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A major challenge to the development of a fundamental understanding of transport and retardation mechanisms of trace metal contaminants (<10 ppm) is their identification and characterization at the nanoscale. Atomic-scale techniques, such as conventional transmission electron microscopy, although powerful, are limited by the extremely small amounts of material that are examined. However, recent advances in electron microscopy provide a number of new analytical techniques that expand its application in environmental studies, particularly those concerning heavy metals on airborne particulates or water-borne colloids. High-angle annular dark field scanning transmission electron microscopy (HAADF-STEM), STEM-energy-dispersive X-ray spectrometry (EDX), and energy-filtered TEM (EFTEM) can be effectively used to identify and characterize nanoparticles. The image contrast in HAADF-STEM is strongly correlated to the atomic mass: heavier elements contribute to brighter contrast. Gold nanocrystals in pyrite and uranium nanocrystals in atmospheric aerosols have been identified by HAADF-STEM and STEM-EDX mapping and subsequently characterized by high-resolution TEM (HRTEM). EFTEM was used to identify U and Fe nanocrystals embedded in an aluminosilicate. A rare, As-bearing nanophase, westerveldite (FeAs), was identified by STEM-EDX and HRTEM. The combined use of these techniques greatly expands the effective application of electron microscopy in environmental studies, especially when applied to metals of very low concentrations. This paper describes examples of how these electron microbeam techniques can be used in combination to characterize a low concentration of heavy metals (a few ppm) on nanoscale particles.

Introduction

Nanoparticles are omnipresent in the low-temperature environments that are characteristic of the near-surface “critical zone” that is of greatest environmental interest. Low temperatures are conducive to the formation and stability of nanoparticles over short to medium time scales (1). Recent interest in the detailed characterization of nanoparticles has included the following: the description of their formation through inorganic (2) and organic pathways (3), the processes of defect and dislocation formation (4), the presence of radioactive elements in aerosols (5), and their impact on global climate change (6). Despite the importance of detailed descriptions of nanoparticles, analytical techniques used to identify nanoscale particles have limited capabilities. Scanning electron microscopy (SEM) can provide morphological information on the submicron scale and elemental information at the micron scale. Atomic force microscopy (AFM) and scanning tunneling microscopy (STM) can provide surface information at the atomic scale; however, chemical data are not provided except in the case of well-controlled, simple experiments (7). Computer simulations using molecular dynamics or Monte Carlo methods also provide important insights into the growth of nanocrystals but provide limited insight for more complex, natural samples (8). Techniques that are useful in analyzing ultrafine atmospheric particles, such as STEM, EELS (electron energy loss spectrometry), AFM, and mass spectrometry, have limitations that have been previously reviewed (9, 10). STEM-EDX and EFTEM have been occasionally used (11, 12), but the particles studied are typically 100–1000 nm in size, and only major element compositions have been determined.

Conventional transmission electron microscopy (CTEM) and associated spectroscopic techniques are the most common tools used in investigating the crystal structure and composition of materials at the <1-nm scale. TEM is generally the most powerful and appropriate technique for investigating the characteristics of nanoparticles, such as colloids (13). Many studies have succeeded in identifying the particles bearing toxic elements using CTEM (14, 15). However, for detecting the low elemental concentrations that are typically of environmental interest, in which almost the entire concentration of the trace metal is located in just a few nanoparticles, it is nearly impossible to find the nanoparticles that contain the metals of interest. When the elemental distribution is widely scattered, the image contrast of CTEM is minimal except under very high magnification, where only a limited number of particles can be examined. However, when the particles of interest consist of relatively heavy elements, as compared with the matrix material, high-angle annular dark field scanning TEM (HAADF-STEM) is a powerful method for finding the nanoparticles of interest, as the contrast of the image is strongly correlated with atomic number and specimen thickness. In the late 1980s and the early 1990s, HAADF-STEM was under active development (16–19). STEM is the reversed optical system of CTEM: the electron path in bright-field STEM (BF-STEM) is the same as when the ray path direction is reversed in CTEM (20, 21). A schematic illustration of a typical STEM system with a field emission gun is shown in Figure 1. Unlike CTEM, HAADF-STEM is based on imaging the incoherent scattering, and the contrast of the image is not reversed by defocusing above and below the point of “just focus” (18, 22). Thermal diffuse scattering (TDS), which is the signal used to produce the image in HAADF-STEM, was previously considered “background intensity” before the use of the annular dark field.
(ADF) detector. With HAADF-STEM, the signal from TDS becomes a powerful source of information.

