

Thin-Foil SEM Analysis of Soil and Groundwater Colloids: Reducing Instrument and Operator Bias

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Scanning electron microscopy (SEM) combined with energy-dispersive X-ray spectrometry (EDS) has been widely used to characterize environmental colloids with little regard for instrumental limitations. Details associated with electron emission source, excitation voltage, and even sample-coating procedures are often omitted from publications because SEM techniques are generally considered routine. In the current study, the common analytical technique of depositing colloidal samples on polycarbonate filters (i.e., conventional analysis) and carbon coating for microanalysis was compared to a thin-foil mounting technique that enhances particle recognition and reduces the nonspecific background (i.e., Bremsstrahlung X-rays) that interferes with compositional analysis of submicron particles. Carbon-coated particle resolution was degraded compared to metal-coated samples, making it difficult to identify smaller particles or discern fine-grained aggregates during EDS analysis. The signal-to-noise ratio for the resulting EDS patterns was highly related to particle size, with smaller particles generating patterns dominated by Bremsstrahlung X-rays. For the thin-foil method, EDS patterns for smaller particles produced better signal-to-noise ratios that were independent of particle size. The ability to easily discriminate particles from the background and collect detailed EDS spectra with minimal analysis time makes this technique ideal for instrumental automation.

Introduction

Studies suggest that the migration of sparingly soluble contaminants, such as radionuclides and hydrophobic organics, may in part be controlled by mobile groundwater colloids (1, 2). The importance of such a vector depends not only on the concentration and mobility of the colloidal phase but also on the affinity of the contaminant for the colloidal phase relative to both the aqueous phase and the stationary soil or aquifer matrix. Information concerning the composition and mineralogy of the mobile phase is critical in developing a mechanistic understanding of the processes controlling generation and subsequent transport of mobile colloids in the field. For instance, processes such as clay dispersion due to the dissolution of Fe-oxide or carbonate cementing agents or the precipitation of secondary colloidal phases due to alterations in groundwater chemistry which are commonly cited to explain colloid behavior in the field are predicated on knowing the composition of the mobile

phase as it relates to the bulk geologic matrix. In addition, synchrotron X-ray fluorescence (SXRF) analysis of mobile colloids has revealed that contaminants may be associated with a specific mineral phase and not generally sorbed to the entire suspension (3), thus confirming the importance of discrete particle techniques for characterizing colloidal constituents.

The inherent bias of destructive analytical techniques, such as total dissolution, and certain noninvasive methods, such as dynamic light scattering, in favor of larger suspension components that may be artifacts of sampling is widely recognized (4–6). For example, the presence of a few large quartz grains may completely dominate the composition of a suspension derived from total dissolution of a nonfiltered groundwater sample but represent little reactive surface to which contaminants may be sorbed. In contrast, discrete particle techniques, such as energy-dispersive X-ray spectrometry (EDS) in the scanning electron microscope (SEM), offer the potential to correlate contaminant loads with a specific mineral fraction.

The SEM has been widely used to characterize the mineralogy, chemical composition, and even particle concentration of dilute colloidal suspensions generated in field (3, 7–13) or laboratory column studies (14–16). Typically, colloidal suspensions are deposited on polycarbonate filters immediately following sampling to reduce post-collection precipitation or aggregation artifacts. Filter sections are then mounted on SEM stubs and carbon or metal coated (Au/Pd) for compositional analysis and micrographic imaging, respectively. In most instances, characterization is limited to qualitative particle surveys describing the dominant particle size, morphology, and composition of “typical” colloids. More-involved analysis schemes developed for identifying clay minerals in the transmission electron microscope (TEM) based on EDS spectra (17) may be inappropriate for the SEM due to complications associated with the various sample-beam-substrate interactions. To overcome such limitations, some research groups have compared X-ray spectra for sample colloids to the spectra for various mineral standards of similar size and composition under the same analytical conditions to “calibrate” instrumental response (7, 15).

