into an unoccupied state or into the continuum within the sample matrix, creating a photoelectron. The absorption edge occurs over an energy region, and therefore, there is usually not a single, precise energy value that defines the absorption edge. The energy reference parameter, E_0 , used for processing EXAFS data is not defined relative to the electron configuration within the solid (i.e., the Fermi level or any other level), but rather it is a parameter that is used to align the experimental spectrum to the theoretically calculated spectrum. A systematic definition of E_0 for all EXAFS spectra from the same adsorption edge is desirable since a shift in E_0 is determined in the fit of theoretical spectra to the experimental spectra. If all data sets have the same E_0 , then all data sets will have the same energy shift. In this case, one variable can be used to model all data sets, rather than using a separate variable for each data set.

Normalizing $\mu(E)$

The EXAFS spectrum is defined as the normalized oscillatory part of the absorption coefficient above the absorption edge. As discussed earlier in "XANES Data Analysis," normalization is done by dividing the measured spectra by the absorption step height at E_0 and subtracting a regressed line determined by the pre-edge region from the entire spectra. The step height determines the amplitude of the EXAFS oscillations and is therefore correlated to the EXAFS parameters such as the amplitude reduction factor (S_0^2), and the coordination number (N). That is, the step height, S_0^2 , and N are all components of one multiplicative amplitude term in the EXAFS equation (Eq. [5]). The uncertainty in the step height determination can be as large as 10%. Therefore, without additional information, a coordination number cannot be determined to greater accuracy than 10%. If one of these three parameters is incorrect, then it is likely that one or both of the other parameters will be fit to an incorrect value to compensate.

Figure 14–19A shows an example of a particularly challenging spectrum from which a reasonable and an unreasonable step height were determined by using different regressions to the pre-edge region for a U L_{III}–edge spectrum with an interfering Y K-edge. Figure 14–19B shows the resulting normalized XANES spectra. Figures 14–19C and 14–19D show the resulting EXAFS spectra, $\chi(k)k$, and the magnitude of the Fourier transform, $|\chi(R)|$, obtained from each of the two normalization procedures. The poor choice for the pre-edge line that includes the Y K-edge results in an approximately 25% greater amplitude of the $\chi(k)k$ spectrum. This 25% deviation will introduce error into S_0^2 and/or N in the final analysis results. Hence, the pre-edge, post-edge, and resulting step height should always be checked before the $\chi(k)$ spectrum is further processed. Normalized XANES spectra can be used in EXAFS analysis to visually check the alignment of the spectra and to look for spectral changes between successive scans from the same sample.

The Basics of Fourier transformations

The Fourier transform (FT) of the EXAFS signal is written as $\text{FT}[\chi(k)k^{kw}]$ indicating that the EXAFS signal written $\chi(k)$ has been multiplied by a k weighting of kw . The units of the Fourier transformed spectrum are $\text{\AA}^{-1-kw}$. The Fourier transform of the EXAFS signal is a function of R and can also be written as $\tilde{\chi}(R)$, where the tilde indicates that a Fourier transform has been applied.

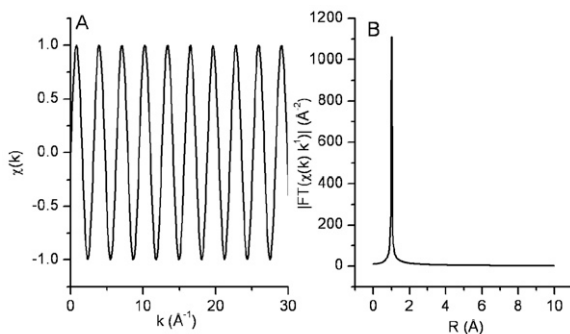


Fig. 14-20. (A) Part of an infinite sine wave with frequency $2k$ and (B) corresponding magnitude of the Fourier transform. The magnitude of the Fourier transform of an infinite sine wave with frequency $2k$ (A) is a delta function at $R = 1.0$ Å (B).

As discussed in the “EXAFS Spectra” section, the information content of the EXAFS spectrum can be described by a sum of sine waves. Each sine wave is characterized by an amplitude and phase that depend on the distance between the absorber atom and the coordinating atoms, and the type of coordinating atoms. Any signal that is a sum of sine waves can be Fourier transformed to separate each frequency component in distance R ; that is, a Fourier transform separates a signal into its Fourier components. Each Fourier component is defined by an amplitude and phase. The most basic example of the Fourier transform is of an infinite sine wave ($k = -\infty$ to $+\infty$) with a single phase. For example, Fig. 14-20 shows part of an infinite sine wave with a phase of $2k$. This signal, with only a single component and infinite data range, is Fourier transformed to an infinitely high and infinitely thin peak (a delta function) at $R = 1.0$ Å (see Fig. 14-20B). Note that as in EXAFS data analysis, a factor of 2 was divided out in the Fourier transform of the infinite sine wave, so that the peak corresponds to R of one instead of two.

The Fourier transformed spectrum becomes harder to interpret with a limited data range, as in a measured EXAFS spectrum. Figure 14-21 shows the Fourier transform of a finite sine wave from $k = 0$ to 10 Å⁻¹ with phase of $2k$ (black curve), another with phase of $3k$ (gray curve), and a third showing the sum of both phases (symbols). The Fourier transform of these finite sine waves are broad peaks (Fig. 14-21B). The broadening is caused by the limited data range of the sine wave. The Fourier transform of the sum of phases $2k$ and $3k$ is identical to the sum of the Fourier transforms of the sine wave with a phase of $2k$ and the sine wave with a phase of $3k$. In fact, any number of unique sine-wave phases can be combined and then separated by using a Fourier transform as long as the difference in the phases is greater than the spatial resolution of the spectra. The property of phase separation by Fourier transformation makes EXAFS analysis a very powerful spectroscopic tool because bonding information from each atomic coordination shell surrounding the absorber atom can often be separated. The Fourier transform separates a signal into its Fourier components and is a complex function containing both real and imaginary parts. The magnitude of the Fourier transform is an envelope of the real and imaginary parts of the Fourier transform.

The signals with phases $2k$ and $3k$ show some overlap and are additive in the real and imaginary parts of the Fourier transform but not in the magnitude of the Fourier transform (Fig. 14-21). The magnitude of the Fourier transform of an EXAFS spectrum contains peaks in R that are related to the interatomic distances. All of the coordinating atoms that are at approximately the same radial distance from the absorber atom (a coordination shell) contribute to one peak in the magnitude of the Fourier transform. In EXAFS fitting analysis, theoretical models are optimized to the data using both the real and imaginary parts of the Fourier transform. This method allows a specific range of phases, corresponding

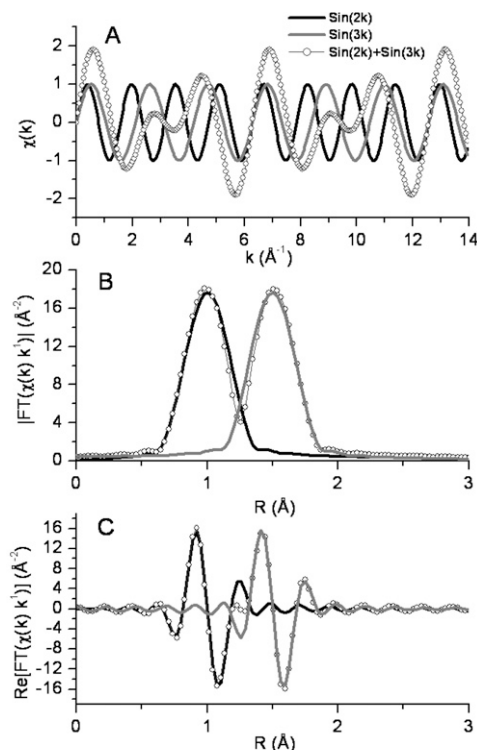


Fig. 14-21. (A) Finite sine wave with phase $2k$ (black), $3k$ (gray), and the sum of signals with phase $2k$ and $3k$ (connected points); and the corresponding (B) magnitude and (C) real parts of the Fourier transform. The Fourier transform was performed using a Kaiser-Bessel window with $k_{\min} = 2.0 \text{ \AA}^{-1}$, $k_{\max} = 12.0 \text{ \AA}^{-1}$, $dk = 3.0 \text{ \AA}$ and a k weight of 1.0. Fourier transform deconvolutes a signal into a sum of sine waves described by an amplitude-term and a phase.

to a specific range of distances about the absorber atom, to be isolated from the other signals in the data.

The magnitude of the Fourier transform of EXAFS spectra is usually shown in publications that model EXAFS spectra. However, EXAFS spectra cannot be correctly interpreted by considering only the Fourier transform magnitude. The magnitude of the Fourier transform is *like* a radial distribution function (RDF) of the atoms about the absorber atom because there is usually a peak corresponding to the first shell of atoms and the R value for the peak is related to the bond length of the first shell. In fact, the magnitude of the EXAFS Fourier transform is *not* a RDF, for several reasons. First, the peak position in the Fourier transform of the EXAFS data does not correspond to the distance between the absorbing atom and its neighboring atoms as they do in a RDF. The shift of the peaks in the Fourier transform depends on the identity of both the absorbing atom and the coordinating atom(s). For example, peaks corresponding to scattering paths from Fe atoms are shifted less than peaks corresponding to scattering paths from O atoms. Second, multiple scattering paths of the photoelectron can produce strong signals in the Fourier transform of the EXAFS data and are not part of a RDF. Third, the interference between two scattering paths of the photoelectron may result in a minimum rather than a peak in the Fourier transform of the EXAFS data. This does not occur in a RDF. Fourth, the amplitude of the peaks in the Fourier transform of the EXAFS data depends on many EXAFS variables (Eq. [5]). A RDF amplitude depends only on the coordination number (N). Finally, the mean free path, $\lambda(k)$, of the photoelectron causes the amplitude of the EXAFS signal to become smaller with increasing radial distance. The amplitude of a RDF depends only on coordi-

nation number. In essence, the magnitude of the Fourier transform of the EXAFS data is a complicated signal that depends only in part on the RDF.

Information Content of a Fourier Transformed Spectrum

The EXAFS signal is a sum of sine waves with an amplitude that depends on wave-number k . To analyze these sine waves in terms of atomic coordination, the EXAFS spectrum is Fourier transformed. Information theory is used to correlate the amount of information in the original EXAFS spectrum to the information in Fourier transform spectrum as determined from the Nyquist theorem

$$N_{\text{IP}} = \frac{2}{\pi} \Delta k \Delta R + 1 \quad [15]$$

where N_{IP} is the number of independent points, Δk is the Fourier transformed data range and ΔR is the region used in EXAFS data analysis (Brillouin, 1962). In general, a robust fitting model uses much fewer variables than N_{IP} . Keeping the number of variables less than N_{IP} is recommended because the information contained within the EXAFS signal is not ideally packed, as described in the later section “Determining Data Range from Fourier Transforms.” A useful goal in EXAFS data analysis is to have twice as many independent points as variables in the fitting model.

Parts of a Fourier Transform

The Fourier transform of any signal is a complex function containing real and imaginary parts. An example of the relationship between these parts and the magnitude of the Fourier transform of the U L_{III} -edge EXAFS data is shown in Fig. 14–22. Figure 14–22E also shows the mirror image of the magnitude of the Fourier transform to illustrate that the magnitude is an envelope function of the real and imaginary parts.

The back Fourier transformed spectra is written as $\tilde{\tilde{\chi}}(k)$, where the double tilde indicates that the Fourier transforms has been applied twice. A common short hand denotes q as a back Fourier transformed k , such that the back Fourier transformed $\chi(k)$ produces a $\chi(q)$ spectrum. Both q and k are measures of wavenumber in units of $\text{\AA}^{-1}$. The back Fourier transform is useful for determining components from the original $\chi(k)$ spectrum that contribute to an R range in the Fourier-transformed spectrum. An example of a back Fourier transform from 1 to 3 $\text{\AA}$ is shown with the original U L_{III} -edge $\chi(k)$ spectrum in Fig. 14–22F. Differences in these spectra illustrate the effect of the initial Fourier transform window shown in Fig. 14–22A and Fourier filtering of the high frequency noise. The high frequency noise is removed from the back Fourier transform because only the frequencies that contribute between 1 and 3 $\text{\AA}$, where the signal/noise ratio is large, were included in the back Fourier transform.

The properties of the back Fourier transform are further illustrated in Fig. 14–23, which shows the back Fourier transforms for four different R ranges. The real part of the back Fourier transform spectra, $\text{Re}[\chi(q)]$, are overlaid with the original EXAFS $\chi(k)$ spectrum in Fig. 14–23B. The real part of $\chi(q)$ spectra at the bottom includes only the low frequency components of the Fourier transform that contribute from 1 to 1.6 $\text{\AA}$ in the Fourier transform. As the back Fourier transform range is increased to include the higher frequency components (e.g., in the range from 1 to 3.2 $\text{\AA}$ in the Fourier transform), the back Fourier transform signal includes more of the detailed shape of the original spectrum. Increasing the back Fourier transform range to 5 $\text{\AA}$ includes most of the high frequency features in the spectra. The back Fourier transformations thus demonstrate the

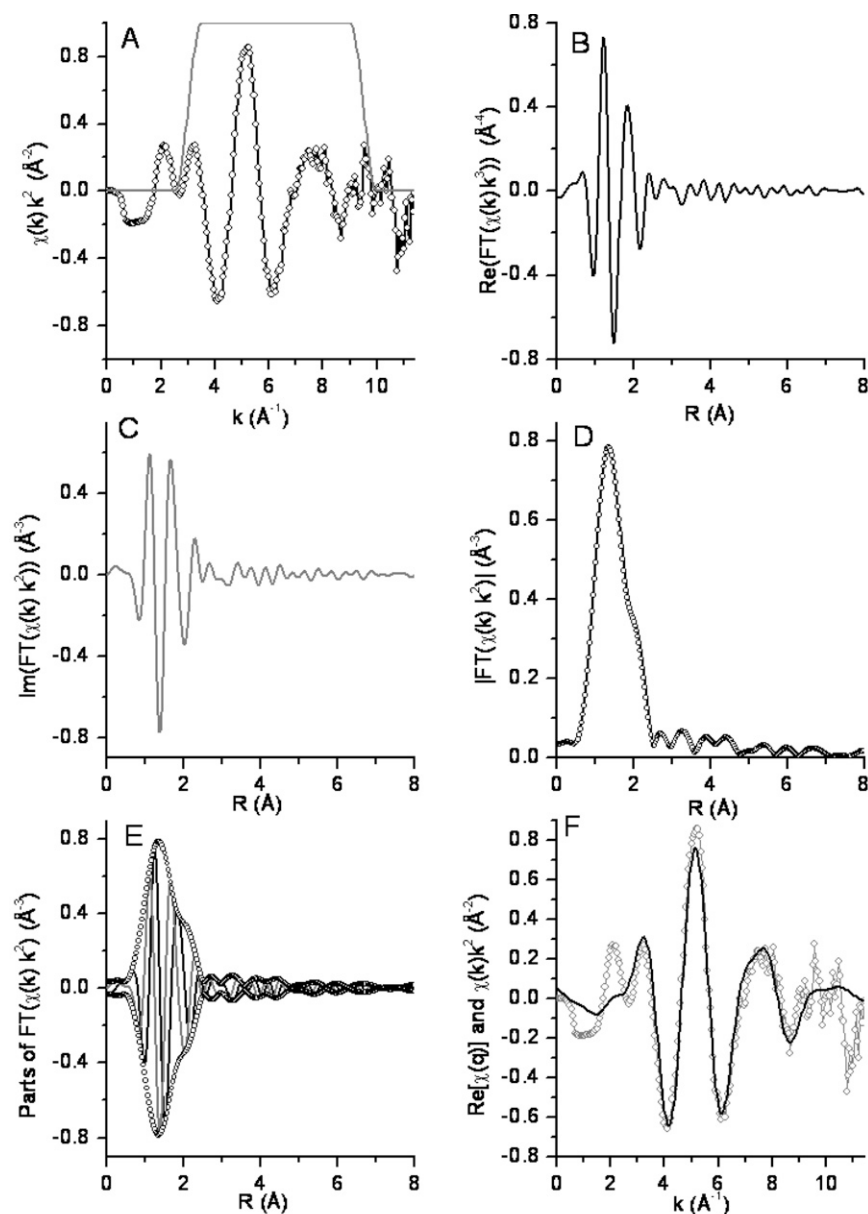


Fig. 14-22. Relationship between the different parts of the Fourier transform of an EXAFS spectrum. (A) Original $\chi(k)k^2$ EXAFS data (symbols) and Fourier transform window (gray line). (B) Real part of the Fourier transform. (C) Imaginary part of the Fourier transform. (D) Magnitude of the Fourier transform. (E) Illustration of the relationship between the real and imaginary parts and the magnitude of Fourier transforms. The magnitude of the Fourier transform is like an envelope function of the real and imaginary parts. (F) Back Fourier transform real part of $\chi(q)$ (solid line) of the EXAFS signal in panel E for $R = 1$ to $3 \text{ \AA}$ is compared to the original $\chi(k)k^2$ signal (connected symbols) to illustrate the effects of the original Fourier transform window function and of high frequency filtering (smoothing by limiting the R range in the back transform).

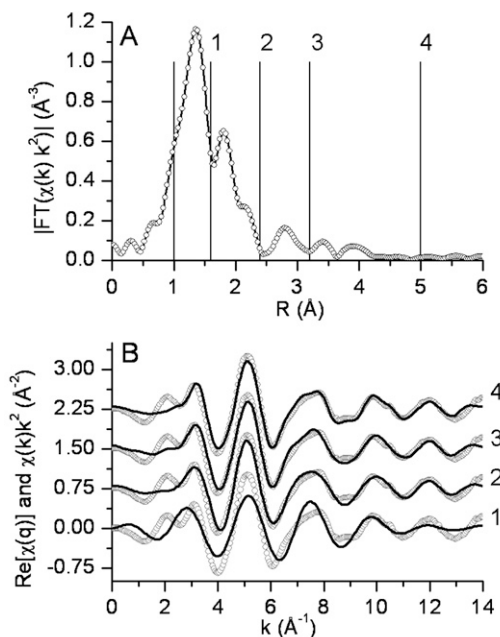


Fig. 14-23. Illustration of back Fourier transformation using different R ranges. (A) Magnitude of the Fourier transform from Fig. 14-20. This spectrum is back Fourier transformed over the different R ranges from 1 to 1.6 Å, 1 to 2.4 Å, 1 to 3.2 Å, and 1 to 5 Å as denoted by the vertical lines labeled 1, 2, 3, and 4, respectively. (B) The original $\chi(k)$ spectra (symbols) is overlaid with the back Fourier transform spectra (solid line) offset from bottom to top with increasing back Fourier transform R ranges. The $\chi(q)$ spectra at the bottom (1) is smooth because it only includes the signal from 1 to 1.6 Å in the Fourier transform. The $\chi(q)$ spectra show more of the original structure in the data as the back Fourier transform range increases from bottom to top. The $\chi(q)$ spectra at the top (4) includes most of the signal and noise in the original spectra.

part of the original $\chi(k)$ spectra that is represented within a discrete region of R by separating different frequency components in the signal.

Fourier Transform Windows

Understanding some mathematical details about Fourier transforms is helpful for practical use in processing EXAFS spectra. The Fourier transform is defined for a spectrum that extends from $-\infty$ to $+\infty$. Because EXAFS spectra have a finite range between $k = 0$ and some larger k value (e.g., 10–15 Å⁻¹), zeros are added to the data outside of this range to pad the dataset from $-\infty$ to $+\infty$. The spectrum to be Fourier transformed contains a sharp change, which is essentially a step function, at the beginning and end of the data because the values of zero outside of the data range change abruptly to some non-zero value within the data set. If a step function was developed by adding together different infinite sine waves, all frequencies of sine waves would be needed so that their signal would cancel to zero before the step, make a sharp transition to a non-zero value at the step, and again cancel to make a flat non-zero value after the step. Hence a Fourier transform of a step function gives signal content at all R values, which is called the Fourier transform ripple. To reduce the effect of the transitions at each end of an EXAFS spectrum, the spectrum is multiplied by a window. The window gradually brings the data up from zero at the beginning and back down to zero at the end of the spectrum. Figure 14-24 shows the effect of a Fourier transform with a gradual window that brings the data up smoothly (Fig. 14-24A) and sharp window that uses two step-like functions to define the Fourier transform data range (Fig. 14-24B). The resulting magnitudes and real parts of the Fourier transforms of the data processed with each window are shown in Fig. 14-24C and 14-24D. These figures illustrate the effect of the window function on the Fourier transform of the spectrum. The spectrum from the sharp window has additional amplitude in the Fourier transform from R values of 0 to 0.8 Å and from 2.2 to 5 Å that is the Fourier transform ripple. The

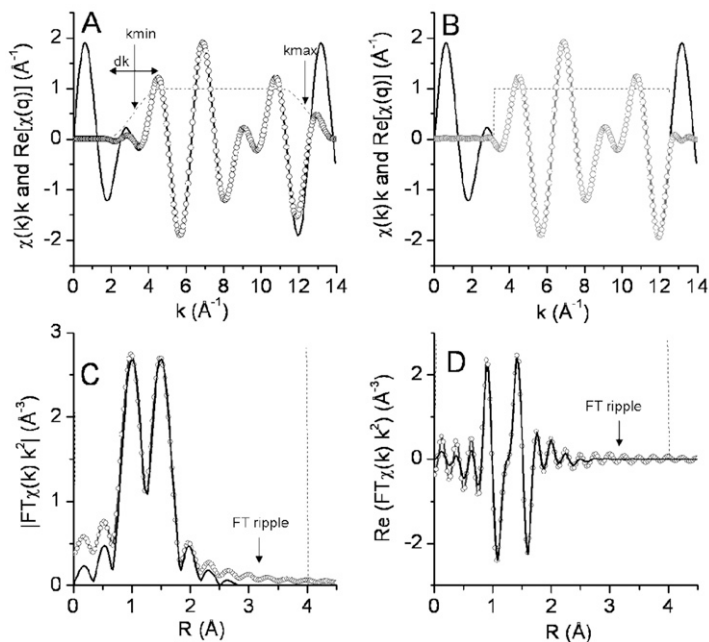


Fig. 14–24. (A, B) The $\chi(k)k$ signal (solid) and back Fourier transform (symbol) for a sine wave of two frequencies of $2k$ and $3k$, along with (A) gradual and (B) sharp Hanning Fourier transform windows (dotted). (C, D) The (C) magnitude and (D) real part of the Fourier transform of the data shown in A (black line) and B (gray symbols). The back Fourier transform window (0–4 Å) is shown by the dashed lines in C and D. The arrows in A show the values for $k_{\min}$ at 3 Å^{-1} and $k_{\max}$ at 12.5 Å^{-1} . The sloped region of the window function is called the window sill, dk . It is centered about $k_{\min}$ and $k_{\max}$ and is 3 Å^{-1} wide.

back Fourier transform of each spectrum is plotted over the original spectra in Fig. 14–24A and 14–24B. The differences between the original and back Fourier transform spectra are caused by the original Fourier transformed window functions. In particular, the oscillation in the EXAFS spectrum from 12 to 14 Å^{-1} shows a gradual decrease to zero in Fig. 14–24A and a sharp decrease to zero in Fig. 14–24B, which in the latter causes the Fourier transform ripple.

Many types of window functions can be used to process EXAFS data, including the commonly used Hanning and Kaiser–Bessel functions. The windows are defined by the same basic parameters: $k_{\min}$ and $k_{\max}$, to specify the beginning and ending k values of the window, and dk , to specify the width of the transition region (Fig. 24A). The value of $k_{\max}$ is a convenient way to limit the Fourier transform to the region of the spectra to be used in the analysis. The Hanning window in Fig. 14–24A shows the transition for a dk value of 2 Å^{-1} ; values of 1 Å^{-1} or 2 Å^{-1} are typical. The Kaiser–Bessel window gradually increases from zero to one and then back to zero over the entire data range. The slope is controlled by the value of dk , with typical values of 3 or 4 Å^{-1} .

Fourier Transform k Weights

The amplitude of EXAFS oscillations diminishes with increasing energy above the absorption edge. Therefore, EXAFS spectra are usually multiplied by k raised to the power of 1 , 2 , or 3 before the Fourier transform is performed. The power of k is called the k weight. A comparison of the EXAFS spectra multiplied with a k weight of 1 , 2 , or 3 is

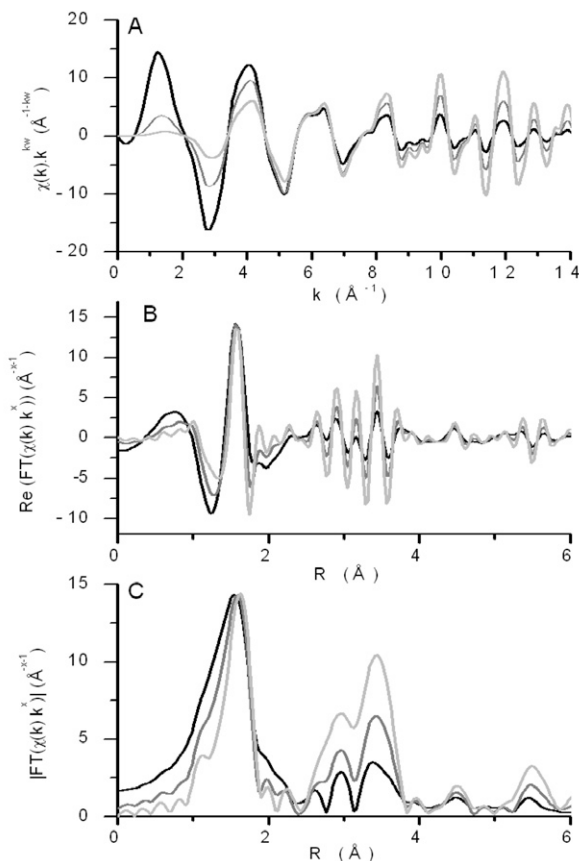


Fig. 14–25. (A) EXAFS spectrum, $\chi(k)k^{k^w}$, and (B) the real part and (C) magnitude of the Fourier transforms for SnO_2 , shown with different values for k weights, x , of 1 (black), 2 (dark gray), and 3 (light gray). Fourier transform data range is $k = 2.4$ to $15.8 \text{ \AA}^{-1}$. The data have been rescaled to the magnitude of the first shell peak near $1.7 \text{ \AA}$ by multiplying the k^1 -weighted data by 34, and the k^2 -weighted data by 6.5.

shown in Fig. 14–25A. By using a k weight of 1, the EXAFS oscillations decrease in amplitude with increasing k . The Fourier transform of this spectrum (Fig. 14–25B and 14–25C) have most spectral weight given to the signal at low k , where the amplitude is greatest. By increasing the k weight, the data at higher k are given relatively more importance, and the Fourier transform will have larger amplitude for the Fourier components that have a larger contribution at higher k values. For example, the theoretical calculation for U–U path and U–O path at $4.0 \text{ \AA}$ is shown in Fig. 14–9. Since the U–U path has a larger signal at k values $>8 \text{ \AA}^{-1}$ as compared with the U–O path, the U–U signal will be more pronounced by using a k weight of 3 in the Fourier transform as compared with the U–O path.

Processing Fourier transforms of EXAFS data using k weights of 1, 2, and 3 is useful for distinguishing between the types of atoms in the first and second shell. For example, Fig. 14–25 show the EXAFS $\chi(k)$ spectra and the Fourier transforms for SnO_2 with different k weights overlaid so the magnitudes of the first shell are scaled to the same height. The rescaled, first-shell signals from 1 to $2 \text{ \AA}$ are similar for all three k weights, but the second-shell signals from 2.5 to $4 \text{ \AA}$ increases with increasing k weight. This difference indicates that the signal in the second shell is from a different type of atom than that of the first shell (Sn rather than O) and is due to the fact that Fourier transform with a k weight of 3 has more emphasis on the data at higher k than in the Fourier transform with a k weight of 1. If the same or similar atoms (e.g., those within one or two atomic numbers) were in

both shells, then the Fourier-transformed data for the different k -weighted spectra would essentially overlay in the regions from 1 to 2 Å and from 2.5 to 4 Å. Thus, comparison of the EXAFS data processed with different k weights is a model-independent procedure that can be used to help determine appropriate atom types for different signals in the different regions of the spectrum. Using all three k weights to model the EXAFS spectrum is a convenient way to ensure that the k dependence of the theory and data are matched.

Determining Data Range from Fourier Transforms

EXAFS data are typically collected over a larger k range than is actually used in the analysis. For example, data on samples containing low concentrations of the absorber element (dilute samples) are typically collected up to $k = 12$ to 14 Å⁻¹. Fourier transform windows can then be used to select a range with adequate signal/noise ratio for analysis.

The effect of noise on the Fourier-transformed signal is similar to the effect of a sharp window in the Fourier transform (Fig. 14–24), as both phenomena cause a ripple effect in the Fourier-transformed signal. This effect on the Fourier transform is useful for determining the usable data range by comparing the real or imaginary part of the Fourier transform, while increasing the upper k range of the data used in the transform. For example, Fig. 14–26A shows U L_{III}-edge $\chi(k)$ data, and Fig. 14–26B shows the real part of the Fourier transform for data ranging from 3 to either 8, 9, or 10 Å⁻¹. The spectra in Fig. 14–26B essentially overlap each other, indicating that these Fourier transforms have essentially the same information content. As the maximum of the Fourier transform range is increased from 10 to 11 or 12 Å⁻¹, significant noise is introduced into the data as shown by the high frequency oscillations in the Fourier transform spectra (Fig. 14–26C). Thus, an upper k limit between 10 and 11 Å⁻¹ is appropriate for these data.

A desirable effect of extending the k range is increased spatial resolution of the Fourier transform signal from neighboring shells of atoms. For example, Fig. 14–27 shows the Fourier transform of a generated EXAFS signal made up of the two phases ($2k$ and $3k$) shown in Fig. 14–21. A Fourier transform with the limited data range from 2 to 7 Å⁻¹ is not sufficient to resolve the two neighboring peaks. In general, signals from two neighboring atomic shells need to be separated by one independent point, $\Delta R = \pi/2\Delta k$ (Eq. [15]), to be resolved in the Fourier transform of the data. In this example, the k data ranges of 2 to 7 Å⁻¹, 2 to 10 Å⁻¹, and 2 to 12 Å⁻¹ result in resolutions in R of ~0.3, 0.2, and 0.15 Å, respectively. Although the peaks in Fig. 14–27 are not visibly separated in the magnitude of the Fourier transform until a resolution of 0.15 Å is achieved, the signals are separated by 0.5 Å and are thus resolvable even with the smallest k range (2–7 Å⁻¹). In summary, optimizing the k range for EXAFS data analysis involves a tradeoff between increasing resolution of neighboring atomic shells and decreasing the contribution of spectral noise to the Fourier transform.

Increasing the k range of the Fourier-transformed spectra increases the resolution of the spectra but does not preferentially allow the shells of atoms at greater radial distances to be resolved. All shells of atoms about the absorbing atom contribute to the EXAFS $\chi(k)$ spectra starting at $k = 0$ (Fig. 14–9). In principle, the amount of information within a given ΔR range and a given Δk range are independent; that is, there is the same amount of information in a spectrum from 1 to 3 Å as there is from 3 to 5 Å. Both of these data ranges have a ΔR range of 2 Å with a Δk range of 10 Å⁻¹ (e.g., $k = 2$ –12 Å⁻¹), such that the information content as given by the Nyquist theorem (Eq. [15]) is the same. In statistical terms with regard to the number of independent points, it does not matter whether the ΔR range of 2 Å is from 1 to 3 Å or from 3 to 5 Å. On the other hand, the XAFS signal is not ideally packed with information. It is much more likely that the signal within the 1 to 3 Å region is dominated by one or two well-separated shells of atoms, whereas the

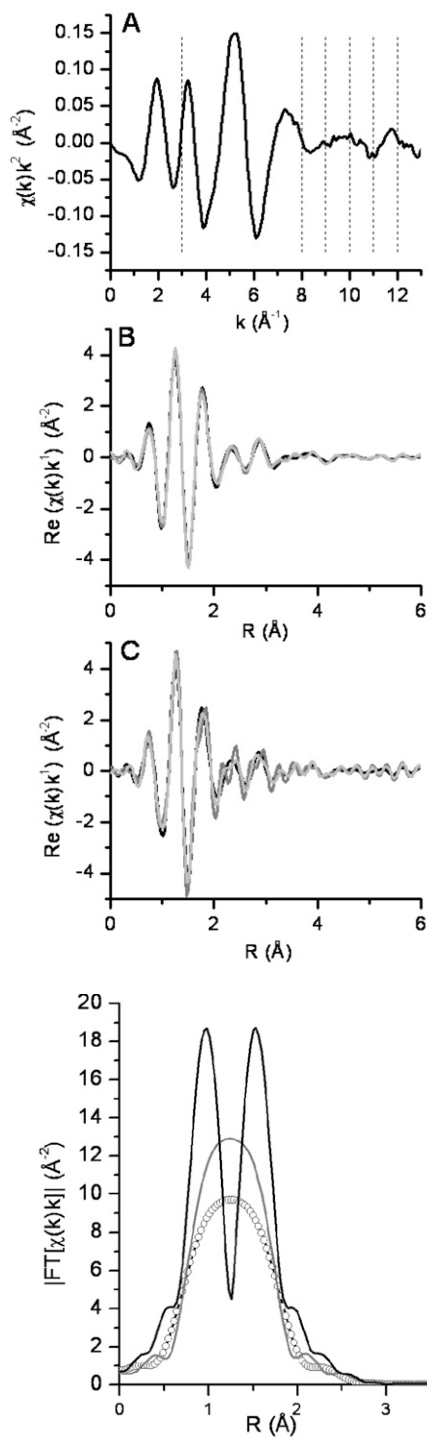


Fig. 14–26. (A) EXAFS $\chi(k)k^2$ data and (B, C) the real part of the Fourier transform obtained by using an increasing data range from 3 to 8 $\text{\AA}^{-1}$ (B: black), 3 to 9 $\text{\AA}^{-1}$ (B: gray), or 3 to 10 $\text{\AA}^{-1}$ (B: light gray) and from 3 to 10 $\text{\AA}^{-1}$ (C: black), 3 to 11 $\text{\AA}^{-1}$ (C: gray), and 3 to 12 $\text{\AA}^{-1}$ (C: light gray).

Fig. 14–27. The magnitude of the Fourier transform of the signal with frequency $2k$ and $3k$ shown in Fig. 14–21, with Fourier transform ranges of 2 to 7 $\text{\AA}^{-1}$ (connected symbols), 2 to 10 $\text{\AA}^{-1}$ (gray curve), and 2 to 12 $\text{\AA}^{-1}$ (black curve). This figure illustrates that the spatial resolution in R space depends on the data range in k space.

region from 5 to 7 Å may contain either no signal (e.g., for a hydrated species), a signal from a mixture of many shells of atoms (e.g., for an adsorbed species), or a signal from well-described shells of atoms (e.g., for a metal or oxide mineral).

Random Noise In EXAFS Spectra

In general, the amplitude of the EXAFS part of an X-ray absorption signal is at best 10% of the total absorption signal. In the example shown in Fig. 14–28A, the edge step is approximately 0.3 (from 0.05 to 0.35), and the largest oscillatory part of the absorption coefficient is approximately 0.02 (8% of the edge step). Ideally, the EXAFS signal is 1000 times greater than the random statistical noise (SN). Hence a noise level $\leq 0.1\%$ of the total signal is desirable. Therefore, if the signal noise is dominated by shot noise, the number of X-rays that need to be measured ($N_{\text{X-ray}}$) is 10^6 according to the relationship:

$$\text{SN} = \frac{1}{\sqrt{N_{\text{X-ray}}}} \quad [16]$$

The number of X-rays can be calculated from the voltage on the ionization detectors through Eq. [11]. The noise in EXAFS spectra can be easily approximated by looking at the EXAFS $\chi(k)k^2$ signal at $k = 10 \text{ Å}^{-1}$. At 10 Å^{-1} , the EXAFS signal is usually weak enough that random fluctuations in the data are visible, and by multiplying the data by a factor of 100 via the k^2 weighting, y axis values can be considered as percentages. Figure

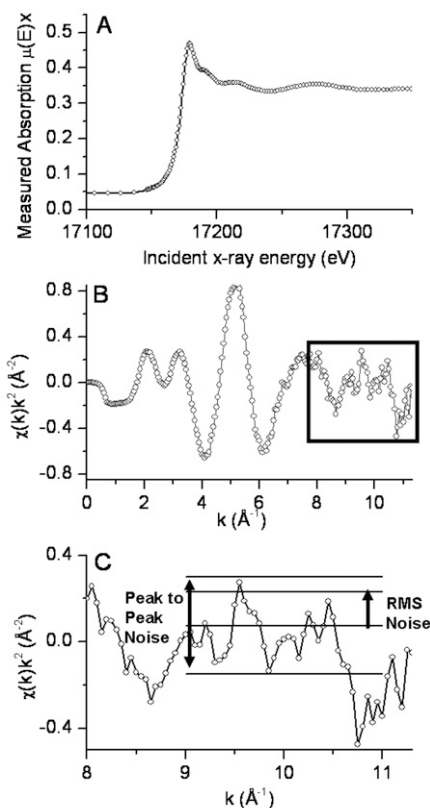


Fig. 14–28. Illustration of noise in $\chi(k)$ EXAFS spectrum. (A) X-ray absorption spectrum. (B) Full $\chi(k)k^2$ EXAFS spectrum. (C) Enlarged view of the $\chi(k)k^2$ data between 8 and 12 Å^{-1} . The peak to peak noise and the root-mean-square (RMS) noise is shown at 10 Å^{-1} . The fluctuations at 10 Å^{-1} can be read in percentage because the spectrum is multiplied by 100 during the k^2 weighting of k^2 at 10 Å^{-1} .

14–28 shows EXAFS data with peak to peak fluctuations at $10 \text{ \AA}^{-1}$ of about 0.45%. The root mean square noise is the peak to peak fluctuations divided by $2\sqrt{2}$, which is 0.16%, indicating that this data set contains about twice the acceptable noise level. To reduce this noise level by a factor of 2, the statistics need to be increased by a factor of 4 (Eq. [16]) by either increasing the counting time or the number of scans to be averaged. Analogous to the count rate effect on noise, the noise level decreases with the number of scans (N_{scans}) by a factor $N_{\text{scans}}^{-1/2}$. Thus, averaging four scans decreases noise by a factor of two relative to a single scan, assuming that each scan has the same counting time and photon flux. Another source of noise in an EXAFS scan is due to systematic noise caused by sample inhomogeneity. If the noise in the sample does not decrease with additional counting as given in Eq. [16], then sample preparation should be considered a likely source of noise.

Background Function

The EXAFS signal is defined as the normalized oscillatory part of the absorption coefficient above an absorption edge. To process the EXAFS signal, the step-like background of the absorption edge itself is removed. The background function is constructed from splines that are connected with knots. A spline is a polynomial of n th order, where order of 1, 2, or 3 correspond to a linear ($y = Ax$), quadratic ($y = Ax + Bx^2$), or cubic ($y = Ax + Bx^2 + Cx^3$) splines. Early background removal procedures manually placed the knots through the EXAFS oscillations (Sayers and Bunker, 1988). A common more recent approach, called AUTOBK, was developed by Newville et al. (1993) and is integrated into the IFEFFIT methods used by ATHENA. This method uses the information content in the low R region of the spectrum to construct the background function. Information theory is used to define the transfer of information from wavenumber (k) to radial distance (R) through a Fourier transform (Eq. [15]). In this approach, the background function is constructed from third-order (cubic) splines. The knots of the spline are not constrained in the optimization of the background function, which gives less importance to the placement of the knots. The number of splines determines the maximum frequency component that is allowed in the background function. This forces the knots to be evenly spaced in photoelectron wavenumber throughout the data region. Information theory is used to constrain the background function to Fourier components that are longer in wavelength than that in the EXAFS spectrum by setting a maximum frequency component in R (called “ R_{bkg} ”), below which contributing Fourier components are considered to be part of the background. Because EXAFS oscillations of longer wavelength correspond to shorter radial distances, and vice versa, the longest wavelength oscillation that is actually part of the structural information arises from the first shell of atoms about the absorber.

In the AUTOBK algorithm, the data range and the value for R_{bkg} determine the number of splines as given by $(2R_{\text{bkg}}\Delta k)/\pi$. In general, R_{bkg} is about one-half the first nearest neighbor distance. This value for R_{bkg} will allow all frequencies up to one-half the first shell distance to be removed by the background. The effectiveness of the background removal is made by comparing the Fourier transform of the $\chi(k)$ data produced with different values of R_{bkg} .