Fundamentally, each atom in the specimen vibrates thermally with a frequency of $10^{12}$ Hz. The atomic vibration is quantized as a phonon. The electron's velocity in the beam of the electron microscope is approximately $1.5 \times 10^{10}$ cm/s. Thus, the time required for the electron to pass through the electron-transparent thickness of the specimen ($<300$ nm) is approximately $7 \times 10^{-17}$ s, which is much shorter than the oscillation period of the atoms. However, the time between successive electrons in an incident electron beam is longer than that of the atomic oscillations. Therefore, the electrons are scattered randomly; the average of the scattered intensity from all configurations of the atoms is incoherent. The TDS profile through spatial frequency analysis can be calculated by the "frozen phonon" method, in which the atoms are allowed to deviate from their original position following a Gaussian distribution (23).

Two kinds of simulation methods have been suggested for calculating the high-resolution HAADF-STEM image: Bethe's eigenvalue method (22) and the multi-slice method (16, 24). The multi-slice method takes tens of hours to create a $512 \times 512$ pixel image as compared with the tens of minutes required with Bethe's method to create the same image. However, an advantage of the multi-slice method is its capability to account for mixed layers in the calculation of specimen potential for electron scattering, which is performed by a linear superposition of the atomic potential of each atom in the specimen. The atomic potential is related to the electron scattering factor (i.e., the electron scattering factor is the Fourier transform of the atomic potential in the approximation). The scattering factor is the amplitude of the intensity of a single scattered electron by a single atom and can be calculated from the electron wave functions of different atoms. For the purpose of this discussion, the most important point is that the TDS is proportional to the mass of the scattering atom. There are many examples of applications of high-resolution HAADF-STEM in material science, for example, the investigation of grain boundaries (25, 26) or the location of a single atom embedded in a metal (27).

EFTEM is another important tool for mapping elemental distributions (28). The slit width of the energy filter is set for major element-specific edges in the electron energy loss spectrum, and the intensity at each pixel is integrated using the Gatan Imaging Filter (GIF). The final elemental map is produced after the subtraction of background as determined by two preedge images. Compared to STEM-EDX, the advantage of EFTEM is the higher energy resolution of the spectrum. The energy resolution in EDX is $>100$ eV, while the energy resolution in EELS is $<0.2$ eV. Thus, spectrum interference can be minimized in EFTEM unless the intense energy loss near-edge structure (ELNES) makes it difficult to construct the background profile. EFTEM is suitable for lighter elements analysis because of the higher intensity in the lower energy region, although low energy in the low-loss region ($<10$ eV) is overlapped by the plasmon loss spectrum, which is related to sample thickness. As EFTEM is based on EELS, the intensity of elemental maps varies depending on the element, edge selection, specimen thickness, and electronic structure of the material. Imaging the elemental map for edge energy higher than 2000 eV is difficult because of the low signal intensity. Generally, the lower energy K- and L-edges are used to produce an elemental image.

In this paper, samples of environmental interest that contain nanoparticles with heavy elements have been investigated using the HAADF-STEM combined with conventional high-resolution TEM (HRTEM). The advantages of using the HAADF-STEM method are demonstrated. The utility of EFTEM for elemental mapping of nanoparticles is also shown by an example in which nanocrystals of iron oxide and of uraninite were identified embedded in an aluminosilicate.

**Experimental Methods**

Observation at the micrometer scale was completed by field emission gun scanning electron microscopy (FESEM; Philips XL30). HRTEM, AEM (analytical electron microscopy), and HAADF-STEM were conducted using a JEOL JEM2010F equipped with a GATAN GIF/PEELS system for EFTEM that was controlled by Gatan Digital Micrograph Version 3.6.4. Three filtered images, two preedge and one postedge, were produced after the subtraction of background as determined by Kirkland et al. (21). TEM specimens were prepared by dispersing the specimen on the holey-carbon mesh or by milling with an Ar ion beam in the Gatan precise ion polishing system (PIPS) after a mechanical polishing to as thin as $\sim 55$ nm. The best performance of the HAADF-STEM for the JEOL JEM-2010F has been demonstrated by James et al. (29). TEM samples were cleaned by plasma using the Fischione model C1020 before STEM observation to minimize contamination. The intensity profile in HAADF-STEM for some elements was simulated with the code developed by Kirkland (21). MacTempas PPC 1.7.8 was also used for HRTEM image simulation, and image processing was completed using Scion Image Beta 4.0.2.