Despite the widespread use of SEM to study mobile colloids, little attention has been placed on the influence of the electron emission source and sample coating on instrument resolution. SEM is generally preferred over TEM because the resulting images can be easily interpreted without a thorough understanding of the theory behind the instrumentation, i.e., colloidal particles look like one expects. However, an understanding of the manner in which the electron beam interacts with the specimen is critical in properly interpreting SEM images and the resolution capabilities of X-ray microanalysis. As the name implies, images are generated in the SEM by rastering the focused electron probe across the surface of a specimen while simultaneously measuring various secondary- and backscattered-electron signals as a function of beam position. Elastic and inelastic scattering spread the incident electron beam within the sample yielding an “interaction volume”, the dimensions of which depend on the electron beam energy and the sample composition (i.e. atomic number), rather than the focused probe size. Various signals responsible for image generation and X-ray microanalysis, as well as several other spectroscopic techniques are generated at different depths within this interaction volume (Figure 1).

The energy of the electron beam has a strong influence on the relative size of the interaction volume, with the depth

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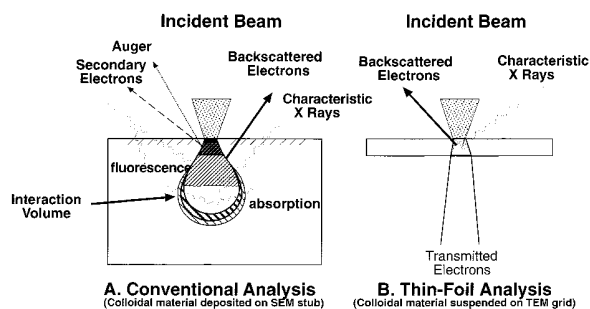


FIGURE 1. Electron/sample interactions for conventional (A) and thin-foil (B) sample mounting techniques.

and lateral dimensions of penetration increasing with increasing beam energy (18). When electrons of adequate energy strike the specimen, "characteristic" X-rays indicative of elemental composition (i.e. atomic number) are produced at intensities proportional to the mass concentration of the given element within a beam/specimen interaction volume. In a sense, fixing a colloid-coated filter to a SEM stub yields a sample of infinite thickness in which much of the X-ray signal may be generated within the substrate (Figure 1A). Lower excitation voltages are used to reduce beam penetration for high-resolution imaging. To generate the X-ray spectra, however, the electron beam voltage must be greater than the X-ray energy for the element of interest. Higher excitation energies, usually 15–20 keV, must be employed for X-ray microanalysis, which results in greater sample penetration. When performing X-ray microanalysis, the dimensions of the interaction volume ($\approx 1 \mu\text{m}^3$) often exceed those of the particle of interest, and energetic electrons can escape the bottom and sides of the particle regardless of the probe size (18, 19).

One method to increase spatial resolution during EDS analysis is to capture or eliminate X-rays generated by electrons passing through the specimen using an analysis architecture similar to the TEM (Figure 1B). Thin-foil analysis methods take advantage of such a configuration by decreasing substrate scattering associated with electrons passing through the specimen that contribute to the signal background. However, such methods have not been applied to the study of mobile groundwater colloids where resolution limits using conventional analysis techniques can severely bias the results. In the current study, various mineral standards, synthetic mineral analogues, and mobile colloids generated in a series of laboratory column studies described in Seaman et al. (15, 20, 21) were characterized using different sample coating and mounting procedures (conventional mounting vs thin-foil) to evaluate their effect on image resolution and X-ray microanalysis results in the SEM.

Materials and Methodology

Three synthetic goethites with varying degrees of Al-substitution (22), a reference kaolinite (Ga-2, Source Clay Minerals Repository), a commercial gibbsite (Alcoa Chemicals, Inc.), and colloids generated in a series of column studies described in Seaman et al. (15, 20) were analyzed by SEM combined with EDS, also known as energy-dispersive X-ray analysis (EDXA). The clay-size fraction ($< 2 \mu\text{m}$) from the bulk kaolinite and gibbsite materials was isolated for analysis by dispersing with Na_2CO_3 (pH 10) followed by centrifugation (23).