Figure 14–29 shows some U L_{III} -edge data with three very different choices for the value of R_{bkg} . In the case of $R_{\text{bkg}} = 0.5 \text{ \AA}$, the background function does not have the flexibility to remove the long frequency in the background. The Fourier transform of this $\chi(k)$ spectrum shows a large peak at $0.5 \text{ \AA}$ that must be due to the background and not to neighboring atoms in the sample, because interatomic distances between atoms are $>1.0 \text{ \AA}$, based on ionic radii of anions being $>1.2 \text{ \AA}$ (Lide, 2003). Optimal background removal ($R_{\text{bkg}} \sim 1.0 \text{ \AA}$ in Fig. 14–29) maximizes the intensity of the first-shell peak while minimizing the amplitudes of peaks at shorter radial distances in the Fourier transform. To the contrary,

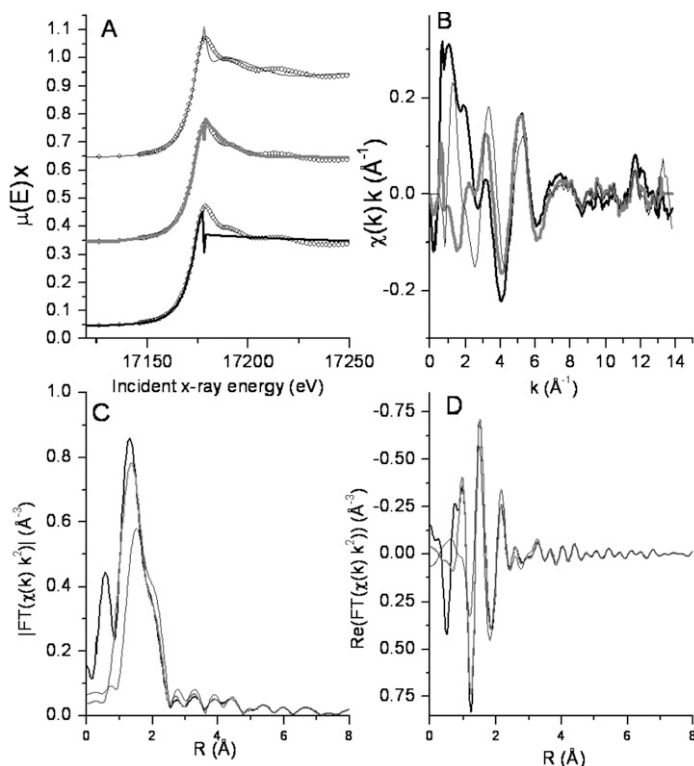


Fig. 14–29. A comparison of Fourier transforms for different choices for R_{bkg} . (A) The U L_{III} -edge data is shown (symbols) three times. The background is shown for R_{bkg} values of 0.5 (bottom: thick black), 1.0 (middle: gray), and 1.5 (top: thin black). (B) The corresponding $\chi(k)k$ data with the same line types. (C, D) The magnitude and the real part of the Fourier transform from 3 to 9.5 $\text{\AA}^{-1}$ of the data shown in B with the same line types.

with an R_{bkg} value of 1.5 $\text{\AA}$, the background follows the oscillatory part of the absorption coefficient above the absorption edge (Fig. 14–29). The amplitude of the signal at $\sim 1.8 \text{ \AA}$ in the Fourier transform of the data has been reduced relative to those of the other Fourier transforms, indicating a R_{bkg} value that is too large.

In practice, the difficult part of defining a background function is in the edge-region of the spectra, where the absorption spectrum changes abruptly and beyond the flexibility of a spline. Examples of the background functions obtained from small changes in R_{bkg} of 0.9, 1.0, and 1.1 $\text{\AA}$ are shown in Fig. 14–30. The resulting $\chi(k)$ spectra are identical in the data range from 3 to 12 $\text{\AA}^{-1}$, but differ in the low k range where the absorption edge changes quickly (Fig. 14–30B). For the initial extraction of $\chi(k)$ from the background, it is a good practice to use the $\chi(k)$ data that comes from the flat region of the absorption spectra (e.g., at $E > 20 \text{ eV}$ above the edge). This unaffected region of the spectra can be identified by comparing the resulting $\chi(k)$ data as shown in Fig. 14–30. In this example, a minimum energy value of 17,209 eV corresponding to a k value of 3.0 $\text{\AA}^{-1}$ should be used in the Fourier transform of the data. Once a structural model has been built to accurately describe this $\chi(k)$ data, this model can be used to test data at lower k or refine the background removal from the edge region. The example in “Aligning Energy Scales of Experimental and Theoretical Spectra” below shows how to optimize the background function once a model has been determined.

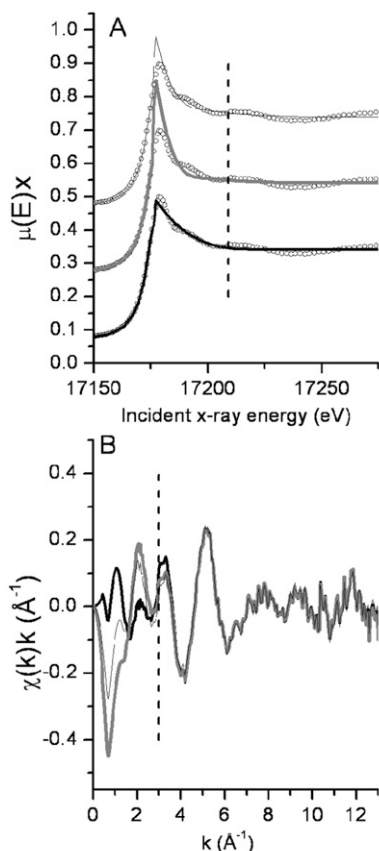


Fig. 14-30. Comparison of (A) background functions and (B) the corresponding $\chi(k)$ data. The U L_{III} -edge data are shown three times in A with different background functions obtained by using R_{bkg} values of 0.9 Å (bottom: thick black), 1.0 Å (middle: thick gray), and 1.1 Å (top: thin black). The resulting $\chi(k)k$ data are shown in B with the corresponding line types. The data are not strongly influenced by the choice of R_{bkg} above the edge region, as indicated by the choice of R_{bkg} above the edge region, as indicated by the dashed line at approximately 3.0 Å^{-1} corresponding to the energy value of 17,209 eV. At this point, the absorption data no longer have the strong curvature of the edge region.

Another parameter that can be varied within the AUTOBK method of background removal is the spectral k weighting used when defining the background function. The background function is optimized when the area above the zero line of the EXAFS oscillations matches the area below the zero line of the oscillation (like a sine wave with equal halves above and below the baseline). Figure 14-30 shows an example of spectra for U in soil with an R_{bkg} value of 1.0 Å and k -weighting values of 1, 2, and 3 used in the background removal process. One way to evaluate the appropriate k weighting to use during background removal is to plot the resulting data with different k weights as in Fig. 14-31. For example, using a k weighting of 3 in the background removal may yield a seemingly reasonable spectrum (balanced on both sides of the baseline) when it is plotted with a k weight of 3 (Fig. 14-31A), but it may be unbalanced and lack oscillations at low k when it is displayed with a k weight of 1 (Fig. 14-31C). In general, using low k -weight values (1 or 2) during background removal will place greater importance on the near edge region of the EXAFS spectrum, so this part of the spectrum is optimized around zero. Higher k -weight values (2 or 3) will place greater emphasis to the higher k region of the spectrum, so the high k -region deviates less from zero. The best value to use for the k weighting of the background removal step should produce a spectrum that is independent of the k weight used to display the background-removed data. In this example (Fig. 14-31), a k weight of 1 or 2 produces a spectrum that is equally balanced when the spectrum is plotted with all three k weights.

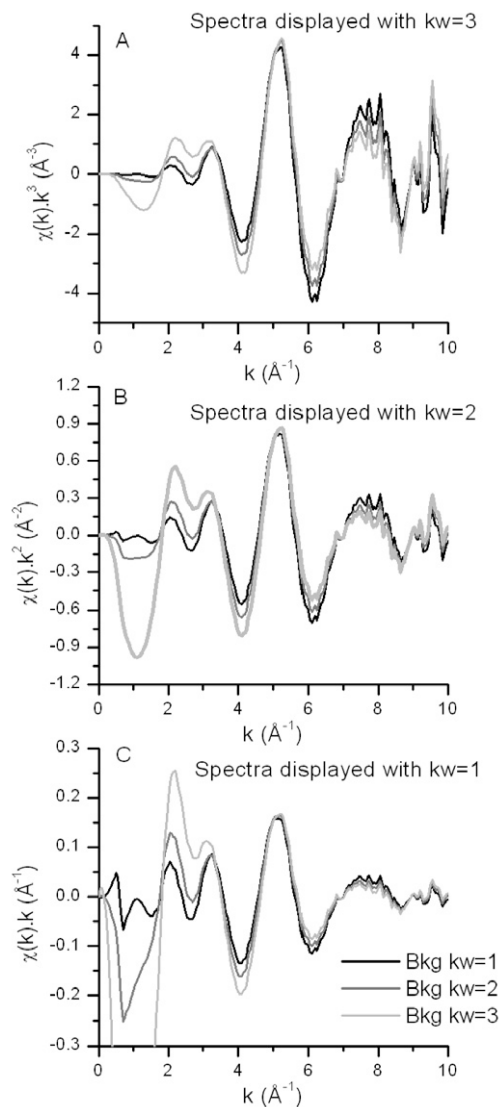


Fig. 14-31. EXAFS $\chi(k)$ spectra produced by using different k weightings (kw) in the background removal procedure. The spectra are displayed with a k weighting of (A) 3, (B) 2, and (C) 1 after background removal using $kw = 1$ (black line), $kw = 2$ (dark gray), and $kw = 3$ (light gray) during the background removal with $R_{\text{bkg}} = 1$.

In general, if the high k region of the spectrum is not sensitive to the k weighting used in background removal (as in Fig. 14-31), then a lower k weight is chosen to keep the edge region closer to zero. If the data below $3 \text{ \AA}^{-1}$ are not used in data analysis, then all three of the spectra in Fig. 14-31 are perfectly reasonable because the spectral region between 3 and $9 \text{ \AA}^{-1}$ is essentially independent of the k weighting used in background removal. Moreover, a Fourier transform of the spectra between 3 and $9 \text{ \AA}^{-1}$ is essentially identical (data not shown). In “Aligning Energy Scales of Experimental and Theoretical Spectra” below, we show how a theory can be used to help define the background function through the edge region. The theory can be used to help define the low k region of the spectrum, and a greater k weighting can be used in the background removal step to help improve the background removal at higher k values.

Some EXAFS software packages do not model the background throughout the edge region. There are several reasons to model the low k region of the EXAFS spectrum. The signal/noise ratio is strongest at low k , and the k dependence of several fitted parameters can be exploited by using the low k region of the spectra to reduce the correlation between these parameters.

EXAFS Fitting—Building Theoretical Models

Modeling EXAFS spectra to determine the average, local-molecular coordination environment of an absorber atom is a multistep process that is best learned through hands-on experience. Therefore, we describe here the process of building a structural model by giving examples for certain steps with specific reference to the EXAFS data analysis programs Athena for background removal and Artemis for optimizing the theoretical model to the measured spectrum (Ravel and Newville, 2005). These programs are user-friendly GUI interfaces to IFEFFIT (Newville 2001). IFEFFIT is an interactive engine that contains the algorithms for fitting the theoretical FEFF calculations (Zabinsky et al., 1995) to the measured spectrum, hence the name IFEFFIT. FEFF is the program that calculates the details of the scattering processes from a cluster of atoms and is explained in more detail below. Table 14–3 lists the data-analysis program names used in our examples and gives a brief summary of their function. These software packages are open-source programs that can be downloaded from a link on the International XAFS Society (www.i-x-s.org [verified 20 Dec. 2007]) or XAFS.ORG (www.xafs.org [verified 20 Dec. 2007]) websites for use on various computer platforms. Currently, Artemis comes with the freely available program FEFF6L (Zabinsky et al., 1995) for computing theoretical EXAFS models. A number of other data analysis programs are available and follow the basic principles described here.

The general approach for determining the average coordination structure around the absorber atom from EXAFS data is to build a structural model for the chemical species being identified, and calculate the theoretical EXAFS spectrum for that model using the FEFF program. Programs such as Artemis adjust the structural parameters in the EXAFS equation (described below) until a least-squares fit is obtained between the theoretical (modeled) and experimental EXAFS spectra. The model is adjusted as needed (e.g., with different atom types) until the best possible fit is obtained between theoretical and experimental spectra.

The Basics of FEFF

To build a model, FEFF requires an initial guess as the starting point for the placement of atoms about the absorber atom in the sample. The initial guess can be in the form of a known crystal structure, or user-defined list of x , y , z coordinates for a cluster of atoms, such as results from density functional theory (DFT) calculations. Using crystal structures

Table 14–3. Computer programs used the EXAFS data analysis examples.

Program	Purpose	Input	Output	Reference
Athena	GUI interface to IFEFFIT for background subtraction, LCF, etc.	Absorption spectra	EXAFS $\chi(k)$ spectra	Ravel and Newville, 2005
Artemis	GUI interface to IFEFFIT and FEFF for fitting a model to measured spectra	EXAFS $\chi(k)$ spectra	Best-fit model and results	Ravel and Newville, 2005
FEFF	Calculates the <i>effective</i> scattering amplitudes [$F_{\text{eff}}(k)$] and phase shifts based on a cluster of atoms	Cluster of atoms	Theoretical EXAFS parameters	Zabinsky et al., 1995
IFEFFIT	An <i>interactive</i> engine that contains the processes for removing background and performing least-square <i>fitting of FEFF</i> theoretical models to measured spectra.	IFEFFIT script	Results from processes	Newville, 2001

```

HOLE 1 1.0 * Sn K edge (29200.0 eV), second number is 90°2
*
  mphase,mpath,mfeff,mchi
CONTROL 1 1 1 1
PRINT 1 0 0 3

RMAX 8.0

*CRITERIA curved plane
*DEBYE temp debye-temp
*NLEG 8

POTENTIALS
*
  ipot Z element
  0 50 Sn
  1 50 Sn
  2 8 O

ATOMS
*
  x y z ipot tag distance
  0.00000 0.00000 0.00000 0 Sn1 0.00000
  0.91429 0.91429 1.59319 2 O1_1 2.05186
  -0.91429 -0.91429 1.59319 2 O1_1 2.05186
  0.91429 0.91429 -1.59319 2 O1_1 2.05186
  -0.91429 -0.91429 -1.59319 2 O1_1 2.05186
  -1.45434 1.45434 0.00000 2 O1_2 2.05675
  1.45434 -1.45434 0.00000 2 O1_2 2.05675
  0.00000 0.00000 3.18638 1 Sn1_1 3.18638
  0.00000 0.00000 -3.18638 1 Sn1_1 3.18638
  3.28293 1.45434 0.00000 2 O1_3 3.59064
  1.45434 3.28293 0.00000 2 O1_3 3.59064
  -3.28293 -1.45434 0.00000 2 O1_3 3.59064

```

Fig. 14–32. Example of a FEFF inp file for SnO₂ showing the HOLE, POTENTIALS and the ATOMS list. The ATOMS list is truncated at 3.6 Å.

or DFT calculations provides a reliable method for producing well-spaced atomic potentials needed for theoretical EXAFS computations, so this approach is used extensively to fit EXAFS data for both crystalline and noncrystalline samples. The program Artemis has a special interface called “Atoms” that converts crystal structure information into a cluster of atoms and provides a list of atom positions in *x*, *y*, *z* coordinates for use in FEFF computations. The Atoms interface allows the user to define a crystal structure by specifying the space group, unit cell dimensions, and the fractional positions of the atoms within the unit cell. This information is available from databases such as CIF files from the Inorganic Crystal Structure Database (ICSD). The Atoms database (Newville et al., 1999a) contains a number of common structures that are already in the correct format. Additional information needed includes the core atom type, the cluster size, and the X-ray absorption edge.

There are three basic sections of the FEFF input file (Fig. 14–32). The first section describes the core “HOLE” being created by X-rays (e.g., inputs of “1” and “4” refer to K and L_{III}–edges, respectively). The second is the independent “POTENTIALS” (“ipot”) list. This list contains the ipot number, atom type, and atomic number of all the atoms in the cluster and is used to specify the unique ion potentials in the FEFF calculation. The FEFF program requires ipot = 0 for the absorber atom. The third section is the position of each “ATOM” with *x*, *y*, and *z* coordinates followed by its ipot number. The Atoms interface also inserts a user defined identifier tag (e.g., O1_1 and O2_2 to distinguish oxygen atoms in different shells), which is intended to make the list easier to read and interpret. FEFF creates muffin tin potentials about the position of the atoms (Zabinsky et al., 1995). The radius of these potentials is defined by the placement of the coordinating atoms. Because the potentials are restricted in size by the coordinating atom, it is important to calculate the theoretical EXAFS signal from a cluster that has a radius *R* that is *larger* than the largest coordinating atom distance used to model the experimental spectrum. Thus, for example, building an EXAFS model for an aqueous species with only one or two shells of atoms should be based on a FEFF calculation of the aqueous structure with many layers of waters to define the size of the muffin tin radii of the outer-shell atoms or a crystalline structure with the correct local geometry about the absorber atom. Specifically, the EXAFS spectra from a hydrated uranyl may only contain two axial oxygen atoms at a distance of 1.8 Å and six equatorial atoms at a distance of 2.4 Å around the uranium atom. The FEFF calculation

based on this cluster will not be accurate because the outer equatorial oxygen atoms do not have well-defined radii since there are no atoms of larger radial distance to define the atom boundaries. The FEFF calculation of a uranyl oxide crystal structure with a cluster size of only 2.4 Å would be similarly incorrect. The FEFF calculation based on the crystal structure of a uranyl oxide to 4 Å including all the atoms beyond the first equatorial oxygen shell will yield improved calculations for the first shell equatorial oxygen atoms to be used to model the hydrated species.

Parameterizing EXAFS Variables

Once a path has been selected for possible inclusion in a structural model, mathematical expressions for the EXAFS parameters (Eq. [5]) are defined. The parameters that are often determined from a fit to the EXAFS spectrum affect either the amplitude of the EXAFS oscillations (N , S_0^2 , σ^2) or the phase of the oscillations (ΔE_0 and ΔR).

There are many approaches that can be used to constrain the EXAFS fitting parameters such that the number of independent points in the measured spectra is greater than the number of variables determined in the model. Example approaches include collecting EXAFS data at multiple edges and co-refining the fitting parameters (Ravel et al., 1999), using independent structural information and bond angles (Frenkel et al., 1994), modeling a mixture of structures (Kelly et al., 1998), determining binding sites (Kelly et al., 2002), integrating results from multiple techniques (Allen et al., 1995), measuring pressure dependence (Frenkel et al., 1997), or temperature dependence (Haskel et al., 2005; Frenkel et al., 2001) of the structures, using multiple edges to obtain structural disorder (Calvin et al., 2002), and using crystal structural information (Ravel et al., 1998). We discuss common approaches for constraining each of the EXAFS fitting parameters:

- **ΔR .** First, ΔR values can be defined by an isotropic expansion–contraction term as αR_{eff} . R_{eff} is a special key word for the effective path length R for each path from the FEFF calculation of the model structure. A single value of α could be determined and applied to all path lengths included in the model, effectively reducing the number of variables for R from the number of paths (i.e., 30) to a single variable. Because this approach assumes that adjustments to all path lengths (interatomic distances) in the structure vary in proportion to one another (α being the proportionality constant), this approach is best used for cubic crystal structures in which physical expansions/contractions are likely to be isotropic. Second, ΔR_i values or $\alpha_i R_{\text{eff}}$ values can be grouped depending on path length or direction. This parameterization gives more freedom to account for an anisotropic expansion–contraction but also requires more variables. Third, ΔR values can be related to unit cell dimensions or atomic moieties. This method requires more sophisticated mathematical expressions that are usually derived from known relationships between interatomic distances in specific crystal structures, and it can be very powerful. Some specialized codes have been written to produce these expressions (Kelly et al., 2001). Fourth, the change in path length parameters can be determined independently for each path.
- **ΔE .** First, one energy shift can be applied to all paths. This is the most common method of defining ΔE , and it accounts for the misalignment of the data with respect to the theoretical calculation. Second, two energy shift values are sometimes used, where the value for the first path is different from the value for all other paths. This method accounts for the energy-alignment issue and for some possible charge imbalance in the FEFF calculation of the first atomic potentials as compared with all other potentials. Third, energy shifts can be assigned to atom types. This method is sometimes used to account for ionic bonds that transfer charge between different types of atoms. In general, these shifts should be less than 2 to 3 eV.
- **σ^2 .** First, σ^2 values can be defined in terms of a Debye or Einstein model (Sevillano et al., 1979) with one or more characteristic temperatures. Second, σ^2 values can be grouped depending on atom type and bond length of a model path. This parameterization assumes that coordination shells involving similar atoms and similar

interatomic distances are similar in thermal and static disorder. Third, σ^2 values can be broken into two components to represent the structural and thermal disorder in the bond lengths. This parameterization can be used with Debye or Einstein models for defining the thermal component, or it can be applied to temperature or pressure dependent spectra. Fourth, a unique σ^2 value can be given to each path, which yields the maximum number of σ^2 parameters in the model.

- S_0^2 . First, one value of S_0^2 is often used for all paths included in the model. Second, S_0^2 values are determined by fitting the measured EXAFS spectrum of a standard of known crystal structure measured under similar conditions as the sample in which unknown species are being identified. Third, theoretical calculations such as those by FEFF 8.4 (Ankudinov et al., 2003) can also be used to determine S_0^2 .
- N . First, the degeneracy of a path, N , is often determined from the coordination number in a known crystal structure or known atomic species. Second, the degeneracy can be independently determined for each single scattering path used in the model. The degeneracy of multiple scattering paths is constrained in terms of the corresponding single scattering paths. Third, coordination numbers can also be constrained by the stoichiometry of a particular ligand attached to the absorber.

Comparing Models Using Statistical Tools

Once a model for the EXAFS parameters has been developed, the variable EXAFS parameters and mathematical variables are adjusted to arrive at a least-squares fit between the experimental and theoretical spectra. The fitting results include the user defined variables, their best-fit values, and the uncertainties in these variables, along with important information about the statistics of the model. Statistical parameters include the number of independent points, N_{idp} , the number of variables N_{var} , which must be less than the number of independent points, the χ^2 (chi-square) and χ^2_{ν} (reduced-chi-square) values, and an R factor. These standard goodness-of-fit parameters are described in more detail in Bevington and Robinson (1992). Use of these statistical parameters for EXAFS analysis is described by Stern et al. (1995). The isolated EXAFS signal is denoted as $\chi(k)$ and should not be confused with the goodness-of-fit parameter χ^2 . Changes in the goodness-of-fit parameter, χ^2_{ν} , are used to compare different EXAFS models.

The R factor is the sum of the squares of the differences between the data and the fit at each data point, divided by the sum of the squares of the data at each corresponding point. It represents the mean square misfit between the data and the fit for both the real and imaginary parts of the Fourier transform and is defined as follows:

$$R \text{ factor} = \frac{\sum_i (\text{data}_i - \text{fit}_i)^2}{\sum_i \text{data}_i^2} \quad [17]$$

In general, R factor values less than 0.05 are considered to reflect a reasonable fit.

The χ^2 value is the sum of the squares of the difference between the predicted fit and the data divided by the uncertainties (ϵ) at each data point (i). It is calculated as

$$\chi^2 = \frac{N_{\text{idp}}}{N_{\text{pts}}} \sum_i \left(\frac{\text{data}_i - \text{fit}_i}{\epsilon_i} \right)^2 \quad [18]$$

where the sum is over each data point in the real and imaginary parts of the Fourier transform, N_{idp} is the number of independent points in the model fit (Eq. [15]), and N_{pts} is the number of data points. To compare models with different numbers of variables, the χ^2_{ν} values are used. It is calculated by the χ^2 divided by the degrees of freedom in the fit, ν .

The degrees-of-freedom in the fit is defined as the number of independent points in the measured spectra minus the number of variables fit in the model.

For a general implementation, the χ^2_ν value is approximately one for an optimized model. This guideline is true for systems with well-defined line shapes and uncertainties that can be quantitatively measured such as statistical noise. It may be possible to quantitatively define nonstatistical errors, but unless they are Gaussian distributed, formal Gaussian statistics will not apply. For XANES and EXAFS analysis, neither the line shapes nor the uncertainties are well defined, resulting in a poorly scaled χ^2_ν value. The uncertainties (ϵ) in EXAFS analysis is based on the amplitude of the EXAFS signal at 15 to 25 Å because there is rarely any contribution from atoms at these distances (Newville et al., 1999b).

The size of the uncertainty ϵ is assumed to be underestimated by the value of χ^2_ν , and this uncertainty is propagated to the uncertainty in the fitting parameters. For example, if the χ^2_ν value of a perfectly reasonable fit is 25 instead of 1, then the uncertainties are increased by $\sqrt{\chi^2_\nu}$ or 5 to rescale the χ^2_ν value to one. This rescaling is automatically performed for each fit, so that large χ^2_ν values result in large uncertainties in the fitted parameters. This methodology does not assign any error to the unknown line shapes in EXAFS spectra, and leads to a conservative uncertainty in the measured parameters.

A statistically significant change in the final $\chi^2_{\nu f}$ value when compared with an initial $\chi^2_{\nu i}$ value is greater than two standard deviations:

$$\frac{\chi^2_{\nu i}}{\chi^2_{\nu f}} - 1 \geq 2\sqrt{\frac{2}{\nu}} \quad [19]$$

This equation implies that significant changes in the χ^2_ν value are on the order of factors of 2. For example, a final smaller χ^2_ν of 40 is better than an initial larger χ^2_ν of 80, but models with χ^2_ν of 40 and 42 are not significantly different.

EXAFS Modeling Examples

Specific examples are provided here to illustrate typical, useful procedures for building a structural model and fitting EXAFS data.

Aligning Energy Scales of Experimental and Theoretical Spectra

One of the steps involved in modeling EXAFS data is aligning the EXAFS spectra to the theoretically calculated spectra. During background removal, the absorption spectrum as a function of energy (E) is transformed into the EXAFS spectrum as a function of wavenumber (k) as defined in Eq. [7]. The wavenumber scale is defined relative to E_0 , which is a user-defined value. The theoretical spectrum is given as a function of wavenumber k , but the energy used for $E_0 = 0$ (and therefore $k = 0$) as defined from the measured spectrum rarely coincides with that determined in the theoretical model. As noted above, a fitted parameter, ΔE_0 can be adjusted in the fit of the theory to the data to account for the mismatch between the user defined position of E_0 on the edge and the theoretically calculated value for $k = 0$. In general, ΔE_0 should be kept as small as possible (a few electronvolts) because shifting the energy scale for the theoretical calculation distorts the theoretical spectrum. Very large values for ΔE_0 (tens of electronvolts) can shift the spectra by a full oscillation in the EXAFS spectrum and lead to erroneous inter-atomic distances (Michalowicz and Vlaic, 1998). By aligning the experimental and theoretical spectra, the theoretical edge energy, E_0 , is defined relative to measured data. This procedure ensures that the E_0 value falls on the edge of the absorption coefficient, thereby preventing a mismatch between

the data and theory by a full oscillation and also preventing distortion of the theoretically calculated spectra.

Here is an example of how to align EXAFS data for SnO_2 to a theoretical spectrum. Figure 14–33A shows spectra based on an initial choice for E_0 and the resulting background function. Figure 14–33B shows the corresponding $\chi(k)$ spectrum. The first-shell fit to the data was obtained using an Sn–O path from FEFF with $N = 4$ and an initial bond length of 2.05 Å. The model was optimized to the data by determining ΔE_0 , ΔR , and σ^2 values, resulting in a ΔE_0 value of 6.2 ± 1.5 eV, a ΔR value of -0.0234 ± 0.015 Å, and a σ^2 value of 0.0015 ± 0.0012 Å². The fit to the data is shown as the magnitude of the Fourier transform in Fig. 14–33C and as $\chi(k)$ spectra in Fig. 14–33D. The next step is to produce a theoretical spectrum with the best-fit values for ΔR and σ^2 of -0.0234 and 0.0015 Å², respectively, while holding the energy shift (ΔE_0) at zero.

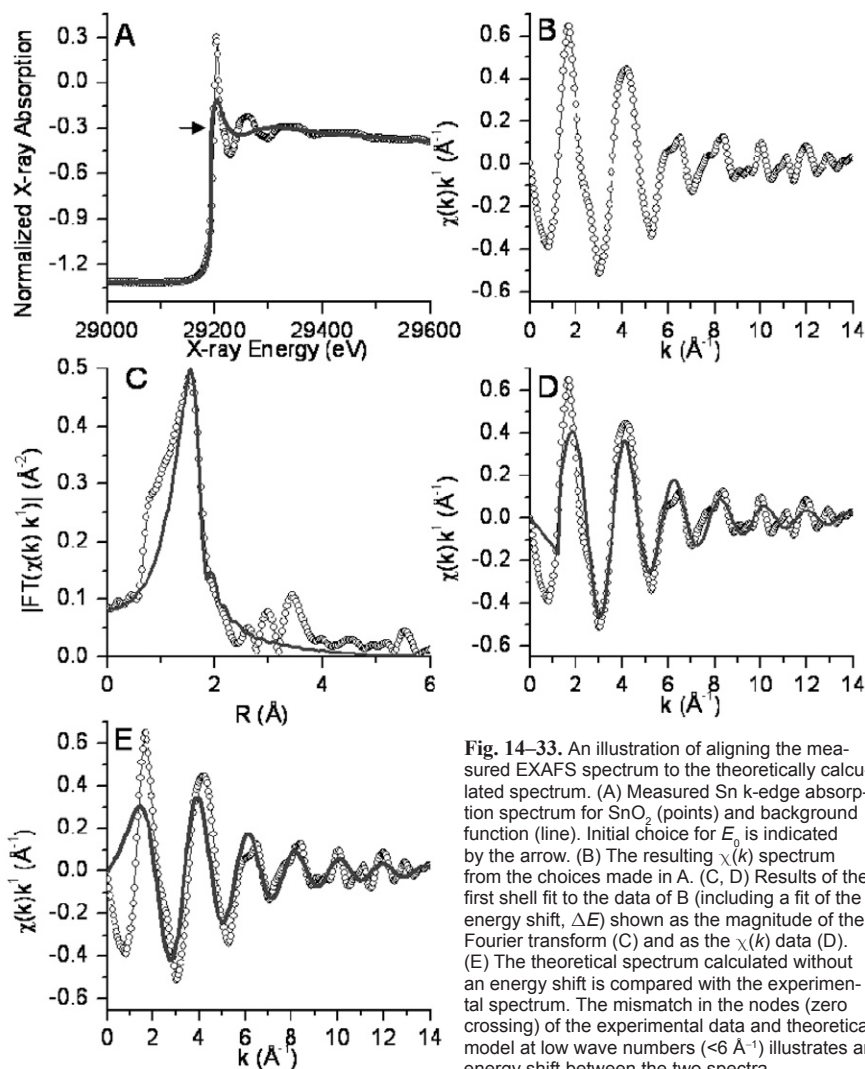


Fig. 14–33. An illustration of aligning the measured EXAFS spectrum to the theoretically calculated spectrum. (A) Measured Sn k-edge absorption spectrum for SnO_2 (points) and background function (line). Initial choice for E_0 is indicated by the arrow. (B) The resulting $\chi(k)$ spectrum from the choices made in A. (C, D) Results of the first shell fit to the data of B (including a fit of the energy shift, ΔE) shown as the magnitude of the Fourier transform (C) and as the $\chi(k)$ data (D). (E) The theoretical spectrum calculated without an energy shift is compared with the experimental spectrum. The mismatch in the nodes (zero crossing) of the experimental data and theoretical model at low wave numbers (<6 Å⁻¹) illustrates an energy shift between the two spectra.

The newly calculated theoretical $\chi(k)$ spectrum (line) is compared to the experimental $\chi(k)$ spectrum (symbol) in Fig. 14–33E. The mismatch between the positions of the nodes of these two spectra at low wavenumbers ($<6 \text{ \AA}^{-1}$) illustrates the energy shift between the two spectra. To align the energy scales, this new theoretical spectrum is used in the background removal, and the value for E_0 , the edge energy, of the measured spectrum is adjusted. The new E_0 and background function is shown in Fig. 14–34A. The aligned theoretical and experimental EXAFS spectra are shown in Fig. 14–34B. A new first shell fit to the aligned spectrum is performed, and the results are shown as the magnitude of the Fourier transform in Fig. 14–34C and the $\chi(k)$ spectra in Fig. 14–34D. The best-fit values obtained by fitting the aligned spectrum are $\Delta E_0 = 3.2 \pm 0.9 \text{ eV}$, $\Delta R = -0.005 \pm 0.009$ and $\sigma^2 = 0.0020 \pm 0.0006 \text{ \AA}^2$. A comparison of ΔR values from fitting of the nonaligned spectrum ($-0.0234 \pm 0.015 \text{ \AA}$) and the aligned spectrum ($-0.005 \pm 0.009 \text{ \AA}$) shows a difference of $0.018 \text{ \AA}$ in ΔR as a result of proper data alignment, which was approximately the magnitude of the uncertainty in the fit for the nonaligned data. Notice that the uncertainty in R was decreased from ± 0.015 to $\pm 0.009 \text{ \AA}$ by properly aligning the spectrum. This example illustrates the importance of aligning the data and the theory to keep ΔE as small as possible (less than a few electronvolts).

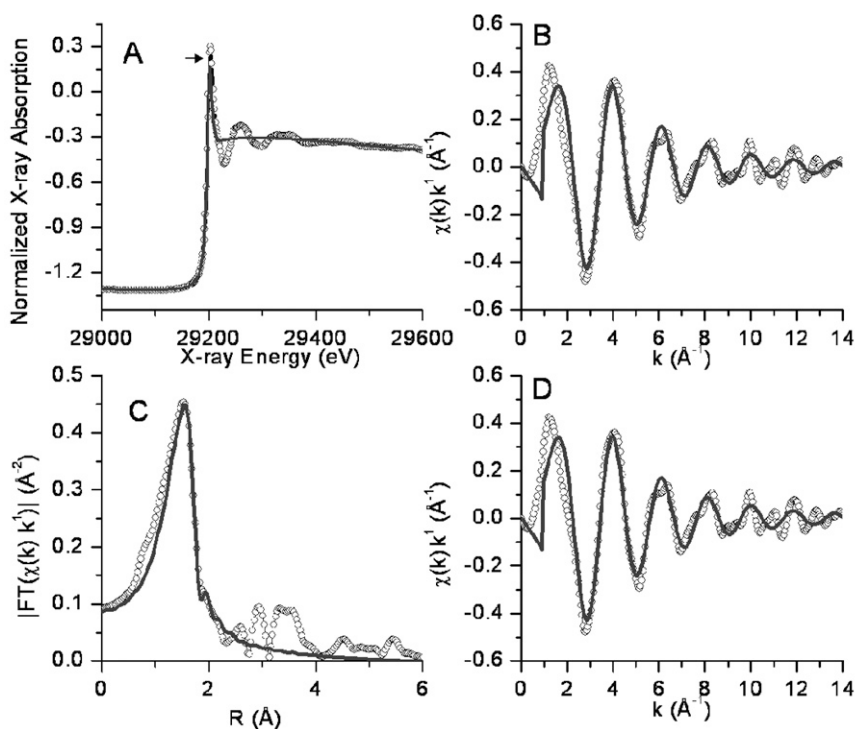


Fig. 14–34. An illustration of the effect of aligning the measured EXAFS spectrum (black circles) to the theoretically calculated spectrum (line). (A) A new choice for E_0 (indicated by the arrow) and the background function used to align the data and the theory as shown in B. (B) A comparison of the measured and the theoretically-calculated spectra without an energy shift ($E_0 = 0 \text{ eV}$). (C and D) Results of a first shell fit (including ΔE) to the newly aligned spectra shown as the magnitude of the Fourier transform (C) and as the $\chi(k)$ data (D).

Modeling a Metal Oxide (SnO₂)

This example steps through the process of modeling EXAFS data for a metal oxide mineral with known crystal structure, SnO₂(c). The absorption data and a proper background removal on aligned spectra were presented in the previous example. The goals of this example are to (i) determine important scattering paths to be included in the EXAFS model, (ii) accurately model the SnO₂ EXAFS spectra to 8 Å, and (iii) discuss typical values for EXAFS parameters.

One of the most useful tools in EXAFS analysis of an unknown system is a comparison of the measured EXAFS spectrum with a theoretical spectrum before adjusting the fitting parameters. The theoretical spectra can be calculated from any defined cluster of atoms. A quick method for generating a theoretical spectrum is to use a defined crystal structure. In this example, we use the known crystal structure (from X-ray diffraction) of SnO₂ to illustrate how varying EXAFS fitting parameters affects the fit between theoretical and experimental spectra. EXAFS data collection and model development for a well-characterized physical standard is a useful starting point for identifying chemical species in an unknown sample.

Once a theoretical spectrum has been generated, as described in “EXAFS Fitting, Building Theoretical Models,” all of the scattering paths can initially be included in the model; for SnO₂, there are 69 paths for a cluster including all atoms to 8 Å. This cluster of atoms is shown in Plate 14–1 (see color insert section). These paths can be summed and compared to the data by setting the EXAFS parameters for S_0^2 to 1.0, ΔE_0 to 0 eV, ΔR to 0 Å, and σ^2 to 0 Å². Summing the theoretical scattering paths with these parameters will create a theoretical spectrum with EXAFS oscillations of greater amplitude than the data because the thermal and statistical disorder in the path lengths (σ^2) has been held at zero and the amplitude reduction factor (S_0^2) has been held to 1.0. A comparison of experimental and theoretical $\chi(k)$ spectra are shown in Fig. 14–35A, and the magnitude of the Fourier transforms is shown in Fig. 14–35B. The $\chi(k)$ spectra show that the data and theory are fairly well aligned in energy, since the measured and theoretical spectra both cross $\chi(k)k = 0$ (nodes) at approximately the same wavenumber for $k < 6$ Å^{−1}. This comparison also shows that the theoretical spectrum is a good match to the measured spectrum in amplitude, since the oscillations vary similarly in relative amplitude for both spectra. Again, the amplitudes are more pronounced in the theoretical spectrum because $\sigma^2 = 0$. Moreover, the interatomic distances (ΔR) are similar for the sample and theoretical structures because both spectra have nodes at approximately the same wavenumber for $k > 6$ Å^{−1}.

The complexity of the model depends on the number of paths used in the model. Therefore it is usually prudent to include only those paths that make a significant contribution to the model. One method for determining the importance of a given path or set of paths is to fit the experimental spectrum with and without including the path(s), then compare the χ^2_ν values (as discussed in the previous section) from the two models. This statistical comparison should be used in cases where it is important to prove that some paths are not essential in the model. In addition, FEFF calculates an approximate amplitude for each path relative to that of the first path. This amplitude is calculated from the magnitude of the paths using a computationally fast, plane-wave approximation. These relative amplitudes can also be used to eliminate paths having a low value, and therefore make only a minor contribution to the EXAFS spectrum. In our example, eliminating all the paths with < 5 , 10, or 15% of the amplitude of the first path reduces the number of paths from 69 to 30, 18, or 8, respectively. The remaining paths can be summed as before, and the theoretical spectrum compared with the measured spectrum (Fig. 14–36). With the 5% amplitude cut-off criterion (only 30 of the original 69 paths retained), all of the structure in

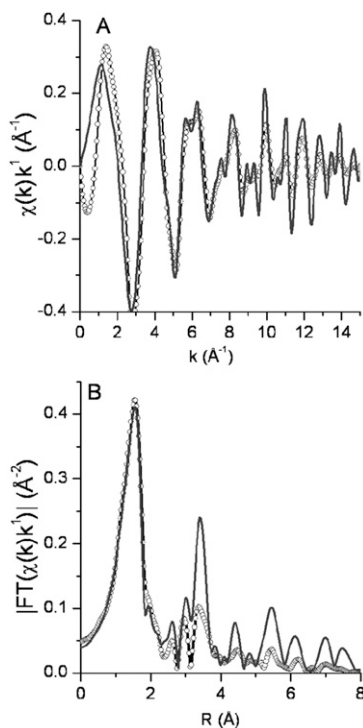


Fig. 14-35. Comparison of the experimental EXAFS data for SnO_2 (symbols) with a theoretically calculated EXAFS spectrum (line) based on the known crystal structure and without optimizing fitting parameters. (A) EXAFS $\chi(k)$ data, and (B) magnitude of the Fourier transform.

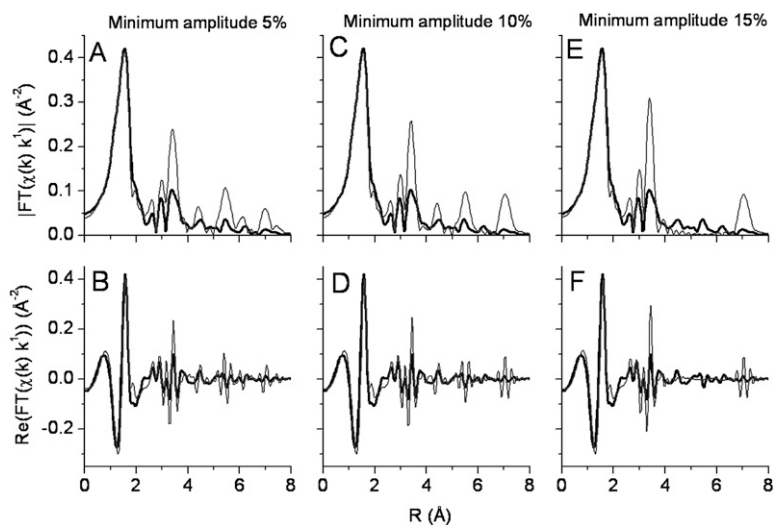


Fig. 14-36. An illustration of the importance of paths calculated by FEFF to be included in the EXAFS model for $\text{SnO}_2(\text{s})$, determined by comparing the experimental data (thick black line) to the sum of paths (thin gray line) including all paths with an approximate amplitude >5, 10, or 15% of the first path. (A, C, E) Magnitude of the Fourier transform. (B, D, F) Real part of the Fourier transform. (A, B) Model includes 30 paths and all features in the experimental data are accounted by the model. (C, D) Model includes 18 paths, and some features in the data at 6 $\text{\AA}$ are missing in the model spectrum. (E, F) Model includes eight paths, and the model spectrum does not follow the data in the region from 4 to 7 $\text{\AA}$.

the theoretical model is retained (Fig. 14–36A and 14–36B). A more careful analysis could likely remove a few more paths, as most of the structure in the theoretical model is accounted by using only 18 of the 30 paths, as illustrated by the 10% amplitude cut-off model in Fig. 14–36C and 14–36D. The 15% amplitude cut-off is too restrictive, since much of the structure present in the data is lost in the theoretical model (Fig. 14–36E and 14–36F). For this example, our model will not depend directly on the number of paths included, so all 30 paths determined from the 5% amplitude cut-off model can be retained.