The mathematical treatment of the fundamental physics of the HAADF-STEM are given by Kirkland (21). Qualitatively, the wave function of a focused probe at any position is...
expressed as an integration of the aberration wave function constrained by the size of the objective aperture. The transmitted wave function of the electrons, after passing through the specimen, has a simple form that is the product of the probe wave function and the transmission function of the specimen. The transmission function of the specimen involves the total projected atomic potential of the specimen and an interaction parameter. The wave function evident on the diffraction plane is the Fourier transformed (FT) form of the transmitted wave function. The integrated square of the absolute value of the wave function is the measured signal.

In STEM mode, a convergent-beam diffraction pattern is formed on the back-focal plane. Thus, the intensity on the detector is the sum of the pure elastic scattered electrons and the pure TDS electrons. Because the elastic scattering must satisfy Black’s condition, that is the reciprocal lattice must cross Ewald’s sphere, the intensity of the zero-order Laue zone (ZOLZ) becomes weak and disappears in the high-frequency region of the diffraction space. As a result, TDS scattering becomes dominant at high angles and is recorded by the HAADF detector. However, signals from the first-order or high-order Laue zones (FOLZ or HOLZ) are possible, and these signals may also be recorded by the HAADF detector. Eventually, the contrast of the HAADF image is a result of the relative intensities of elastic scattering and TDS on the HAADF detector.

**Results**

**Intensity Simulation.** Intensity profiles of single atoms, which will be used in the following examples, are shown in Figure 2 in order to quantify the contrast variation in HAADF-STEM. The contrast in HAADF-STEM has an inverse intensity as compared with the contrast profile near Scherzer focus in normal TEM mode (Figure 2a). Image contrast in HAADF-STEM becomes brighter as the atomic mass of the element in the particle becomes heavier (Figure 2b). The contrast in a HAADF-STEM image is proportional to the square of the atomic number.

**Au Nanoparticles.** This sample is from a Carlin-type gold deposit in Twin Creeks, NV (30). An arsenic-bearing pyrite formed as the rim around each pyrite grain. The presence of gold in the arsenic-bearing rim has been known from bulk chemical analyses (30), but the form of the gold had not been determined, that is, whether it was incorporated into the Au-bearing pyrite or existed as a separate phase. In TEM, the conventional EDX spectrum indicated that some regions consisted of Au, Fe, and S. Although the EDX system can be used as a sub-nanometer probe, the EDX profile usually contains some background signal from surrounding areas: Fe and S in this case. In contrast, the HAADF-STEM image clearly revealed the heterogeneous occurrence of heavy element nanoparticles less than 30 nm in diameter embedded in the pyrite matrix that consisted of relatively lighter elements (Figure 3a). According to the contrast in simulated HAADF-STEM (Figure 2b), the particles must contain Au. The EDX mapping clearly revealed that Au nanoparticles occur in the pyrite matrix (Figure 3b). HREM image (Figure 3c) and selected area electron diffraction confirmed the presence of nanoscale crystalline gold particles.

**Uraninite Nanocrystals in an Atmospheric Aerosol.** Here we show the results of an analysis of the distribution of a trace (1–10 ppm) radioactive element, uranium, in airborne particulates, mainly from coal-fired power plants. The aerosol samples were collected from a 2-μm-sized Teflon filter that was used to sample the air over Detroit (5). A large amount of soot was observed adhering to the surface of the minerals constituting the dust, such as quartz and gypsum. Using conventional TEM techniques, the BF image (Figure 4a) did not clearly reveal the structure or composition of the nanoparticles. However, the HAADF-STEM image of the same area clearly showed the heterogeneous distribution of nanoparticles, <10 nm, that contain heavy elements (Figure 4b). STEM elemental mapping confirmed the presence of uranium (Figure 4c). These nanoparticles were subsequently identified as uraninite, UO2(x), using HRTEM (5). In addition, these uraninite nanocrystals were encased by well-crystallized graphite (Figure 4d). The encapsulation of UO2 in a graphite cage may retard the oxidation of tetravalent uranium to the more mobile hexavalent form (5).