Conventional SEM-EDS Analysis. Dilute suspensions were deposited on $0.1 \mu\text{m}$ pore-size polycarbonate filters, and sections of the filters were secured to SEM stubs with carbon tape and then coated with either evaporated carbon or Au/Pd prior to X-ray microanalysis or imaging, respectively, to reduce sample charging in the beam. Imaging and

microanalysis were conducted using two instruments: (1) a Hitachi S 800 SEM equipped with a FE electron source and a Tracor 5500 EDS system, and (2) a JEOL JSM 6400 SEM (tungsten filament) equipped with a Noran Voyager EDS system. The carbon-coated filters were systematically scanned and EDS analysis was performed randomly on approximately 25 particles or particle aggregates per filter at an excitation voltage of 20 keV. This analytical procedure will be referred to as "conventional" SEM analysis. Micrograph images were taken of representative particles on the carbon-coated filters to illustrate resolution compared to metal-coated samples.

Thin-Foil Analysis. For thin-foil analysis, the filtered colloids were resuspended in DI water by sonication and deposited on TEM grids. The colloid-coated grids were then mounted on a special sample stage above a "deep hole" carbon block trap that captures transmitted electrons passing through the sample. This "TEM-like" configuration will be referred to as "thin-foil" analysis. EDS spectra for particles of various sizes were collected at an excitation voltage of 20 keV and a current of 1 nA using the JEOL JSM 6400 tungsten filament SEM described above. It is important to note that the particles were not coated with a conductive material prior to analysis. The same thin-foil sample mounts were also analyzed using the automated Noran Voyager EDS system. During automated analysis, backscattered electrons were used to identify particles based on the atomic-weight contrast from the low-molecular weight carbon substrate, i.e., TEM grid. After recognizing and defining the morphology (dimensions) of particles within a field of view, the automated system collected 5-s EDS spectra at an excitation voltage of 20 keV and a current of 1 nA.

Results and Discussion

Identification of clay-size minerals in the SEM based on morphology is difficult because soil clays and environmental colloids are often irregularly eroded, variable in composition, coated with other mineral or organic phases, and rarely resemble synthetic analogues or mineral standards (15, 17, 24, 25). Analytical parameters such as the electron emission source and the sample-coating procedures can affect resolution during imaging and X-ray microanalysis. The brightness of an electron source, current density per solid angle, is an important indicator of the ability to maintain current within a finely focused probe required for high resolution. Therefore, the brightness of various electron sources and resolving power decreases as follows: field emission (FE) > LaB_6 > thermionic tungsten (18).

Sample coating procedures can impact particle resolution during X-ray microanalysis, regardless of the instrument emission source. Electron micrographs of colloids generated in a series of column studies described in Seaman et al. (15, 20) are presented in Figure 2. When the filters are coated with Au/Pd for imaging, numerous distinct small rod-shaped particles or aggregates dominate the filters (Figure 2A). Larger particles displaying platelike morphologies indicative of layer silicates are also present but in much smaller numbers and generally coated or aggregated with the more numerous rod-shaped particles. When samples from the same filter are carbon coated for microanalysis, the visual resolution at a given keV is degraded compared to metal-coated samples. Larger particles take on a more amorphous appearance, and it becomes difficult to distinguish smaller colloids from the filter background (Figure 2B).

As the particle size decreases and excitation voltage increases, electron beam penetration increases, and a greater portion of the signal originates in the substrate, thus contributing to signal noise and degrading image quality. Particles readily visible at the slow scan rates associated with recording a photomicrograph may become virtually invisible to the operator as the scan rate is increased for identifying