There are five EXAFS parameters that must be defined for each path included in the theoretical model. These parameters (as defined by the EXAFS equation for each path, Eq. [5]) are ΔR , ΔE_0 , σ^2 , S_0^2 , and N . Even with 30 paths instead of 69, the number of variables would exceed the number of independent points (Eq. [15]) if all of these parameters are allowed to vary independently for each path. Thus, constraints must be applied to limit the number of independent variables in the fitting analysis.

The purpose of modeling the data set for SnO_2 , for which the crystal structure is known, is to most accurately reproduce the experimental EXAFS spectrum out to 8 Å in the Fourier transform. This model can then be applied to a data set for samples containing SnO_2 (or other unknown Sn species) at a low concentration (Bare et al., 2005). Using a EXAFS $\chi(k)$ data range (Δk) and Fourier transform modeling range (ΔR) of approximately 2 to 13.5 Å⁻¹ and 1 to 8 Å, respectively, the data set contains 56 independent points (Eq. [15]). Thus, fitting approximately one-half the number of independent points (28) as variables would be completely reasonable for the amount of data. In general, a well-described spectrum has twice as many independent points as variables determined in the model of the spectrum. Based on the high degree of matching between the phase of the data and the theoretical model (Fig. 14–35A and 14–35B), and the cubic crystal symmetry of SnO_2 (s), ΔR is justifiably parameterized by the isotropic expansion–contraction expression, αR_{eff} . In defining σ^2 , we expend most of the parameters by grouping σ^2 based on atom type and distance, resulting in eight independent σ^2 values. One S_0^2 and one ΔE term is fit for all paths, and N is constrained to the degeneracy values based on the crystal structure of SnO_2 . This model is fully described in Table 14–4 and uses 16 parameters.

The best-fit value for each EXAFS parameter is given in Table 14–5, and a comparison of the data and model fit is shown in Fig. 14–37. For $\Delta R = \alpha R_{\text{eff}}$, the fitted α value was -0.0001 ± 0.0007 , leading to ΔR values of <0.001 Å. Note that ΔR represents the shift applied to the interatomic distance for a given photoelectron path in the starting (theoretical) model that is needed to fit the experimental data. Fitted values for ΔR should be <0.1 Å; otherwise the theoretical model should be refined to yield values of R_{eff} closer to the final value. The energy shift parameter is <5 eV, indicating that the EXAFS data and theoretical EXAFS are adequately aligned. Values of σ^2 are generally between 0.003 and 0.020 Å², which are reasonable values for a metal oxide at room temperature. Thermal and structural disorder in bond lengths usually becomes larger with increasing bond length. The S_0^2 value of 1.1 ± 0.1 falls within the typical range of 0.7 to 1.0.

For a soil or sediment sample containing Sn contamination as SnO_2 , the extensive EXAFS model developed here (Table 14–4) can be used as a starting point for fitting data for the unknown species. If Sn species other than SnO_2 (c) are dominant, the structural model developed here can be refined, for example, by decreasing the number of paths if the fitting results of higher-shell coordination distances and atoms are not reasonable. It is both the success and the failure of a given model that give clues to the species present in the sample. Highly constrained models, as shown here for SnO_2 , provide a rigorous test for the crystalline SnO_2 structure within a sample of unknown Sn speciation.

Table 14-4. EXAFS parameterization of SnO_2 . All changes in path length (ΔR) relative to each reference path length (R_{eff}) obtained from the crystal structure were determined in terms of an expansion parameter (σ) used as a multiplier on R_{eff} . The σ^2 values were grouped depending on the path length and atom types. Linear combinations of these energy shifts were used to describe the multiple scattering paths. One S_0^2 value and one ΔE_0 value was also determined in the fit, and N (by default) was equal to the degeneracy of each path determined from the crystal structure of SnO_2 . There are a total of 16 parameters in this model.

Path	N	R_{eff} $\text{\AA}$	σ^2 $\text{\AA}^2$
Sn-O ₁	4	2.052	$\sigma^2\text{o1}$
Sn-O ₂	2	2.057	$\sigma^2\text{o1}$
Sn-Sn ₁	2	3.186	$\sigma^2\text{sn1}$
Sn-O ₃	4	3.591	$\sigma^2\text{o3}$
Sn-Sn ₂	8	3.709	$\sigma^2\text{sn2}$
Sn-Sn ₂ -O ₁	8	3.909	$\sigma^2\text{sn2o1}$
Sn-Sn ₂ -O ₂	8	3.909	$\sigma^2\text{sn2o1}$
Sn-O ₅	8	4.241	$\sigma^2\text{o5}$
Sn-Sn ₃	4	4.737	$\sigma^2\text{sn3}$
Sn-O ₇	8	4.801	$\sigma^2\text{o7}$
Sn-Sn ₄	8	5.709	$\sigma^2\text{sn4}$
Sn-Sn ₅	8	5.837	$\sigma^2\text{sn4}$
Sn-Sn ₅ -O ₁	8	5.841	$\sigma^2\text{sn4}$
Sn-Sn ₅ -O ₄	8	5.841	$\sigma^2\text{sn4}$
Sn-O ₁ -Sn ₅ -O ₁	4	5.850	$\sigma^2\text{sn4}$
Sn-O ₄ -Sn ₅ -O ₄	4	5.850	$\sigma^2\text{sn4}$
Sn-Sn ₆ -Sn ₁	4	6.373	$\sigma^2\text{sn6}$
Sn-Sn ₁ -Sn ₆ -Sn ₁	2	6.373	$\sigma^2\text{sn6}$
Sn-Sn ₇	4	6.700	$\sigma^2\text{sn7}$
Sn-Sn ₇ -O ₂	4	6.700	$\sigma^2\text{sn7}$
Sn-Sn ₇ -O ₆	4	6.700	$\sigma^2\text{sn7}$
Sn-Sn ₇ -O ₆ -O ₂	4	6.700	$\sigma^2\text{sn7}$
Sn-O ₆ -Sn ₇ -O ₂	4	6.700	$\sigma^2\text{sn7}$
Sn-O ₂ -Sn ₇ -O ₆ -O ₂	4	6.700	$\sigma^2\text{sn7}$
Sn-O ₆ -Sn ₇ -O ₆ -O ₂	4	6.700	$\sigma^2\text{sn7}$
Sn-Sn ₈	8	7.419	$\sigma^2\text{sn8}$
Sn-Sn ₈ -Sn ₂	16	7.419	$\sigma^2\text{sn8}$
Sn-Sn ₂ -Sn-Sn ₂	8	7.419	$4\sigma^2\text{sn2}$
Sn-Sn ₂ -Sn ₈ -Sn ₂	8	7.419	$\sigma^2\text{sn8}$
Sn-Sn ₉	16	7.658	$\sigma^2\text{sn9}$

Determining a Second Shell Atom Type

This example illustrates how to build a molecular-moiety model to determine the type of atoms in the second coordination shell around the absorber atom. The data comes from a series of sediment samples containing U(VI) in equilibrium with Fe oxides and different microbial components. The first shell of atoms contains both axial and equatorial oxygen atoms of the uranyl cation (UO_2^{2+}). A representation of the uranyl is shown in Plate 14-2A (see color insert section). Based on matrix properties, the second shell could contain O, C, P, Fe, and/or

Table 14-5. The EXAFS results for σ^2 values for SnO_2 . S_0^2 was determined to be 1.07 ± 0.05 . The model constrained the distances R in terms of an expansion-contraction parameter of $-0.0001(7)$ relative to the XRD values. The degeneracy of each path (N) is based on the crystal structure of SnO_2 . The best fit value for $\Delta E_0 = 3.4 \pm 0.2$ eV.

σ^2 values	($\times 10^3 \text{ \AA}^2$)
σ^2_{o1}	3.4 ± 0.3
σ^2_{sn1}	3.8 ± 0.3
σ^2_{o3}	38 ± 18
σ^2_{sn2}	5.2 ± 0.2
σ^2_{sn2o1}	2.6 ± 1.2
σ^2_{o5}	9.4 ± 3.4
σ^2_{sn3}	7.0 ± 1.3
σ^2_{o7}	6.0 ± 3.5
σ^2_{sn4}	7.7 ± 0.8
σ^2_{sn6}	8.6 ± 2.8
σ^2_{sn7}	6.2 ± 2.3
σ^2_{sn8}	8.6 ± 1.8
σ^2_{sn9}	9.0 ± 3.2

U atoms. A bidentate representation of the uranyl bound to PO_4 , CO_3 , and FeO_6 groups are shown in Plate 14-2B through 14-2D. The FEFF calculations for this molecular-moiety model are based on crystalline structures of andersonite, meta-autinite, and richetite. Before the final structural models shown here were determined, 30 to 50 different possible combinations of these atoms at different distances were tested, illustrating that EXAFS modeling of complex geochemical systems can require much trial and error before arriv-

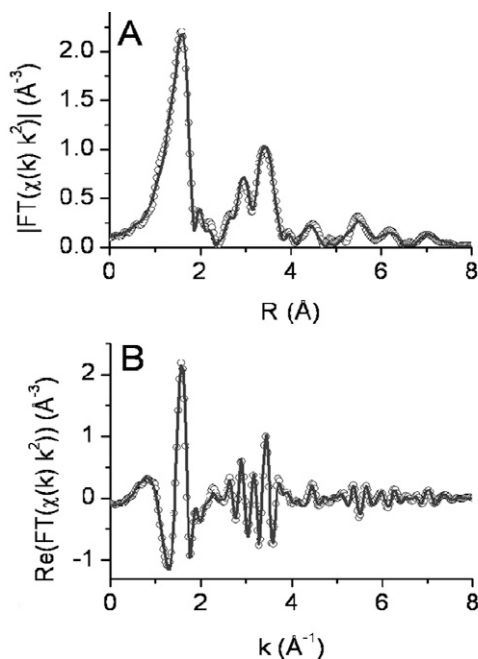


Fig. 14-37. (A) Magnitude and (B) real part of the Fourier transform of the $\text{SnO}_2(\text{s})$ data (symbols) and final model fit (solid line).

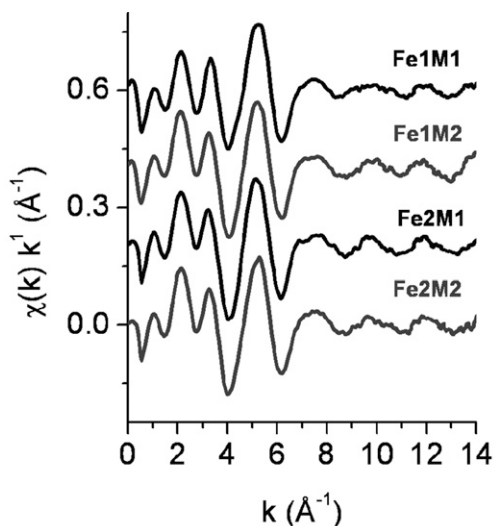


Fig. 14–38. Uranium L_{III} -edge EXAFS spectra for four similar samples.

ing at the best model. Knowledge of the chemical system (e.g., through complementary data, as discussed under XANES analysis above) helps to refine the EXAFS model. The three example EXAFS models described below contain combinations of C and Fe, P and Fe, or Fe and Fe as the second and third shell atoms. The EXAFS $\chi(k)$ spectra from the series of four samples are shown in Fig. 14–38. The EXAFS model is described in Table 14–6. In the model, the value of S_0^2 was set to 1.0 as determined by fitting a standard uranyl compound of known crystal structure with a best-fit value of 1.0 ± 0.1 . One energy shift parameter (ΔE) was determined for all paths in the fit. The number of axial oxygen atoms was set at 2, based on the known structure of uranyl. The interatomic distance (ΔR) and the disorder term (σ^2) for the axial oxygen atoms were determined in the fitting analysis of the measured spectrum. The distance (ΔR), the number (N), and the disorder term (σ^2) for the equatorial oxygen atoms, C/Fe/P atoms (X) at $R_{\text{eff}} = 2.9 \text{ \AA}$, and Fe atoms at $R_{\text{eff}} = 3.5 \text{ \AA}$ were determined. This model has a total of 12 variables, which is slightly less than the 15 independent points in the measured spectrum for the data range ($3\text{--}11 \text{ \AA}^{-1}$) and fit range ($1\text{--}3.6 \text{ \AA}$) used.

Before such a fit is performed, it is best to sum the paths of the model to get an overall spectrum and also to compare where the signal for each individual shell makes a contri-

Table 14–6. Description of the EXAFS model used to determine the second shell neighbor type listed as X (path U– X).

Path	N	R_{eff}	ΔR	σ^2	ΔE
		$\text{\AA}$	$\text{\AA}$	$\text{\AA}^2$	eV
U–Oax	2	1.8	ΔR_{oax}	σ^2_{oax}	ΔE_{o1}
U–Oeq	Noeq	2.4	ΔR_{oeq}	σ^2_{oeq}	ΔE_{o1}
U–Oax1–Oax2	2	3.6	$2\Delta R_{\text{oax}}$	$2\sigma^2_{\text{oax}}$	ΔE_{o1}
U–Oax1–Oax1	2	3.6	$2\Delta R_{\text{oax}}$	$4\sigma^2_{\text{oax}}$	ΔE_{o1}
U–Oax1–U–Oax2	2	3.6	$2\Delta R_{\text{oax}}$	$2\sigma^2_{\text{oax}}$	ΔE_{o1}
U– X	N_x	2.9	ΔR_x	σ^2_x	ΔE_{o1}
U–Fe	N_{fe}	3.5	ΔR_{fe}	σ^2_{fe}	ΔE_{o1}

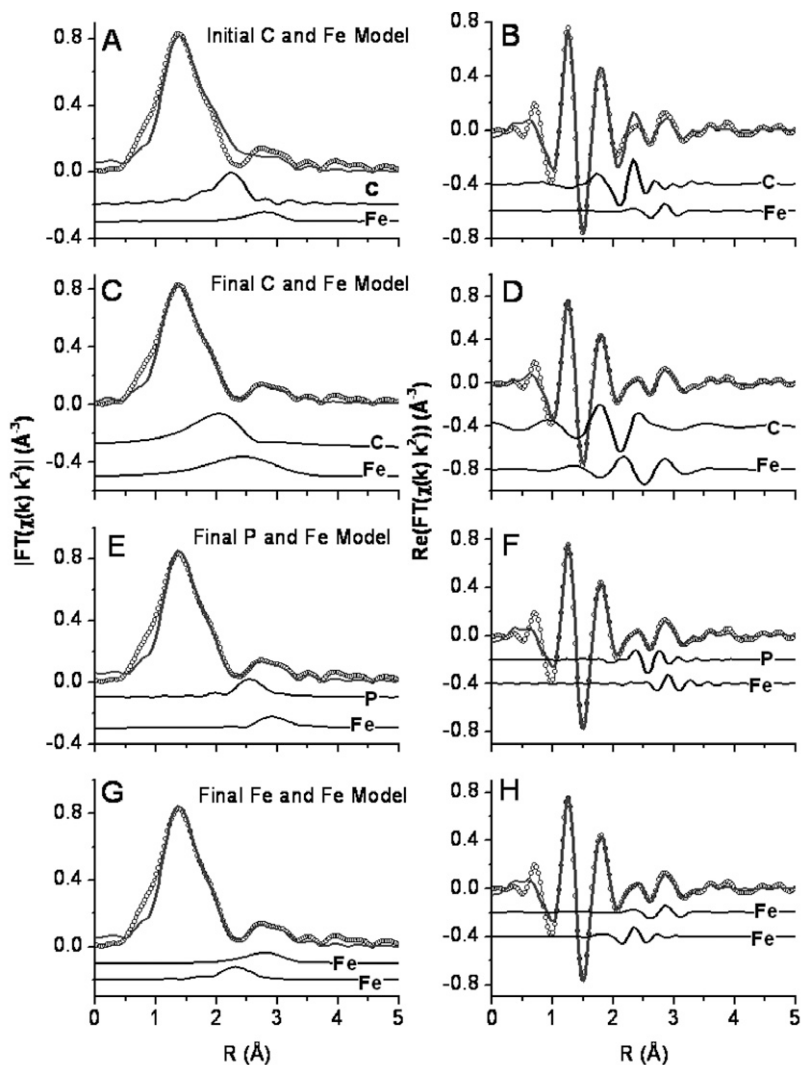


Fig. 14-39. Uranium L_{III} -edge EXAFS data. (A, B) magnitude and real part of the EXAFS spectra from one of the data sets in Fig. 14-38 along with a model (sum of paths before optimization) showing the initial placement of the second (C) and third (Fe) shells. Magnitude and real part of the EXAFS spectrum from one of the data sets along with an optimized model showing the EXAFS contributions from Fe along with different atoms for X (C, P, or Fe) in Table 14-6: (C, D) C and Fe, (E, F) P and Fe, and (G, H) Fe and Fe. The contribution to the model from the U-Fe and U-X paths are shown as lines beneath the data.

bution to the EXAFS structure of the real or imaginary part of the spectrum. As shown in Fig. 14-39A and 14-39B for the C-Fe model, this assessment helps assign a path to each spectral feature in the measured EXAFS data that has a shape similar to that of the theoretical spectrum generated from the sum of paths. If this preliminary analysis is not done, then the optimization of the theory to the experimental spectra can lead to highly variable or nonsensical parameters.

The shell parameters (Nx , Rx , and σ^2x) for $X = C$, P , or Fe were set at values of $Nc = 3$, $Rc = 2.85 \text{ \AA}$, and $\sigma^2c = 0.003 \text{ \AA}^2$; $Np = 1$, $Rp = 3.05 \text{ \AA}$, and $\sigma^2p = 0.000 \text{ \AA}^2$; and $Nfe = 1$, $Rfe = 2.80 \text{ \AA}$, and $\sigma^2fe = 0.013 \text{ \AA}^2$, respectively. These values are reasonable for a bidentate group bound to the uranyl as shown in Plate 14–2. Knowledge of the atomic structures typical of the absorbing atom is essential to judging reasonable proposed structures. Notice that the path lengths are slightly different for C vs. P vs. Fe , even though the signal from each element falls in the same region of the Fourier transform (the initial spectra is only shown for the C – Fe model in Fig. 14–39A and 14–39B). This relationship exists because the energy-dependent phase shift $[\varphi(k)]$ (Eq. [5]) is needed for computing bond lengths from EXAFS data, and $\varphi(k)$ depends on the atom type. EXAFS data analysis programs account for these phase shifts when computing interatomic distances. The sum of the contributions from the C and Fe paths at the starting values are shown explicitly in Fig. 14–39A and 14–39B.

The magnitude and real part of the Fourier transform obtained after optimizing the fit of the unknown EXAFS parameters in Table 14–6 are shown in Fig. 14–39C and 14–39D for the C – Fe model, Fig. 14–39E and 14–39F for the P – Fe model, and Fig. 14–39G and 14–39H for the Fe – Fe model. The fit results for the C shell parameters were 24 ± 53 for Nc , $2.9 \pm 0.8 \text{ \AA}$ for Rc , and $0.025 \pm 0.030 \text{ \AA}^2$ for σ^2c . Clearly, the Nc and σ^2c values are too high for a bidentate-bonded carbonate group, where $Nc \leq 3$ and $\sigma^2c < 0.01 \text{ \AA}^2$ are reasonable. There can only be six equatorial oxygen atoms coordinated to uranyl, and each bidentate carbonate group needs to have two equatorial oxygen atoms, so there cannot be more than three bidentate carbonate groups (Plate 14–2C). The best-fit results for the P shell parameters Np , Rp , and σ^2p were 0.5 ± 1.4 , $3.06 \pm 0.15 \text{ \AA}$, and $0.0006 \pm 0.020 \text{ \AA}^2$, respectively. This model is closer at giving reasonable structural values, but the σ^2 value is unreasonably small. In general, the values for σ^2 become bigger with increasing bond length, similar to the bond becoming weaker for a longer bond. The best-fit results for the Fe shell parameters were 0.4 ± 1.3 (Nfe), $2.80 \pm 0.09 \text{ \AA}$ (Rfe), and $0.006 \pm 0.030 \text{ \AA}^2$ (σ^2fe). Only this model produces reasonable results for all three of the EXAFS parameters, and yields a good fit to the experimental EXAFS spectra as shown in Fig. 14–39G and 14–39H.

The modeling performed here was done by optimizing the theoretical spectra to the measured spectra using all three k weights in the Fourier transform. Without using all three k weights in the modeling, the correlations between the EXAFS parameters are larger, and it is difficult to identify neighboring atom types.

Modeling a Data Series

Now that we have a satisfactory model for the atom type in the second coordination shell (U – X ; Table 14–6), suppose that we want to reduce the uncertainty in our measurement of the number of neighboring atoms. One method for achieving a better estimate of N in the U – X shell is to model a series and only allow the coordination numbers for Fe atoms within the second and third shells to vary independently. This procedure begins by modeling each of the four spectra independently and comparing the best-fit values for the EXAFS parameters. Parameters that are found to overlap within their uncertainties can be constrained to a single value in the multiple data set fit. For our spectra shown in Fig. 14–38, all other values, including $Noeq$, ΔE_0 , all ΔR values, and all σ^2 values, can be constrained to be the same for all four data sets. By considering the data series together in fitting analysis, the number of independent points dramatically increases, and the Fe coordination numbers can be determined within a third of one atom. The best-fit values for the second shell Fe atoms were 0.5 ± 0.2 , 0.5 ± 0.2 , 0.6 ± 0.3 , and 0.6 ± 0.3 for data sets Fe1M1 through Fe1M4 shown in Fig. 14–40A and 14–40B, where the final model fits are overlaid on the data for this series of samples. This example shows how EXAFS data from

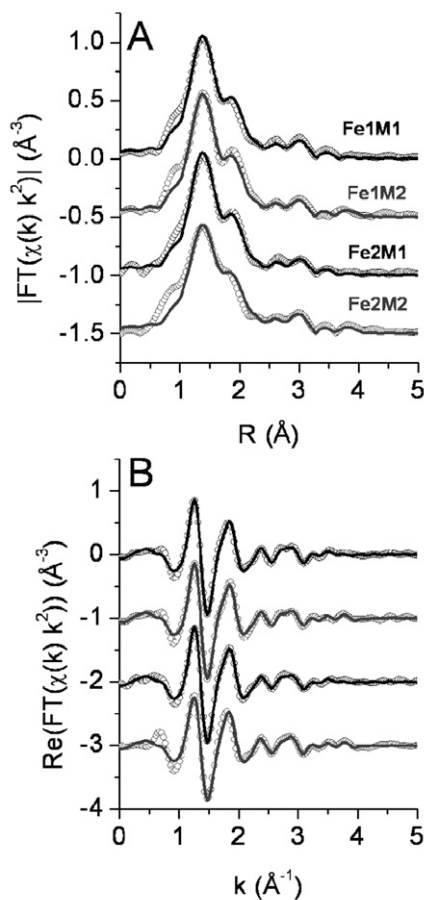


Fig. 14–40. Magnitude and real part of the Fourier transform of the U L_{III} -edge EXAFS data (open circles) and best-fit model (lines) optimized for each spectrum in the entire data series.

a chemically similar set of samples improves statistical power when performing EXAFS fitting analysis, once a reasonable structural model has been developed.

EXAFS Modeling of Complex, Multicomponent Systems

One of the principle objectives of this chapter is to develop generalized approaches for modeling a series of spectra that include trace elements within soil samples. Here we outline one approach based on the analysis methods explained in the examples. If it is expected that the trace element interacts strongly with Fe (e.g., in an Fe oxide) or some other metal within the soil, it is recommended that absorption spectra are collected for both elements to develop a self-consistent structural model for both metals. However, recognize that if the major elemental concentration in the sample is much greater than that of trace elements, then the signal from the majority absorber will contain only a fraction of the minority atoms in its nearby coordination shells.

The first step is to decide which standard spectra should be collected. These could be fine-grained metal powder or minerals of known crystalline structure mixed with BN. It is common to use the standard with a known crystal structure to determine an appropriate value for S_0^2 . Other commercially available minerals standards might be included to help gain insight into the soil spectra. Many of these standard spectra are freely available from

the EXAFS standards database (Newville et al., 1999a). Additional synthesized standards should also be considered.

Once the data have been collected and processed, a generalized modeling approach is needed. One of the most useful initial approaches is to compare the measured spectra to each other without any theoretical models. Comparison of XANES and EXAFS $\chi(k)$ spectra are valuable for determining similarities and differences. As shown in Fig. 14–38, these spectra were a subset of the initially collected data, but were determined from comparison of the $\chi(k)$ spectra to be very similar. The next step usually involves processing each of the spectra so that the usable k range is determined (Fig. 14–26). Then the Fourier transform of the spectrum with different k weights (of 1, 2, and 3) can be compared. The spectra are scaled so that the first peak height is about the same for all three spectra. As shown in Fig. 14–25, differences in second and third shell atom types as compared with the first shell types can be determined from relative changes in the Fourier transform processed with different k weights. General trends for each spectrum and between spectra can give insights into how the species are related within each of the samples.

The next step is to model the standard spectra that have known crystal structures. These models can be built, as shown with SnO_2 in the above sections. From these models, we gain insight to the typical values of EXAFS parameters (N , ΔR , σ^2 , ΔE , S_0^2) for different species.

The EXAFS spectra for the soil samples with unknown crystalline or noncrystalline structures can be modeled with simple molecular-moiety models similar to those described above in the example of “Determining a Second Shell Atom Type” (Plate 14–2). In this step the simplest model that describes the EXAFS data for all three k weights is determined. The structural parameters of this model are compared with the known structural models from the previous step to gain additional insight into the species present in the soil sample.

Once a simple model has been determined for the soil sample, the atomic distribution can be compared with the known crystal structures. From this comparison, it might be possible that one of the known crystal structural models can be used to model the spectra from the undefined species, or the known crystal structural model may need to be slightly modified to describe the species within the sample. Possible methods for using the crystal structural model to describe an adsorbed or coprecipitated species include fixing all of the EXAFS parameters from the crystalline model, then multiplying all paths in the soil sample by one additional fractional parameter (f) so that the degeneracy of each path is given by fN , where N is the degeneracy of the path determined from the crystal structure. This model can be used to test whether the measured spectra represents a fractional component of the crystalline mineral phase in the sample (Bare et al., 2005). Another modeling approach adds an additional constant disorder term (σ_d^2) to each of the paths. This approach would test the measured spectra for a fractional component that is more disordered than the crystal structure of the standard. It is also possible that the unknown species includes a fraction of poorly crystalline particles. If so, then the second and third shell N values will be too large compared with the first shell values. These coordination numbers could be relaxed (e.g., use fN only for the higher-shell paths) for modeling the unknown species. In addition, nanoparticles can cause the interatomic distances to become smaller than for the bulk structure, so that these parameters could also need adjustment, as in O’Loughlin et al. (2003).

It is also possible that the major and minor element spectra may be related to each other in some way so that the spectra from both edges can be co-refined. In particular, the Major–Minor path from the major element spectra should have the same distance and σ^2 values as the Minor–Major path from the minor element spectra. Physically the Minor–

Major pairs in the sample are represented by only one distance and one σ^2 value. They do not depend on the absorber atom. The EXAFS spectrum is the average local atomic distribution of all the absorber atoms within the sample. It is possible, for example, that with only one minor-element atom for every 50 major-element atoms within a sample, the major-element edge spectra would not detect the minor-element signal. Nevertheless, it is best to make the measurement and then determine if there is a measurable effect. Even in this case, the major-element spectrum is useful for describing the host material with which the minor-element might be associated (adsorbed or coprecipitated), particularly if X-ray diffraction cannot be used to characterize crystalline phases in the soil sample.

There are many different models that can be used to accurately describe one EXAFS spectrum. The most robust model uses the fewest variables and is constrained to make physical sense for the system. If the model is based on a known crystal structure, then the model might include many paths but only a few variables. If the model is based on molecular-moiety approach, then the model might include only a few of the most important paths with a relatively large number of variables. Both of these methods for building EXAFS models can be appropriate for different questions proposed by the scientific objectives behind the measurements. Understanding the chemical species possible for a given element and its geochemical properties within the sample of interest are fundamental to building robust EXAFS models.

DATA REPORTING

The International XAFS Society (IXS) has formed the IXS Standards and Criteria Subcommittee to generate criteria for reporting spectral analyses. These documents are available at www.i-x-s.org. Following is a brief summary of the information that should be presented within a manuscript when reporting XAFS analysis.

Data collection procedures need to be reported or referenced. The acknowledgment section should include the funding source for the synchrotron, and the methods section should include a reference for the beamline. Parameters pertaining to the energy selection method (monochromator type, monochromator slits), harmonic X-ray elimination (detune percentage, type of mirror), focusing optics (KB mirrors, zone plates), X-ray filters (for fluorescence measurements), and detector types (ionization chambers, solid-state detectors), including the fill gases for ionization chambers, are relevant for almost all experiments. The profile (spot size, shape) of the X-ray beam incident on the sample is usually relevant. Key sample parameters are the type of sample, sample preparation methods, and the mode of data collection (transmission or fluorescence).

The data processing procedures (deglitching, smoothing, alignment, normalization, background removal, fitting) should be given, including references to software packages written by your peers for your benefit. The structural model should be described, including a method for accounting for each of the EXAFS parameters (N , R , σ^2 , S_0^2 , ΔE_0) for each path included in the model. The fitting results for all parameters determined in the fit to the measured spectra should be given with uncertainties, and those uncertainties should be determined from the optimization of each parameter. Generalized uncertainties (such as those stated in a table heading) are not meaningful. The total number of variables determined in the model fit and the number of independent points in the measured spectrum, including the data range (Δk) and the fit range (ΔR) should be given. If several model fits are compared, then the procedure used to determine the quality of the model needs to be given or referenced, including how to determine the significance of two different statistical measurements of fit quality. Plots of the EXAFS data overlaid with the model fit must be shown.

The EXAFS $\chi(k)$ spectra (data and fit) alone is sufficient, but it is better to also include at least one part (magnitude, real, or imaginary) of the Fourier transform. Alternatively, both the magnitude with either the real or imaginary parts of the Fourier transform are sufficient. All EXAFS data spectra should start at k or R of 0 and end beyond the fitting range used in the analysis. The EXAFS spectra should be labeled with values on both axes, and the axis labels should include the k weight of the spectra shown.

SUMMARY

X-ray absorption spectroscopy is a powerful tool for characterizing both crystalline and non-crystalline chemical and mineralogical forms (species) of many different elements occurring in trace or abundant concentrations in soil and mineral samples. This technique complements X-ray diffraction analysis of the more abundant soil minerals and is sensitive to minority components undetectable by X-ray diffraction. Theories of both XANES and EXAFS spectra have been developed to the point where quantitative information on average local molecular bonding can be derived. Because XANES and EXAFS features arise from different physical phenomena, each of these regions of the XAS spectrum can be treated as independent (and complementary) sources of structural information for chemical species. Results from both XANES and EXAFS must be consistent if a given structural model is accurate.

A major advantage of XAS over other analytical techniques for soils is its applicability to whole (moist) soil samples with little or no pretreatment requirements. Care must be taken in sample preservation and preparation for XAS analysis to ensure that the molecular state of the element of interest is not significantly altered between the time of sampling and the time of data collection at a synchrotron facility. Presently, the greatest limitation to fully quantifying the chemical species of an element in a complex soil matrix is our ability to select and synthesize or isolate purified standards that have molecular-scale properties identical to the species of an element expected in a soil. Nevertheless, coupling synchrotron techniques such as micro-X-ray diffraction and micro-XAS with bulk-sample XAS analysis to identify the structural features that make soil minerals and noncrystalline solids unique is a powerful tool.

Acknowledgments

The authors acknowledge Kimberly Hutchison, Amanda Morris, and Yu-Ting Liu for laboratory work associated with some of the data presented, and Dr. Suzanne Beauchemin for insightful comments on the PCA section. We acknowledge Kenneth Kemner, Mandy Michalsen, and Simon Bare for some of the example spectra. Support for SDK and BR provided by the Environmental Remediation Science Program, Office of Biological and Environmental Research, Office of Science, U.S. Department of Energy (DOE), under contract W-31-109-Eng-38 and for DH provided by the North Carolina Agricultural Research Service (NC-ARS). This work was carried out in part at MRCAT at the Advanced Photon Source (APS) at Argonne National Laboratory, and at Beamlines X-11A, X-11B, and X-15B at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. MRCAT operations are supported by the Department of Energy and the MRCAT member institutions. Use of the APS and NSLS is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract Numbers DE-AC02-06CH11357 and DE-AC02-98CH10886.

REFERENCES

- Allen, P.G., J.J. Bucher, D.L. Clark, N.M. Edelstein, S.A. Ekberg, J.W. Gohdes, E.A. Hudson, N. Kaltsoyannis, W.W. Lukens, M.P. Neu, P.D. Palmer, T. Reich, D.K. Shuh, C.D. Tait, and B.D. Zwick. 1995. Multinuclear NMR, Raman, EXAFS, and X-ray-diffraction studies of uranyl carbonate complexes in near-neutral aqueous-solution: X-ray Structure of $[\text{C}(\text{NH}_2)_3]_6[(\text{UO}_2)_3(\text{CO}_3)_6] \cdot 6.5\text{H}_2\text{O}$. *Inorg. Chem.* 34:4797–4807.
- Ankudinov, A.L., A.I. Nesvizhskii, and J.J. Rehr. 2003. Dynamic screening effects in x-ray absorption spectra. *Phys. Rev. B* 67:115120.
- Ankudinov, A.L., B. Ravel, J.J. Rehr, and S.D. Conradson. 1998. Real-space multiple-scattering calculation and interpretation of X-ray absorption near-edge structure. *Phys. Rev. B* 58:7565–7576.
- Bare, S.R., S.D. Kelly, W. Sinkler, J.J. Low, F.S. Modica, S. Valencia, A. Corma, and L.T. Nemeth. 2005. Uniform catalytic site in Sn-beta-zeolite determined using X-ray absorption fine structure. *J. Am. Chem. Soc.* 127:12924–12932.
- Bartlett, R.J., and B.R. James. 1993. Redox chemistry of soils. *Adv. Agron.* 50:151–208.
- Beauchemin, S., D. Hesterberg, and M. Beauchemin. 2002. Principal component analysis approach for the modeling of sulfur K-XANES spectra of humic acid. *Soil Sci. Soc. Am. J.* 66:83–91.
- Beauchemin, S., D. Hesterberg, J. Chou, M. Beauchemin, R.R. Simard, and D.E. Sayers. 2003. Speciation of phosphorus in P-enriched agricultural soils using XANES spectroscopy and chemical fractionation. *J. Environ. Qual.* 32:1809–1819.
- Benfatto, M., P. D'Angelo, S. Della Longa, and N.V. Pavel. 2002. Evidence of distorted fivefold coordination of the Cu^{2+} aqua ion from an x-ray-absorption spectroscopy quantitative analysis. *Phys. Rev. B* 65: Art. No. 174205.
- Bertsch, P.M., and D.B. Hunter. 2001. Applications of synchrotron-based X-ray microprobes. *Chem. Rev.* 101:1809–1842.
- Bertsch, P.M., D.B. Hunter, S.R. Sutton, S. Bajt, and M.R. Rivers. 1994. In situ chemical speciation of uranium in soils and sediments using micro X-ray absorption spectroscopy. *Environ. Sci. Technol.* 28:980–984.
- Bevington, P.R., and D.K. Robinson. 1992. Data reduction and error analysis for the physical sciences. 2nd ed. McGraw-Hill, New York.
- Brillouin, L. 1962. Science and information theory. Academic Press, New York.
- Brown, G.E., Jr. 1990. Spectroscopic studies of chemisorption reaction mechanisms at oxide–water interfaces. p. 309–364. *In* M.F. Hochella, Jr. and A.F. White (ed.) Mineral–water interface geochemistry. Reviews in Mineralogy 23. Mineralogical Soc. of America, Washington, DC.
- Brown, G.E., and G.A. Parks. 2001. Sorption of trace elements on mineral surfaces: Modern perspectives from spectroscopic studies, and comments on sorption in the marine environment. *Int. Geol. Rev.* 43:963–1073.
- Brown, G.E., and N. Sturchio. 2002. An overview of synchrotron radiation applications to low temperature geochemistry and environmental sciences. p. 1–115. *In* P.A. Fenter et al. (ed.) 2002. Applications of synchrotron radiation in low-temperature geochemistry and environmental science. Reviews in Mineralogy and Geochemistry 49. Mineralogical Soc. Am., Washington, DC.
- Calvin, S., E.E. Carpenter, B. Ravel, V.G. Harris, and S.A. Morrison. 2002. Multiedge refinement of extended X-ray-absorption fine structure of manganese zinc ferrite nanoparticles. *Phys. Rev. B* 66: Art. No. 224405.
- Compton, A.H.A. 1923. Quantum theory of the scattering of X-rays by light elements. *Phys. Rev.* 21:483–502.
- Cross, J.O., and A.I. Frenkel. 1998. Use of scattered radiation for absolute energy calibration. *Rev. Sci. Instrum.* 70:38–40.
- Cullity, B.D. 1978. Elements of X-ray diffraction. Addison-Wesley, Reading, MA.
- Davis, L.E. 1976. Handbook of Auger electron spectroscopy: A reference book of standard data for identification and interpretation of Auger electron spectroscopy data. Physical Electronics Industries, Inc., Edina, MN.
- de Groot, F. 2005. Multiple effects in X-ray spectroscopy. *Coord. Chem. Rev.* 249:31–63.
- Duff, M.C., D.B. Hunter, I.R. Triay, P.M. Bertsch, D.T. Reed, S.R. Sutton, G. Shea-McCarthy, J. Kitten, P. Eng, S.J. Chipera, and D.T. Vanniman. 1999. Mineral associations and average oxidation states of sorbed Pu on tuff. *Environ. Sci. Technol.* 33:2163–2169.
- Elam, W.T., B. Ravel, and J.R. Sieber. 2002. A new atomic database for X-ray spectroscopic calculations. *Radiat. Phys. Chem.* 63:121–128.
- Felmy, A.R., D.C. Girvin, and E.A. Jenne. 1984. MINTEQA—A computer program for computing aqueous equilibria. Rep. by Battelle Pacific Northwest Laboratories for the USEPA, Athens, GA.
- Fendorf, S.E. 1999. Fundamental aspects and applications of x-ray absorption spectroscopy in clay and soil science. p. 19–67 *In* D.G. Schulze et al. (ed.) Synchrotron methods in clay science. CMS

- Workshop Lectures, Vol. 9. Clay Minerals Society, Aurora, CO.
- Fendorf, S.E., and D.L. Sparks. 1996. X-ray absorption fine structure spectroscopy. p. 357–375. *In* D.L. Sparks (ed.) *Methods of soil analysis*. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI.
- Fendorf, S.E., D.L. Sparks, G.M. Lamble, and M.J. Kelley. 1994. Applications of x-ray absorption fine structure spectroscopy to soils. *Soil Sci. Soc. Am. J.* 58:1583–1595.
- Fenter, P.A., M.L. Rivers, N.C. Sturchio, and S.R. Sutton (ed.) 2002. Applications of synchrotron radiation in low-temperature geochemistry and environmental science. *Reviews in Mineralogy and Geochemistry* 49. Mineralogical Soc. Am., Washington, DC.
- Fernández-García, M., C.M. Alvarez, and G.L. Haller. 1995. XANES-TPR study of Cu-Pd bimetallic catalysts: Application of factor analysis. *J. Phys. Chem.* 99:12565–12569.
- Frenkel, A.I., C.W. Hills, and R.G.A. Nuzzo. 2001. A view from the inside: Complexity in the atomic scale ordering of supported metal nanoparticles. *J. Phys. Chem. B* 105:12689–12703.
- Frenkel, A.I., E.A. Stern, A. Voronel, M. Qian, and M. Newville. 1994. Solving the structure of disordered mixed salts. *Phys. Rev. B* 49:11662–11674.
- Frenkel, A.I., F.M. Wang, S. Kelly, R. Ingalls, D. Haskel, E.A. Stern, and Y. Yacoby. 1997. Local structural changes in KNbO_3 under high pressure. *Phys. Rev. B* 56:10869–10877.
- Gustafsson, J.P. 2007. Software download page for Visual Minteq. Available at <http://www.lwr.kth.se/English/OurSoftware/vminteq/> (accessed 31 July 2007, verified 17 Dec. 2007).
- Haskel, D., J.C. Lang, Z. Islam, A. Cady, G. Srajer, M. van Veenendaal, and P.C. Canfield. 2005. Atomic origin of magnetocrystalline anisotropy in $\text{Nd}_2\text{Fe}_{14}\text{B}$. *Phys. Rev. Lett.* 95: Art. No. 217207.
- Heald, S.M. 1988a. Design of an EXAFS experiment. p. 87–118. *In* D.C. Koningsberger and R. Prins (ed.) *X-ray absorption: Principles, applications, techniques of EXAFS, SEXAFS and XANES*. John Wiley & Sons, New York.
- Heald, S.M. 1988b. EXAFS with synchrotron radiation. p. 119–161. *In* D.C. Koningsberger and R. Prins (ed.) *X-ray absorption: Principles, applications, techniques of EXAFS, SEXAFS and XANES*. John Wiley & Sons, New York.
- Hesterberg, D., D.E. Sayers, W. Zhou, G.M. Plummer, and W.P. Robarge. 1997. X-ray absorption spectroscopy of lead and zinc speciation in a contaminated groundwater aquifer. *Environ. Sci. Technol.* 31:2840–2846.
- Hesterberg, D., W. Zhou, K.J. Hutchison, and D.E. Sayers. 2001. Bonding of Hg(II) to reduced organic sulfur in humic acid as affected by S/Hg ratio. *Environ. Sci. Technol.* 35:2741–2745.
- Joly, Y. 2001. X-ray absorption near edge structure calculations beyond the muffin-tin approximation. *Phys. Rev. B* 63:125120–125129.
- Kelly, S., R. Ingalls, F. Wang, B. Ravel, and D. Haskel. 1998. X-ray-absorption fine-structure study of the B1-to-B2 phase transition in RbCl . *Phys. Rev. B* 57:7543–7550.
- Kelly, S.D., K.M. Kemner, J.B. Fein, D.A. Fowle, M.I. Boyanov, B.A. Bunker, and N. Yee. 2002. X-ray absorption fine structure determination of pH-dependent U-bacterial cell wall interactions. *Geochim. Cosmochim. Acta* 66:3855–3871.
- Kelly, S.D., E.T. Rasbury, S. Chattopadhyay, A.J. Kropf, and K.M. Kemner. 2006. Evidence of a stable uranyl site in ancient organic-rich calcite. *Environ. Sci. Technol.* 40:2262–2268.
- Kelly, S.D., E.A. Stern, and R. Ingalls. 2001. Determining crystalline atomic positions using XAFS, a new addition to the UWXAFS analysis package. *J. Synchrotron Radiat.* 8:311–313.
- Kemner, K.M., J. Kropf, and B.A. Bunker. 1994. A low-temperature total electron yield detector for X-ray absorption fine structure spectra. *Rev. Sci. Instrum.* 65:3667–3669.
- Khare, N., D. Hesterberg, S. Beauchemin, and S.L. Wang. 2004. XANES determination of adsorbed phosphate distribution between ferrihydrite and boehmite in mixtures. *Soil Sci. Soc. Am. J.* 68:460–469.
- Khare, N., D. Hesterberg, and J.D. Martin. 2005. Investigating phosphate surface precipitation in single and binary mixtures of Fe- and Al-oxide minerals using XANES. *Environ. Sci. Technol.* 39:2152–2160.
- Koningsberger, D.C., and R. Prins (ed.) 1988. *X-ray absorption: Principles, applications, techniques of EXAFS, SEXAFS and XANES*. John Wiley & Sons, New York.
- Li, G.G., F. Bridges, and C.H. Booth. 1995. X-ray absorption fine-structure standards—A comparison of experiment and theory. *Phys. Rev. B* 52:6332–6348.
- Lide, D.R. (ed.) 2003. *CRC handbook of chemistry and physics*. 84th ed. CRC Press, Boca Raton, FL.
- Malinowski, E.R. 2002. *Factor analysis in chemistry*. 3rd ed. John Wiley & Sons, New York.
- Manceau, A., L. Charlet, M.C. Boisset, B. Didier, and L. Spadini. 1992. Sorption and speciation of heavy metals on hydrous Fe and Mn oxides. From microscopic to macroscopic. *Appl. Clay Sci.* 7:201–223.