**Nanoparticles Embedded in Phyllosilicates.** The alteration of uraninite, UO2(x), at the Oklo-OkeÂlobondo uranium deposits in Gabon, where nuclear fission chain reactions were sustained as small “natural” reactors 2 billion years ago, has been studied extensively as an analogue for the corrosion of used nuclear fuel in a geologic repository (31, 32). Close examination of samples from near the reactor core showed that a clay surrounds the uraninite. On the basis of a chemical analysis obtained from EDX analysis, the aluminosilicate contains a small amount of U and Fe. To investigate the distribution of these elements, elemental mapping using EETEM was performed on this area. For the uranium N-edge (381 eV) image, an energy “window” with a width of 30 eV was set with 406 eV as the postedge and 331 and 361 eV as the preedges to build the background. The EETEM images of zero-loss and of uranium N-edge at the boundary between uraninite and aluminosilicate (Figure 5) clearly show the distribution of the uraninite nanocrystals. Note that the zero-loss image (Figure 5a) does not provide any chemical information, but the filtered zero-loss image eliminates any inelastically scattered electrons (i.e., a little noise is removed). Because the aluminosilicate was sensitive to the electron irradiation, zero-loss imaging did not reveal the structure of the aluminosilicate; however, nanocrystals of uraninite (evident in the U-map; Figure 5b) embedded in aluminosilicate matrix were resolved. In addition, rounded nanocrystals, 10–30 nm, were also found within the aluminosilicate at a distance of 0.5 μm from the boundary between the aluminosilicate and the uraninite (Figure 6). An Fe map obtained by EETEM (Figure 6b) clearly revealed that the nanoparticles consist of Fe. The energy slit of 30 eV width was set with 734 eV as the postedge and 658 and 688 eV.
FIGURE 3. (a) HAADF-STEM image of pyrite in gold ore. Many bright contrast nanoparticles are embedded in pyrite. (b) The high magnification micrograph with an inset of STEM-EDX maps of the square region, outlined in white, showing that the nanoparticles consist of Au. (c) HRTEM image of a gold nanocrystal.

FIGURE 4. (a) BF-CTEM image of carbonaceous matter in atmospheric aerosol from Detroit. (b) HAADF-STEM image of the region outlined in white in panel (a). There are many particles with bright contrast (indicated by arrows) in the carbonaceous matter. (c) The HAADF-STEM image with EDX map of uranium in carbonaceous matter. The resolution of the EDX map is less than that of the STEM image due to the shorter data acquisition time because the specimen drifted as the carbonaceous matrix decomposed under the electron beam. (d) HRTEM image of uraninite nanocrystals in a matrix of carbonaceous matter composed of well-crystallized carbon.
as the preedges for the iron L-edge. In general, structural information, such as that obtained from an electron diffraction pattern, is needed to fully characterize the crystals. However, the smallest selected area aperture is about 175 nm in diameter, which is not small enough to obtain the diffraction pattern from nanocrystals less than a few tens of nanometers. For cases in which nanocrystals are abundant, their combined diffraction patterns and resulting rings of diffraction maxima can be used to identify the phase. Unfortunately, the concentration of nanocrystals in most natural materials is much too low to obtain such a “ring” pattern. In this case, a convergent-beam electron diffraction pattern was used to identify the phase as magnetite.

**Arsenic Nanocrystals.** Trace occurrences of arsenic from either anthropogenic or natural sources require precise identification of the form and, hence, the source of As. The exact form of the As plays a determinate role in its release into groundwater (33). In this example, we have identified an arsenic-bearing phase existing as nanoscale particles at the grain boundaries of feldspar crystals in a pegmatite in New Hampshire (33). On the basis of HRTEM with EDX, the nanocrystals and the surrounding area consist essentially of As and Fe. Obtaining a distinct contrast in HAADF-STEM mode was difficult because of the small difference in atomic mass between As and Fe. However, elemental maps of Fe and As (area outlined by white square in Figure 7a) could be made in BF-STEM mode. Some polycrystals were distinguished using BF-STEM, and they consisted of both As and Fe. Fe was also present in the matrix of the polycrystals. One of the particles in the mapped region was characterized by HRTEM (Figure 7b). Indexing of the FFT (fast Fourier transform) image confirmed the identification of a rare mineral, westerveldite, FeAs (Figure 7c).

Each of these briefly described examples has demonstrated that HAADF-STEM is a powerful technique for the investigation and identification of nanoscale materials (either crystalline or amorphous) that contain relatively heavy elements at extremely low bulk concentrations. The combination of HAADF-STEM, STEM-EDX mapping, and HRTEM are generally underutilized in investigations of nanoparticles in environmental samples. EFTEM is another useful method for creating an elemental map without using a focused electron beam; it also finds special application in the investigation of nanoscale particles consisting of lighter elements. Generally speaking, the EFTEM signal decreases as the specimen becomes thicker, whereas the signal from STEM-EDX increases with increasing the specimen thickness. With EFTEM, the lower energy edges of lighter elements are preferably used in order to increase the intensity and decrease the exposure time. Short exposure times can minimize the effect of specimen drift and damage caused by the electron beam. When there is a relatively large difference in the atomic mass among the constituent elements, HR HAADF-STEM can be used to provide information on the elemental distribution at the near-atomic scale. Once the nanoparticles are located by HAADF-STEM, characterization can be completed using HRTEM.

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