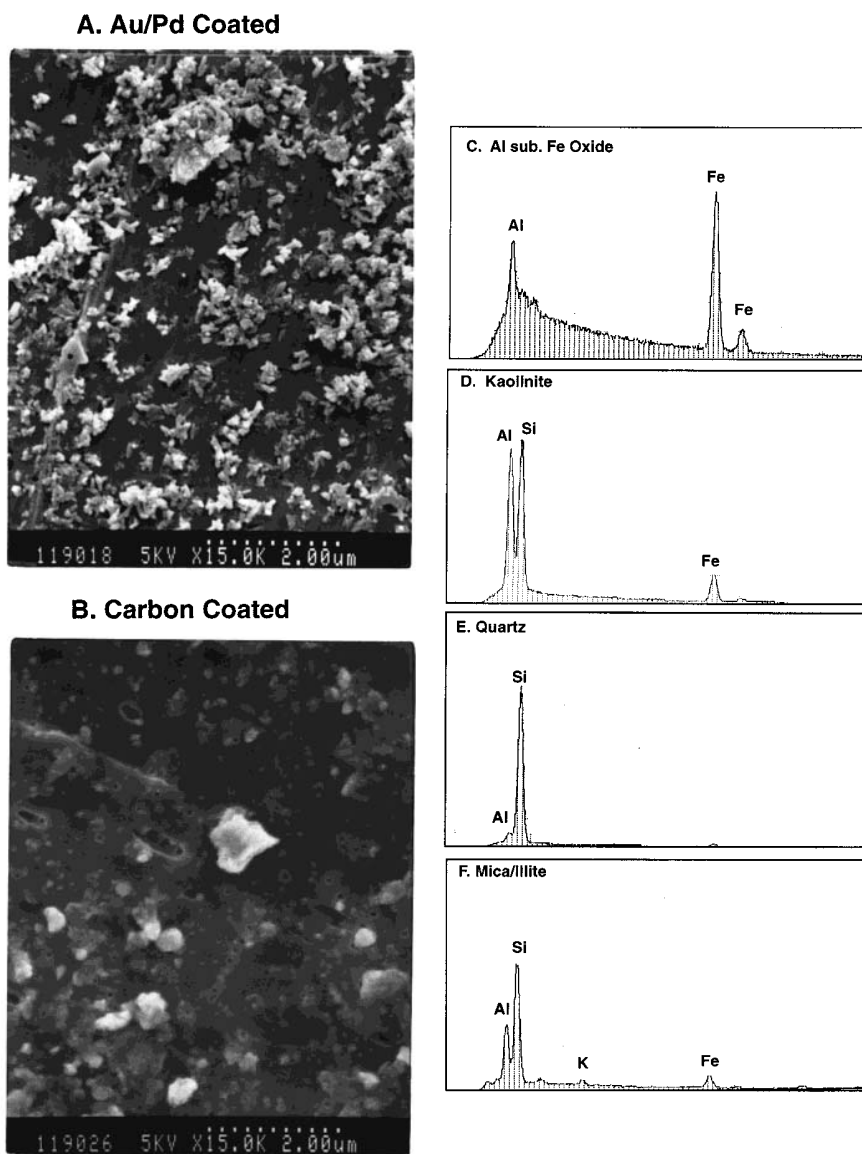


FIGURE 2. Electron micrographs for mobile colloids collected by Seaman et al. (15), deposited on polycarbonate filters, and then metal coated (Au/Pd) for particle imaging (A) and carbon coated for EDS analysis (B). Select particle types derived from SEM/EDS analysis: (C) Fe- and Al-rich phase (Al-sub. goethite); (D) Si:Al, 1:1 ratio (kaolinite); (E) Si-rich phase (quartz); and (F) Al:Si, 1:2 phase with detectable K (mica). Particle categories were confirmed by selected area electron diffraction in the TEM.

particles during random surveys (18), severely biasing sample analysis in favor of larger colloids. In addition, Figure 2B was taken at an instrument voltage of 5 keV, much lower than necessary for microanalysis, $\approx 15\text{--}20$ keV. Smaller colloidal particles, aggregates, and even surface coatings on larger particles can be essentially invisible to the operator during compositional analysis but sufficiently resolved when samples are metal coated and imaged at lower electron voltages. As a consequence, published micrographs generated using metal-coated samples, such as Figure 2A, often reflect much greater visual resolution than when compositional analysis was actually performed (Figure 2B), making it impossible to assign any specific composition to the published images. Noting such resolution problems, several research groups have used TEM as the primary discrete particle analysis technique (26–28) or have combined TEM analysis techniques, such as electron diffraction and X-ray microanalysis, to confirm conclusions drawn from SEM surveys (15, 29).