- Manceau, A., M. Schlegel, D. Chateigner, B. Lanson, C. Bartoli, and W. Gates. 1999. Application of polarized EXAFS to fine-grained layered minerals. p. 69–114. *In* D.G. Schulze et al. (ed.) *Synchrotron methods in clay science*. CMS Workshop Lectures, Vol. 9. Clay Minerals Society, Aurora, CO.
- Manceau, A., N. Tamura, M.A. Marcus, A.A. MacDowell, R.S. Celestre, R.E. Sublett, G. Sposito, and H.A. Padmore. 2002. Deciphering Ni sequestration in soil ferromanganese nodules by combining X-ray fluorescence, absorption, and diffraction at micrometer scales of resolution. *Am. Miner.* 87:1494–1499.
- McMaster, W.H., N. Kerr Del Grande, J.H. Mallett, and J.H. Hubbell. 1969. Compilation of X-ray cross sections. Lawrence Livermore Natl. Lab. Rep. UCRL-50174, Section II Revision I. National Technical Information Services L-3. U.S. Dep. of Commerce, Washington, DC.
- Michalowicz, A., and G. Vlaic. 1998. Multiple solutions in data fitting: A trap in EXAFS structural analysis and some ideas to avoid it. *J. Synchrotron Radiat.* 5:1317–1320.
- Morra, M.J., S.E. Fendorf, and P.D. Brown. 1997. Speciation of sulfur in humic and fulvic acids using X-ray absorption near-edge structure (XANES) spectroscopy. *Geochim. Cosmochim. Acta* 61:683–688.
- Murray, G.C., and D. Hesterberg. 2006. Iron and phosphate dissolution during abiotic reduction of ferrihydrite-boehmite mixtures. *Soil Sci. Soc. Am. J.* 70:1318–1327.
- Myneri, S.C.B. 2002. Soft X-ray spectroscopy and spectromicroscopy studies of organic molecules in the environment. p. 485–579. *In* P.A. Fenter et al. (ed.) *Applications of synchrotron radiation in low-temperature geochemistry and environmental science*. Reviews in Mineralogy and Geochemistry 49. Mineralogical Soc. Am., Washington, DC.
- Newville, M., P. Livins, Y. Yacoby, J.J. Rehr, and E.A. Stern. 1993. Near-edge X-ray-absorption fine structure of Pb: A comparison of theory and experiment. *Phys. Rev. B* 47:14126–14131.
- Newville, M., S.A. Carroll, P.A. O'Day, G.A. Waychunas, and M. Ebert. 1999a. A web-based library of XAFS data on model compounds. *J. Synchrotron Radiat.* 6:276–277.
- Newville, M., B.I. Boyanov, and D.E. Sayers. 1999b. Estimation of uncertainties in XAFS data. *J. Synchrotron Radiat.* 6:264–265.
- Newville, M. 2001. IFEFFIT: Interactive EXAFS analysis and FEFF fitting. *J. Synchrotron Rad.* 8:322–324.
- Niemeyer, J., and J. Thieme. 1999. Reactions of clay particles in aqueous dispersions studied by x-ray microscopy. p. 207–239. *In* D.G. Schulze et al. (ed.) *Synchrotron methods in clay science*. CMS Workshop Lectures, Vol. 9. Clay Minerals Society, Aurora, CO.
- O'Loughlin, E.J., S.D. Kelly, R.E. Cook, R. Csencsits, and K.M. Kemner. 2003. Reduction of uranium(VI) by mixed iron(II)/iron(III) hydroxide (green rust): Formation of UO_2 nanoparticles. *Environ. Sci. Technol.* 37:721–727.
- Osán, J.B., S. Török, and K.W. Jones. 1997. Study of chemical state of toxic metals during the life cycle of fly ash using X-ray absorption near-edge structure. *X-ray Spectrom.* 26:37–44.
- Prietzl, J., J. Thieme, U. Neuhausler, J. Susini, and I. Kögel-Knabner. 2003. Speciation of sulphur in soils and soil particles by X-ray spectromicroscopy. *Eur. J. Soil Sci.* 54:423–433.
- Ravel, B., E. Cockayne, M. Newville, and K.M. Rabe. 1999. Combined EXAFS and first-principles theory study of Pb1-xGexTe . *Phys. Rev. B* 60:14632–14642.
- Ravel, B., and M. Newville. 2005. ATHENA, ARTEMIS, HEPHAESTUS: Data analysis for X-ray absorption spectroscopy using IFEFFIT. *J. Synchrotron Radiat.* 12:537–541.
- Ravel, B., E.A. Stern, R.I. Vedrinskii, and V. Kraizman. 1998. Local structure and the phase transitions of BaTiO_3 . *Ferroelectrics* 206:407–430.
- Rehr, J.J., and R.C. Albers. 1990. Scattering-matrix formulation of curved-wave multiple-scattering theory: Application to X-ray-absorption fine structure. *Phys. Rev. B* 41:8139–8149.
- Rehr, J.J., and R.C. Albers. 2000. Theoretical approaches to X-ray absorption fine structure. *Rev. Mod. Phys.* 72:621–654.
- Rehr, J.J., and A. Ankudinov. 2005. Progress in the theory and interpretations of XANES. *Coord. Chem. Rev.* 249:131–140.
- Rehr, J.J., A. Ankudinov, and S.I. Zabinsky. 1998. New developments in NEXAFS/EXAFS theory. *Catal. Today* 39:263–269.
- Ressler, T., J. Wong, J. Roos, and I.L. Smith. 2000. Quantitative speciation of Mn-bearing particulates emitted from autos burning methylcyclopentadienyl)manganese tricarbonyl-added gasolines using XANES spectroscopy. *Environ. Sci. Technol.* 34:950–958.
- Sayers, D.E., E.A. Stern, and F.W. Lytle. 1971. New technique for investigating non-crystalline structures: Fourier analysis of the extended X-ray absorption fine structure. *Phys. Rev. Lett.* 27:1204–1207.
- Sayers, D.E., and B. Bunker. 1988. Data analysis. p. 211–256. *In* D.C. Koningsberger and R. Prins (ed.) *X-ray absorption: Principles, applications, techniques of EXAFS, SEXAFS and XANES*. John Wiley & Sons, New York.

- Schulze, D.G., and P.M. Bertsch. 1995. Synchrotron X-ray techniques in soil, plant, and environmental research. *Adv. Agron.* 55:1–66.
- Schulze, D.G., S.R. Sutton, and S. Bajt. 1995. Determining manganese oxidation state in soils using x-ray absorption near-edge structure (XANES) spectroscopy. *Soil Sci. Soc. Am. J.* 59:1540–1548.
- Sevillano, E., H. Meuth, and J.J. Rehr. 1979. Extended X-ray absorption fine structure Debye-Waller factors. I. Monoatomic crystals. *Phys. Rev. B* 20:4908–4911.
- Shaltout, A., H. Ebel, and R. Svagera. 2005. Update of photoelectric absorption coefficients in the tables of McMaster. *X-ray Spectrom.* 35:52–56.
- Sham, T.K., and M.L. Rivers. 2002. A brief overview of synchrotron radiation. p. 117–147. *In* P.A. Fenter et al. (ed.) *Reviews in Mineralogy and Geochemistry* 49. Mineral. Soc. Am., Washington, DC.
- Stern, E.A. 1978. Structural determination by X-ray absorption. *Contemp. Phys.* 19:239–310.
- Stern, E.A., and S.M. Heald. 1979. X-ray filter assembly for fluorescence measurements of X-ray absorption fine structure. *Rev. Sci. Instrum.* 50:1579–1583.
- Stern, E.A., and S.M. Heald. 1983. Basic principles and applications of EXAFS. p. 995–1014. *In* E.E. Koch (ed.) *Handbook of synchrotron radiation*. Vol. 10. North-Holland, Amsterdam.
- Stern, E.A., M. Newville, B. Ravel, Y. Yacoby, and D. Haskel. 1995. The UWXAFS analysis package: Philosophy and details. *Physica B* 208 & 209:117–120.
- Stöhr, J. 1992. *NEXAFS Spectroscopy*. Springer-Verlag, New York.
- Teo, B.K. 1986. *EXAFS spectroscopy: Basic principles and data analysis*. Springer-Verlag, New York.
- Tokunaga, T.K., S.R. Sutton, S. Bajt, P. Nuessle, and G. Shea-McCarthy. 1998. Selenium diffusion and reduction at the water-sediment boundary: Micro-XANES spectroscopy of reactive transport. *Environ. Sci. Technol.* 32:1092–1098.
- Tröger, L., D. Arvanitis, K. Baberschke, H. Michaelis, U. Grimm, and E. Zschech. 1992. Full correction of the self-absorption in soft-fluorescence extended X-ray absorption fine structure. *Phys. Rev. B* 46:3283–3289.
- Wasserman, S.R. 1997. The analysis of mixtures: Application of principal component analysis to XAS spectra. *J. Phys. IV France*, 7–C2:203–205.
- Wasserman, S.R., P.G. Allen, D.K. Shuh, J.J. Bucher, and N.M. Edelstein. 1999. EXAFS and principal component analysis: A new shell game. *J. Synchrotron Radiat.* 6:284–286.
- Waychunas, G.A., C.C. Fuller, J.A. Davis, and J.J. Rehr. 2003. Surface complexation and precipitate geometry for aqueous Zn(II) sorption on ferrihydrite: II. XANES analysis and simulation. *Geochim. Cosmochim. Acta* 67:1031–1043.
- Webb, S. 2007. SixPACK (Sam's Interface for XAS Package). Stanford Synchrotron Radiation Source, Stanford, CA. Available at <http://www-ssrl.slac.stanford.edu/~swebb/sixpack.htm> (accessed 31 July 2007, verified 17 Dec. 2007).
- Xia, K., F. Weesner, W.F. Bleam, P.R. Bloom, U.L. Skjellberg, and P.A. Helmke. 1998. XANES studies of oxidation states of sulfur in aquatic and soil humic substances. *Soil Sci. Soc. Am. J.* 62:1240–1246.
- Zabinsky, S.I., J.J. Rehr, A. Ankudinov, R.C. Albers, and M.J. Eller. 1995. Multiple-scattering calculations of X-ray-absorption spectra. *Phys. Rev. B* 52:2995–3009.
- Zachara, J.M., C.C. Ainsworth, G.E. Brown, J.G. Catalano, J.P. McKinley, O. Qafoku, S.C. Smith, J.E. Szecsody, S.J. Traina, and J.A. Warner. 2004. Chromium speciation and mobility in a high level nuclear waste vadose zone plume. *Geochim. Cosmochim. Acta* 68:13–30.

Structural Allocation of Clay Mineral Elemental Components

A.D. KARATHANASIS, University of Kentucky, Lexington

The composition of soil and clay fractions provides important information about the nature of their major and minor mineral constituents, as well as their physical and chemical properties and behavior. However, complete solid-phase characterization is a tedious process requiring identification and quantification of the minerals, elemental composition determination, partitioning the elemental components to the minerals of known structural composition, and estimation of the structural formulas of the minerals whose composition is unknown (Kelley, 1945, 1955). Even with the most advanced instrumentation and the most careful analysis, these characterizations are at best approximations. Therefore, it is essential that all mineral and elemental quantifications be checked by more than one method, and all assumptions made for elemental allocations be justifiably reasonable to assure relative confidence in the solid-phase characterization. Current X-ray diffraction (XRD) and thermal analysis (TGA) techniques can provide mineral quantifications with an accuracy of better than $\pm 3\%$, depending on the mineralogical composition of the sample (Karathanasis and Hajek, 1982; Wilson, 1987; Amonette and Zelazny, 1994). Improvements in elemental analysis procedures and instrumentation, including inductively coupled plasma (ICP), X-ray fluorescence (XRF), nuclear magnetic resonance (NMR), infrared (IR), and Mössbauer spectroscopies, as well as microprobe analysis techniques, can provide elemental composition data within $\pm 0.1\%$ of the actual concentration (Vainerdi and Uken, 1979; Johnson and Maxwell, 1981; Jackson, 1985; Amonette and Zelazny, 1994; Sparks, 1996).

In spite of these advances, elemental allocation to various mineral phases comprising a soil clay sample is always a challenging task because of the accuracy required for both the mineral and elemental composition. The simplest case involves relatively high purity monomineralic samples. The task becomes much more difficult with increasing number of mineral phases, especially when these minerals are of variable or unknown chemical composition. Certain minerals, such as kaolinite, gibbsite, feldspar, and quartz, in spite of small variations, can be assumed for practical purposes as having relatively constant chemical composition. However, other common clay minerals, such as micas, smectites, vermiculites, and chlorites exhibit a wider range in structural composition, depending on the extent of lattice isomorphic substitutions and ion exchange loading. Furthermore, even though the calculations implicitly assume the presence of single homogeneous mineral phases, soil clay minerals frequently contain minor impurities in the form of sorbed or structural cations, deformed or fragmented structural units, and interstratified or mixed-layer components, all of which cause significant variations in chemical composition and charge distribution. In spite of recent advances in lattice imaging techniques, which may

detect such heterogeneities, it is advisable to use caution in the interpretation of structural formula compositions. Nevertheless, when accounting carefully for these limitations, structural formula calculations at least provide invaluable comparative information relative to the chemical composition, charge distribution, weathering trends, and geological origin of minerals (Kelley, 1955) and as such can be used to infer much about the reactivity of soil clays.

STRUCTURAL COMPOSITION OF LAYER SILICATE MINERALS

Most clay minerals are essentially hydrous aluminosilicates with variable substitutions of Al by Mg and Fe, and some alkali and alkaline earth elements in their structure. Hydrogen is usually present as structural hydroxyl or sorbed water. These substitutions cause a wide range in chemical composition, primarily in the layer silicate group of minerals. Their structure involves mainly Si tetrahedra and Al octahedra connected through shared oxygen atoms to form two-dimensional layer silicates in 1:1 (kaolinite-type), 2:1 (smectite- or mica-type), or 2:1 with interlayer hydroxide (chlorite-type, formerly referred to as “2:1:1”) ratios (Bailey, 1980; Dixon and Weed, 1989). The central atoms in the tetrahedral sheet are usually Si, Al, or Fe^{3+} , while in the octahedral sheet they vary, from the most common Al, Fe^{3+} , Fe^{2+} , or Mg to less common atoms, such as Zn, Cr, Ni, or Li. Various combinations of central atoms in the tetrahedral and octahedral positions result in a net negative layer charge, which is balanced by interlayer cations. When all or almost all of the available cation sites in the octahedrally coordinated sheets are filled mainly by divalent cations, such as Fe^{2+} or Mg, they produce a trioctahedral series of minerals. When only two-thirds of the available sites are occupied mainly by trivalent cations, such as Al or Fe^{3+} , they form a dioctahedral series of minerals. Table 15-1 lists the common layer silicate mineral groups found in soils and their theoretical structural compositions. These ideal formulas provide a statistical picture of the occurrence of central atoms in tetrahedral, octahedral, or interlayer positions, which are used as models for calculating structural formulas of layer silicate minerals having unknown chemistry.

There are two main approaches to determine the occupancy of the central atoms in the structure of layer silicate minerals and calculate their structural formula: (i) using elemental compositions determined from bulk chemical analyses alone and (ii) using analytical

Table 15-1. Ideal structural composition formulae for common soil phyllosilicate minerals.

Layer	Coordination	Unit cell formula†
1:1	dioctahedral	$T_4M_4O_{10}(\text{OH})_8$
1:1	trioctahedral	$T_4M_6O_{10}(\text{OH})_8$
2:1	dioctahedral	$T_8M_4O_{20}(\text{OH})_4X$
2:1	trioctahedral	$T_8M_6O_{20}(\text{OH})_4X$
Interlayered‡		
2:1	di-dioctahedral	$T_8M_4O_{20}(\text{OH})_4[M_4(\text{OH})_{12}]X$
2:1	di-trioctahedral	$T_8M_4O_{20}(\text{OH})_4[M_6(\text{OH})_{12}]X$
2:1	tri-dioctahedral§	$T_8M_6O_{20}(\text{OH})_4[M_4(\text{OH})_{12}]X$
2:1	tri-trioctahedral	$T_8M_6O_{20}(\text{OH})_4[M_6(\text{OH})_{12}]X$

† T = tetrahedral sites, M = octahedral sites, X = unit cell charge.

‡ Formerly called “2:1:1” phyllosilicates because they contain 2 tetrahedral sheets, one octahedral sheet, and an additional octahedral sheet in the interlayer region.

§ Apparently there are no known phyllosilicates in this category, but the ideal formula is shown for illustration purposes.

characterization of certain elements by special chemical techniques (e.g., acid dissolution, IR, NMR, Mossbauer spectroscopy) to supplement bulk chemical analysis.

STRUCTURAL FORMULAS FROM BULK CHEMICAL ANALYSIS

In most traditional elemental analyses of soil or clay fractions the goal is to account for elemental constituents so that when expressed on an oxide basis the sum is near 100%. To achieve such a recovery it is necessary at the very least, particularly for clay fractions, to make the following elemental determinations: Si, Al, Fe^{3+} , Fe^{2+} , Mg, Ca, K, Na, Mn, and Ti. For some samples additional determinations of less common elements, such as, Li, P, or F, may also be necessary. Water content evolved below 100°C (sorbed) and between 100 and 1000°C (structural) is usually measured by thermogravimetric analysis (Karathanasis, 2008, this volume) or alternatively the analysis can be performed on an “ignited” (i.e., heated to >1000°C) basis (Norrish and Hutton, 1969) where all water is removed. For samples containing smectite- or vermiculite-type minerals, the sorbed H_2O range is below 220°C, and the structural OH water range is usually defined as between 220 and 1000°C. Knowing the amount of sorbed and structural water is especially critical for determining the formulas of smectites and interlayered smectitic minerals.

Assuming that the structure of the mineral is charge-balanced the total cation charge should equal the total anion charge for the characteristic mineral group (Table 15–1). This electroneutrality principle constitutes the basis of most structural formula calculation procedures from mass chemical analysis. The two main methods used rely on either a fixed number of anionic charges or a fixed central cation occupancy in tetrahedral and octahedral sites of the mineral structure. The first method assumes an ideal anionic structural network whose negative charge is balanced by incorporating the central cations, based on chemical analysis, to maintain electroneutrality. While in most cases the total anion content is assumed to be made up by O^{2-} and OH^- , some minerals may contain F^- or Cl^- in their structure, which contribute to the total anionic charge. Because the divalent oxygen charges in a mineral structure are usually more stable than OH^- or other anionic charges, most structural formula calculation procedures are based on a fixed number of O^{2-} charges, while the OH^- charges are assumed to have ideal composition or are determined directly from thermogravimetric analysis. The presence of additional anionic components is accounted for as part of the total O^{2-} charge balance. For example, the anionic content of a 2:1 mineral with 24 O^{2-} charges, ideal OH^- composition, and the presence of F and Cl can be expressed as $\text{O}^{2-}_{24-x-y}\text{F}^-_x\text{Cl}^-_y$ (Newman and Brown, 1987). The second method is based on the assumption of ideal occupancy of all tetrahedral and octahedral positions by central cations, whose positive charge is balanced by the ideal or determined anionic charges of the respective mineral group to maintain electroneutrality. Assuming that the sample is monomineralic and the analysis is accurate, both methods should produce nearly identical structural formulas. The presence of other minerals, particularly layer silicates, or considerable amounts of impurities (i.e., organic matter) in the sample may result in significant variations in the calculated distribution of central cations in the structure.

The Fixed Negative Charge Method

The basic requirements of this method are (Ross and Hendricks, 1945; Kelley, 1945, 1955):

- Silicon and Al are the only central atoms occupying all possible tetrahedral sites. This assumption may not always be valid because several Fe-rich minerals, such as nontronites and some vermiculites, may contain Fe^{3+} in tetrahedral sites (Gates et al., 2002; Cardile and Slade, 1988).

- Silicon occupies only tetrahedral positions.
- Aluminum fills the remaining tetrahedral sites and the rest is allocated to octahedral or interlayer positions.
- The other cations are assigned either to octahedral or interlayer positions.
- At least one type of sites (usually tetrahedral) should have ideal occupancy.

Assuming a monomineralic sample, this method involves the following steps:

1. Normalize the elemental oxide composition of the mineral to 100%.
2. Divide the percentage concentration of each oxide by its molecular weight and multiply by the total number of metal atoms in the oxide to obtain the respective mass equivalent.
3. Multiply the result by the normal charge of the metal to obtain the charge equivalent for each cation.
4. Sum all the charge equivalents.
5. Calculate the proportionality factor (R) by dividing the number of the assumed fixed negative charges in the structure of the mineral with the sum of charge equivalents.
6. Calculate the occupancy of atoms per unit cell by multiplying each charge equivalent with the proportionality factor (R) and dividing by the valence of the cation.
7. Allocate the atoms in the unit cell by assuming ideal occupancy of 4.0 central atoms in the tetrahedral positions of 1:1 minerals, and 8.0 central atoms for 2:1 minerals (including the “2:1:1” chlorite-type minerals).
8. Assign all Si atoms to tetrahedral sites and fill the remaining tetrahedral positions with Al to make up the difference from ideal occupancy.
9. In 1:1 and 2:1 minerals, assign the remaining Al atoms and all Fe^{3+} , Fe^{2+} , and Mg atoms to octahedral sites, assuming that they are not present as counter ions or impurities in the sample.
10. In “2:1:1” or chlorite-type minerals, the nature of the hydroxy-interlayer component (Al, Fe, Mg) should be established before the allocation. For Al-hydroxy-interlayered minerals, all the Fe and Mg atoms are allocated to octahedral sites, and the difference from ideal occupancy (4.0 for dioctahedral, and 6.0 for trioctahedral) is made up by Al.
11. The remaining Al, along with the extra OH, are assigned to the interlayer component.
12. The rest of the cations are assumed to be counter ions balancing the charge deficit of the unit cell, which is calculated as follows. Assuming a theoretical distribution of 32 tetrahedral and 12 octahedral charges (total of 44 negative charges) in a 2:1 dioctahedral mineral, the total surface charge (X^-) is estimated as the difference between the total calculated positive tetrahedral charges from 32 plus the difference between the total calculated positive octahedral charges from 12. In chlorite-type minerals, the calculation also involves the charge of the hydroxy-interlayer component.
13. Significant discrepancies between the calculated net unit cell charge (X^-) and the counter ion charge usually indicates errors in the calculation of the structural formula (Newman and Brown, 1987; Čičel and Komadel, 1994).

Structural Formula Calculation Excluding H_2O Content

Tables 15–2 and 15–3 demonstrate the calculation of structural formulas for a 1:1 mineral (Georgia kaolinite) and a 2:1 mineral (Wyoming montmorillonite), when the H_2O

Table 15-2. Structural formula calculation for a Georgia kaolinite sample based on ideal hydroxyl composition and 28 total negative charges.†

Elemental oxides	Weight %	Normalized weight %‡	Molecular weight	Number of cations in oxide	Cation valence	Charge equivalents	Proportionality factor (R)§	Cation valence	Atoms per unit cell
SiO ₂	45.20	53.33	60.06	1	4	3.5518	4.4516	4	3.95
Al ₂ O ₃	39.20	46.25	101.95	2	3	2.7219	4.4516	3	4.04
Fe ₂ O ₃	0.17	0.20	159.70	2	3	0.0075	4.4516	3	0.01
MgO	0.08	0.09	40.32	1	2	0.0045	4.4516	2	0.01
CaO	0.06	0.07	56.08	1	2	0.0025	4.4516	2	0.005
Na ₂ O	0.03	0.04	61.99	2	1	0.0013	4.4516	1	0.005
K ₂ O	0.02	0.02	94.19	2	1	0.0004	4.4516	1	0.002
TiO ₂	1.21	—	—	—	—	—	—	—	—
H ₂ O	13.30	—	—	—	—	—	—	—	—
Total	99.27	100.00	—	—	—	6.2899	—	—	—

† Structural formula: $(\text{Si}_{1.95}\text{Al}_{0.05})(\text{Al}_{1.99}\text{Fe}^{3+}_{0.01})\text{O}_{10}(\text{OH})_8$. Layer charge: tetrahedral = 0.05⁻; octahedral = 0.00; total = 0.05⁻. Exchange cation charge: 0.02 + 0.01 + 0.005 + 0.002 = 0.037⁻.

‡ Normalized to 100%, considering TiO₂ as anatase impurity and ideal structural hydroxyl H₂O content.

§ R = 28/6.2899 = 4.4516

Table 15-3. Structural formula calculation for a Clay Spur Wyoming montmorillonite sample based on ideal hydroxyl composition and 44 oxygen negative charges.†

Elemental oxides	Weight %	Normalized weight %‡	Molecular weight	Number of cations in oxide	Cation valence	Charge equivalents	Proportionality factor (R)§	Cation valence	Atoms per unit cell
SiO ₂	59.60	64.67	60.06	1	4	4.3070	7.1398	4	7.69
Al ₂ O ₃	22.17	24.05	101.95	2	3	1.4154	7.1398	3	3.37
Fe ₂ O ₃	4.32	4.69	159.70	2	3	0.1762	7.1398	3	0.42
MgO	2.73	2.96	40.32	1	2	0.1468	7.1398	2	0.52
CaO	0.14	0.15	56.08	1	2	0.0053	7.1398	2	0.02
Na ₂ O	3.18	3.45	61.99	2	1	0.1113	7.1398	1	0.79
K ₂ O	0.03	0.03	94.19	2	1	0.0006	7.1398	1	0.004
TiO ₂	0.09	—	—	—	—	—	—	—	—
H ₂ O	6.02	—	—	—	—	—	—	—	—
Total	98.28	100.00	—	—	—	6.1626	—	—	—

† Structural formula: $(\text{Si}_{1.69}\text{Al}_{0.31})(\text{Al}_{1.06}\text{Fe}^{3+}_{0.02}\text{Mg}_{0.53})\text{O}_{20}(\text{OH})_4 \cdot \text{Na}_{0.79}\text{Ca}_{0.03}\text{K}_{0.004}$. Layer charge: tetrahedral = 32 - (7.69 × 4 + 0.31 × 3) = 0.31⁻ (or 8.00 - 7.69 = 0.31⁻); octahedral = 12 - (3.06 × 3 + 0.42 × 2) = 0.52⁻ (or 4.00 - 3.06 - 0.42 = 0.52⁻). Total layer charge = 0.31 + 0.52 = 0.83⁻. Tetrahedral/total ratio = 0.31/0.83 = 37%. Exchange cation charge: 0.79 + (0.02 × 2 + 0.004) = 0.834⁻.

‡ Normalized to 100%, considering TiO₂ as anatase impurity and ideal structural hydroxyl H₂O content.

§ R = 44/6.1626 = 7.1398.

content is unknown, and therefore assumed to be ideal. The calculation is based on 28 ideal total negative charges $[\text{O}_{10}(\text{OH})_8]$ for the kaolinite structure and 44 ideal total negative charges $[\text{O}_{20}(\text{OH})_4]$ for the montmorillonite structure. The ideal number of negative charges for each mineral is divided by the sum of charge equivalents to calculate the respective proportionality factor (R). The distribution of atoms per unit cell is calculated by multiplying each charge equivalent by R and dividing by the valence of the cation. Since the procedure assumes structural electroneutrality, the overall cationic charge should be balanced by the total anionic charge, allowing for rounding-off errors only. An anion–cation balance check is advisable to ensure error-free structural formula calculations (Jackson, 1985; Čičel and Komadel, 1994).

In the calculated structural formula for the Georgia kaolinite sample (Table 15–2) some minor substitutions of Si by Al in the tetrahedral sheet, and Al by Fe and Mg in the octahedral sheet produce a low negative charge, which is counter balanced by small amounts of Ca, Na, and K. The charge of the tetrahedral unit is 15.95^+ compared with the ideal 16.00^+ , thus leaving a charge imbalance of 0.05^- . The charge of the octahedral unit is 12.02^+ compared to the ideal 12.00^+ , thus leaving a charge imbalance of 0.02^+ . Therefore, the net negative charge of the mineral is 0.03^- , which is balanced by the total charge of 0.03^+ of the counter ions. In the calculated formula for the Wyoming montmorillonite (Table 15–3), the tetrahedral charge is 31.69^+ compared with the ideal 32.00^+ , leaving a net negative charge of 0.31^- , while the octahedral charge is 11.45^+ compared with the ideal of 12.00^+ , leaving a net negative charge of 0.55^- . Therefore, the total negative charge of the mineral is 0.83^- , which is balanced by 0.83^+ charges of the counter ions.

Structural Formula Calculation Including H_2O and Other Anions

This approach is based on 48 negative charges, considering that the total anionic content in the formula of a 2:1 mineral is $\text{O}^{2-}_{24-x-y-z}$, where x and y are other anions, and z is OH water. This method can be used when dehydroxylation measurements have been made by thermal analysis. Therefore, the sum of charge equivalents in this case includes the individual charge equivalents of OH water, and other anions present (e.g., F, Cl, P). The proportionality factor (R) is calculated by dividing 48 by the total sum of charge equivalents and the distribution of atoms per unit cell is determined as in the previous method (Tables 15–2 and 15–3). Table 15–4 illustrates this procedure in calculating the structural formula of a biotite mineral sample (Scenario B) and compares it with the structural formula of the same mineral calculated by the previous method (Scenario A), which excludes H_2O and other anions. The R_B factor is calculated by dividing 44 with the partial sum of charge equivalents consisting of cationic components only, while the R_A factor is determined by dividing 48 with the total sum of charge equivalents. The two formulas compare favorably in their tetrahedral, octahedral, and counter ion composition, but differ considerably in their anionic composition. However, electroneutrality is maintained in both cases, with a cation and anion balance of ~ 44.00 charges (Newman and Brown, 1987).

Structural Formula Calculation with Fixed O^{2-} but Variable OH^- Charges

Structural formulae of 2:1 hydroxy-interlayered minerals can be calculated on the basis of 56 total negative charges $[\text{O}_{20}(\text{OH})_4(\text{OH})_{12}]$, using either of the two procedures described above (Tables 15–2 through 15–4). However, because the degree of interlayering and the composition of the hydroxy-interlayer component is variable, the calculated formula may be erroneous and in some cases unsuitable. This is of particular concern in partially hydroxy-interlayered minerals, which may have considerably lower hydroxyl

Table 15–4. Structural formula calculation for a biotite sample based on 44 equivalent charges with ideal H₂O (Scenario A) vs. 48 total charges including H₂O plus F (Scenario B).†

Elemental oxides	Normalized weight	%	Molecular weight		Number of cations in oxide		Cation valence		Charge equivalents	Scenario A‡	Scenario B§
SiO ₂	40.65	÷	60.06	×	1	×	4	=	2.7073	5.83	5.84
Al ₂ O ₃	19.88	÷	101.95	×	2	×	3	=	1.1700	3.36	3.36
Fe ₂ O ₃	—	—	—	—	—	—	—	—	—	—	—
FeO	7.89	÷	71.85	×	1	×	2	=	0.2196	0.95	0.95
MgO	15.68	÷	40.32	×	1	×	2	=	0.7778	3.35	3.35
CaO	0.97	÷	56.08	×	1	×	2	=	0.0346	0.15	0.15
K ₂ O	9.46	÷	94.19	×	2	×	1	=	0.2009	1.73	1.73
Partial sum									5.1102		
H ₂ O	2.90	÷	18.00	×	1	×	2	=	0.3222		2.78
F	2.57	÷	19.00	×	1	×	1	=	0.1353		1.17
Total sum	100.00								5.5677		

† Formula A: $K_{1.73}(Si_{5.83}Al_{2.17})(Al_{1.19}Fe^{2+}_{0.95}Mg_{3.35})O_{20}(OH)_4 \cdot Ca_{0.15}$. Formula B: $K_{1.73}(Si_{5.84}Al_{2.16})(Al_{1.20}Fe^{2+}_{0.95}Mg_{3.35})O_{20.05}(OH)_{2.78}F_{1.17} \cdot Ca_{0.15}$.

‡ Scenario A formula composition based on $R_A = 44 \div 5.1102 = 8.6102$.

§ Scenario B formula composition based on $R_B = 48 \div 5.5677 = 8.6212$.

content in the interlayer component. Assuming ideal OH[−] content in such cases usually results in an overestimation of the tetrahedral Si atoms in the unit cell, frequently exceeding the ideal occupancy of 8.0. To resolve this issue Kirkland and Hajek (1972) proposed a procedure based on 40 fixed O^{2−} charges, which allows for the OH[−] content of the mineral to vary according to the cationic composition of the interlayer. In this procedure, the proportionality factor (R) = $40/(C - 2H)$, where C is the total sum of charge equivalents and H is the charge equivalent for OH water. The allocation of atoms per unit cell is conducted in a manner similar to the above methods, with the following additional considerations for an Al-hydroxy-interlayered mineral.

1. All Si and enough Al is assigned to the tetrahedral sheet to fill the 8.0 ideal positions.
2. All Fe and Mg and enough Al is assigned to the octahedral sheet to fill the ideal 4.0 (dioctahedral) or 6.0 (trioctahedral) positions.
3. Twenty O^{2−} and 4 OH[−] are assigned to the 2:1 layer.
4. The remaining Al and OH are assigned to the hydroxy-interlayer.
5. All the counter ions are assigned to exchangeable positions.

Table 15–5 compares structural formula calculation procedures for a chlorite mineral based on 56 total negative charges (Scenario A) vs. 40 fixed O charges (Scenario B). The proportionality factor R_A is calculated by dividing 56 by the partial sum of charge equivalents excluding H₂O, while R_B is based on the equation described above. The two formulas compare favorably, with only minor differences in cationic and anionic composition and in charge balance. However, this is because chlorite exhibits the maximum degree of hydroxy-interlayering containing a complete interlayer sheet. Partially hydroxy-interlayered smectites and vermiculites, which are predominant minerals in the clay fractions of many acid soils, have hydroxy-interlayer composition ranging from $Al(OH)_{2.0}^{+1}$ to $Al(OH)_{2.9}^{+0.1}$.

Table 15-5. Structural formula calculations for a dioctahedral chlorite mineral based on 56 total charges (Scenario A) vs. 40 fixed oxygen charges (Scenario B).†

Elemental oxides	Normalized weight %	Molecular weight	Number of cations in oxide	Cation valence	Charge equivalents	Scenario A‡	Scenario B§
SiO ₂	40.60 ÷	60.06 ×	1 ×	4	=	2.7040	7.11
Al ₂ O ₃	43.10 ÷	101.95 ×	2 ×	3	=	2.5365	8.90
Fe ₂ O ₃	1.05 ÷	159.70 ×	2 ×	3	=	0.0394	0.14
MgO	0.80 ÷	40.32 ×	1 ×	2	=	0.0397	0.14
CaO	0.60 ÷	56.08 ×	1 ×	2	=	0.0214	0.11
Partial sum						5.3410	
H ₂ O	13.85 ÷	18.00 ×	2 ×	1	=	1.5389	16.19
Total sum	100.00					6.8800	

† Formula $R_A = (\text{Si}_{7.09}\text{Al}_{0.91})(\text{Al}_{1.77}\text{Fe}^{3+}_{0.14}\text{Mg}_{0.14})\text{O}_{20}(\text{OH})_4[\text{Al}_{4.23}(\text{OH})_{12}]\text{Ca}_{0.11}$. Formula $R_B = (\text{Si}_{7.11}\text{Al}_{0.89})(\text{Al}_{3.72}\text{Fe}^{3+}_{0.14}\text{Mg}_{0.14})\text{O}_{20}(\text{OH})_4[\text{Al}_{4.29}(\text{OH})_{12.19}]\text{Ca}_{0.11}$.

‡ $R_A = 56/5.3411 = 10.4847$.

§ $R_B = 40/[6.8810 - (2 \times 1.5389)] = 10.5202$.

(Kirkland and Hajek, 1972; Karathanasis et al., 1983; Karathanasis, 1988b). Using the 56 total negative charge approach to calculate the structural formula of such minerals usually results in excessive Si tetrahedral occupancy, and therefore, unacceptable compositions.

For Mg-hydroxy-interlayered minerals containing a “brucite” layer, in Step 2 above, the remaining Al following the tetrahedral allocation and enough Mg is assigned to the octahedral sheet to fill the ideal 4.0 (dioctahedral) or 6.0 (trioctahedral) positions. In Step 4, the remaining Mg and OH are assigned to the hydroxyl-interlayer.

The Fixed Cation Occupancy Method

This method assumes that all central cations occupy a fixed number of positions in the structure as dictated by the type of the mineral. Thus, this number could be 8 or 10 for di- or trioctahedral 1:1 minerals, respectively, and 12 or 14 for di- or trioctahedral 2:1 minerals, respectively. For “2:1:1” hydroxy-interlayered minerals, the number of positions could range from 16 in dioctahedral to 20 in trioctahedral structures. The stepwise procedure followed by this method for calculating the structural formula of a mineral involves the following steps:

1. Normalize the elemental oxide composition of the mineral to 100%.
2. Divide the percentage concentration of each oxide by its molecular weight and multiply by the total number of metal atoms in the oxide to obtain the mass equivalent.
3. Calculate the partial sum of mass equivalents for the cations occupying tetrahedral and octahedral positions.
4. Calculate the proportionality factor (R) by dividing the total number of central cations corresponding to the mineral group with the partial sum of their mass equivalents.
5. Calculate the occupancy of atoms in the unit cell by multiplying their respective mass equivalent with the proportionality factor (R) and dividing by their formal valence.
6. Allocate the atoms in the unit cell assuming 4.0 tetrahedral central atoms per unit cell for 1:1 minerals, and 8.0 for 2:1 minerals, including hydroxy-interlayered minerals.

7. Assign all Si to tetrahedral positions and fill the remaining positions for ideal occupancy with Al.
8. Assign the remaining Al atoms and all Fe^{2+} , Fe^{3+} , and Mg to octahedral positions.
9. Consider all remaining cations as counter ions balancing the unit cell charge.

Table 15–6 demonstrates the structural formula calculation for a Clay Spur Wyoming montmorillonite sample following the fixed central cation occupancy method. The formula appears to be identical to that calculated by the fixed negative charge method for the same mineral (Table 15–3). This is unusual and probably due to the high purity of the sample, as in most cases the two methods yield structural formulae with slight differences in the number of central atoms occupying octahedral positions because of admixed impurities. The fixed cation occupancy method by definition results in ideal occupancy, while the fixed negative charge method usually results in lower or higher than ideal occupancy. Grim and Guven (1978) compared the two methods for 152 smectite samples and found an average octahedral cation occupancy of 4.14 by the fixed negative charge method compared with the ideal 4.00 of the fixed central cation occupancy method. Another difference between the two methods is their sensitivity to impurities. While the fixed negative charge method may indicate excessive amounts of free Si, Al, or Fe in the sample, resulting in overpopulation of tetrahedral or octahedral positions, the fixed central atom occupancy method is insensitive to such admixtures due to its ideal occupancy presumption. Central atom occupancy differences of 0.10 to 0.15 between the two methods in the calculated structural formulae of 2:1 minerals may indicate the presence of nonmonomineralic samples, with as much as 5 to 6% free Si, Al, or Fe oxides (Grim and Guven, 1978).

STRUCTURAL FORMULA CALCULATION FOR NONMONOMINERALIC SAMPLES

If necessary, any one of the above methods can also be used for the calculation of the structural formula of a variable-composition mineral in a sample with multiple mineral components. However, this is generally undesirable and problematic. If the mineral suite of the soil or clay fraction consists of several minerals with relatively constant composition and only one variable mineral, the common procedure followed is to initially allocate the appropriate elemental constituents to the known composition minerals and then assign the remaining elements to the unknown composition mineral (Kirkland and Hajek, 1972; Karathanasis and Hajek, 1983; Karathanasis et al., 1983). In samples containing more than

Table 15–6. Structural formula calculation for the Clay Spur, Wyoming montmorillonite sample (Table 15–3) based on 12 central atom positions.†

Elemental oxides	Weight %		Molecular weight		Number of metal cations		Mass equivalents		$R^\ddagger$		Atoms per unit cell
SiO_2	64.67	÷	60.06	×	1	=	1.0768	×	7.1399	=	7.69
Al_2O_3	24.05	÷	101.95	×	2	=	0.4718	×	7.1399	=	3.37
Fe_2O_3	4.69	÷	159.70	×	2	=	0.0587	×	7.1399	=	0.43
MgO	2.96	÷	40.32	×	1	=	0.0734	×	7.1399	=	0.52
Partial sum							1.6807				
CaO	0.15	÷	56.08	×	1	=	0.0027	×	7.1399	=	0.02
Na_2O	3.45	÷	61.99	×	2	=	0.1113	×	7.1399	=	0.79
K_2O	0.03	÷	94.19	×	2	=	0.0006	×	7.1399	=	0.004
Total	100.00										

† Structural formula: $(\text{Si}_{7.69}\text{Al}_{0.31})(\text{Al}_{3.06}\text{Fe}^{3+}_{0.43}\text{Mg}_{0.52})\text{O}_{20}(\text{OH})_4\text{Na}_{0.79}\text{Ca}_{0.02}\text{K}_{0.004}$. Layer charge: = 0.31^- (tetrahedral) + 0.52^- (octahedral) = 0.83^- total. Exchangeable cation sum = 0.834^+ .

‡ $R = 12/1.6807 = 7.1399$.

one mineral with variable chemical composition, the least variable mineral is assumed to have the composition of a reference mineral of the same kind, and the remaining elements are partitioned to the composition of the most variable mineral. However, considerable variations in the composition of the latter mineral may arise depending on how accurately the assumed composition of the reference mineral approximates the composition of the least variable mineral. Therefore, it is important that the selection of the reference mineral is justified from other available characterization data (e.g., geology, XRD, optical microscopy) to minimize these variations. In minerals considered to be part of a weathering sequence, precursor minerals (i.e., micas) found in soil parent materials may be analyzed and used as reference minerals in calculating the structural composition of their presumed weathering products (e.g., vermiculites, smectites) (Karathanasis, 1988a, 1988b). In addition, it is necessary to estimate the relative abundances of the individual mineral components within the admixture with as high a degree of accuracy as possible. Often this can be done using XRD and other techniques in combination (e.g., IR, TGA), as outlined below.

Tables 15–7, 15–8, and 15–9 demonstrate the calculation of the structural formula of an Al-hydroxy-interlayered vermiculite (HIV) mineral present in a multimineralic sample using the method of 40 fixed O charges (Kirkland and Hajek, 1972) (Table 15–5). The mineralogical composition of the sample based on XRD and thermal analysis (Karathanasis and Hajek, 1982) included 2% quartz, 2% K-feldspars, 38% kaolinite, 15% illite, and 43% hydroxy-interlayered vermiculite. The elemental oxide composition of the sample was determined by XRF analysis (Karathanasis, 1996) and the OH water by thermogravimetric analysis (Karathanasis, 2008, this volume). The first step involves the partitioning of the proper elemental oxide constituents to each mineral (except HIV), according to their percentage concentration in the sample (Table 15–7), and their theoretical or reference elemental composition (Table 15–8). The sum of the elemental oxides allocated to these minerals is subtracted from the original elemental oxide composition of the sample and the difference normalized to 100% constituting the elemental oxide composition of HIV (Table 15–7). Following the allocation of tetrahedral and octahedral atoms assuming ideal occupancies of 8.0 and 4.0, respectively, for the dioctahedral HIV mineral, four of the calculated OH are allocated to the octahedral layer, and the remaining Al and OH are partitioned in the hydroxy-interlayered component (Table 15–9). The total negative charge of the mineral is 1.35^- , of which 0.76^- originates from the tetrahedral sheet and 0.59^- from the octahedral sheet. This negative charge is balanced by 0.60^+ charges from the Ca counter ions and 0.75^+ charges from the Al-hydroxy-interlayer component, thus maintaining electroneutrality in the structure.

Table 15–7. Structural allocation of elemental oxide components to minerals comprising the clay fraction based on elemental oxide compositions reported in Table 15–8.†

Elemental oxide	Weight %	Quartz	K-feldspar	Kaolinite	Illite	HIV‡	Normalized to 100%
SiO ₂	51.70	2.00	1.29	17.67	8.05	22.69	52.77
Al ₂ O ₃	32.81	—	0.37	15.01	4.05	13.38	31.12
Fe ₂ O ₃	2.05	—	—	—	0.48	1.57	3.65
MgO	1.59	—	—	—	0.35	1.24	2.88
CaO	0.85	—	—	—	—	0.85	1.97
K ₂ O	1.54	—	0.34	—	1.20	0.00	—
H ₂ O	9.46	—	—	5.32	0.87	3.27	7.61
Total	100.00						100.00

‡ HIV elemental oxide composition calculated by difference and normalized to 100%.

Table 15-8. Elemental oxide composition and structural formulae of some common clay minerals.

Mineral	Structural formula	SiO ₂	Al ₂ O ₃	FeO	Fe ₂ O ₃	MgO	K ₂ O	Na ₂ O	CaO	H ₂ O [†]
Kaolinite	Si ₂ Al ₂ (OH) ₄	46.5	39.5	—	—	—	—	—	—	14.0
Illite	K _{0.7} (Si _{3.5} Al _{0.5})(Al _{1.6} Fe _{0.2} Mg _{0.2})O ₁₀ (OH) ₂	53.7	27.0	—	3.2	2.3	8.0	—	—	5.8
Quartz	SiO ₂	100.0	—	—	—	—	—	—	—	—
Gibbsite	Al(OH) ₃	—	65.4	—	—	—	—	—	—	34.6
Orthoclase	KAlSi ₃ O ₈	64.7	18.4	—	—	—	16.9	—	—	—
Albite	NaAlSi ₃ O ₈	68.7	19.5	—	—	—	—	11.8	—	—
Goethite	FeOOH	—	—	—	89.9	—	—	—	—	10.1
Chlorite	(Si _{3.0} Al _{1.0})(Mg _{6.0} Al _{1.0} Fe _{1.0})O ₁₀ (OH) ₈	30.7	17.4	12.2	—	27.5	—	—	—	12.2
Muscovite	K(AlSi ₃)(Al ₂)O ₁₀ (OH) ₂	45.3	38.4	—	—	—	11.8	—	—	4.5
Biotite	K(AlSi ₃)(Mg ₂ Fe)O ₁₀ (OH) ₂	40.2	11.4	15.9	—	18.0	10.5	—	—	4.0
Vermiculite	(Si _{3.84} Al _{2.16})(Al _{0.22} Fe _{0.13} Mg _{5.65})O ₁₀ (OH) ₄ Ca _{0.9} K _{0.03}	44.0	15.1	—	1.3	28.5	0.2	—	6.4	4.5
Montmorillonite	(Si _{7.50} Al _{0.50})(Al _{1.37} Fe _{0.75} Mg _{1.21})O ₁₀ (OH) ₄ Ca _{0.36}	59.4	19.3	—	7.9	6.4	—	—	2.7	4.3

† Structural hydroxyl water.

Table 15-9. Structural formula calculation for the HIV mineral based on 40 fixed oxygen charges.†

Elemental oxides	Weight %	Molecular weight	Number of atoms in oxide	Cation valence	Charge equivalents	R [‡]	Cation valence	Atoms per unit cell
SiO ₂	52.77	÷ 60.06	× 1	4	× 3.5145	8.2423	÷ 4	7.24
Al ₂ O ₃	31.12	÷ 101.95	× 2	3	× 1.8315	8.2423	÷ 3	5.03
Fe ₂ O ₃	3.65	÷ 159.70	× 2	3	× 0.1371	8.2423	÷ 3	0.38
MgO	2.88	÷ 40.32	× 1	2	× 0.1429	8.2423	÷ 2	0.59
CaO	1.97	÷ 56.08	× 1	2	× 0.0726	8.2423	÷ 2	0.30
H ₂ O	7.61	÷ 18.00	× 2	1	× 0.8456	8.2423	÷ 1	6.97
Total	100.00				6.5442			

† Structural formula: (Si_{7.24}Al_{5.03})(Al_{3.03}Fe_{0.38}Mg_{0.59})O₃₀(OH)₄[Al_{1.24}(OH)₄]₁Ca_{0.30}. Layer charge: tetrahedral = 32 - (7.24 × 4 + 0.76 × 3) = 0.76⁻; octahedral = 12 - (3.03 × 3 + 0.38 × 3 + 0.59 × 2) = 0.59⁻. Total layer charge = 0.76⁻ + 0.59⁻ = 1.35⁻. Interlayer charge: Hydroxide charge = (1.24 × 3) - 2.97 = 0.75⁺; exchange cation charge = 0.30 × 2 = 0.60⁺. Total charge: 0.75⁺ + 0.60⁺ = 1.35⁺.

‡ R = 40/[6.5442 - (2 × 0.8456)] = 8.2423.

EFFECT OF ADMIXTURES ON THE STRUCTURAL FORMULA CALCULATION

The calculation of accurate structural formulae is based on the following assumptions:

- The elemental chemical analysis is correct.
- The sample used for the analysis is monomineralic.
- The mineralogical composition of the sample is precisely determined.
- The allocation of the elemental oxides to the mineral in question is accurate.

Inaccurate chemical analysis and incorrect allocation of elemental constituents due to errors in the mineralogical composition or the presence of impurities can significantly affect the distribution of chemical constituents in the structure and result in nonrepresentative structural formulae.

Errors in elemental oxide analysis may lead to inaccurate structural formulae, particularly if the analytical uncertainty is associated with multivalent (Si, Al, Fe³⁺) and multisite (Al, Fe) cations. Matrix effects due to abundance of multivalent cations, such as Si, in elemental analytical determinations should also be taken into consideration because they usually result in excessive Si occupancies in the structural formula of 2:1 minerals. Distorted distributions of octahedral central atoms may also be caused by questionable Fe³⁺/Fe²⁺ elemental oxide composition data. These analytical uncertainties are usually propagated to questionable distributions and proportions of the calculated cations, particularly those with multiple valence and coordination (Giaramita and Day, 1990).

The presence of admixtures mainly affects the octahedral distribution of central cations, which is typically the most sensitive indicator of the accuracy of the structural formula. Generally, the total number of octahedral cations is lower than ideal if the admixing components contain atoms of formal valence ≥ 3 and higher than ideal for admixtures containing atoms with formal valence ≤ 3 (Čičel and Komadel, 1994). Furthermore, the sensitivity of total octahedral occupancy to admixtures of other layer silicates, feldspars, or zeolites is usually lower than that of free oxides (e.g., quartz, goethite, cristobalite, gibbsite) because of the balancing effect of the multielement error distribution in the former compared with one element in the latter. The sensitivity to free oxides also increases with increasing valence of the element and with decreasing mass equivalent.

As a rule of thumb, a value of tetrahedral Si occupancy of >8.0 unambiguously indicates the presence of free Si in the sample. In such samples, the amount of free SiO₂ present may be at least 2% for montmorillonites and much greater for beidellites (Grim and Guven, 1978). For tetrahedral Si occupancy of <8.0 , the only indicator of free SiO₂ in the sample is the lower than ideal octahedral cation occupancy. Generally, an excess of 1% SiO₂ in smectite samples may increase the tetrahedral occupancy by 0.04 and decrease the octahedral occupancy by 0.01 to 0.02. For octahedral occupancies differing by more than 1% of the ideal (0.04 for dioctahedral, 0.06 for trioctahedral), a reassessment of the SiO₂ is advisable. Since Al may occupy both tetrahedral and octahedral positions, increases in free SiO₂ result in increasing transfers of Al from tetrahedral to octahedral sites. This makes multiple analyses (i.e., XRD, IR) all the more important to assist in determining whether or not admixtures exist.

The presence of free Al or Fe oxide admixtures in the sample may decrease the Si atoms in the tetrahedral sheet and increase the total octahedral occupancy. Based on studies with smectites, an excess of 1% Al₂O₃ or Fe₂O₃ in the sample may decrease the tetrahedral occupancy by 0.08 and 0.05, respectively, while causing respective increases of 0.02

to 0.03 in the octahedral occupancy (Kelley, 1955; Grim and Guven, 1978). Excessive increases in octahedral occupancy, particularly of Al, may also indicate the presence of Al-hydroxy-interlayering components, especially when accompanied by increases in structural OH water (Karathanasis, 1988b). Layer silicate admixtures of up to 3% may not significantly affect the distribution of central atoms in the structural formula, but may cause considerable imbalance in the structural OH composition. Experiments with smectite-type minerals suggest that the presence of kaolinite admixture in the sample has the greatest impact, causing a Si tetrahedral occupancy change of about 0.01 to 0.02, and 0.005 octahedral occupancy change for each percentage unit of impurity. In contrast, the impact of similar illite admixtures was <0.005 for either tetrahedral or octahedral occupancy. Generally, the total number of octahedral cation occupancy calculated by the fixed negative charge method is a useful indicator of the accuracy of the structural formula (Čičel and Komadel, 1994). For dioctahedral 2:1 minerals values of 4.00 ± 0.05 are generally acceptable, and for trioctahedral values of 6.00 ± 0.06 are generally acceptable. In monoionic samples prepared by saturation with a particular counter ion to balance the charge, excessive charge estimates outside the range of the specific mineral type may indicate inadequate washing of the free salts.

It is recommended that a standard convention be used with significant figures in the calculation process to avoid excessive deviations in the structural formula composition. Typically, the elemental oxide concentrations are expressed with one or two decimals, depending on the accuracy of the chemical analysis used. The molecular weight of the elemental oxides and the occupancy of atoms per unit cell in the structural formula are expressed with two decimals. The proportionality factor and the charge/mass equivalents are expressed with at least four decimals to maintain the required charge electroneutrality in the formula.

STRUCTURAL FORMULAE FROM OTHER SPECIAL CHEMICAL TECHNIQUES

In conjunction with bulk chemical analysis, other special chemical techniques have been used to refine mineral structural formulae, particularly with respect to the distribution of central atoms within the mineral structure. The most classical of these approaches involves acid dissolution, while more innovative techniques include IR, NMR, Mössbauer spectroscopy (Osthaus, 1956; Farmer, 1974; Wilson et al., 1984; Kinsey et al., 1985; Amonette et al., 1994), XRD, and EXAFS (Gates et al., 2002).

Acid Dissolution Techniques

The most common procedure involves dissolving a sample with 6 M HCl at 95°C over 24 to 48 h and analyzing the dissolved phases for Al, Fe, Mg, and Ca (Brindley and Youell, 1951; Novák and Čičel, 1978). The end of the dissolution period is usually marked by the disappearance of the layer lattice $\text{Si}_{(t)}\text{—O—Al}_{(o)}$ infrared band at 529 cm^{-1} (Čičel and Novák, 1977). Original approaches relied on the premise that the rate of cation dissolution follows the sequence: exchangeable cations > octahedral cations > tetrahedral cations, but evidence based on NMR and Mössbauer spectroscopy suggests similar dissolution rates between octahedral and tetrahedral cations (Luca and MacLachlan, 1992; Tkáč et al., 1994; Komadel et al., 1996). Nevertheless, the technique provides useful supplementary information about the quantitative distribution of octahedral and exchangeable cations in the structure of 2:1 and hydroxy-interlayered minerals, particularly smectites and chlorites (Novák and Čičel, 1978; Čičel et al., 1990).

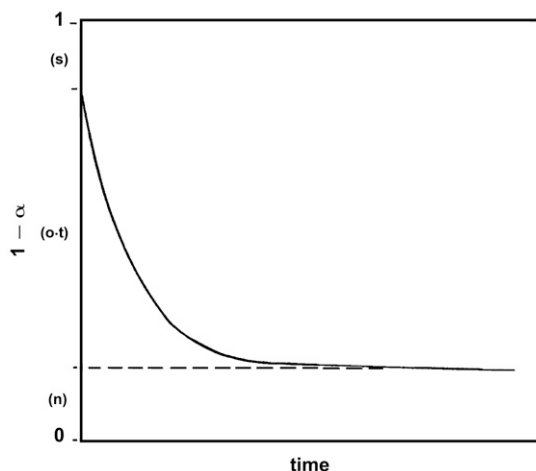


Fig. 15-1. A typical acid dissolution curve for a structural cation in a layer silicate mineral showing the readily soluble fraction (s), the insoluble fraction (n), and the dissolved octahedral + tetrahedral ($o + t$) fraction.

According to this procedure, the soluble (s), octahedral + tetrahedral ($o + t$), and the insoluble (n) fraction for each cation are estimated from its respective dissolution curve, plotted as $1 - a$ vs. time, where $a = n + s$, and $1 - a = o + t$ (Fig. 15-1). The soluble (s) fraction, defined by the intercept of the dissolution curve with the $1 - a$ axis at time 0, usually contains exchangeable Ca, Mg, Na, and K, as well as readily soluble Fe and Al oxides and hydroxides (e.g., goethite, gibbsite). Some of the Mg, Fe, and Al may be weakly bound to edge octahedral sites. An accurate distinction between octahedrally coordinated Fe vs. Fe associated with free oxides or hydroxides can be made by Mössbauer spectroscopy, but such a distinction for Al by NMR is not reliable (Morris et al., 1990; Čičel et al., 1992). The insoluble fraction (n), consisting of the residual phases left at the end of the dissolution experiment (usually kaolinite, quartz, hydrous poorly crystalline Si, and anatase impurities), is estimated from the difference between the dissolved and the soluble fraction (Fig. 15-1). Therefore, the dissolved fraction is estimated from the equation $(o + t) = 1 - a = 1 - (s + n)$. All $\text{Mg}_{(o+t)}$ and $\text{Fe}_{(o+t)}$ is allocated to octahedral positions, unless Mössbauer spectroscopy data can be used to distinguish between tetrahedral and octahedral Fe coordination. The Al distribution between tetrahedral and octahedral sites is calculated by magic angle NMR spectroscopy following a computer optimization procedure that takes into consideration the tetrahedral/octahedral Fe and Mg occupancy. In the absence of NMR data, the Al occupancy in the octahedral sheet is calculated from the difference between 4.0 and the sum of Fe and Mg, assuming ideal octahedral occupancy for a dioctahedral smectite. The remaining Al is allocated to the tetrahedral sheet along with any tetrahedrally coordinated Fe measured by Mössbauer spectroscopy. Finally, the Si occupancy is estimated from the difference between 8.0 and the sum of tetrahedral Al + Fe, assuming ideal tetrahedral occupancy for smectites.

Table 15-10 demonstrates the calculation of the structural formula for a smectite sample following an acid dissolution experiment, which produced the following data: readily soluble fractions (s) for $\text{Ca}_{(s)} = 1.00$, $\text{Mg}_{(s)} = 0.05$, $\text{Fe}_{(s)} = 0.06$, and $\text{Al}_{(s)} = 0.05$. The respective insoluble fractions (n) were $\text{Ca}_{(n)} = 0.00$, $\text{Mg}_{(n)} = 0.10$, $\text{Fe}_{(n)} = 0.12$, and $\text{Al}_{(n)} = 0.15$. Assuming (i) the same dissolution rate for octahedral and tetrahedral cations, (ii) that all Fe is in octahedral coordination, (iii) that the exchange phase is monoionic (Ca), and (iv) that the soluble fractions of Mg, Fe, and Al are associated with octahedral edge sites, the following distribution of structural cation fractions is obtained: $\text{Mg}_{(o)} = 0.90$, $\text{Fe}_{(o)} = 0.88$,

Table 15–10. Structural formula calculation for a Ca-saturated Wyoming montmorillonite sample from acid dissolution data (modified from Čechil and Komadel, 1994).†

Elemental oxide	Normalized weight %	Molecular weight	Number of cations in oxide		Cation valence	Charge equivalents	$R^\ddagger$	Cation valence	Atoms per unit cell
SiO ₂	70.65								7.56
Al ₂ O _{3(t)}	1.55								0.44
Al ₂ O _{3(o)}	18.30	÷ 101.95	×	2	×	3	= 1.0770		3.02
Fe ₂ O _{3(o)}	4.22	÷ 159.70	×	2	×	3	= 0.1584	× 8.8889	÷ 3 = 0.47
MgO _(o)	2.31	÷ 40.32	×	1	×	2	= 0.1146	× 8.8889	÷ 2 = 0.51
Partial sum						1.3500			
CaO _(ex)	2.97	÷ 56.08	×	1	×	2	= 0.1060	× 8.8889	÷ 2 = 0.47
Total	100.00								

† Structural formula: $(\text{Si}_{7.56}\text{Al}_{0.44})(\text{Al}_{3.02}\text{Fe}^{3+}_{0.47}\text{Mg}_{0.51})\text{O}_{20}(\text{OH})_4\text{Ca}_{0.47}$. Layer charge: tetrahedral = $32 - (7.56 \times 4 + 0.44 \times 3) = 0.44^-$; octahedral = $12 - (3.02 \times 3 + 0.47 \times 3 + 0.51 \times 2) = 0.51^-$. Total charge = $0.44^- + 0.51^- = 0.95^-$. Exchange cation charge: $0.47 - 2 = 0.94^+$.

‡ $R = 12/1.3500 = 8.8889$.

$\text{Al}_{(o+t)} = 1.0 - 0.15 = 0.85$, $\text{Ca}_{(ex)} = 1.00$. The corresponding concentrations of these fractions on a percentage basis, along with the measured concentration of dissolved Si normalized to 100%, are listed in Table 15–10. The concentration of Al was partitioned to tetrahedral and octahedral sites following an independent determination of tetrahedral Al coordination by magic angle NMR. Following the calculation of mass equivalents, the proportionality factor is calculated by dividing the 12 octahedrally coordinated O by the partial sum of the mass equivalents associated with the octahedral cations. The distribution of atoms per unit cell is calculated by multiplying each mass equivalent with the proportionality factor, and dividing by the respective valence of the cation. The calculated structural formula is within the acceptable range for smectite minerals with a reasonable balance between the 0.95^- structural charges and the 0.94^+ counter ion charges. However, a back calculation of the dissolved Si corresponding to the 7.56 cation occupancy in the tetrahedral sheet reveals that only 51.08% of SiO₂ would have been adequate ($7.56 \times 60.06/8.8889 = 51.08$), suggesting an excess of 19.57% of free SiO₂ in the sample. On the other hand, this amount may be overestimated because of the potential overlapping in the calculation of Al_(t) by acid dissolution and NMR, and/or underestimation of the Al_(o) determination by NMR spectroscopy due to paramagnetic interference by Fe (Morris et al., 1990; Komadel et al., 1993). Gates et al. (1996) showed that reduction of Fe³⁺ to Fe²⁺ substantially decreases this interference. They mentioned that this might prove a useful technique to estimate tetrahedral Al content in smectites, but they did not present this work in their study.

Other Special Chemical Techniques

The octahedral distribution of central atoms in the structure of 2:1 layer silicates can also be estimated by IR spectroscopy (Slonimskaya et al., 1986; Madejová et al., 1992; Salil et al., 1997; Post and Borer, 2002; Gates et al., 2002; Madejová, 2003). This approach utilizes the different vibrational frequencies of structural OH groups associated with different octahedral cations. The integrated intensity of each characteristic band is measured in the 3400 to 3700 cm⁻¹ region and the octahedral cation distribution is calculated from the decomposed IR spectra (Fig. 15–2). Recent studies (Vantelon et al., 2001, 2003; Gates, 2005) suggest that the bending bands between 500 and 1000 cm⁻¹ or the combination

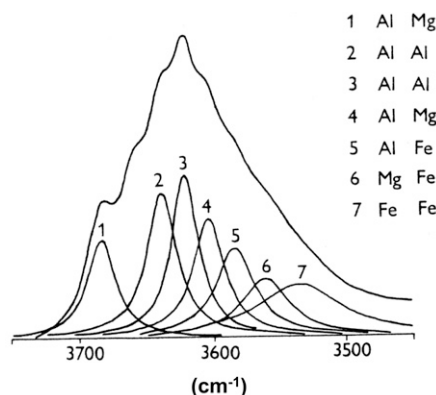


Fig. 15-2. Deconvolution of infrared spectra in the 3500 to 3700 cm^{-1} band for a montmorillonite sample, showing Al–Al, Al–Mg, Al–Fe, Mg–Fe, and Fe–Fe bands (Madejová et al., 1992).

bands near 4500 cm^{-1} may provide better resolution, at least for dioctahedral clays. Since each OH group is coordinated with two central atoms, each integral intensity value represents one-half of the concentration of the respective octahedral cation. For example, the octahedral distribution of Al is the result of integral intensity contributions (I) of Al–Al, Al–Fe³⁺, Al–Fe²⁺, and Al–Mg coordinations with structural OH. Therefore, the concentration of octahedral Al = $2(I)_{\text{Al-Al}} + (I)_{\text{Al-Fe}^{3+}} + (I)_{\text{Al-Fe}^{2+}} + (I)_{\text{Al-Mg}}$. The octahedral distribution of central atoms is performed assuming ideal octahedral occupancy, where $\text{Al} + \text{Fe}^{3+} + \text{Fe}^{2+} + \text{Mg} = 4.0$ for dioctahedral 2:1 minerals. A comparison of octahedral central atoms distributions in some 2:1 layer silicate minerals calculated from bulk chemical analysis, acid dissolution, and IR spectra suggests a closer agreement between bulk chemical analysis and IR techniques. The main limitation of IR techniques is that it is applicable only to monomineralic samples because most octahedrally coordinated silicate minerals produce IR spectral bands in the range of 3500 to 3700 cm^{-1} (Farmer, 1974; Čičel and Komadel, 1994; Madejová and Komadel, 2001; Madejová, 2003), although recent research has indicated that the combination band region can also be useful in determining Al and Fe distributions (Gates, 2005).

Nuclear magnetic resonance techniques are also sensitive in determining the coordination of central structural atoms, as well as the type and number of atoms associated with that coordination (Wilson et al., 1984; Kinsey et al., 1985). Recent advances involving the combination of NMR with a magic angle spinning (MAS) technique permit the determination of tetrahedral/octahedral Al ratios, and consequently, estimation of the tetrahedral Al occupancy in the structure (Goodman and Stucki, 1984; Nadeau et al., 1985; Woessner, 1989; Lausen et al., 1999). The main advantage of this technique is that it provides a direct measurement of the composition of the tetrahedral sheet, assuming that Si and Al are the only tetrahedral central atoms. Limitations include a high equipment cost and signal interferences from increasing amounts of Fe in the sample or the presence of other tetrahedrally coordinated minerals (Morris et al., 1990). Generally, for samples with relatively low Fe content, the tetrahedral central atom distributions obtained by MAS–NMR are more reliable than those derived from bulk chemical analysis. In most cases, discrepancies between the two methods have been attributed to excessive amounts of free SiO_2 determined by bulk chemical analysis (Woessner, 1989). In samples containing Fe³⁺ in tetrahedral coordination, Fe reduction may improve the estimate of tetrahedral Al occupation by NMR (Gates et al., 1996).

The distribution, coordination, and oxidation state of Fe atoms in mineral structures have also been determined by ⁵⁷Fe Mossbauer spectroscopy (Goodman and Nadeau, 1988;

Amonette et al., 1994). This technique improves considerably the accuracy of the Fe atom distribution in the structural formula calculated by bulk chemical analysis, while at the same time eliminates errors from Fe oxide or hydroxide impurities in the sample (Murad, 1987; Goodman et al., 1988; Lear et al., 1988). Experiments have shown that Fe bound to goethite determined by Mossbauer spectroscopy is correlated well with the amount of readily soluble Fe measured from acid dissolution techniques (Komadel et al., 1993). The technique has also been useful in providing evidence for tetrahedral Fe coordination in 2:1 layer silicate minerals and for distinguishing between Fe^{2+} and Fe^{3+} distributions (Luca and MacLachlan, 1992; Gates et al., 2002).

Combinations of bulk chemical analysis, IR, NMR–MAS, and Mossbauer spectroscopy have also been used to investigate the structural composition of mixed layer minerals, such as illite–smectite, and chlorite–smectite (Lindgreen et al., 2002). Recently more precise quantifications and structural partitioning of Fe^{2+} and Fe^{3+} atoms have been obtained with new innovative techniques, such as synchrotron micro-XANES (Dyar et al., 2002), electron energy-loss near-edge spectroscopy (van Aken and Liebscher, 2002), and polarized EXAFS (Gates et al., 2002). These innovative approaches combined with bulk chemical analyses can provide the comprehensive characterization data needed for the calculation of structural formulas depicting with greater accuracy the real composition of the mineral, and thus leading to improved mineralogical and geological interpretations.

REFERENCES

- Amonette, J.E., and L.W. Zelazny (ed.) 1994. Quantitative methods in soil mineralogy. SSSA Misc. Publ. SSSA, Madison, WI.
- Amonette, J.E., F.A. Khan, H. Gan, J.W. Stucki, and A.D. Scott. 1994. Quantitative oxidation-state analysis of soils. p. 83–113. *In* J.E. Amonette and L.W. Zelazny (ed.) Quantitative methods in soil mineralogy. SSSA Misc. Publ. SSSA, Madison, WI.
- Bailey, S.W. 1980. Structures of layer silicates. p. 1–123. *In* G.W. Brindley and G. Brown (ed.) Crystal structure of clay minerals and their X-ray identification. Miner. Soc., London.
- Brindley, G.W., and R.F. Youell. 1951. A chemical determination of the tetrahedral and octahedral aluminum in a silicate. *Acta Crystallogr.* 4:495–497.
- Cardile, C.M., and P.G. Slade. 1988. Structural studies of vermiculites with different iron contents by ^{57}Fe Mössbauer spectroscopy. *Neues Jahrbuch Fur Mineralogie-Monatshefte.* 7:297–308.
- Čičel, B., and P. Komadel. 1994. Structural formulae of layer silicates. p. 114–135. *In* J.E. Amonette and L.W. Zelazny (ed.) 1994. Quantitative methods in soil mineralogy. SSSA Misc. Publ. SSSA, Madison, WI.
- Čičel, B., P. Komadel, E. Bednáriková, and J. Madejová. 1992. Mineralogical composition and distribution of Si, Al, Fe, Mg, and Ca in the fine fraction of some Czech and Slovak bentonites. *Geol. Carpathica. Ser. Clays* 43:3–7.
- Čičel, B., P. Komadel, and J. Hronský. 1990. Dissolution of the fine fraction of Jelšovský Potok bentonite in hydrochloric and sulphuric acids. *Ceramics-Silikaty* 34:41–48.
- Čičel, B., and I. Novák. 1977. Dissolution of smectites in hydrochloric acid—I. Half-time of dissolution as a measure of reaction rate. p. 163–171. *In* J. Konta (ed.) Proc. 7th Conf. Clay Mineral and Petrol. Karlovy Vary. 1976. Charles Univ., Prague, Czechoslovakia.
- Dixon, J.B., and S.B. Weed. 1989. Minerals in soil environments. 2nd ed. SSSA Book Ser. 1. SSSA, Madison, WI.
- Dyar, M.D., E.W. Lowe, C.V. Guidotti, and J.D. Delaney. 2002. Fe^{3+} and Fe^{2+} partitioning among silicates in metapelites: A synchrotron micro-XANES study. *Am. Mineral.* 87:514–522.
- Farmer, V.C. 1974. The layer silicates. p. 331–363. *In* V.C. Farmer (ed.) The infrared spectra of minerals. Miner. Soc., London.
- Gates, W.P. 2005. Infrared spectroscopy and the chemistry of dioctahedral smectites. p. 126–168. *In* T. Klopogge (ed.) Vibrational spectroscopy of layer silicates and hydroxides. Clay Miner. Society Workshop Series. Vol. 14. Clay Miner. Soc., Boulder, CO.
- Gates, W.P., P.G. Slade, A. Manceau, and B. Lanson. 2002. Site occupancy by iron in nontronites. *Clays Clay Miner.* 50:223–239.
- Gates, W.P., J.W. Stucki, and R.J. Kirkpatrick. 1996. Structural properties of reduced Upton montmorillonite. *Phys. Chem. Miner.* 23:535–541.

- Giaramita, M.J., and H.W. Day. 1990. Error propagation in calculations of structural formulas. *Am. Mineral.* 75:170–182.
- Goodman, B.A., and P.H. Nadeau. 1988. Identification of oxide impurity phases and distribution of structural iron in some diagenetic illitic clays as determined by Mössbauer spectroscopy. *Clay Miner.* 23:301–308.
- Goodman, B.A., P.H. Nadeau, and J. Chadwick. 1988. Evidence for the multiphase nature of bentonites from Mössbauer and EPR spectroscopy. *Clay Miner.* 23:147–159.
- Goodman, B.A., and J.W. Stucki. 1984. The use of nuclear magnetic resonance (NMR) for the determination of tetrahedral aluminum in montmorillonite. *Clay Miner.* 19:663–667.
- Grim, R.E., and N. Guven. 1978. *Bentonites*. Elsevier, Amsterdam, the Netherlands.
- Jackson, M.L. 1985. *Soil chemical analysis, advanced course*. 2nd. ed. Published by the author, Madison, WI.
- Johnson, W.M., and J.A. Maxwell. 1981. *Rock and mineral analyses, Chemical analysis*. 2nd ed. Vol. 27. Wiley-Interscience, New York.
- Karathanasis, A.D. 1988a. Soil solution: A sensitive index of mineral stability in pedogenic environments. p. 157–195. *In* S.S. Augustithis (ed.) *Weathering, its products and deposits*. Theophrastus Publ. S.A., Athens, Greece.
- Karathanasis, A.D. 1988b. Compositional and solubility relationships between hydroxyinterlayered soil smectites and vermiculites. *Soil Sci. Soc. Am. J.* 52:1500–1508.
- Karathanasis, A.D. 1996. X-ray fluorescence spectrometry. p. 161–224. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Karathanasis, A.D. 2008. Thermal analysis. p. 117–160. *In* A.L. Ulerý and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Karathanasis, A.D., F. Adams, and B.F. Hajek. 1983. Stability relationships in kaolinite, gibbsite, and Al-hydroxyinterlayered vermiculite soil systems. *Soil Sci. Soc. Am. J.* 47:1247–1251.
- Karathanasis, A.D., and B.F. Hajek. 1982. Revised methods for rapid quantitative determination of minerals in soil clays. *Soil Sci. Soc. Am. J.* 46:419–425.
- Karathanasis, A.D., and B.F. Hajek. 1983. Transformation of smectite to kaolinite in naturally acid soil systems. Structural and thermodynamic considerations. *Soil Sci. Soc. Am. J.* 47:158–163.
- Kelley, W.P. 1945. Calculating formulas for the fine grained minerals on the basis of chemical analysis. *Am. Mineral.* 30:1–26.
- Kelley, W.P. 1955. Interpretation of chemical analysis of clays. *Clays Clay Miner.* 1:92–94.
- Kinsey, R.A., R.J. Kirkpatrick, J. Hower, K.A. Smith, and E. Oldfield. 1985. High resolution aluminum-27 and silicon-29 nuclear magnetic resonance spectroscopic study of layer silicates, including clay minerals. *Am. Mineral.* 70:537–548.
- Kirkland, D.L., and B.F. Hajek. 1972. Formula derivation of Al-interlayered vermiculite in selected soil clays. *Soil Sci.* 114:317–322.
- Komadel, P., J. Madejová, M. Janek, W.P. Gates, R.J. Kirkpatrick, and J.W. Stucki. 1996. Dissolution of hectorite in inorganic acids. *Clays Clay Miner.* 44:228–236.
- Komadel, P., J.W. Stucki, and B. Čičel. 1993. Readily HCl-soluble iron in the fine fractions of some Czech bentonites. *Geol. Carpathica. Ser. Clays* 44:11–16.
- Lausen, S.K., H. Lindgreen, H.J. Jakobsen, and N.C. Nielsen. 1999. Solid-state Si-29 MAS NMR studies of illite and illite-smectite from shale. *Am. Mineral.* 84:1433–1438.
- Lear, P.R., P. Komadel, and J.W. Stucki. 1988. Mössbauer spectroscopic identification of iron oxides in nontronite from Hohen Hagen, Federal Republic of Germany. *Clays Clay Miner.* 36:376–378.
- Lindgreen, H., V.A. Drits, B.A. Sakharov, H.J. Jakobsen, A.L. Salyn, L.G. Dainyak, and H. Kroyer. 2002. The structure and diagenetic transformation of illite-smectite and chlorite-smectite from North Sea Cretaceous-Tertiary chalk. *Clay Miner.* 37:429–450.
- Luca, V., and D.J. MacLachlan. 1992. Site occupancy in nontronite by acid dissolution and Mössbauer spectroscopy. *Clays Clay Miner.* 40:1–7.
- Madejová, J. 2003. FTIR techniques in clay mineral studies. *Vib. Spectrosc.* 31:1–10.
- Madejová, J., and P. Komadel. 2001. Baseline studies of The Clay Minerals Society source clays: Infrared methods. *Clays Clay Miner.* 49:410–432.
- Madejová, J., P. Komadel, and B. Čičel. 1992. Infrared spectra of some Czech and Slovak smectites and their correlation with structural formulas. *Geol. Carpathica. Ser. Clays* 43:9–12.
- Morris, H.D., S. Bank, and P.D. Ellis. 1990. ²⁷Al NMR spectroscopy of iron-bearing montmorillonite clays. *J. Phys. Chem.* 94:3121–3129.
- Murad, E. 1987. Mössbauer spectra of nontronites: Structural implications and characterization of associated iron oxides. *Z. Pflanzenernähr. Bodenk.* 150:279–285.
- Nadeau, P.H., V.C. Farmer, W.J. McHardy, and D.C. Bain. 1985. Compositional variations of the

- Unterrupstoth beidellite. *Am. Mineral.* 70:1004–1010.
- Newman, A.C.D., and G. Brown. 1987. The chemical constitution of clays. p. 1–128. *In* A.C.D. Newman (ed.) *Chemistry of clays and clay minerals*. Mineral Soc. Monogr. 6. Miner. Soc., London.
- Norrish, K., and J.T. Hutton. 1969. An accurate X-ray spectroscopic method for the analysis of a wide range of geologic samples. *Geochim. Cosmochim. Acta* 33:431–453.
- Novák, I., and B. Čížel. 1978. Dissolution of smectites in hydrochloric acid—II. Dissolution rate as a function of crystallochemical composition. *Clays Clay Miner.* 26:341–344.
- Osthaus, B.B. 1956. Kinetic studies on montmorillonites and nontronite by acid dissolution technique. *Clays Clay Miner.* 4:301–321.
- Post, J.L., and L. Borer. 2002. Physical properties of related illites, beidellites, and mixed-layer illite-beidellites from southwestern Idaho, and their infrared spectra. *Appl. Clay Sci.* 22:77–91.
- Ross, C.S., and S.B. Hendricks. 1945. Minerals of the montmorillonite group. USGS Paper 205(B). U.S. Gov. Print. Office, Washington, DC.
- Salil, M.S., J.P. Shrivastava, and S.K. Pattanayak. 1997. Similarities in the mineralogical and geochemical attributes of detrital clays of Maastrichtian Lameta Beds and weathered Deccan basalt, Central India. *Chem. Geol.* 136:25–32.
- Slonimskaya, M.V., G. Besson, L.G. Daynyak, C. Tchoubar, and V.A. Drits. 1986. Interpretation of the IR spectra of celadonites and glauconites in the region of OH-stretching frequencies. *Clay Miner.* 21:377–388.
- Sparks, D.L. (ed.) 1996. Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Tkáč, I., P. Komadel, and D. Müller. 1994. Acid treated montmorillonites—A ^{29}Si and ^{27}Al MAS NMR study. *Clay Miner.* 29:11–19.
- Vainerdi, R.E., and E.A. Uken. 1979. Modern methods of geochemical analysis. Plenum Press, New York.
- van Aken, P.A., and B. Liebscher. 2002. Quantification of ferrous/ferric ratios in minerals: New evaluation schemes of Fe L-23 electron energy-loss near-edge spectra. *Phys. Chem. Miner.* 29:188–200.
- Vantelon, D., E. Montarges-Pelletier, L.J. Michot, V. Briois, M. Pelletier, and F. Thomas. 2003. Iron distribution in the octahedral sheet of dioctahedral smectites. An Fe K-edge X-ray absorption spectroscopy study. *Phys. Chem. Miner.* 30:44–53.
- Vantelon, D., M. Pelletier, and L.J. Michot. 2001. Fe, Mg, and Al distribution in the octahedral layer of montmorillonites. An infrared study in the OH-bending region. *Clay Miner.* 36:369–379.
- Wilson, M.J. (ed.) 1987. A handbook of determinative methods in clay mineralogy. Blackie, New York.
- Wilson, M.A., P.F. Barron, and A.S. Campbell. 1984. Detection of Al coordination in soils and clay fractions using ^{27}Al magic angle spinning NMR. *J. Soil Sci.* 35:210–217.
- Woessner, D.E. 1989. Characterization of clay minerals by ^{27}Al nuclear magnetic resonance spectroscopy. *Am. Mineral.* 74:203–215.