The bias of SEM in favor of larger colloidal particles is not necessarily limited to imaging and particle selection for

microanalysis. Typical EDS patterns representing four of the six mobile colloid categories identified in Seaman et al. (15) are included in Figure 2: (C) Fe-rich phase with varying amounts of Al (Al-sub. goethite); (D) Si:Al, 1:1 ratio (kaolinite); (E) Si-rich phase (quartz); and (F) 2:1 aluminosilicate phase containing detectable levels of K (mica/illite). The difference in spectral quality for the Fe oxides compared to the other three particle categories is quite obvious, reflecting the larger average particle sizes observed for those mineral classes. The high EDS background observed for the smaller particles and aggregates reflects the fact that the electron interaction volume exceeds the particle dimensions regardless of emission source. Similar high EDS backgrounds are evident for the more numerous, smaller mobile colloids collected in the field by Kaplan et al. (3) and Gschwend et al. (7). Smaller colloidal materials, such as the Al-rich goethite colloids (Figure 2C) identified by Seaman et al. (15), may be overlooked or EDS patterns representing multiple discrete particulates may be incorrectly attributed to one phase, such as an Fe-rich clay, rather than an Fe-oxide coated kaolinite. The smaller

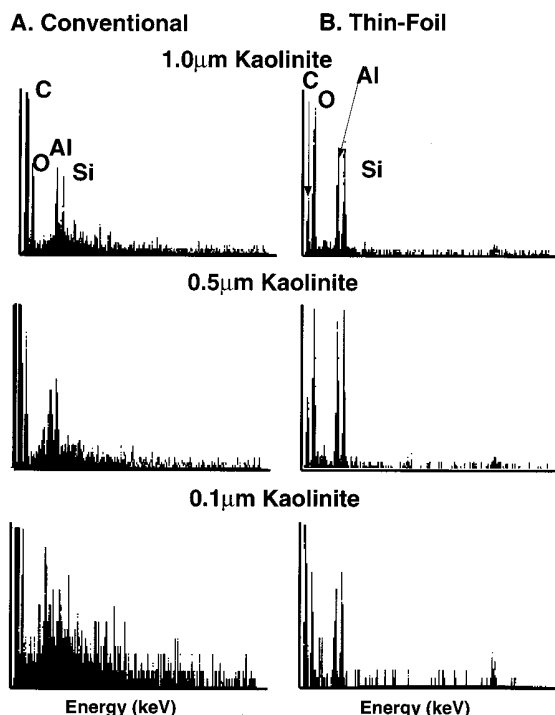


FIGURE 3. EDS spectra for reference kaolinite as a function of particle size using the conventional (A) and thin-foil (B) mounting techniques.

goethite particles were readily identified in Seaman et al. (15) not due to improved instrument resolution over previous studies, but because they completely dominated the column-generated suspensions. Consequently, the reliance on SEM/EDS as the sole nondestructive identification and characterization technique may neglect small, high surface area minerals, resulting in the incorrect identification of the mobile solid phase actually responsible for contaminant transport.

EDS results must be interpreted with caution as elements representing multiple discrete solid phases may be incorrectly attributed to one mineral phase. Iron present in detectable levels for most of the particle categories (e.g., Figure 2D,F) identified in Seaman et al. (15) was attributed to distinct Fe oxides aggregated to the larger particles; however, TEM analysis was necessary to draw that conclusion. Concentration of the suspension at the filter surface during deposition can induce aggregation artifacts (30). Heavy filter loadings are likely to yield X-ray data indicative of multiple particles or aggregates due to the large specimen interaction volume in the SEM. In addition, typical X-ray patterns reflecting assigned particle categories are often omitted from publications due to space constraints, making it impossible to critically evaluate the degree of noise present within spectra, suggest other mineral categories that account for observed element ratios, or assess potential resolution problems.

Particle resolution problems when conducting X-ray microanalysis are less critical in the TEM because spreading of the electron beam is much reduced due to the very high excitation voltages and the thin-foil nature of the sample (31). The transmission-like configuration improves EDS spectral quality because transmitted electrons are captured without generating various artifact signals from the polycarbonate filter, SEM mount, or nearby colloids. In the current study, the colloid samples were resuspended on TEM grids and placed on the modified grid holder above an electron trap such that transmitted electrons fail to induce X-ray generation within the substrate. The spectra for the kaolinite standard as a function of particle size and analysis technique,