Analysis of Layer Charge, Cation and Anion Exchange Capacities, and Synthesis of Reduced Charge Clays

DAVID LAIRD & PIERCE FLEMING, USDA-ARS, National Soil Tilth Laboratory, Ames, Iowa

The quantity and nature of charge on surfaces of clay minerals are primary criterion for the classification of clay minerals (Bailey et al., 1971). Furthermore, surface charge together with surface area, and the nature of the saturating cations are primary properties controlling the reactivity of clays in both natural environments and industrial applications. Surface charge exerts a major influence on the ability of clays to retain cations (cation exchange capacity [CEC]); the selectivity exhibited by clays during cation exchange reactions (Maes and Cremers, 1977; Sawhney, 1969; Shainberg et al., 1987; Laird and Shang, 1997); the hydration (MacEwan and Wilson, 1980), swelling (Slade et al., 1991; Laird et al., 1995; Chiou and Rutherford, 1997; Laird, 1999) and rheology of clays; the sorption affinity of clays for organic molecules; and the ability of clay surfaces to catalyze various organic reactions.

Charge on the surfaces of clay minerals is derived from isomorphous substitution of cations within the crystal structure of the clay mineral and from ionizable groups on external surfaces of the minerals (Johnston and Tombácz, 2002). Surface charge arising from isomorphous substitution is termed *permanent charge* because these charge sites are independent of solution pH, ionic strength, and valence of the counter-ions. Permanent charge sites are primarily found on the surfaces of 2:1 phyllosilicates and zeolites. Kaolinites and other 1:1 phyllosilicates may have some permanent charge, but if so it is very small and difficult to unequivocally demonstrate. Surface charge arising from ionizable groups (often called “broken bonds” or “dangling bonds”) on external surfaces of minerals is termed *variable charge* because the magnitude of the charge varies with pH, ionic strength, and the presence of ligands capable of reacting with surface groups. Variable charge sites are found on the lateral edges of phyllosilicates and the exposed surfaces of metal oxyhydroxides, poorly crystalline aluminosilicate clays, and most primary minerals.

Terminology relating to surface charge can be confusing, especially so because some terms refer to a bulk property of a sample while other terms refer to a specific property of a particular mineral phase. The term *surface charge*, or more correctly *surface charge density* (moles of charge per square meter), is used both when referring to an average property of a sample that contains multiple mineral phases and for a specific property of a single mineral phase. *Specific charge density* (moles of charge per kilogram) is directly related to surface charge density through the specific surface area (meters squared per kilogram) of the sample or mineral. *Cation exchange capacity* is not a measure of a clay surface property; it is a measure of a sample's ability to retain exchangeable cations relative to

the mass of the sample (centimoles of charge per kilogram). In contrast, *layer charge* is a property of a specific mineral phase. Layer charge is expressed as moles of charge per mole of formula unit ($\text{mol}_c \text{mol}_{fu}^{-1}$) or per mole of unit cell ($\text{mol}_c \text{mol}_{uc}^{-1}$). Layer charge is related to the specific charge density of a mineral phase through the formula unit weight (kg mol_{fu}^{-1}) of the mineral. However, layer charge cannot be directly related to CEC values because the positive charge associated with nonexchangeable cations (e.g., K^+ in illitic layers) is excluded from CEC values, whereas the negative charge balanced by such cations is included in layer charge values. Furthermore, natural clay samples often contain multiple organic and inorganic phases, and interactions between phases can have a substantial effect on CEC values. For example, metal oxyhydroxide coatings on clay surfaces can mask numerous potential cation exchange sites.

Theoretically, surface charge density of a clay sample can be determined by dividing measured CEC values by measured specific surface area values. However, this approach is not recommended. A critical assumption of this approach is that the methodology used for measuring surface area probes the same surfaces that are available for cation exchange reactions. The N_2 adsorption method for determining surface area clearly violates this assumption because N_2 only probes external surfaces, while exchangeable cations come from both internal and external surfaces. Methods used for measuring total surface area of clays are generally based on vapor-phase adsorption of either water or one of various polar organic molecules. Unfortunately polar molecules tend to cluster around adsorbed cations and surface charge sites rather than spreading uniformly over clay surfaces; hence, the vapor phase adsorption of polar molecules is strongly influenced by surface charge density (Chiou and Rutherford, 1997; Laird, 1999).

The best way to determine surface charge density for relatively pure samples of 2:1 phyllosilicates is first to determine layer charge and then divide the layer charge by the surface area per unit cell. For many studies, layer charge is a more useful quantity than surface charge density, as layer charge is used in classification of layer silicates and provides a convenient relative measure of surface charge that can be easily related to cation exchange, hydration, swelling, and other properties of clays. Cation exchange capacity and anion exchange capacity (AEC) are generally more appropriate measures of charge properties for samples with mixed mineralogy and samples dominated by low activity and variable charge minerals.

In this chapter we present analytical methods for determining surface charge properties of clay minerals and clay-rich samples. All of the methods presented here are based on well-established procedures; however, we have specifically tested each method and have made numerous modifications in an effort to enhance their utility with clay samples. The methods presented here are by no means exclusive. Our hope is that the reader will find the methods described here to be broadly applicable, reasonably robust, and relatively easy to use. We also have attempted to point out the advantages and disadvantages of each procedure, false or questionable assumptions, and potential problems that may lead to inaccurate results.

LAYER CHARGE

Layer charge is a property of a specific mineral phase and is therefore expressed as moles of charge per mole of formula unit or moles of charge per mole of unit cell. Furthermore, the term layer charge only has meaning when discussing the surface charge of a 2:1 phyllosilicate (smectite, vermiculite, illite, or one of the various micas). Other terms such as surface charge density or specific charge density should be used when dis-

cussing surface charge properties of other clay minerals or when discussing charge properties of a sample that contains several mineral phases. Theoretically, the term layer charge refers only to the permanent charge of a 2:1 phyllosilicate. In practice, however, there is often ambiguity as to whether a reported value of layer charge represents the permanent charge or the total charge of a clay mineral.

The two dominant methods for determining layer charge, the alkylammonium and structural formula methods, are describe in this chapter. Several other methods of estimating layer charge have been described. Petit et al. (1999) suggested using Fourier transform infrared (FTIR) analysis of NH_4 -saturated clays. Relative peak areas for the $\nu_4\text{NH}_4$ IR band ($\sim 1400\text{ cm}^{-1}$) and another structural band (e.g., νSiO about 1050 cm^{-1}) were related to layer charge. Bujdak et al. (2001) used ultraviolet-visible spectroscopy to relate layer charge of smectites to conformational changes of intercalated organic dyes. Christidis and Eberl (2003) proposed comparisons of measured X-ray diffraction patterns for K-saturated ethylene-glycol solvated samples to theoretically calculated X-ray diffraction patterns. All of these approaches have merit but ultimately must be calibrated relative to a primary method.

Alkylammonium Method

Weiss (1963) first observed a linear relationship between basal spacing and layer charge of alkylammonium saturated 2:1 phyllosilicates. Lagaly and Weiss (1969) later used this relationship as the basis for the "alkylammonium method" of determining layer charge. Various authors (Lagaly and Weiss, 1971; Stul and Mortier, 1974; Rühlicke and Kohler, 1981; Stanjek and Friedrich, 1986; Laird et al., 1989; Olis et al., 1990) have proposed refinements in the alkylammonium method.

Significant sample preparation is required for the alkylammonium method. The first step is the stoichiometric exchange of inorganic interlayer cations with various straight chain n -alkylammonium cations (Eq. [1]).



Typically samples are prepared using alkylammonium cations with C chain lengths (n_c) ranging from 6 (hexylammonium) to 18 (octadecylammonium) carbons. Then the samples must be washed free of excess alkylammonium cations, prepared as oriented specimens, dried, and analyzed by X-ray diffraction. Lagaly and Weiss (1969) observed that alkylammonium cations assume monolayer, bilayer, pseudotrilayer, or paraffin-type configurations in the interlayer space of 2:1 phyllosilicates (Fig. 16-1) depending on n_c and the layer charge of the clay. Characteristic basal spacings of 13.6, 17.6, and 22 Å distinguish the monolayer, bilayer, and pseudotrilayer configurations, whereas basal spacings for samples with paraffin-type configurations increase linearly with n_c . Lagaly and Weiss (1969) proposed three different techniques for estimating layer charge from basal spacing- n_c relationships. The three techniques are based on (i) the critical carbon chain length associated with mono-bilayer transitions, (ii) the critical carbon chain length associated with bilayer-pseudotrilayer transitions, and (iii) the slope of the n_c -basal spacing relationship for samples exhibiting paraffin-type interlayer alkylammonium configurations. The latter two techniques have not been widely used, and questions about their accuracy have been raised (Mermut, 1994). On the other hand, determinations of layer charge based on mono-bilayer transitions have been widely used and give consistent results. The accuracy of the results, however, has been questioned.

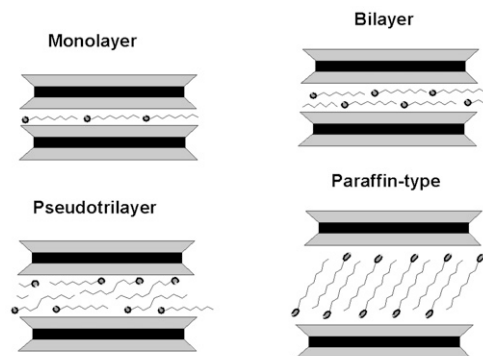


Fig. 16-1. Configurations of alkylammonium cations in the interlayer of 2:1 phyllosilicates (Lagaly and Weiss, 1969). In this figure the horizontal-striped polygons represent the 2:1 phyllosilicate layers, the zigzag lines represent the alkyl tails of the alkylammonium cations, and the small circles represent the positively charged ammonium head group of the alkylammonium cations.

The traditional approach for estimating layer charge is to assume that alkylammonium-clay derivatives shift from a monolayer to a bilayer configuration when the average planar area occupied by an interlayer alkylammonium (A_c) exceeds the average planar area available per charge site (A_e) in the interlayer space of a 2:1 phyllosilicate. Lagaly and Weiss (1971) estimated values of A_c in Angstroms squared from

$$A_c = 5.67(n_c) + 14 \quad [2]$$

and values of A_e from

$$A_e = \frac{[d(100)d(010)]}{2\sigma} \quad [3]$$

where $d(100)d(010)$ is the planar area per unit cell and σ is layer charge ($\text{mol}_c \text{mol}_{fu}^{-1}$). Assuming that $d(100)d(010) = 46.5 \text{ \AA}^2$, as is typical for dioctahedral 2:1 phyllosilicates, then layer charge is determined from

$$\sigma = \frac{23.25}{5.67(n_c^*) + 14} \quad [4]$$

where n_c^* is the critical carbon chain length associated with the mono-bilayer transition (i.e., the carbon chain length of the alkylammonium cation that causes the clay to shift from a monolayer to a bilayer configuration).

Most, if not all, mono-bilayer transitions do not occur abruptly; rather, mono-bilayer transitions occur gradually over a range of alkylammonium ions. Samples with monolayer and bilayer configurations are distinguished by having characteristic basal spacings of 13.6 and 17.6 Å, respectively, and by a rational series of higher order X-ray diffraction (XRD) reflections. X-ray diffraction patterns for samples with $d(001)$ values between 13.6 and 17.6 Å will generally have broad and relatively low intensity 001 peaks and an irrational series of higher order peaks. Such data indicate random interstratification of monolayer and bilayer domains. The traditional approach is to use MacEwan's peak migration curve to estimate proportions of the sample with monolayer and bilayer configurations for each n_c^* and to construct histograms depicting the distribution of layer charge (Lagaly and Weiss, 1976; Lagaly, 1981). Mean layer charge values for a sample are calculated from the weighted average layer charge values using the histograms.

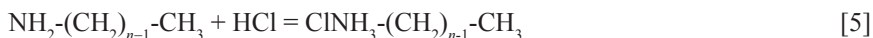
The traditional approach for estimating layer charge based on mono-bilayer transitions yields reproducible estimates of mean layer charge. However, the traditional approach has been shown to be highly inaccurate by direct comparison of values of layer charge determined by the traditional alkylammonium method with values of layer charge determined by the structural formula method (Laird et al., 1989; Laird, 1994). The difference between total charge (effectively determined by the structural formula method) and permanent charge (determined by the alkylammonium method) cannot explain why alkylammonium values of layer charge are as much as 40% below structural formula values for high-charged clays and actually exceed structural formula values for low-charge clays. The cause of the discrepancy between the structural formula and alkylammonium values of layer charge is probably related to inaccurate assumptions in the traditional approach about the packing density of alkylammonium cations in the interlayers (Laird, 1994). Regardless of the cause, it is known that the traditional approach for estimating layer charge based on mono-bilayer transitions seriously underestimates the permanent charge of most 2:1 phyllosilicates.

In the traditional approach, gradual mono-bilayer transitions are interpreted as evidence for layer charge heterogeneity (Lagaly and Weiss, 1976; Lagaly, 1981). While layer charge heterogeneity will theoretically cause gradual mono-bilayer transitions, gradual transitions may also be caused by local (nanometer scale) rearrangement of alkylammonium ions in the interlayers (Laird, 1994). Because of this problem, the accuracy of numerous published histograms reporting to show layer charge heterogeneity is questionable, and use of the alkylammonium method to infer layer charge heterogeneity is not recommended.

The great advantage of the alkylammonium method is the ability to determine the layer charge of smectites and vermiculites in samples that contain more than one mineral phase. This is possible because XRD peaks for alkylammonium-expanded smectites and vermiculites are independent of XRD peaks for quartz, kaolinite, and other mineral phases that may be present in a sample. Furthermore, it is at least conceptually possible to simultaneously determine the layer charge of both smectite and vermiculite in the same sample using the alkylammonium method. However, extreme caution must be exercised in interpreting XRD patterns of alkylammonium-treated samples that contain illite. Alkylammonium cations displace K from the interlayers of illite (Mackintosh and Lewis, 1968; Mackintosh et al., 1971, 1972), and the alkylammonium-expanded illites yield low-angle XRD peaks that are easily mistaken as evidence of vermiculite (Laird et al., 1987). The problem is exacerbated because the low-angle expanded illite peaks can be an order of magnitude more intense than the 10-Å illite peaks observed in XRD patterns of Mg-saturated samples of the same clay. Furthermore, little or no loss in intensity of the 10-Å peaks may be apparent in XRD patterns of the alkylammonium-treated clays exhibiting low-angle expanded illite peaks. This occurs because alkylammonium cations primarily expand poorly ordered illite domains and may not expand the highly crystallized illite domains that dominantly contribute intensity to the 10-Å peak. One clue suggesting the presence of expanded illite is substantial variation in intensity of the low-angle XRD peaks with alkylammonium C chain length. Chemical analysis for the amount of K⁺ displaced from a sample by an alkylammonium treatment is a definitive test for the presence of alkylammonium-expanded illite. As a general rule, whenever a sample contains illite, one should assume that low-angle peaks in XRD patterns for alkylammonium-treated samples are caused by expanded illite rather than vermiculite.

Synthesis of Alkylamine Hydrochlorides

Various alkylammonium chlorides are needed for the alkylammonium method of determining the layer charge of 2:1 phyllosilicates. Most of the needed alkylammonium chlorides are not commercially available and must be prepared by protonation of their respective alkylamines, which are commercially available. The reaction



proceeds spontaneously whenever acid is added to an aqueous or alcoholic solution of an alkylamine. Rühlicke and Kohler (1981) proposed a method that includes bubbling HCl vapor through ethanolic solutions of the various alkylamines followed by desolvation in a rotary vacuum evaporator to prepare crystals of the various alkylammonium chlorides. On the other hand, Lagaly (1994) indicated that it is unnecessary to prepare the alkylammonium chlorides in advance, and recommended that alkylammonium chloride exchange solutions be prepared directly by titration of the appropriate alkylamines as needed. In our experience, the direct preparation of alkylammonium chloride exchange solutions is easy and fast. The only apparent advantage of first crystallizing the alkylammonium chlorides is that impurities will be removed during the procedure. As long as alkylamines of reasonable purity are available, the direct procedure is recommended. For the sake of completeness, both the direct and the crystallization procedures are described below.

Direct Preparation of Alkylammonium Chloride Solutions

Apparatus

- 250-mL glass beaker
- pH paper
- Combination magnetic stirrer-hot plate

Reagents

- 95% ethanol
- 2 M HCl
- Alkylamines with carbon chain lengths ranging from 5 to 18.

Procedure

1. Place a mark on the 250-mL glass beaker to indicate a 100-mL volume.
2. Add the appropriate quantity of alkylamine (see Table 16-1).
3. Add 50 mL of 95% ethanol. Slowly heat the sample while stirring until all of the alkylamine is dissolved in the ethanol. The short chain alkylamines will readily dissolve in the ethanol at room temperature, while the longer chain alkylamines will require mild heat. If the alkylamines creep up the sides of the beaker while heating, place a watch glass on top of the beaker to reflux the ethanol and wash the alkylamines back into the solution.
4. Slowly add the 2 M HCl until the endpoint (pH 6.5) is reached. The approximate volume of 2 M HCl needed is shown in Table 16-1. To avoid overshooting the endpoint, add the last 1 mL of HCl dropwise while frequently checking the pH by transferring a drop of the mixed solution to a small piece of pH paper. The pH will change rapidly from >9 to <7 when the endpoint is reached. If the endpoint is overshoot, the solution may be back titrated with a dilute solution of the appropriate alkylamine dissolved in ethanol.
5. Dilute the solution to a final volume of 100 mL using deionized water and store in a closed container.

Table 16–1. Quantities of the various alkylamines and HCl required for direct preparation of 100 mL of alkylammonium exchange solutions.

Carbon chain length	Alkylamine g	2 M HCl mL	Molarity of final solution
5	4.36	25	0.5
6	5.06	25	0.5
7	5.76	25	0.5
8	6.46	25	0.5
9	7.16	25	0.5
10	7.86	25	0.5
11	1.71	5	0.1
12	1.85	5	0.1
13	1.99	5	0.1
14	2.13	5	0.1
15	2.27	5	0.1
16	1.21	2.5	0.05
17	1.28	2.5	0.05
18	1.35	2.5	0.05

Comments

Because a great excess of alkylammonium cations will be present in the solutions during the cation exchange reaction, a high degree of accuracy is unnecessary when making the final dilution. We are uncertain how stable the prepared alkylammonium solutions are, but as a matter of caution we recommend using the solutions within 2 mo of their preparation.

Preparation of Alkylamine Hydrochloride Crystals

Apparatus

- 500-mL beaker
- Large porcelain Buchner funnel
- Medium cellulose filter paper
- Laboratory freezer capable of -20°C
- pH paper

Reagents

- 95% ethanol
- Concentrated HCl
- Acetone
- Alkylamines with carbon chain lengths ranging from 5 to 18.

Procedure

1. Weigh 25 g of alkylamine into a 500-mL beaker.
2. Add 25 mL of 95% ethanol.
3. Slowly heat the solution on a hotplate while stirring until the alkylamine is dissolved. During heating, place a watch glass on top of the beaker and reflux the ethanol.
4. Slowly add concentrated HCl while stirring until the endpoint (pH 6.5) is reached. The approximate volume of concentrated HCl needed is shown in Table 16–2. To avoid overshooting the endpoint, add the last 1 mL of HCl dropwise while frequently checking the pH by transferring a drop of the mixed solution to a small piece of pH paper. The pH will change rapidly from >9 to <7 when the endpoint is reached. If the endpoint is overshoot, the solution may be back titrated with a dilute solution of the appropriate alkylamine dissolved in ethanol.

5. Place the sample in the laboratory freezer (-20°C).
6. Attach a sidearm flask to a low vacuum source and attach the Buchner funnel to the sidearm flask. Place a piece of medium or fast cellulose filter paper in the Buchner funnel.
7. After the alkylamine hydrochlorides have crystallized, stir the sample with a small spatula to break up the crystals. Then decant as much solvent as possible onto the filter paper in the Buchner funnel. Complete the transfer of the alkylammonium crystals onto the filter paper using a small spatula.
8. After the alkylammonium chloride crystals are thoroughly dry they may be transferred to an amber glass bottle for indefinite storage at room temperature.
9. Alkylammonium chloride exchange solutions can easily be prepared by weighing the appropriate quantity (see Table 16–2) of crystallized alkylammonium chloride salts into a 150-mL bottle, adding 25 mL of 95% ethanol and 75 mL of deionized water.

Comments

The alkylammonium chlorides exist as both oils and as crystals. Patience and skill are required to prepare high quality crystals. As a general rule, the longer chain alkylammonium chlorides are easier to crystallize than the shorter chain alkylammonium chlorides. The amount and polarity of the solvent in an alkylammonium solution has a large influence on the tendency of the alkylammonium chlorides to crystallize. Therefore, use the smallest amount of ethanol required to solubilize the alkylamine in Step 2 and use concentrated HCl for the titration in Step 4 to minimize the amount of water added to the solution. If the alkylammonium chloride will not crystallize at -20°C , then remove the solvent with a rotary vacuum evaporator. Then add a small amount of isopropyl alcohol and heat the sample to remove the alkylammonium chloride residue from the round bottom flask. Crystallize the alkylammonium chlorides in a -20° freezer and remove the solvent by vacuum filtration as described above. The crystallized alkylammonium chlorides will partially or totally dissolve in ethanol if allowed to warm to room temperature. Therefore, ethanol must be

Table 16–2. Approximate quantity of concentrated HCl required to convert 25 g of the various alkylamines into their respective alkylammonium chlorides and the quantity of alkylammonium chloride required to prepare exchange solutions.

Carbon chain length	Concentrated HCl	Alkylammonium chloride needed to prepare 100 mL of exchange solutions	Molarity of exchange solutions
	mL	g	
5	23.9	6.18	0.5
6	20.6	6.88	0.5
7	18.1	7.58	0.5
8	16.1	8.29	0.5
9	14.5	8.99	0.5
10	13.2	9.69	0.5
11	12.2	2.08	0.1
12	11.2	2.22	0.1
13	10.4	2.36	0.1
14	9.8	2.50	0.1
15	9.2	2.64	0.1
16	8.6	1.39	0.05
17	8.2	1.46	0.05
18	7.7	1.53	0.05

removed from the crystals quickly while the sample is still cold. Some of the alkylammonium chloride will pass through the filter paper with the ethanol, even under the best circumstances. However, impurities are also leached out of the sample during the filtration procedure. During a recent test of this procedure, we obtained a yield of 73% for dodecylammonium chloride.

Preparation of Alkylammonium-Saturated Clay Samples

The preparation of alkylammonium-saturated clays involves treating the clay sample with enough alkylammonium cations to replace all of the exchangeable inorganic cations, washing excess alkylammonium cations out of the sample, preparing an oriented sample on a glass slide, drying the sample, and then analyzing the sample by X-ray diffraction. To facilitate the exchange reaction, Rühlicke and Kohler (1981) recommended that samples be Na-saturated before they are treated with the alkylammonium chloride solutions. We have not confirmed the need for prior Na saturation, but the recommendation seems reasonable. To avoid rehydration of the treated clays, Lagaly (1981) recommended sealing the samples in glass capillaries and then analyzing the samples with a Debye-Scherrer camera. However, many labs do not have Debye-Scherrer cameras, and we have found that satisfactory results can be obtained using oriented samples on glass slides and a powder X-ray diffractometer if the samples are handled carefully.

Apparatus

- X-ray diffractometer
- Centrifuge with angle head rotor
- High-density polypropylene or polycarbonate centrifuge tubes with lids (e.g., Oak Ridge centrifuge tubes).
- Laboratory oven
- Desiccator
- Laboratory balance
- Vortex mixer

Reagents

- 1 M NaCl. Dissolve 58.44 g of reagent-grade NaCl in 1 L of deionized water.
- Alkylammonium chloride exchange solutions (see previous section).
- 95% ethanol

Procedure

1. Weigh 0.15 g of clay sample into a centrifuge tube.
2. Add 25 mL of 1 M NaCl and disperse the clay by vigorous stirring.
3. Centrifuge the sample at 3000 g for 10 min; then decant the supernatant.
4. Repeat Steps 2 and 3 two times.
5. Add 25 mL of 95% ethanol and disperse the clay by vigorous stirring.
6. Centrifuge the sample at 3000 g for 10 min; then decant the supernatant.
7. Repeat Steps 5 and 6.
8. Add 3 mL of the appropriate alkylammonium solution and disperse the clay by vigorous stirring.
9. Incubate the sample in the closed centrifuge tube for 12 h at 65°C.
10. Centrifuge the sample at 3000 g for 10 min. then decant the supernatant.
11. Repeat Steps 8, 9, and 10 twice for smectites and three times for vermiculites.

12. Add 25 mL of 95% ethanol and disperse the clay by vigorous stirring.
13. Incubate the sample for 1 h at 65°C.
14. Centrifuge the sample at 3000 g for 10 min. then decant the supernatant.
15. Repeat Steps 12, 13, and 14 three times.
16. Add 1 mL of 95% ethanol and disperse the sample by stirring with a spatula.
17. Using a Pasture pipette, transfer the clay suspension to a glass microscope slide, and allow the ethanol to evaporate so that a film of clay is deposited on the glass slide.
18. Dry the sample at 65°C for 2 h, and then transfer the slide while still hot to a desiccator.
19. Analyze the sample by X-ray diffraction between 2 and 20° 2 θ using Cu K α radiation. A stream of dry air or dry N₂ should be blown onto the sample during the XRD analysis to prevent rehydration.

Comments

Treatment of 2:1 phyllosilicates with alkylammonium cations makes the surfaces and the interlayers hydrophobic, so one may question the need for efforts to avoid rehydration after the slides are prepared. We tested the ability of six samples to rehydrate by adding several drops of water on top of the prepared slides. Comparison of XRD patterns for the dry and wet samples indicated that three of the six wet samples had expanded sufficiently to cause an error of about 8% in estimation of layer charge. Providing that samples are properly dried and stored in a desiccator before XRD analysis, brief exposure of the samples to ambient humidity during the XRD analysis will probably cause negligible error.

Interpretations of XRD Data for Alkylammonium Saturated Clays

As discussed in the introduction to this section, several approaches for estimating layer charge from results of XRD analysis of alkylammonium-saturated clays have been proposed. In the authors' experience, the traditional approach yields reproducible, but inaccurate estimates of layer charge. Furthermore, the traditional approach requires preparation and analysis of samples saturated with each of 13 different alkylammonium chlorides. The simplified empirical approach proposed by Olis et al. (1990) only requires preparation and analysis of samples saturated with one or two different alkylammonium chlorides. The simplified approach, however, was calibrated using values of layer charge determined by the traditional approach and therefore also seriously underestimates the mean layer charge of most clays. Neither of these approaches is recommended.

In our laboratory, we have found a strong correlation between layer charge as determined by the structural formula method and the ratio of $d(001)$ to $n_c^{0.577}$ for samples that have basal spacings intermediate between monolayer and bilayer configurations. Based on this correlation the following equation is recommended for estimating mean layer charge, σ (mol_c mol_{tu}⁻¹), from results of XRD analysis of alkylammonium saturated clay samples:

$$\sigma = -0.3485 + 0.1915(d/n_c^{0.577}) \quad [6]$$

where d is the measured basal spacing in Angstroms, and n_c is the carbon chain length for the saturating alkylammonium cation. A critical requirement for use of Eq. [6] is that the measured basal spacing be between 14 and 17 Å. We have found that Eq. [6] yields similar estimates of layer charge when used for samples of the same clay saturated with different alkylammonium cations as long as the measured basal spacings are within the mono-bi-

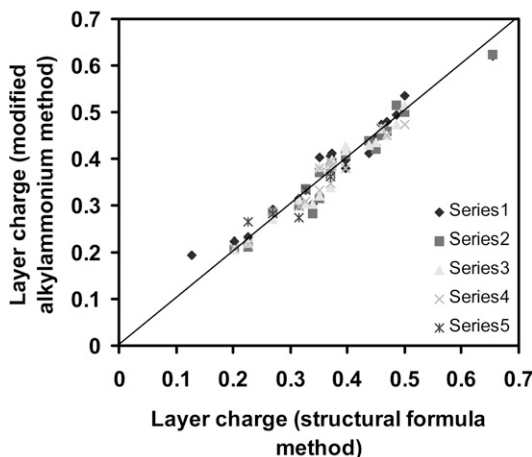


Fig. 16–2. Relationship between values of layer charge ($\text{mol}_c \text{mol}_{\text{fu}}^{-1}$) determined by the structural formula method and the modified alkylammonium methods (using Eq. [6]). Each series of vertically stacked points are independent solutions of Eq. [6] for the same clay sample saturated with alkylammonium ions having different C chain lengths.

layer transition region. More accurate values of layer charge are obtained by averaging results for samples saturated with several different alkylammonium cations.

Comments

Shown in Fig. 16–2 is the calibration curve on which Eq. [6] is based. The calibration curve includes 84 data points for 22 different clay samples. Typically three or four data points are included for each clay. The primary advantage of using Eq. [6] is that layer charge can be estimated from an XRD pattern of a single alkylammonium-treated clay sample. However, the appropriate choice of alkylammonium cations to use with the analysis varies because of the criterion that the measured d -spacing be between 14 and 17 Å. With vermiculites n_c should be in the 5 to 8 range, with high charge smectites n_c should be in the 6 to 12 range, and with low charge smectite n_c should be in the 10 to 18 range. The recommended approach is empirical because it has been calibrated against values of layer charge determined by the structural formula method. For the same reason, estimates of layer charge determined by the recommended approach are estimates of total charge not permanent charge. The procedure gives no information about the heterogeneity of the layer charge distribution.

Structural Formula Method

The structural formula method of determining layer charge of 2:1 phyllosilicates is the “gold standard.” However, the structural formula method is theoretically valid only when used with highly accurate total chemical analysis of monomineralic samples. The presence of more than one mineral phase in a sample, sample contamination, inaccuracies in chemical analysis of a sample, and inaccurate assumptions made during structural formula calculations all adversely affect the accuracy of estimates of layer charge obtained by the structural formula method. Because of these limitations, the use of the structural formula method is generally only appropriate for high-quality reference samples and is not recommended for most soil clay samples. Procedures for total chemical analysis of clays and structural formula calculations are given in Chapter 15 of this volume (Karathanasis, 2008). Following is a brief discussion of how layer charge estimates are derived from structural formulas and potential sources of error in such calculations.

Layer Charge Estimates From Structural Formulae

Estimates of layer charge, tetrahedral charge, and octahedral charge may be obtained from structural formulae. Layer charge is calculated as the net anionic charge per formula unit after tetrahedral and octahedral cation charges have been deducted:

$$\sigma = F + \sum_i z_i G_i \quad [7]$$

where σ is the layer charge ($\text{mol}_c \text{mol}_{\text{fu}}^{-1}$), F is the framework anionic charge (usually assumed to equal $-22.000 \text{ mol}_{\text{ac}} \text{mol}_{\text{fu}}^{-1}$), z_i is the valence of structural cation i , and G_i is the formula unit stoichiometric coefficient for cation i . The layer charge is numerically equal but opposite in sign to the interlayer cation charge (the sum of interlayer cation valences times interlayer cation stoichiometric coefficients). The tetrahedral charge is formally calculated as the difference between the tetrahedral anionic charge (typically assumed to be -16.000) and the sum of charges for tetrahedral cations. Similarly, the octahedral charge is formally calculated as the difference between the octahedral anionic charge (assumed to be -6.000) and the sum of charges for octahedral cations. An example of these calculations is given in Table 17–3.

The use of a -22.000 framework anionic charge in structural formula calculations carries the implicit assumption that the individual layers are infinite and without defects, in other words, that all of the charge balancing cations are associated with permanent charge sites and that the variable charge is equal to zero. Because variable charge is not considered, structural formula calculations based on a -22.000 framework anionic charge effectively use charge balancing cations associated with both variable and permanent charge sites to estimate permanent charge. Because the magnitude of this error is prescribed by the magnitude of the variable charge, values of layer charge determined by the structural formula method are actually better estimates of total charge than of permanent charge (Laird et al., 1989).

The above-mentioned error in structural formula calculations could be avoided if additional information were available. For example, if one knew a priori that octahedral occupancy was exactly 2.000 for a dioctahedral mineral or 3.000 for a trioctahedral mineral, then the framework anionic charge could be treated as a variable in structural formula calculations and any deviation of the calculated framework anionic charge from the ideal -22.000 would indicate the average magnitude of the variable charge per formula unit. Unfortunately there is no reason to believe that the octahedral occupancy of a real sample is exactly 2.000 or 3.000, and any deviation from the idealized values will cause substantial errors in estimated values of the variable charge.

Table 16–3. Example calculations of layer charge, interlayer cation charge, tetrahedral charge, and octahedral charge from a structural formula.

Structural formula	Interlayer ($\text{Ca}^{2+}_{0.241}$)	Tetrahedral ($\text{Si}^{4+}_{3.781} \text{Al}^{3+}_{0.219}$)	Octahedral ($\text{Al}^{3+}_{1.167} \text{Fe}^{3+}_{0.602} \text{Mg}^{2+}_{0.215}$)	Anionic framework ($[\text{O}_{10}(\text{OH})_2]$)
Layer charge				
$-22.000 + 4 \times 3.781 + 3 \times 0.219 + 3 \times 1.167 + 3 \times 0.602 + 2 \times 0.215 = -0.482 \text{ mol}_c \text{mol}_{\text{fu}}^{-1}$				
Interlayer cation charge				
$2 \times 0.241 = 0.482 \text{ mol}_c \text{mol}_{\text{fu}}^{-1}$				
Tetrahedral charge				
$-16.000 + 4 \times 3.781 + 3 \times 0.219 = -0.219 \text{ mol}_c \text{mol}_{\text{fu}}^{-1}$				
Octahedral charge				
$-6.000 + 3 \times 1.167 + 3 \times 0.602 + 2 \times 0.215 = -0.263 \text{ mol}_c \text{mol}_{\text{fu}}^{-1}$				

Sample Contamination Effects

Some level of inaccuracy is inevitable in chemical analysis of a mineral phase. Inaccuracies may result from the presence of small amounts of mineral phases other than the desired phase in the sample, contamination of the sample by salts, and inaccuracies in the chemical analysis. Furthermore, several assumptions generally need to be made when calculating structural formulae from a chemical analysis. The accuracy of such assumptions is by no means assured. Therefore, it is important to understand how inaccuracies in a structural formula affect values of layer charge, octahedral charge, and tetrahedral charge derived from the structural formula.

Estimates of both tetrahedral and octahedral charge are substantially affected by sample contamination or other errors in the structural formula when the framework anionic charge is assumed to be -22.000 (Laird, 1994). For example, a 1% contamination of SiO_2 decreases tetrahedral charge and increases octahedral charge by approximately 8%. A 1% contamination of Al_2O_3 increases tetrahedral charge and decreases octahedral charge by about 17%. Iron and Mg^{2+} contamination increase tetrahedral charge at the expense of octahedral charge. If ferrous Fe is falsely assumed to be ferric Fe, tetrahedral charge will be overestimated and octahedral charge will be underestimated. Fortunately, estimates of total charge are relatively insensitive to sample contamination. A 1% contamination of SiO_2 , Al_2O_3 , MgO , or Fe_2O_3 causes only a 1% decrease in the estimate of total charge. False assumptions about the oxidation state of structural Fe also have a negligible effect on estimates of total charge. Estimates of total charge, however, are extremely sensitive to even a small amount of contamination by the index cation. A 1% contamination of CaO (commonly as CaCO_3 or CaCl_2) in a Ca-saturated smectite causes the estimated total layer charge to increase 20 to 30%. Therefore, it is imperative that samples be free of all spurious sources of index cations (such as free carbonates) and that they be thoroughly washed before chemical analysis.

CATION EXCHANGE CAPACITY AND ANION EXCHANGE CAPACITY

Sumner and Miller (1996) did an excellent job of describing methods for measuring cation exchange capacity (CEC) of soils. They also described many of the problems and potential sources of error associated with soil CEC measurements. However, CEC methods that were designed for soils may not work well when used with clay samples, due to the unique properties of clays. The methods described in this section are specifically designed for the determination of CEC and anion exchange capacity (AEC) of clay samples.

Fixation of index ions by clay minerals can adversely affect the accuracy of CEC and AEC measurements. It is well known that some clay minerals (vermiculites, illites, and some smectites) have the capacity to fix K^+ , NH_4^+ , Rb^+ , and Cs^+ . Here the term *fixation* indicates that these cations become trapped in dehydrated interlayers and are not readily available for cation exchange. Air-drying and particularly oven drying clays saturated with these cations generally increases the extent of fixation. Another type of fixation occurs when Li- or Mg-saturated dioctahedral–octahedrally charged smectites are heated to approximately 200°C . At this temperature, Li^+ and Mg^{2+} are small enough and energetic enough to penetrate the structure of dioctahedral 2:1 phyllosilicates and occupy vacant octahedral positions, reducing the layer charge. In a related phenomenon, Jaynes et al. (1992) noted that when Mg-hectorite (a trioctahedral mineral) is heated some of the interlayer Mg^{2+} will displace octahedral Li, reducing the layer charge. Generally this type of fixation occurs when samples are heated to $>200^\circ\text{C}$, but may occur to a lesser extent at lower temperatures (Hrobáriková et al., 2001). A third type of fixation occurs when an oxyanion (e.g.,

PO_4) or a transition metal (e.g., Cu) reacts with a clay surface through ligand exchange or specific adsorption reactions. These types of reactions generally occur on variable charge sites. Clearly, conditions that may cause fixation of an index ion by any of these mechanisms should be avoided during CEC or AEC analyses.

Colloidal dispersion is another issue of importance in measuring CEC of clay samples. When Na- or Li-saturated clays are dispersed in distilled water, it may be impossible to settle the clay by centrifugation. Even K- and NH_4 -saturated clays can be extremely difficult to settle by centrifugation when dispersed in distilled water. Because of dispersion, significant amounts of the sample may be accidentally thrown out with the rinse water when clays saturated with monovalent cations are washed with deionized water to remove excess salt. Smectites are generally more dispersive than other clays and tend to be selectively discarded relative to other mineral phases during washing. This phenomenon can have an adverse effect on accuracy of subsequent mineralogical or chemical analyses, including CEC and AEC measurements.

Hydrolysis is another potential problem associated with CEC and AEC measurements. As a clay sample is rinsed with or dialyzed against deionized water, hydronium ions produced from the hydrolysis of water molecules will progressively replace the exchangeable cations. Measuring the electrical conductivity each time the rinse or dialysis water is changed may not provide a clear endpoint because significant hydrolysis can occur before excess salt is completely removed (Okazaki et al., 1963). To avoid hydrolysis problems during CEC analysis of soils, samples are commonly weighed after the final rinse with a dilute salt solution of the index cation to determine the amount of water retained by the sample. The quantity of index ions estimated to be in the residual water is then subtracted from the measured quantity of index ions displaced from the sample by washing with another salt solution. For clay samples, this practice is problematic because the assumption that the concentration of index ions in the residual water is equal to the concentration of index ions in the bulk final rinse solution is not necessarily valid. For smectite-rich samples, a significant portion of the water retained by the sample following centrifugation is either in the interlayers of smectite quasicrystals or in diffuse double layers between quasicrystals or other clay particles. Both excess cations and anions are excluded from smectite interlayers (Posner and Quirk, 1964), and neither the cation nor the anion concentrations in diffuse double layers are equal to that of an equilibrated bulk solution.

Accurately determining the oven-dry weight of clay samples is a larger problem than commonly appreciated. The common practice of heating a subsample to 105°C for 2 h will not remove all of the water from many clays. Indeed, some tightly bound water is impossible to get out without heating samples to temperatures exceeding 500°C . Unfortunately structural water from some disordered minerals and some components of soil organic matter are lost at temperatures as low as 250°C . Furthermore, many clays are hygroscopic and will rapidly readsorb water from the atmosphere if allowed to cool on a lab bench after heating. On a practical level, heating clay samples to 200°C for 2 h and cooling them in a desiccator before weighing appears to be a reasonable compromise for determining oven-dry weights of most samples.

The type of cations on the exchange complex when the oven-dry weight is determined also influences the CEC of a clay sample. For example, consider an idealized montmorillonite with a layer charge of $0.33 \text{ mol}_c \text{ mol}_{\text{fu}}^{-1}$ $[\text{M}_{0.33}^{+}(\text{Si}_{4.00})(\text{Al}_{1.66}\text{Mg}_{0.33})\text{O}_{10}(\text{OH})_2]$. The formula unit weight is $363.2 \text{ g mol}_{\text{fu}}^{-1}$ when this idealized montmorillonite is Mg-saturated and 381.8 when it is Ba-saturated. A pure sample of this idealized clay would have a CEC of $91 \text{ cmol}_c \text{ kg}^{-1}$ when Mg-saturated and $86 \text{ cmol}_c \text{ kg}^{-1}$ when Ba-saturated. This effect of saturating cations on CEC values increases with the CEC of the clay and with the differ-

ence in equivalent weights of the exchangeable cations. Theoretically, the difference can be as large as 30%, comparing the CEC of a high-charged vermiculite saturated with Cs^+ to the CEC of the same vermiculite saturated with Li (assuming no fixation of either the Cs^+ or Li^+).

Because of the problems discussed above, no perfect method for measuring CEC or AEC exists. Furthermore, the optimum method varies depending on the nature of the sample and the question being addressed. If a sample contains soluble salts or slightly soluble minerals such as gypsum, the soluble components must be removed before any attempt to measure CEC or AEC is made. Carbonates are also often removed from clay samples before CEC analysis, but for some studies the removal of carbonates may not be appropriate. The reader is referred to Chapter 2 (Soukup et al., 2008, this volume), where procedures to remove excess salt and carbonates are described. Following are three different methods which work reasonably well for samples containing carbonates, neutral to mildly acidic samples dominated by permanent charge minerals, and samples dominated by variable charge minerals, respectively.

Sodium Acetate Method

The sodium acetate method is intended for samples containing carbonates. Any soluble salts or slightly soluble minerals such as gypsum must be removed before this method can be used. In this method, samples are Na-saturated by washing with a sodium acetate (NaOAc) pH = 8.2 solution, washed free of excess salt using water–isopropyl alcohol solutions, and then the retained Na is displaced using NH_4Cl .

Reagents

- **Saturating solution, 1 M NaOAc pH = 8.2.** Dissolve 136.08 g of $\text{NaOAc}\cdot 3\text{H}_2\text{O}$ in 750 mL of deionized water. Adjust the pH to 8.2 by adding acetic acid (HOAc) or NaOH . Dilute to 1 L with deionized water.
- **First rinsing solution, 80% IPA.** Combine 800 mL of isopropyl alcohol (IPA) with 200 mL of deionized water.
- **Second rinsing solution, 100% IPA.** Isopropyl alcohol.
- **Replacing solution, 0.1 M NH_4Cl .** Dissolve 5.35 g NH_4Cl and dilute to 1 L in deionized water.

Procedure

1. Accurately weigh 0.2 g of air-dry clay into a 30-mL centrifuge tube with a secure lid (Oak Ridge tube). At the same time accurately weigh 0.5 g of the same air-dry clay into a preweighed aluminum drying pan. The sample in the aluminum drying pan should be heated at 200°C for 2 h, cooled in a desiccator, and weighed again to determine the water content of the air-dry clay sample. This oven-dried sample is only used to calculate the water content correction factor, F .
2. Add 15 mL of the saturating solution (1 M NaOAc). Carefully vortex or stir the sample to achieve homogenous suspension. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and discard the supernatant. Care must be taken to avoid losing any of the sample during the mixing and decantation procedures.
3. Repeat Step 2 two more times.
4. Add 15 mL of the first rinsing solution (80% IPA). Carefully vortex or stir the sample to achieve homogenous suspension. Centrifuge the sample in an angle head

rotor at 3000 g for 5 min. Decant and discard the supernatant. If the sample is not sufficiently consolidated after the centrifugation step, it can be recentrifuged at a higher speed.

5. Repeat Step 4 several times, each time measuring the electrical conductivity of the supernatant solution. When the conductivity of the supernatant solution drops below the conductivity of NaOAc saturated IPA (about $6 \mu\text{S cm}^{-1}$) switch to the second rinsing solution (100% IPA). Continue rinsing the sample until the conductivity of the supernatant solution drops below $1 \mu\text{S cm}^{-1}$ (in our lab 100% IPA has a conductivity $<0.1 \mu\text{S cm}^{-1}$).
6. After the final rinse step, allow the sample to air-dry by placing the open container in a fume hood.
7. Add 15 mL of the replacing solution. Carefully vortex or stir the sample to achieve homogenous suspension. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and save the supernatant into a 100-mL volumetric flask.
8. Repeat Step 7 three more times, adding the supernatant to the same 100-mL volumetric flask each time.
9. Bring the volumetric flask to 100 mL with deionized water.
10. Analyze the sodium content by inductively coupled plasma–atomic emission spectrometry (ICP–AES), atomic adsorption spectrophotometry, or other suitable method.

Calculations

Water content correction factor (F) is determined using another sample of the same clay in Step 1.

$$F = (\text{weight of oven-dry clay and aluminum pan} - \text{weight of aluminum pan}) / (\text{weight of air-dry clay and aluminum pan} - \text{weight of aluminum pan})$$

C = Na concentration (mg L^{-1}) in the 100-mL volumetric flask (Step 10)

W = Weight (g) of air-dry clay added to centrifuge tube (Step 1)

$$\text{CEC} = (C \times 0.435) / (WF) \text{ (cmol}_c \text{ kg}^{-1}\text{)}$$

Comments

The pH is adjusted to 8.2 to minimize dissolution of carbonates during the Na saturation procedure. The rinsing steps (4 and 5) are a potential source of error, either because not all of the excess Na^+ was removed or because H^+ ions (from hydrolysis of water) replace some of the exchangeable Na^+ . Sodium acetate is only slightly soluble in 100% IPA; hence, it is necessary to use a mixed water–IPA solution for the first few rinses. Otherwise it will be nearly impossible to remove all of the excess NaOAc. One hundred percent IPA is used for the second rinsing solution to avoid hydrolysis. Because conductivity meters can vary, measure the conductivity of both 100% IPA and a saturated solution of NaOAc in IPA. Switch from the first rinsing solution (80% IPA) to the second rinsing solution (100% IPA) when the conductivity of the supernatant first falls below that of NaOAc-saturated IPA. Stop the rinsing with 100% IPA when the conductivity of the supernatant is stable at a conductivity slightly above that of 100% IPA. If too much water is used in the first rinsing solution, the clay will disperse and it will be nearly impossible to centrifuge out of suspension. In Step 6 air-drying the samples removes residual IPA, which if present in the final

solution may adversely affect the accuracy of Na analysis by ICP–AES or other means. NH_4Cl is used as the replacing solution because Na contamination of reagent-grade NH_4Cl is usually very small, whereas Na contamination in other salts (e.g., CaCl_2 and MgCl_2) may be substantial. When the replacing solution is added to the sample, some carbonates in the sample may dissolve, releasing NH_3^+ gas and Ca^{2+} or Mg^{2+} ions to the solution. The Ca^{2+} or Mg^{2+} ions released will be more effective at displacing the retained Na^+ than the original NH_4^+ . Even though reagent-grade NH_4Cl should be nearly free of Na^+ contamination, a blank containing 60 mL of replacing solution and 40 mL of deionized water should be analyzed along with the samples. A disadvantage of the sodium acetate method is that AEC cannot be readily determined because acetate is specifically adsorbed by some clays.

Magnesium-Ammonium Method

The Mg-NH_4 method is generally suitable for neutral or mildly acidic samples dominated by permanent charge clays. The procedure should not be used if the sample $\text{pH} > 7.0$, especially if the sample contains free carbonates. Also, the procedure should not be used with samples dominated by variable charge clays because ionic strength and pH are not effectively controlled.

Reagents

- **Saturating solution, 0.5 M MgCl_2 .** Dissolve 101.65 g of reagent-grade $\text{MgCl}_2 \cdot 6(\text{H}_2\text{O})$ in 1 L of deionized water.
- **First rinsing solution, 80% IPA.** Combine 800 mL of isopropyl alcohol with 200 mL of deionized water.
- **Second rinsing solution, 100% IPA.** Isopropyl alcohol.
- **Replacing solution 0.1 M NH_4NO_3 .** Dissolve 8.00 g of reagent-grade NH_4NO_3 in 1 L of deionized water.

Procedure

1. Weigh 0.2 g of air-dry clay into 30-mL centrifuge tube with a secure lid (Oak Ridge tube). At the same time weigh 0.5 g of the same air-dry clay into a preweighed aluminum drying pan. The sample in the aluminum drying pan should be heated at 200°C for 2 h, cooled in a desiccator, and weighed again to determine the water content of the air-dry clay sample. This oven-dried sample is only used to calculate the water content correction factor, F .
2. Add 15 mL of the saturating solution (0.5 M MgCl_2). Carefully vortex or stir the sample to achieve homogenous suspension. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and discard the supernatant. Care must be taken to avoid losing any of the sample during the mixing and decantation stages.
3. Repeat Step 2 three more times.
4. Add 15 mL of the first rinsing solution (80% IPA). Carefully vortex or stir the sample to achieve homogenous suspension. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and discard the supernatant. If the sample is not sufficiently consolidated after the centrifugation step it can be recentrifuged at a higher speed.

5. Repeat Step 4 several times, each time measuring the electrical conductivity of the supernatant solution. When the conductivity of the supernatant solution drops below the conductivity of NaOAC saturated IPA ($\sim 6 \mu\text{S cm}^{-1}$), switch to the second rinsing solution (100% IPA). Continue rinsing the sample until the conductivity of the supernatant solution drops below $1 \mu\text{S cm}^{-1}$ (in our lab 100% IPA has a conductivity $< 0.1 \mu\text{S cm}^{-1}$).
6. After the final rinse step, allow the sample to air-dry by placing the open container in a fume hood.
7. Add 15 mL of the replacing solution ($0.1 \text{ M NH}_4\text{NO}_3$) to the air-dried sample. Carefully vortex or stir the sample to achieve a homogenous suspension. Centrifuge the sample in an angle head rotor at $3000 g$ for 5 min. Decant and save the supernatant into a 100-mL volumetric flask.
8. Repeat Step 7 three more times, adding the supernatant to the same 100-mL volumetric flask each time.
9. Bring the volumetric flask to 100 mL with deionized water.
10. Analyze the Mg content by atomic adsorption spectrophotometry, or other suitable method. If AEC is desired, Cl^- should be determined by ion chromatography.

Calculations

Water content correction factor (F) is determined using another sample of the same clay in Step 1.

$$F = (\text{weight of oven dry clay and aluminum pan} - \text{weight of aluminum pan}) / (\text{weight of air-dry clay and aluminum pan} - \text{weight of aluminum pan}).$$

$$C_1 = \text{Mg concentration (mg/L) in the 100-mL volumetric flask from Step 10.}$$

$$C_2 = \text{Cl concentration (mg/L) in the 100-mL volumetric flask from Step 10.}$$

$$W = \text{Weight (g) of air-dry clay added to centrifuge tube in Step 1.}$$

$$\text{CEC} = (C_1 \times 0.823) / (WF) \text{ (cmol/kg)}$$

$$\text{AEC} = (C_2 \times 0.282) / (WF) \text{ (cmol/kg)}$$

Comments

The great advantage of the Mg-NH_4 method is its simplicity. The disadvantage is that it should not be used with calcareous samples or samples with significant quantities of variable charge. The Mg-NH_4 method uses unbuffered saturating and replacing solutions, and therefore determines the CEC and AEC at the sample pH. Although $\text{MgCl}_2 \cdot 6(\text{H}_2\text{O})$ is fairly soluble in IPA, use of a mixed water-IPA solution for the first few rinses is recommended to help remove excess MgCl_2 . One hundred percent IPA is used for the second rinsing solution to avoid hydrolysis. In Step 6 air-drying the samples removes residual IPA, which if present in the final solution may adversely affect the accuracy of Mg^{2+} analysis by ICP-AES or other means. NH_4NO_3 is used as the replacing solution because Mg^{2+} contamination of reagent-grade NH_4NO_3 is usually very small, whereas Mg^{2+} contamination in other salts (e.g., CaNO_3) may be substantial. Even though reagent-grade NH_4NO_3 should be nearly free of Mg^{2+} contamination, a blank containing 60 mL of replacing solution and 40 mL of deionized water should be analyzed along with the samples.

Compulsive Exchange Method

The compulsive exchange method was originally proposed by Gillman (1979) for determining CEC and AEC of soil samples dominated by variable charge minerals. Gillman and Sumpter (1986) proposed several modifications to the method and stated that it could be used to measure CEC and AEC of almost all soils. In the procedure, a sample is first washed with BaCl_2 to saturate cation exchange sites with Ba^{2+} and the anion exchange sites with Cl^- . Then a known volume of low ionic strength MgSO_4 is added. The exchange reaction between Mg^{2+} and Ba^{2+} is driven to completion by precipitation of nearly insoluble BaSO_4 . The Cl^- on the anion exchange sites is replaced by SO_4^{2-} . Cation exchange capacity is determined by measuring the decrease in Mg in the replacing solution, and AEC is determined by measuring the amount of Cl^- released into the replacing solution. Both pH and ionic strength are carefully controlled in the procedure, which is critical for measuring CEC and AEC of variable charge minerals. The procedure described below includes several minor changes from the modified method proposed by Gillman and Sumpter (1986), which are intended to make the method more suitable for clay samples.

Reagents

- **Saturating solution, 0.1 M BaCl_2 .** Dissolve 24.43 g of $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1 L of deionized water. Adjust the pH to 6.0 by adding 0.1 M HCl or saturated $\text{Ba}(\text{OH})_2$.
- **Equilibration solution, 0.002 M BaCl_2 .** Pipette 20 mL of the 0.1 M BaCl_2 saturating solution into a 1-L volumetric flask and dilute to 1 L with deionized water.
- **Replacing solution, 0.005 M MgSO_4 .** Dissolve 0.63 g of anhydrous MgSO_4 in 1 L of deionized water.
- **EC reference solution, 0.0015 M MgSO_4 .** Transfer 30 mL of the replacing solution (0.005 M MgSO_4) to a 100-mL volumetric flask and dilute to 100 mL with deionized water.
- **pH adjusting solutions.** Small amounts of 0.1 M HCl, saturated $\text{Ba}(\text{OH})_2$, and 0.1 M H_2SO_4 are needed to adjust pH.

Procedure

1. Accurately weigh between 0.1 and 1 g of air-dry clay into a preweighed 30-mL centrifuge tube with a secure lid (e.g., Oak Ridge tube). The optimum amount of clay to use (w) increases as the CEC of the sample decreases ($w = 10.5/\text{CEC}$). At the same time accurately weigh and place 0.5 g of the same air-dry clay into a preweighed aluminum drying pan. The sample in the aluminum drying pan should be heated at 200°C for 2 h, cooled in a desiccator, and weighed again to determine the water content of the air-dry clay sample. This oven-dried sample is only used to calculate the water content correction factor, F .
2. Add 15 mL of the saturating solution (0.1 M BaCl_2). Carefully stir the sample to break up any large aggregates then close the lid tightly and shake the sample for 1 h. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and discard the supernatant. Care must be taken to avoid losing any of the sample during the mixing and decantation stages.
3. Add 15 mL of the saturating solution (0.1 M BaCl_2).
4. Carefully stir the sample to break up any large aggregates. Then close the lid tightly and shake the sample for 1 h. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Measure the pH of the supernatant. If the pH differs from 6.0 by more

than 0.1 pH units, adjust the pH to 6.0 by adding 0.1 M HCl or saturated $\text{Ba}(\text{OH})_2$. Do not decant.

5. Repeat Step 4 until the pH is stable at 6.0. Then centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and discard the supernatant. Care must be taken to avoid losing any of the sample during the mixing and decantation stages.
6. Add 15 mL of the equilibrating solution (0.002 M BaCl_2). Carefully stir the sample to break up any large aggregates. Close the lid tightly and shake the sample for 1 h. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and discard the supernatant. Care must be taken to avoid losing any of the sample during the mixing and decantation stages.
7. Repeat Step 6 two times. If AEC will be determined, save the supernatant from the last equilibration for Cl analysis.
8. Weigh the sample tube after the last equilibrating solution has been removed. This weight is needed to determine the amount of residual 0.002 M BaCl_2 solution.
9. Add 15 mL of the replacing solution (0.005 M MgSO_4). Carefully stir the sample to break up any large aggregates. Then close the lid tightly and shake the sample for 1 h. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Do not decant.
10. Measure the pH of the supernatant solution. If the pH is >6.0 add 0.1 M H_2SO_4 dropwise to lower the pH to 6.0 (± 0.1).
11. Measure the electrical conductivity of the supernatant solution above the clay and the electrical conductivity of the 0.0015 M MgSO_4 EC reference solution. If the conductivity of the supernatant solution is less than that of the reference solution add 0.005 M MgSO_4 in 0.50-mL increments until the conductivity of the supernatant solution equals or slightly surpasses that of the reference solution. Record the volume of 0.005 M MgSO_4 added. If the electrical conductivity of the supernatant solution is more than the reference solution, add deionized water dropwise until electrical conductivity of the supernatant solution closely approaches that of the reference solution.
12. Carefully stir the sample to break up any large aggregates. Then close the lid tightly and shake the sample overnight (>12 h). Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Measure the pH and the electrical conductivity of the supernatant solution. If the pH is greater than 6.0 (± 0.1) or the electrical conductivity differs significantly from that of the reference solution, repeat Steps 10 to 12.
13. Weigh the sample to determine the weight of the centrifuge tube plus clay plus supernatant solution. This weight is needed to estimate the total volume of supernatant solution.
14. Centrifuge the sample in an angle head rotor at 3000 g for 5 min. Decant and save the supernatant for analysis.
15. Measure the concentration of Mg in the supernatant by ICP–AES or other suitable means. Measure the concentration of Mg in the 0.005 M MgSO_4 solution using the same method and the same standards as are used for analysis of Mg in the supernatant solution. Analyze the supernatant solution for Cl by ion chromatography if AEC will be determined.

Calculations

Water content correction factor (F) is determined using another sample of the same clay in Step 1.

$$F = (\text{weight of oven dry clay and aluminum pan} - \text{weight of aluminum pan}) / (\text{weight of air-dry clay and aluminum pan} - \text{weight of aluminum pan})$$

$$W_1 = \text{Tare weight of the centrifuge tube (Step 1)}$$

$$W_2 = \text{Weight (g) of centrifuge tube plus the air-dry clay (Step 1)}$$

$$W_3 = \text{Weight (g) of the centrifuge tube plus the clay plus the residual 0.002 M BaCl}_2 \text{ solution (Step 8)}$$

$$W_4 = \text{Weight (g) of the centrifuge tube plus the clay plus the total supernatant solution (Step 13)}$$

$$V_1 = \text{Total volume (mL) of 0.005 M MgSO}_4 \text{ added (Steps 9 and 11)}$$

$$V_2 = \text{Total volume (mL) of the supernatant solution } (V_2 = W_4 - W_2)$$

$$V_3 = \text{Volume (mL) of residual BaCl}_2 \text{ equilibration solution } (V_3 = W_3 - W_2)$$

$$C_1 = \text{Concentration of Mg (mg L}^{-1}\text{) in the 0.005 M MgSO}_4 \text{ solution (step 15)}$$

$$C_2 = \text{Concentration of Mg (mg L}^{-1}\text{) in the supernatant solution (Step 15)}$$

$$C_3 = \text{Concentration of Cl (mg L}^{-1}\text{) in the residual 0.002 M BaCl}_2 \text{ equilibration solution (Step 7)}$$

$$C_4 = \text{Concentration of Cl (mg L}^{-1}\text{) in the supernatant solution (Step 15)}$$

$$\text{CEC} = (C_1 V_1 - C_2 V_2) / [121.53 F (W_2 - W_1)] \text{ (cmol}_c \text{ kg}^{-1}\text{)}$$

$$\text{AEC} = (C_4 V_2 - C_3 V_3) / [354.53 F (W_2 - W_1)] \text{ (cmol}_c \text{ kg}^{-1}\text{)}$$

Comments

The primary advantage of the compulsive exchange method is that pH and ionic strength are carefully controlled. Controlling pH and ionic strength is critical for measuring CEC and AEC of variable charge clays, but is not necessary within reasonable limits for permanent charge clays. Another benefit of the compulsive exchange method is that the samples do not have to be rinsed with ethanol or isopropyl alcohol to remove excess salts; hence, hydrolysis problems are avoided. The primary disadvantage of the compulsive exchange method is that it is complicated and labor intensive. Another disadvantage of the compulsive exchange method is that both the total volume of the supernatant solution (V_2) and the volume of residual 0.002 M BaCl₂ equilibration solution (V_3) are estimated gravimetrically. This is problematic because the clay sample retains an undetermined amount of interlayer and near surface water, which should not be included in these volumes, and because the weight of the sample increases both when Ba replaces the original cations on the exchange complex and later when Mg replaces the Ba and the BaSO₄ precipitate is not removed. Fortunately, the magnitude of these errors is relatively small, and the use of air-dry weight instead of oven-dry weight in calculating V_2 and V_3 partially compensates for these errors.

The pH is arbitrarily set at 6.0 in the described procedure. However, the pH may be adjusted over a limited range depending on the nature of the sample and the question being addressed. Use of pH values below 5.0 are not recommended because Al released through hydrolysis of the clay will compete with Mg for the exchange sites. Gillman and Sumpster (1986) claimed that the procedure can be used with calcareous and even saline soils. We have not tested the upper pH limit for the method, but we strongly suspect that the formation of relatively insoluble Ba and Mg carbonates and hydroxides and complex ions such as MgOH⁺ will severely degrade the accuracy of the method under alkaline conditions. Therefore, we do not recommend the use of the method above pH 7.0.

In the described method, the ionic strength ($I = 0.5\sum C_i Z_i^2$) is set at 0.006. Gillman (1979) chose an ionic strength of 0.006 because it is close to the ionic strength of many soils. Reducing the ionic strength much below 0.006 will require the addition of more of the replacing solution and will decrease the accuracy of the analysis. The accuracy will also decrease if the ionic strength is raised too high.

PREPARATION OF REDUCED CHARGE SMECTITES

Knowledge of the Hofmann and Klemen (1950) effect and the Greene-Kelly (1953, 1955) test led both Calvet and Prost (1971) and Brindley and Ertem (1971) to propose that Li plus heat treatments could be used to prepare a series of nearly isostructural clays that differed primarily in layer charge. Since then such reduced charge smectites have been widely used to study the effects of layer charge on hydration, swelling, and sorption of organic molecules by smectites. Although reduced charge smectites are generally prepared using reference smectites rather than soil clays, the procedure has proven extremely valuable in many fundamental studies of clay properties and is therefore described here.

Apparatus

- Centrifuge with an angle head rotor and either high density polyethylene or polycarbonate centrifuge tubes with lids (Oak Ridge tubes)
- Zirconium or platinum crucibles
- Laboratory oven capable of operation at 250°C

Reagents

- **One molar LiCl.** Dissolve 42.39 g of reagent-grade LiCl in 1 L of deionized water.
- **One molar NaCl.** Dissolve 58.44 g of reagent-grade NaCl in 1 L of deionized water.
- **Mixed LiCl–NaCl saturating solution.** Volumetrically combine the 1 M LiCl and 1 M NaCl solutions in various proportions. For example, to prepare a 10% Li solution combine 10 mL of the 1 M LiCl and 90 mL of the 1 M NaCl solutions. To avoid irreversible collapse of clay layers it is recommended that the Li not exceed 50%.
- **Ethanol washing solution.** Mix 0.1 mL of the mixed LiCl–NaCl saturating solutions with 99.9 mL of 95% ethanol.

Procedure

1. Weigh 0.5 g of dry clay into a 30-mL centrifuge tube. Add 20 mL of the desired mixed LiCl–NaCl saturating solution. Stir vigorously until a homogeneous suspension is achieved. Centrifuge at 3000 g for 5 min. Decant and discard the supernatant.
2. Repeat step one a total of three times.
3. Add 20 mL of the appropriate ethanol washing solution to the centrifuge tube. Stir vigorously until a homogeneous suspension is achieved. Centrifuge at 12,000 g for 10 min. Decant and discard the supernatant.
4. Perform Step 3 a total of three times.
5. After the last rinse in ethanol, transfer the clay to a crucible. Place the samples under a fume hood until any residual ethanol has evaporated.
6. Heat the samples at 250°C for 24 h.
7. After cooling, the samples may be transferred to an agate mortar and crushed.
8. The dry powders may be stored indefinitely in a sealed container.

Comments

The addition of a small amount of the appropriate mixed LiCl–NaCl saturating solution to the ethanol washing solution is necessary to avoid hydrolysis and the associated displacement of Na and Li from the exchange complex during the ethanol washing phase of the procedure. We have found that Li- and Na-saturated smectites can be settled by centrifugation at 12,000 g for 10 min from 95% ethanol suspensions. However, it is much more difficult to settle Na- and Li-smectites by centrifugation if methanol is used instead of ethanol or if the water content of the ethanol is >5%.

REFERENCES

- Bailey, S.W., G.W. Brindley, W.D. Johns, R.T. Martin, and M. Ross. 1971. Summary of national and international recommendations on clay mineral nomenclature. *Clays Clay Miner.* 19:129–132.
- Brindley, G.W., and G. Ertem. 1971. Preparation and solvation properties of some variable charge montmorillonites. *Clays Clay Miner.* 19:399–404.
- Bujdak, J., M. Janek, J. Madejová, and P. Komadel. 2001. Methylene blue interactions with reduced-charge smectites. *Clays Clay Miner.* 49:244–254.
- Calvet, R., and R. Prost. 1971. Cation migration into empty octahedral sites and surface properties of clays. *Clays Clay Miner.* 19:175–186.
- Chiou, C.T., and D.W. Rutherford. 1997. Effects of exchanged cation and layer charge on the sorption of water and EGME vapors on montmorillonite clays. *Clays Clay Miner.* 45:867–880.
- Christidis, G.E., and D.D. Eberl. 2003. Determination of layer-charge characteristics of smectites. *Clays Clay Miner.* 51:644–655.
- Gillman, G.P. 1979. A proposed method for the measurement of exchange properties of highly weathered soils. *Aust. J. Soil Res.* 17:129–139.
- Gillman, G.P., and E.A. Sumpter. 1986. Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Aust. J. Soil Res.* 24:61–66.
- Greene-Kelly, R. 1953. The identification of montmorillonoids in clays. *J. Soil Sci.* 4:233–237.
- Greene-Kelly, R. 1955. Dehydration of montmorillonite minerals. *Mineral. Mag.* 30:604–615.
- Hofmann, U., and R. Klemen. 1950. Verlust der Austauschfähigkeit von Lithium-Ionen an Bentonite durch Erhitzung. *Z. Anorg. Allg. Chem.* 262:95–99.
- Hrobáriková, J., J. Madejová, and P. Komadel. 2001. Effect of heating temperature on Li-fixation, layer charge and properties of fine fractions of bentonites. *J. Mater. Chem.* 11:1452–1457.
- Jaynes, W.F., S.J. Traina, J.M. Bigham, and C.T. Johnston. 1992. Preparation and characterization of reduced-charge hectorites. *Clays Clay Miner.* 40:397–404.
- Johnston, C.T., and E. Tombácz. 2002. Surface chemistry of soil minerals. p. 37–67. *In* J.B. Dixon and D.G. Schulze (ed.) *Soil mineralogy with environmental applications*. SSSA Book Ser. 7. SSSA, Madison WI.
- Karathanasis, A.D. 2008. Structural allocation of clay mineral elemental components p. 465–484. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Lagaly, G. 1981. Characterization of clays by organic compounds. *Clay Miner.* 16:1–21.
- Lagaly, G. 1994. Layer charge determination by alkylammonium ions. p. 1–46. *In* A.R. Mermut (ed.) *Layer charge characteristics of 2:1 silicate clay minerals*. CMS Workshop Lectures 6. The Clay Minerals Society, Chantilly, VA.
- Lagaly, G., and A. Weiss. 1969. Determination of the layer charge in mica-type layer silicates. p. 61–80. *In* L. Heller (ed.) *Proceedings of the International Clay Conference, Tokyo, 1969*. Vol.1. Israel Univ. Press, Jerusalem, Israel.
- Lagaly, G., and A. Weiss. 1971. Anordnung und Orientierung kationischer Tenside auf Silicatoberflächen, IV. Anordnung von *n*-Alkylammoniumionen bei niedrig geladenen Schichtsilicaten. *Kolloid-Z.u.Z. Polymere.* 243:48–55.
- Lagaly, G., and A. Weiss. 1976. Problems in layer-charge determination of montmorillonites. *Clay Miner.* 11:173–187.
- Laird, D.A. 1994. Evaluation of the structural formula and alkylammonium methods of determining layer charge. p. 79–103. *In* A.R. Mermut (ed.) *Layer charge characteristics of 2:1 silicate clay minerals*. CMS Workshop Lectures 6. The Clay Minerals Society, Chantilly, VA.
- Laird, D.A. 1999. Layer charge influences on the hydration of expanding 2:1 phyllosilicates. *Clays Clay Miner.* 47:630–636.

- Laird, D.A., and C. Shang. 1997. Relationship between cation exchange selectivity and crystalline swelling in expanding 2:1 phyllosilicates. *Clays Clay Miner.* 45:681–689.
- Laird, D.A., C. Shang, and M.L. Thompson. 1995. Hysteresis in crystalline swelling of smectites. *J. Colloid Interface Sci.* 171:240–245.
- Laird, D.A., A.D. Scott, and T.E. Fenton. 1987. Interpretation of alkylammonium characterization of soil clays. *Soil Sci. Soc. Am. J.* 51:1659–1663.
- Laird, D.A., A.D. Scott, and T.E. Fenton. 1989. Evaluation of the alkylammonium method of determining layer charge. *Clays Clay Miner.* 37:41–46.
- MacEwan, D.M.C., and M.J. Wilson. 1980. Interlayer and intercalation complexes of clay minerals. p. 197–248. *In* G.W. Brindley and G. Brown (ed.) *Crystal structures of clay minerals and their X-ray identification*. Mineralogical Society, London.
- Mackintosh, E.E., and D.G. Lewis. 1968. Displacement of potassium from micas by dodecylammonium chloride. *Trans. Int. Congr. Soil Sci.* 9th 2:695–703.
- Mackintosh, E.E., D.G. Lewis, and D.J. Greenland. 1971. Dodecylammonium–mica complexes: I. Factors affecting the exchange reactions. *Clays Clay Miner.* 19:209–218.
- Mackintosh, E.E., D.G. Lewis, and D.J. Greenland. 1972. Dodecylammonium–mica complexes: II. Characterization of the reaction products. *Clays Clay Miner.* 20:125–134.
- Maes, A., and A. Cremers. 1977. Charge density effects in ion exchange: I. Heterovalent exchange equilibria. *J. Chem. Soc. Faraday Trans. 1* 73:1807–1814.
- Mermut, A.R. 1994. Problems associated with layer charge characterization of 2:1 phyllosilicates. p. 79–103. *In* A.R. Mermut (ed.) *Layer charge characteristics of 2:1 silicate clay minerals*. CMS Workshop Lectures 6. The Clay Minerals Society, Chantilly, VA.
- Okazaki, R., H.W. Smith, and C.D. Moodie. 1963. Hydrolysis and salt-retention errors in conventional cation exchange capacity procedures. *Soil Sci.* 96:205–209.
- Olis, A.C., P.B. Malla, and L.A. Douglas. 1990. The rapid estimation of the layer charges of 2:1 expanding clays from a single alkylammonium ion expansion. *Clay Miner.* 25:39–50.
- Petit, S., D. Righi, J. Madejova, and A. Decarreau. 1999. Interpretation of the infrared NH_4^+ spectrum of the NH_4^+ -clays: Application to the evaluation of the layer charge. *Clay Miner.* 34:543–549.
- Posner, A.M., and J.P. Quirk. 1964. The adsorption of water from concentrated electrolyte solutions by montmorillonite and illite. *Proc. R. Soc. A* 278:35–56.
- Rühlicke, G., and E.E. Kohler. 1981. A simplified procedure for determining layer charge by the n -alkylammonium method. *Clay Miner.* 16:305–307.
- Sawhney, B.L. 1969. Cesium uptake by layer silicates: Effect on interlayer collapse and cation exchange capacity. p. 605–611. *In* L. Heller (ed.) *Proc. Int. Clay Conf.*, Tokyo, Japan. Sept. 1969. Israel Univ. Press, Jerusalem, Israel.
- Shainberg, I., N.I. Alperovitch, and R. Keren. 1987. Charge density and Na-K-Ca exchange on smectites. *Clays Clay Miner.* 35:68–73.
- Slade, P.G., J.P. Quirk, and K. Norrish. 1991. Crystalline swelling of smectites samples in concentrated NaCl solutions in relation to layer charge. *Clays Clay Miner.* 39:234–238.
- Soukup, D.A., B.J. Buck, and W. Harris. 2008. Preparing soils for mineralogical analyses. p. 13–32. *In* A.L. Ulery and L.R. Drees (ed.) *Methods of soil analysis. Part 5. Mineralogical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Stanjek, H., and R. Friedrich. 1986. The determination of layer charge by curve-fitting of lorentz- and polarization-corrected X-ray diagrams. *Clay Miner.* 21:183–190.
- Stul, M.S., and W.J. Mortier. 1974. The heterogeneity of the charge density in montmorillonites. *Clays Clay Miner.* 22:391–396.
- Sumner, M.E., and W.P. Miller. 1996. Cation exchange capacity and exchange coefficients. p. 1201–1229. *In* D.L. Sparks et al. (ed.) *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- Weiss, A. 1963. Mica-type layer silicates with alkylammonium ions. *Clays Clay Miner.* 10:191–224.

Subject Index

- Ab initio simulations, XANES, 422–423
- Absorbed electrons, electron microprobe, 336
- Absorption bands, diffuse reflectance spectroscopy analysis, 378–380
- Absorption correction, 350–351
- Absorption edges, 392–394
- Absorption, X-rays, 291, 389–393
- Abundant soil minerals, 387
- Accelerating voltage, 261, 349
- Accelerator, 248
- Accessory minerals, optical properties of, 183–185
- Acetone replacement, thin section preparation, 200–201, 248
- Acid dissolution technique, clay mineral allocation, 477–479
- Acid-base accounting, 42
- Acidity, sulfide oxidation, 42
- Adhesive force, AFM, 316
- AEC. *see* Anion exchange capacity
- Aegirine-augite, optical properties of, 179
- AFM. *see* Atomic force microscopy
- Agarose coating procedure, TEM, 251
- Aggregating agents, removing, 25
- Air drying samples, alteration and, 10
- Albite, 98, 175, 475
- Aligning spectra, EXAF, 424–425
- Alkaline extractants, 57–59
- Alkylamine hydrochlorides, synthesis, layer charge, 490–495
- Alkylammonium cations, configurations, 488
- Alkylammonium method, clay minerals layer charge, 487–489
- Allophanes, 47, 63–65
- Alteration, air drying soil samples, 10
- Aluminosilicate materials, short-range-order, 22
- Aluminosilicates, 63–65
- Aluminum
- AOD treatment, 50–54
 - EDS patterns and, 271
 - hydroxylamine hydrochloride treatment, 56–57
 - pyrophosphate extraction, 48–50
 - treatment reagents, Al, Fe, Mn, Si dissolution, 63–65
- Alunite, 140–141
- Ammonium dihydrogen phosphate, WDS diffraction, 345
- Ammonium oxalate dissolution. *see* AOD treatment
- Amorphous AL, treatment reagents, Al, Fe, Mn, Si dissolution, 63–65
- Amorphous materials, 47, 63–65
- Amorphous Mn, 63–65
- Amphiboles, 98, 178, 180
- Amorphous Fe, 63–65
- Analcime, 98, 186
- Analcite, 186
- Analytical line, electron microprobe, 348–351
- Anatase, d-spacings, 98
- Andesine, optical properties of, 175
- Anion exchange capacity, 486
- Anisotropic minerals, 163
- Anisotropic optical properties, 166
- Anorthite, 98, 175
- Antigorite, 98, 140, 177
- AOD treatment, 50–54
- Apatite, optical properties of, 185–186
- Aperture setting, SEM, 286–287
- Aquatic natural organic matter, in-solution AFM, 320
- Aragonite, d-spacings, 98
- Aragonite, optical properties of, 181
- Archaeology, micromorphology and, 192–193
- Archiving soil samples, 10–11
- Argonne National Laboratory, 401
- Artemis analysis program, 452
- Athena analysis program, 452
- Atomic force microscopy, 299–328
 - air and solution imaging, 302–308
 - applications, 316–328
 - artifacts, common, 306–309
 - biomineralization, 323–326
 - carbonates and, 324–326
 - chemical and biological force, 326–327
 - components, 299–300

- Atomic force microscopy, *continued*
 force measurements, 311–314
 forces between tip and sample, 300–302
 inter- and intramolecular forces, measuring, 309–316
 microbial mediated mineral dissolution, AFM, 321–323
 operating principles, 299–302
 phase imaging, 304–306
 tips and, 309–311, 324
- Atomic numbers, 291, 350
- Atoms
 list, FEFF, 442
 schematic, 83
 X-ray absorption spectroscopy, 388–389
- Auger boring, soil sampling and, 5
- Auger electron production, 390
- Auger electrons, EDS patterns, 291
- Augite, d-spacings, 98
- Augite, optical properties of, 179
- Authigenic phyllosilicates, morphology of, 293
- AUTOBK algorithm, 437
- Averaging spectra, EXAF, 424–425
- Background counts, WDS spectrum, 349
- Background function, EXAF, 437–441
- Backscatter
 distribution maps, electron and X-ray, 363
 electron detector, electron microprobe, 342, 359, 361
 electron images, SEM, 279–280
 electrons, electron microprobe, 336
- Bacteria, biofilms, 316–319
- Barium, 359, 361
- Beam current, electron microprobe, 349
- Beam sizes, SEM, 286–287
- Beam voltage, scanning electron microscopy, 284–286
- Beamline setup, 402–408
- Becke line, 164–165
- Beryl, 183–184
- Biaxial optical properties, 166
- Biofilm structures, TMAFM, 316–319
- Biological activity, TEM, 238
- Biological force microscopy, atomic force microscopy, 326–327
- Biomineralization, AFM, 323–326
- Biotite, 98, 176–177, 475
- Birefringence, 165, 213
- Birnessite, 63–65
- Boreholes, soil sampling and, 5
- Bragg's law, 342–343, 403
- Bridged grain microstructure, 212
- Buffer, fixative and rinsing solution, embedding technique, 252
- Bytownite, optical properties of, 175
- Cacodylate buffer, 248
- Calcites, 98, 140–141, 181
- Calcium, 359, 361, 479
- Calculations
 AOD treatment, 53
 CBD procedure, 62
 hydroxylamine hydrochloride treatment, 56–57
 pyrophosphate extraction, 50
 tiron and alkaline extractants, 58–59
 total potential acidity, 43–44
 X-ray absorption, 411–413
- Calibration, monochromators, 405
- Cameras, transmission electron microscopy, 263
- Cantilever, atomic force microscopy, 299–300, 302
- Cantilever's spring constant, AFM, 313–314
- Capillary adhesion, Atomic force microscopy, 301
- Carbon build-up, electron microprobe, 346
- Carbonates
 analysis techniques, 17–18
 clusters, biomineralization, AFM, 324–326
 dissolution techniques, 36–41
 d-spacings, 98
 optical properties of, 180–181
 quantification, thermal analysis, 150–151
 removal from samples, 16
 testing, 16–17
- Cassiterite, optical properties of, 183–184
- Cation exchange capacity, 485–486
- Cation saturation, 91–92, 289–290
- CBD procedure, 59–65
- Centrifugation, samples, 249
- Centrifugation, sand, separation of, 28
- Ceramic tile mount, XRD, 89
- Chamosite, d-spacings, 98
- Channel microstructure, 212
- Characteristic X-rays, electron microprobe, 337
- Charging, scanning electron microscopy, 270
- Chemical characterization sequence, electron microprobe, 355–356, 362
- Chemical contents, TEM, 236
- Chemical force microscopy, atomic force microscopy, 326
- Chemicals, hazardous and safety advices, 248
- Chlorites
 d-spacings, 98–101
 elemental oxide composition and structural formula, 475
 optical properties of, 176–178
 TG weight loss regions, 140
- Chromaticity coordinates, 373
- Chromium, EDS patterns and, 271
- Chrysotile, 98, 177
- Citrate-bicarbonate-dithionite procedure, 59–65
- Citrate-dithionite, dissolution, carbonates, 39–41