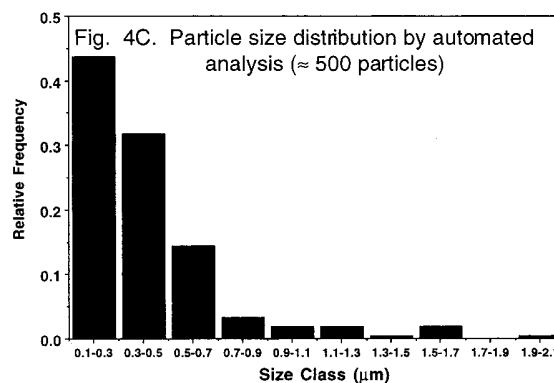
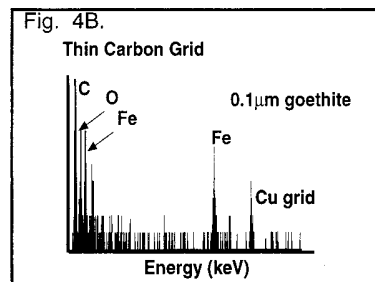
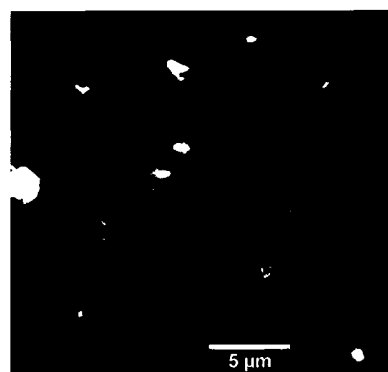


FIGURE 4. Backscattered electron image (A), EDS spectra (B), and particle size distribution (C) for synthetic goethite mounted using the thin-foil technique and analyzed using an automated routine.

conventional vs thin-foil, are presented in Figure 3. A significant Bremsstrahlung background that increases with decreasing particle size can be seen for the kaolinite using the conventional mounting procedure (Figure 3A). Therefore, analysis of submicron particulates in the SEM is limited by both visual resolution during analysis (Figure 2B) and the reduction in EDS spectral quality as particle size decreases (Figures 2C–F and 3A). In contrast, only a minor increase in the background is observed for kaolinite with decreasing particle size in the thin-foil analysis scheme (Figure 3B). Similar results were observed for each of the colloidal samples that were analyzed.

Backscattered electrons can be used to identify particles against the low-molecular weight carbon substrate (i.e., TEM grid). The high-contrast, BE signal for thin-foil mounting, such as the image presented for synthetic goethite (Figure 4A), lends itself to instrument automation of microanalysis and particle sizing. Particles appear as bright featureless regions against the dark substrate background. After recognizing and defining the morphology (dimensions) of particles within a field of view, the automated system returns to each particle and collects an EDS spectrum (Figure 4B). Often the thin-foil method does not require sample coating

because of the reduced potential for charging since fewer electrons are deposited in the sample and substrate. The thin-foil technique offers much greater particle size resolution (Figure 4C) compared to dynamic light scattering; however, the method is still sensitive to the same aggregation artifacts associated with conventional SEM analysis which confound both particle sizing and the interpretation of EDS results.

Compared to conventional particulate analysis in the SEM, the thin-foil method improves particle resolution, elemental detection limits, and overall spectral quality, i.e., greater signal-to-noise ratio that is less dependent on particle size. Particle recognition is less sensitive to excitation voltage, instrumental emission source, or sample coating method, thus providing better particle size and morphology data. Such improvements in resolution make it possible to automate analysis so that a more statistically relevant number of particles are characterized. The technique does, however, require a modified sample holder that may not be commercially available for all instruments. Ideally, the particles must be well dispersed for microanalysis, whether using the thin-foil or conventional analysis methods. The resuspension of particles collected on filters may induce aggregation artifacts in addition to those resulting from the initial filtration. The operator must still define particle categories when conducting compositional analysis, which requires the use of other analytical techniques, such as EDS and selected-area electron diffraction in the TEM. Unfortunately, samples mounted using the thin-foil technique provide poor micrographic images, one of the primary reasons researchers choose SEM methods over the greater resolution afforded by EDS analysis in the TEM. Regardless of the SEM analysis method, the instrumental limits of such analyses must be recognized so that small, high-surface area minerals that may be responsible for facilitating contaminant migration are not overlooked.

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