- Classification, thermoanalytical and calorimetric methods, 117–118
- Clay
- dehydration kinetics, 153
 - flocculation, 27–28
 - freeze-drying suspension, 29–30
 - separation of, particle-size fractionation, 27
- Clay mineral allocation components
- admixtures, effect on calculation, 476–477
 - cation exchange capacity, 497–506
 - compulsive exchange method, 503–506
 - fixed negative charge method, 467–468
 - ion exchange capacity, 497–506
 - IR spectroscopy and, 479–480
 - layer silicates, structural composition, 466–467
 - magnesium-ammonium method, 501–502
 - nuclear magnetic resonance techniques, 480–481
 - reduced charge smectites, 506–507
 - sodium acetate method, CEC, AEC, 501–502
 - structural formulas, 467–473
- Clay minerals, 485–497
- Clay Spur Wyoming montmorillonite, 469, 473
- Clay-size fractions, 238–242
- Cleavage, 171, 293
- Clinoptilolite, d-spacings, 98
- Clinzoisite, optical properties of, 185–186
- Clods, sampling, 9
- Coating samples, 270–272, 355
- Collodion, 248
- Colloidal dispersions, clay minerals, 498
- Colloidal forces, Atomic force microscopy, 300
- Colloids, waste water, 250
- Color notations and calculations, DRS, 373, 377
- Color stimulus, 373
- Compton scattering, 389
- Compulsive exchange method, CEC, AEC, clay minerals, 503–506
- Computer diffraction modeling, XRD, 105–108
- Computer image analysis, micromorphology, 219–220
- Constant height imaging, 302
- Constructive interference, X-rays, 343
- Contact-mode atomic force microscopy, 302
- Continuous X-rays, 337
- Continuum concept, DRS, 378–379
- Contrast, 278–279, 283, 288
- Coordination number, 395
- Copper, EDS patterns and, 271
- Cores, diameters, 5
- Counting times, WDS, 350
- Cristobalite, optical properties of, 173
- Crusts, micromorphology and, 192
- Crystallite size, XRD patterns, 102
- Crystals, 165–166, 260, 381, 381–382
- Data sets, types of, electron microprobe, 355–356
- Dehydrated halloysite, 101
- Depositional crusts, micromorphology, 192
- Depth of field, SEM, 286–287
- Depth of penetration, SEM, 285
- Detuning, X-ray absorption spectroscopy, 404
- Dichloroethane, 1,2, 248
- Differential scanning calorimetry, 124–126
- combined XRD and TO techniques, 129
 - DSC patterns, 143–144
 - soils in clay, 142
 - thermal gravimetric analysis and, 144–148
- Differential thermal analysis, 121–124, 141
- Differential X-ray diffraction, 108–112
- Diffraction materials, WDS, 345
- Diffraction, 345
- Diffractometers, X-ray, 93–94
- Diffuse reflectance spectroscopy, 367–383
- absorption bands, analysis, 378–380
 - apparatus, 373–374
 - elucidation of crystal properties, 381–382
 - identification of mineral species, 380–381
 - mixed powder analysis, 372–373
 - principles of, 367–373
 - quantitative analysis, 382–383
 - sample holders, 376
 - sample preparation, 375–376
 - standards, 374–375
- Digital image analysis, 227–233
- Dilution, X-ray absorption spectroscopy, 411
- Diocahedral mica, diagnostic d-spacings, 99
- Diopside, optical properties of, 179
- Dipoles forces, Atomic force microscopy, 300
- Directional reflection. *see* regular reflectance
- Directional-hemispherical reflectance, DRS, 372
- Dissolution techniques, 33–71
- carbonates, 36–41
 - extractable aluminum, iron, and silicon, 47–65
 - gypsum, 35–36
 - halides and sulfates, 34–35
 - reagents, 65
 - sulfides, hydrogen peroxide soluble, 41–44
- Distance component, AFM, 314
- Documentation, sampling and, 6–7
- Dolomite, 98, 140–141, 181
- Double tip, atomic force microscopy, 308
- Dry mounting techniques, 87
- Drying, 10
- DSC. *see* Differential Scanning Calorimetry
- Dyes and stains, micromorphology samples, 209–210
- Edge energy, normalization, 426
- Edge regions, changes, 393–394

EDS

- analysis of soluble salts, gypsum, and carbonates, 17–18
- atomic numbers and, 291
- electron microprobe and, 341–342
- interpretation, spectra, 289–292
- line of sight requirements, 284
- patterns, sample coating interference, 271
- quantitative analysis, electron microprobe and, 351–353

Electrical double-layer forces, Atomic force microscopy, 301

Electron binding energy, 389

Electron detectors, electron microprobe, 341

Electron microprobe, 335–363

- composition analysis, chemicals in soil, 362
- composition, Iron-Manganese minerals, 357–363
- data sets, types of, 355–356, 358, 363
- description of, 339–347
- development of, 335
- EDS, WDS spectrometry, quantitative analysis and, 351–353
- electron beam and matter interaction, 335–346
- elemental distribution map, 362
- experimental modification studies, 357
- geochemical analysis of minor elements, 357
- optics system, 339–340
- qualitative analysis, 347–348
- quantitative analysis, 348–353, 363
- sample requirements and preparation, 353–355
- scanning electron microscope and, 335
- in situ cation exchange of alteration minerals, 357
- soil studies, 356–363
- spectra, Iron-Manganese concretion, 359–360
- vacuum systems, 346
- weathering, compositional change studies, 356–357

Electron penetration, SEM, 285

Electron probe microanalyzer, 269. *see* Electron microprobe

Electron transitions, 390

Electron yield detectors, 407–408

Electroneutrality principle, 466–467

Electrons, 336–337

Electrostatic forces, Atomic force microscopy, 300

Elemental distribution map, 362

Elemental reference standards, 350

Embedded samples, from hydrated pastes and gels, 249–251

Emission volumes, SEM, 284

Endellite, 140

Energy dispersive X-ray spectrometry, 269, 281–282, 342–343

Energy reference parameter, 426

Energy scales, aligning spectra, EXAFS, 445–447, 445–447

Energy shift parameter, XANES, 418

Enstatite, 98, 179

Environmental scanning electron microscope, 269–270

Epidote, 185–186

EPMA. *see* Electron microprobe

Epoxy resins, sample preparation and, 203, 205–207

Epsomite, d-spacings, 98

Equations

EXAFS, 396–399

intensity ratio method, XRD, 104

internal standard method, XRD, 105

Kubelka-Munk function, 372–373

Lambert Absorption Law, 368

phyllosilicate minerals, ideal composition formula, 466

Scherrer, crystallite size, XRD, 102

stereological relationships, 215–216

X-ray, photon, wavelength relationship, 338

Eukaryotic cells, linkage chemistry, 309–311

Evolved gas, thermal analysis, applications, 155–156

EXAFS

see also X-ray absorption spectroscopy

background functions, 437–441

comparing models, statistics and, 444–445

data analysis programs, examples, 452

data processing, 423–426

data reporting, 458–459

fitting, building models, 441–445

modeling, 445–458

parameterizing variables, 443–444

random noise in spectra, 436–437

second shell atom type with sediments, determining, 451–455

sediment data series, modeling, 455–456

spectra, 394–399

Experimental modification studies, electron microprobe, 357

Extractants, fractionation and, 67–71

FEFF analysis program, 441–443, 452

Feldspars

atomic force microscopy imaging, 306

d-spacings, 98

EDS and, 292

K, EDS data, 282

K, weathering, 294

optical properties of, 174–175

Ferrihydrite, 47, 63–65

Fertilizers, impact on TEM, 239–240

Field observations, sampling, 6–7

Field-dissolved samples, AFM, 322

Fitting range, XAS, 417

Fixation of index ions, clay mineral, 497–498

Flocculation, clay, 27–28

- Flow-through proportional ionization detector, 346
- Fluorapatite, d-spacings, 98
- Fluorescence
 - correction, 350–351
 - detectors, X-ray absorption spectroscopy, 406–407
 - mode samples, X-ray absorption spectroscopy, 410
 - resonance energy transfer, AFM, 314
 - sample geometry and detector, 391
 - X-rays, 291, 337
- Focus, scanning electron microscopy and, 288
- Force components, AFM, 311–316
- Force measurements, AFM, 311–314
- Force microscopy, 326–327
- Force-distance curve, AFM, 327
- Formwar, 248
- Fourier transformations
 - basics, 426–429
 - comparison of, 438
 - data range, determining, 434–436
 - information content of, 429
 - k* weights, 432–434
 - parts of, 429–431
 - windows, 431–432
- Fractionation, 22–29, 65–71
- Free oxides, 47
- Freeze-drying, 29–30, 201–202
- Friable soils, samples, micromorphology, 197–198
- FULLPAT program, 106–107
- Functionalization, tips, atomic force microscopy, 309–311
- Furnaces, thermal analysis, 120, 122, 136–139
- Gains ripples, atomic force microscopy, 307–308
- GALOPER program, 105–106
- Garnets, optical properties of, 183–184
- Geochemical analysis, minor elements, electron microprobe, 357
- Geochemical context, TEM, 238
- Geomorphic documentation, 6–7
- Gibbsite, 63–65, 98–99, 140–141, 149–150, 475
- Glass bead, linkage chemistry, 309–311
- Glass slide mounts, 88–89
- Glitch removal, EXAF, 423–424
- Global data, micromorphology, 214–215
- Glutaraldehyde, 248
- Glycerol solvation, XRD, 92–93
- Goethite, 63–65
 - d-spacings, 98–99
 - DTA or DSC temperature regions and reactions, 141
 - elemental oxide composition and structural formula, 475
 - optical properties of, 187
 - quantification, thermal analysis, 149–150
 - TG weight loss regions, 140
- Gold, EDS patterns and, 271
- Grain counts, petrographic microscope techniques, 170–172
- Grain morphologies, EDS and, 290
- Granular microstructure, 212
- Graphical extrapolated onset and return technique, 144–148
- Gravitational settling, sand, 28–29
- Grids, selecting, TEM, 246–247
- Ground water NOM, atomic force microscopy, 320
- Groundmass material, 212
- Gypsum, 99
 - analysis techniques, 17–18
 - d spacings, major and diagnostic, 98–99
 - dissolution techniques, 35–36
 - DTA or DSC temperature regions and reactions, 141
 - quantification, thermal analysis, 152
 - removal from samples, 15–16
 - testing for, 14–15
 - TG weight loss regions, 140
- Halides, dissolution techniques, 34–36
- Halloysite, 65, 99, 101, 140–141, 150–151
- Harmonic rejection, X-ray absorption spectroscopy, 403–405
- Hausmannite, 63–65
- Hazardous chemical designations, 248
- H-bonding forces, Atomic force microscopy, 300
- Heat, electron microprobe and, 337
- Heating rate, thermal analysis, 138–139
- Hectorite, 140
- Hedley fractionation scheme, 66–67
- Height mode TMAFM imaging, 306
- Hematite, 63–65, 98, 187
- Heulandite, d-spacings, 98
- High soluble salt samples, XRD, 90–91
- Hooke's law, force component AFM, 311–313
- Hornblende, 98, 181, 294
- Human activity, transmission electron microscopy sampling, 238
- Hydrated oxides, 47
- Hydraulic conductivity, micromorphology, 193
- Hydrogen peroxide, organic matter, removing from samples, 18–21, 45–46
- Hydrolysis, clay minerals, 498
- Hydrophobic forces, Atomic force microscopy, 300
- Hydrous oxides, treatment reagents, 63–65
- Hydroxides, 47
- Hydroxy-interlayers, 63–65, 99–101
- Hydroxylamine hydrochloride, manganese, 54–57
- Hysteresis, AFM, 315
- IFEFFIT analysis program, 452
- Illite, 475
- Ilmenite, 98, 185, 187–188

- Image acquisition, micromorphology, 230
- Image analysis. *see* Digital image analysis
- Imaging methods, scanning electron microscopy, 278–283
- Imogolite, 47, 63–65
- Impregnation, electron microprobe samples, 354
- Inelastic collisions, SEM, 280
- Infillings, 213
- Intensity ratio method, XRD, 104
- Interactions, electron beams, 335–337
- Interference colors, 165
- Interferences, WDS, 349
- Interlayer cation charge, calculations, clay minerals, 496
- Intermolecular forces, AFM, 300–302, 309–316
- Internal standard method, XRD quantification, 105
- Interstratification, XRD patterns and, 101–102
- Intramolecular forces, AFM, 300–302, 309–316
- Ion exchange, mineral studies TEM, 245–246
- Ionization detector, flow-through proportional, 346
- Ionization detector, X-ray absorption spectroscopy, 405–406
- Iridium, EDS patterns and, 271
- Iron
- air dying samples and, 10
 - AOD treatment, 50–54
 - BSE image, 359, 361
 - CBD procedure, 59–65
 - EXAFS signals, stacked theoretical calculations, 397
 - hydroxylamine hydrochloride treatment, 56–57
 - pyrophosphate extraction, 48–50
 - treatment reagents, Al, Fe, Mn, Si dissolution, 63–65
- Iron nodules, acid mine drainage and, 239
- Iron oxides, 21–22, 322
- Iron sulfide, oxidation of, 42
- Iron-Manganese concretion, electron microprobe spectra, 359–360, 359–360
- Isoamyl acetate, 248
- Isotropic minerals, 163
- Isotropic optical properties, 166
- Jarosite, 98, 140
- Johannson focal point, count rates, 344
- Johannson spectrometer design, 344
- Kaoline vermiforms, secondary electron imaging, 281
- Kaolinite, 63–65
- d-spacings, 98–99
 - DTA or DSC temperature regions and reactions, 141
 - EDS and, 292
 - elemental oxide composition and structural formula, 475
 - optical properties of, 178–179
 - quantification, thermal analysis, 150–151
 - structural formula calculation, 469
 - TG weight loss regions, 140
- K-feldspar, 282, 294
- Kinetic characteristics, thermal analysis, 152–154
- Kubelka-Munk theory, 369–373, 379
- Kubiena tins, 197–198
- Labradorite, optical properties of, 175
- Lambert Absorption Law, 368
- Lateral frictional forces, Atomic force microscopy, 302
- Latex beads, linkage chemistry, atomic force microscopy tips, 309–311
- Lattice fringe images, TEM, 260–261
- Layer charge, clay minerals and, 486–496
- Lead citrate, 248
- Lechatelierite, optical properties, 173–174
- Lennard-Jones potential energy function, 300–301
- Lepidocrocite, 63–65
- Light, cross- and plane-polarized, 163–167
- Linear combination fittings, 416–417
- Liner combination fitting, XANES, 418–421
- Linkage chemistry, atomic force microscopy tips, 309–311
- Lithiophorite, 63–65
- Lithium fluoride, WDS, 345
- London dispersion forces, Atomic force microscopy, 300
- Low-salt samples, mounting for XRD, 85–90
- LRWhite medium, hard, 248
- LRWhite resin, 253
- Macromorphology, soil, 7–8
- Maghemite, 63–65
- Magnesite, 140–141
- Magnesium, 10, 351
- Magnesium-Ammonium method, CED, AEC, 501–502
- Magnetite, optical properties, 185, 187–188
- Manganese
- AOD treatment, 50–54
 - BSE image, 359, 361
 - CBD procedure, 59–65
 - dissolution, hydroxylamine hydrochloride, 54–57
 - hydroxylamine hydrochloride treatment, 56–57
- Manganite, 63–65
- Marcasite, d-spacings, 98
- Massive microstructure, 212
- Maximum potential acidity, 42
- Membrane filter wet mount, XRD, 89–90
- Membrane supports, TEM, 247
- Metals
- chemical extractants and reaction mechanisms, 69
 - composition, electron microprobe analysis, 357–363
 - sequential extraction schemes in soil, sediment, environmental samples, 70
- Metamorphic aluminosilicates, optical properties, 180, 182–183

- Methacrylate, sample preparation and, 203
- Methanol, 248
- Methodology. *see* Procedures
- Mg-smectite, TG weight loss regions, 140
- Mg-smectite vermiculite, temperature regions and reactions, 141
- Mg-vermiculite, 140–141
- Micas, 98, 101, 140, 345
- Michel-Levy chart, interference colors, 165–166
- Microbes, linkage chemistry, AFM tips, 309–311
- Microbial mediated mineral dissolution, AFM, 321–323
- Microcline, 98, 175
- Micromass material, 212–213
- Micromorphology, 191–220
 - see also* Digital image analysis
 - air and oven-drying samples, 200
 - applications, 191–194
 - biological studies, 193
 - cohesive and loose material sampling techniques, 199
 - computer image analysis, 219–220
 - cutting and lapping samples, 207–209
 - defined, 191, 227
 - description procedures, 214
 - dyes and stains, 209–210
 - impregnating resins, thin sample preparation, 202–207
 - lighting and imaging systems, building, 229–230
 - measurement techniques, quantification, 216–219
 - multiscale analysis, 228
 - point counting, 216–217
 - sample orientation, 196
 - sample size, 195
 - samples, replicates, 196
 - sampling, 194–199
 - soil block preparation, 228–229
 - soil structure and, 192
 - thin sections, 199–210, 228–229
 - water removal techniques, 200–202
- Microscope, petrographic, 162–167
- Microstructure, describing, 211
- Microstructure studies, clay size sampling and, 238–240
- Microstructure types, 212
- MIF, 103–104
- Mineral characterization programs, XRD, 105–106
- Mineral intensity factor, 103–104
- Mineral quantification programs, XRD, 106–107
- Mineral shards, atomic force microscopy tips, 309–311
- Mobile colloidal fractions, sampling, 242–244
- Models, building, EXAFS fitting, 441–445
- Modular sample holders, X-ray absorption spectroscopy, 412–413
- Monazite, optical properties of, 186
- Monochromators, 403, 405
- Montmorillonite, 98, 469, 473, 475
- Morphology, particle, 292–296
- MPA. *see* Maximum potential acidity
- Muck fulvic acid, atomic force microscopy, 320
- MUDMASTER program, 105–106
- Multiple scattering, 368
- Multiple tips, atomic force microscopy, 307
- Munsell soil color charts, 367
- Muscovite, 98, 178–179, 292, 475
- Nanotubes, linkage chemistry, AFM, tips, 309–311
- Natural organic matter, AFM, 319–321
- Net potential acidity, 42
- Neutralization potential, 42
- NEWMOD program, 106–108
- Nickel, 271
- Nickel oxide, XAS spectra, 392
- Noncontact-mode atomic force microscopy, 302–303
- Noncrystalline materials, 47
- Normalization, XANES spectra, 414–416
- NPA. *see* Net potential acidity
- Nucleic acid, linkage chemistry, AFM tips, 309–311
- Nutrient testing, composite sampling methods, 5–6
- Octahedral charge calculations, clay minerals, 496
- Oligoclase, 175
- Olivine, 178–179
- Olivine, ZAF correction, 351
- Opal, 172–173
- Opaline Si, 63–65
- Operating distance, SEM, 286
- Optical interference, AFM, 315
- Optical microscope, electron microprobe, 340–341
- Optical scans, electron microprobe, 359
- Optical settings, transmission electron microscopy, 262
- Organic colloids, waste water, 243
- Organic matter
 - fixation, TEM, 251–252
 - removing from samples, 18–21, 44–47
 - sorbed, AFM, 319–321
 - staining, TEM, 256–259
 - transmission electron microscopy and, 236
- Organic monolayer, linkage chemistry, AFM tips, 309–311
- Organic soils, sampling, 9
- Oriented past mounts, XRD, 90
- Oriented powder sample mount, 88
- Oriented samples, dilute suspensions, TEM, 247–249
- Oriented suspension mounts, 88
- Oriented X-ray diffraction pattern models, 107–108
- Orthoclase, 98, 175, 475
- Osmium, staining, TEM, 256
- Osmium tetroxide, 248

- Oven-dry weight, clay minerals, 498–499
- Overvoltage, EDS, effects, 291
- Oxidation reactions, thermogravimetry samples, 120–121
- Oxides, 47, 98, 474–475
- Oxygen, EXAFS signals, stacked theoretical calculations, 397
- Oxyhydroxides, 47

- P fractions schemes, 66–67
- Palladium, EDS patterns and, 271
- Palygorskite, d-spacings, 98
- Parent materials, documentation of, 7
- Particle morphology, EDS, 292–296
- Particle single scattering albedo, 371
- Particle-size, 22–29, 86, 410–411
- Pb-stearate, WDS diffraction, 345
- Peak fitting, XANES, 422
- Pedofeatures, 213, 230–231, 273–274
- Pedon description, sampling and, 7–9
- Pentaerythritol, WDS, 345
- Permanent charge, 485
- Permanent mounts, petrographic microscope techniques, 168–169
- Permits, site sampling and, 3
- Perthitic microcline particle, weathering, EDS, 294
- Petrographic microscope techniques, 161–189
 - grain counts, 170–172
 - heavy mineral separation, 168
 - microscope, about, 162–167
 - mineral descriptions, 172–189
 - particle size, 188–189
 - permanent mounts, 168–169
 - procedures, 167–170
 - sample preparation, 167–168
 - slide preparation, 168
 - technological advances, 189
 - XRD and, 168
- Phase imaging, 304–306, 317–319
- Phosphate buffer, 248
- Phosphates, 98, 141
- Phosphorus
 - chemical extractions, 66–67
 - electron microprobe analysis of sediment, 357
 - EXAFS signals, stacked theoretical calculations, 397
 - extractants for sequential fractionation, 67
 - fractionation of, 65–71
 - fractionation schemes, soil and sediment, 66, 68
 - quantification, thermal analysis, 152
- Photodiode detector, atomic force microscopy, 299–300
- Photodiode shift voltage, AFM, 312–313
- Photons, 389
- Phyllosilicate minerals, structural composition formulae, 466
- Phyllosilicates
 - discriminating among, XRD, 99–101
 - expansible, 91
 - expansible, d-spacings, 98
 - interstratification, XRD identification, 101–102
 - optical properties of, 176
 - particle morphology, 293
- Piezoelectric scanner, atomic force microscopy, 299–300
- Pixels, scanning electron microscopy beam size, 286–287
- Plagioclase feldspars, optical properties, 174–175
- Plagioclase, weathering, EDS, 294
- Plane-polarized light, minerals and, 163–164
- Platinum, EDS patterns and, 271
- Platy microstructure, 212
- PMT. *see* Petrographic microscope techniques
- Point counting, micromorphology, 216–217
- Polyester resins, 202–207
- Polymerization, 253
- Polysaccharide, linkage chemistry, AFM tips, 309–311
- Polysaccharide, staining with, 258
- Polyvinyl formal, 248
- Poorly crystalline materials, 47
- Population, means and estimates, sampling, 5
- Pore studies, 219–220
- Positive relief, 164
- Potassium acid phthalate, WDS diffraction, 345
- Potassium, calculations, clay-sized mica, 67
- Potassium feldspars, optical properties, 174–175
- Potential per-oxide acidity, 42–43
- PPA. *see* Potential per-oxide acidity
- Preservation, soil samples, 9–11
- Pressed powder pellets, electron microprobe samples, 354–355
- Probes, soil sampling and, 5
- Procedures
 - reflectance spectra, diffuse reflectance spectroscopy, 376–377
 - alkylammonium chloride solutions, 490–491
 - alkylammonium hydrochloride crystals, 491–493
 - alkylammonium-saturated clay samples, 493–494
 - AOD treatment, 52–53
 - carbonates, dissolution techniques, 36–41
 - carbonates, testing for and removing, 16–17
 - cation saturation, XRD, 91
 - CBD procedure, 61–62
 - ceramic tile mounts, glycerol solvation, 92–93
 - chemical characterization sequence, electron microprobe, 355–356
 - clay, particle-size fractionation, 25–29
 - clay suspension, freeze-drying, 29–30
 - compulsive exchange method, CEC, AEC, 503–506

Procedures, *continued*

- cutting and lapping samples, micromorphology thin samples, 207–209
- description, micromorphology samples, 214
- differential X-ray diffraction, sample mounting, 110–111
- epoxy resin impregnation, micromorphology thin samples, 205–207
- Fe, removal, hydroxylamine hydrochloride technique, 56–57
- fixed negative charge, clay mineral allocation, 467–468
- freeze-drying suspension, clay, 29–30
- gypsum, testing for, 14–15
- halides, dissolution techniques, 34–36
- homoionic clay, dry mounting, 92
- impregnating resins, micromorphology thin sections, 202–207
- iron oxide removal from samples, 21–22
- Iron, removal, 56–57
- lighting and imaging systems, micromorphology, 229–230
- magnesium-ammonium method, CED, AEC, clay minerals, 501–502
- membrane filter mounts, glycerol solvation, 93
- membrane filter mounts, XRD, 90
- mineral identification, XRD, 97–99
- Mn, removal, hydroxylamine hydrochloride technique, 56–57
- NEWMOD model, oriented X-ray diffraction patterns, 107–108
- organic matter, removal with hydrogen peroxide, 18–21
- organic matter, removal with sodium hypochlorite, 20–21, 46–47
- particle-size fractionation, 25–29
- petrographic microscope techniques, 167–170
- point counting, micromorphology, 216–217
- pyrophosphate extraction, 49–50
- quantification, micromorphology, 214–220
- reduced charge semectites, clay minerals, 506–507
- reducible Mn, 56–57
- short-range-order aluminosilicate materials from samples, 22
- Si, removal, hydroxylamine hydrochloride technique, 56–57
- sodium acetate, CEC, AEC, clay minerals, 499–501
- sodium saturation, sand and clay, 27
- soil block, micromorphology, 228–229
- soil sampling, 8–9
- thin sections, micromorphology, 228–229
- tiron and alkaline extractants, 58–59
- water removal, micromorphology samples, 200–202
- Propylene oxide, 248
- Proteins, linkage chemistry, AFM tips, 309–311
- Pseudocrystals, WDS, 345

- Purge gas, thermal analysis, 137
- Pyrite, 98, 140, 187
- Pyrolusite, 63–65
- Pyrophosphate extraction, 48–50
- Pyroxenes, d-spacings, 98, 178
- Qualitative analysis, electron microprobe, 347–348
- Quantification, micromorphology and, 214–220
- Quantitative analysis, electron microprobe, 348–353
- Quartz
 - d-spacings, 98
 - DTA or DSC temperature regions and reactions, 141
 - EDS imaging, 284
 - elemental oxide composition and structural formula, 475
 - kinetic plots, differential scanning, 153
 - optical properties of, 172–173
 - quantification, thermal analysis, 151–152
 - surface structure categories, 294–296
 - WDS diffraction, 345
- Radial distribution function, EXAFS, 428
- Rastered beams, scanning electron microscope, 278
- Rayleigh scattering, 389
- Rebinning, EXAF, 423
- Reduced samples, TEM, 254–255
- Reflectance, 367
- Reflectance spectra, DRS, 376–380, 376–380
- Reflected light, petrographic microscope techniques, 171
- Refractive index, 163
- Regular reflectance, 367
- Relative humidity, XRD and, 96–97
- Relaxation processes, 390
- Relief, refractive index, 164
- Resins, sample preparation and, 202–207
- Resistant minerals (weathering), 162
- Rh harmonic rejection mirror, 405
- Riebeckite, d-spacings, 98
- Rietveld method, XRD, 107
- Road cuts, sampling methods and, 4–5
- Rock fragments, optical properties of, 188
- ROCKJOCK program, 106–107
- Rubidium acid phthalate, WDS diffraction, 345
- Runoff, documentation of, 7
- Rutile, 98, 183–184
- Safety, chemicals and, 248
- Salts, soluble, 14–18
- Sample coating, 270–272
- Sample mounting, XRD , low salt, 85–90
- Sample preparation
 - coating, scanning electron microscopy, 270–276
 - diffuse reflectance spectroscopy, 375–376
 - electron microprobe, 353–355

Sample preparation, *continued*

- petrographic microscope techniques, 167–168
- scanning electron microscopy, 270–276
- thin sections, micromorphology, 199–209
- X-ray absorption spectroscopy, 408–414

Sample stage, electron microprobe, 341

Sampling

- archiving and, 10–11
- burrowing animals and, 8–9
- collection, 6–9
- composite methods, 5–6
- disturbance during, 4–5
- initial preparation of, 10–11
- micromorphology, 194–199
- mobile colloidal fractions, TEM, 242–244
- number of, 6
- objectives, 1–2
- preservation of, 9–11
- procedures and equipment, 8–9
- purpose of, 1–3
- representative pedons, 6–9
- site selection, 2–3
- soils, 1–11
- strategies, 3–6
- thermal analysis, preparation, 132–135
- transmission electron microscopy, 237–244
- transporting methods, 9–10
- types of, 4–6
- variability, 2

Sand, removal from samples, 28–29

Sanidine, optical properties, 175

Scaling factors, constraints, XANES, 418

Scan parameters, X-ray absorption spectroscopy, 408

Scanning electron microscopy, 269–297

- see also* EDS; X-rays
- about, 269–270
- beam size, 286–287
- beam voltage, 284–286
- EDS interference and, 271–272
- electron microprobe and, 335
- image resolution, 283
- imaging methods, 278–283
- low concentration detection, 275
- mineral identification, 289–296
- operating distance, 286
- operating parameters for, 276–287
- sample examination, 287–289
- sample preparation, 270–276
- schematic diagram, 277
- secondary electron images, 280–281
- single mineral grains, examination, 272–273
- staining and, 275–276
- thin sections and polished blocks, 274–275
- X-ray signal capture, 281–284

Scattering, 368, 389, 395–396

SCD, 33–34, 33–34

Schematic diagrams

- alkylammonium cations, 488
- Argonne National Laboratory, 401
- atom, 83
- atomic force microscope, 300
- backscattered electron detector, electron microprobe, 342
- beamline optical enclosure, 402
- Bohr model of an atom, 388
- capillary forces, atomic force microscopy, 301
- cross section, powder layer, 369
- differential scanning calorimetry, 125
- differential thermal analysis, 122
- dry powder sample mounting, XRD, 88
- electron microprobe, 339
- electron shell and important X-ray lines, 338
- Everhart-Thomley secondary electron detector, 341
- EXAFS experimental setup, 391
- flow-through proportional ionization detector, 346
- force curve, 312
- force-distance curve, AFM, 312–313
- illumination geometries, diffuse reflectance, 374
- Johannson spectrometer, 344
- petrographic microscope, 163
- phase imaging TMAFM, 305
- scanning electron microscope, 277
- secondary electron imaging, SEM, 280
- Si(Li) energy-dispersive spectrometry detector, 343
- TG-MS combination analysis, 131
- thermogravimetry, 119
- thermomechanical analysis, 127
- tip size and shape, atomic force microscopy, 306–307
- X-ray diffractometer, 85
- X-ray tube, 82
- XRD by regularly spaced planes of atoms in a crystal, 83

Scherrer equation, 102

SE. *see* Secondary electrons, electron microprobe

Secondary electron imaging, SEM, 280–281, 285

Secondary electrons, electron microprobe, 336

Second-derivative minima, 379, 381

Sediments, EXAFS data modeling, 455–456

Semectites, 148–149, 242

SEM-EDS, analysis of soluble salts, gypsum, and carbonates, 17–18

Separation components, AFM, 314

Sepiolite, d-spacings, 98

Sequential extraction schemes, metals in soil, sediment, environmental samples, 70

Serpentines, 98, 178

Settling times, specific gravity and, 24

Shadowing artifact, atomic force microscopy, 307

- Shell, atoms, 395
- Short-range order materials, 47
- Short-range-order aluminosilicate materials, removal, 22
- Siderite, 140, 181
- Si(Li) energy-dispersive spectrometry detector, 342–343
- Silica, morphologies, EDS, 295
- Silicon, hydroxylamine hydrochloride treatment, 56–57
- Silt, removal, 28–29
- Silver, 271
- Single grain microstructure, 212, 272–273
- Slits, X-ray absorption spectroscopy, 403
- Smear mounts, 90
- Smectites, 99–101
- Sodium acetate method, CEC, AEC, 499–501
- Sodium carbonate, TG weight loss regions, 140
- Sodium hypochlorite, organic matter, removing, 20–21, 46–47
- Soils
 - chemical characterization analysis, 362
 - clods, sampling and, 9
 - describing thin sections, micromorphology, 210–214
 - heterogeneity, transmission electron microscopy and, 235
 - macromorphology, 7–8
 - management, impacts on clay fractions, 240
 - modification in structures, sampling and, 4–5
 - organic, sampling, 9
 - pits, sampling methods, 4
 - sampling, 1–11
 - sequential extraction schemes, 70
 - structures, describing, 211
 - structures, micromorphology and, 192
 - ultrastructural organization, 240–242
- Soluble salts, 14–18
- Solvation forces, atomic force microscopy, 300
- Solvent exchange and embedding technique, TEM, 251–252
- Sorbed natural organic matter, AFM, 319–321
- Spacial context, TEM, 237
- Specific charge density, 485
- Sphene, optical properties, 183–185
- Spurr resin, 248, 253
- Stacked Cr K-edges, 394
- Stannic oxide, EXAFS and, 446–451
- Stereological relationships, micromorphology, 215–216
- Stereology, 215
- Stern-Heald detector, 407
- Stilbite, optical properties, 186
- Stoke's law, particle settling within liquid, 23–25
- Stream fulvic acid, atomic force microscopy imaging, 320
- Strengite, 98, 140
- Stress, clay water content, TEM, 245
- Structural allocation, clay mineral elemental components, 465–481
- Structural crusts, micromorphology, 192
- Structural formula method, layer charge in clay minerals, 495–496
- Structural formulas
 - 2:1 hydroxy-interlayered minerals, 470–472
 - admixtures, effect on calculation, 476–477
 - dioctahedral chlorite minerals, 471–472
 - fixed cation occupancy method, 472–473
 - fixed negative charge method, 467–468
 - Kaolinite, Georgia, 468–469
 - Montmorillonite, Clay Spur Wyoming, 468–469
 - nonmonomineralic samples, 473–475
 - water content excluded, 468–470
 - water content included with other Anions, 469–470
- Subangular blocky microstructure, 212
- Sulfates, 34–36, 98, 180, 182
- Sulfides, 41–44, 98
- Surface charge density, 485–486
- Suspension mounts, 88
- TA. *see* Thermal analysis
- Talc, 140, 176–177
- Tantalum, 271
- TappingMode atomic force microscopy, 303–304, 306, 316–319
- TEM. *see* Transmission electron microscopy
- Temperature, thermogravimetry samples, 120
- Temporary mounts, petrographic microscope techniques, 169–170
- Tetrahedral charge calculations, clay minerals, 496
- Texture, transmission electron microscopy, 260
- TFD4 solution, 248
- TG. *see* Thermogravimetry
- TG-DSC techniques, 128–129
- TG-DTA, 128–129
- TG-DTG analysis, 128–129
- TG-EGA techniques, 130–132
- TG-FTIR combination analysis, 131–131-2
- TG-MS combination analysis, 130
- Thallium acid phthalate, WDS, 345
- Thermal analysis, 117–155
 - classification, methods, 117–155
 - combined techniques, 128–132
 - data processing, 139
 - differential scanning calorimetry, 124–126
 - differential thermal analysis, 121–124
 - evolved gas analysis, applications, 155–156
 - gravimetric analysis, 140–143
 - heating rate, effects, 138–139
 - instrumentation principles, 119–132
 - kinetic characteristics, 152–154

- Thermal analysis, *continued*
- lesser techniques, 132
 - mineral reactions, 118–119
 - operating conditions, 136–139
 - qualitative interpretations, 139–144
 - quantitative interpretations, 144–156
 - sample preparation, 132–135
 - sample size and packing, 135–136
 - TG and DSC patterns, calculations, 144–148
 - thermogravimetry, 119–121
 - thermogravimetry samples, 127–128
 - thermomechanical analysis, 126–127
 - thermomechanical analysis applications, 154–155
- Thermal gravimetric analysis, 140–148
- Thermal optical analysis, 127–129
- Thermal reactions, 118–119
- Thermogravimetry, 119–121, 140
- Thermomechanical analysis, 126–127, 154–155
- Thermo-optical analysis, 127–128
- Thickness effects, X-ray absorption spectroscopy, 410
- Thin sections, scanning electron microscopy, 274–275
- Tin oxide, EXAFS data, 446
- Tin oxide, EXAFS modeling, 448–451
- Tip size and shape, atomic force microscopy, 306–307
- Tips, Atomic force microscopy, 306–311
- Tiron extractants, 57–59
- Titanium, AOD treatment, 50–54
- TMAFM. *see* TappingMode atomic force microscopy
- Topographic quadrangles, documentation of, 6
- Total Potential Acidity, dissolution techniques, 43–44
- Trace metals, fractionation „, sequential extraction, 65–71
- Transmission electron microscopy, 235–264
- agarose coating procedure, 251
 - applications, 260–261
 - cameras and, 263
 - embedded samples, hydrated pastes and gels, 249–251
 - image formation, 259
 - mobile colloidal fractions, sampling, 242–244
 - observations, 259–264
 - operating conditions, 261–263
 - organic matter fixation, 251–252
 - oriented samples, dilute suspensions, 247–249
 - reduced samples, 254–255
 - reduced samples, conditions for, 254–255
 - sample supports, 246–247
 - sampling, 237–244
 - soil heterogeneity and, 235
 - specimen preparation, 244–259
 - staining organic components, 256–259
 - ultrathin sectioning techniques, 255
- Transmission mode samples, X-ray absorption spectroscopy, 409–410
- Tremolite, d-spacings, 98
- Tremolite-actinolite, 181
- Trenches, sampling methods, 4
- Tridymite, optical properties, 172–173
- Trioctahedral mica, diagnostic d-spacings, 99
- Truncating, EXAF, 424–425
- Tube sampling methods, 5
- Twinning, petrographic microscope techniques, 171
- Types of sampling, 4–6
- Ultrastructural organization in soils, 240–242
- Ultrathin sectioning, TEM, 255
- Uranium, EXAFS signals, stacked theoretical calculations, 397
- Uranyl acetate, 248
- Vacuum systems, electron microprobe, 346
- Van der Waals forces, Atomic force microscopy, 300–301
- Variability, soils and, 2
- Variable deflection mode, 302
- Variscite, d-spacings, 98
- VdW force, 302–303
- Vegetation, documentation of, 7
- Vermiculites, 98–101, 148–149, 475
- Viscoelastic properties, AFM, 315–316
- Visible light, electron microprobe and, 337
- Voids, describing, 211–212
- Volcanic glass, optical properties, 173–174
- Volume reflection. *see* diffuse reflectance
- Volume single-scattering albedo, 371
- Water content, TEM, 236
- Water, documentation, sampling and, 7
- Water potential equilibration, 249–251
- Water-soluble minerals, dissolution techniques, 34–36
- Wavelength-dispersive spectrometer, 335, 342–343, 348–349
- Wavellite, d-spacings, 98
- Weatherable minerals, 162
- Weathering, 293–294, 356–357
- Wet mounting techniques, 88–90
- Wet samples, TEM, 245
- Wet sieving, sand and silt separation, 29
- White X-rays, 281
- Working distance, SEM, 286–287
- XANES. *see* X-ray absorption spectroscopy
- XAS. *see* X-ray absorption spectroscopy
- X-ray absorption spectroscopy, 387–459. *see also* X-rays
- absorption edges, 392–393
 - absorption measurements, 399–414
 - component analysis, XANES, 421–422
 - data analysis, 414–423
 - detectors, 405–408

- X-ray absorption spectroscopy, *continued*
edge energy and normalization, 424–425
EXAF spectra, 394–399
EXAFS background functions, 437–441
EXAFS experimental setup, 391
EXAFS fitting, building models, 441–445
fine structure data analysis, 423–458
Fourier transformations, 426–441
principles, 388–399
project designs, 399–400
sample mounting, 413–414
sample preparation, 408–414
sample preservation, 409
scan parameters, 408
spectra, 393–394
synchrotron radiation facilities, 400–402
Xanes data analysis, 414–423
- X-ray diffraction. *see* XRD
- X-ray powder diffraction analysis, 84
- X-rays
absorption, 291, 389–393
absorption coefficient, 390–392
absorption process, 394–395
characteristic, 337
constructive interference, 343
continuous, 337
detectors, limitations, SEM, 282
diffraction crystals by atomic numbers, 345
diffractometers, 93–94
discrimination, 342–343
edge energies, 392–393
electron microprobe, 337–339
fluorescence, 291, 388, 390
fluorescent, 337
generation, 82, 337–339
image resolution and, SEM, 283
ion particles, 283
maps, 281
nomenclature, 337–339
photomicrographs, SEM, 283
photon, wavelength relationship, 338
scattering, 389
schematic, sealed-off filament, 82
signal capture, SEM, 281–284
silicon particles, 283
spectrometers, electron microprobe, 341–346
- XRD
alkylammonium saturated clay data, interpreting, 494–495
analysis of soluble salts, gypsum, and carbonates, 17–18
ceramic tile wet mount, 89
computer diffraction modeling, 105–108
crystallite size and, 102
differential scanning calorimetry and, 129
differential X-ray diffraction, principles and applications, 108–112
diffraction data, nature of, 95
diffraction patterns, soil clays, 96
diffractometers, 93–94
dry mounting techniques, 87
glass slide wet mounts, 88–89
high-soluble salt samples, 90–91
intensity ratio method, 104
internal standard method, 105
interstratification, patterns and, 101–102
membrane filter wet mount, 89–90
mineral characterization programs, 105–106
mineral identification from, 95–102
mineral intensity factor, 103–104
mineral quantification programs, 106–107
occurrence of, 82–83
oriented past (smear) mounts, 90
oriented powder sample mount, 88
oriented suspension mounts, 88
petrographic microscope techniques, 168
principles of, 82–84
quantification, limits and approaches, 102–105
relative humidity and, 96–97
Rietveld method, 107
sample examinations, 93–95
sample preparation, 84–93
sample storage, 87
scans, conducting, 95
schematic, 85
soil mineral identification, 81–112
theoretical considerations, 103–104
wavelength and, 94
wet mounting techniques, 88–90
- ZAF correction factor, 350, 353
Zeolites, 98, 185–186
Zircon, optical properties, 183–184
Zoisite, optical properties, 185–186