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Forensic Applications of the Transmission Electron Microscope

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KEYWORDS

Transmission electron microscopy (TEM), selected area electron diffraction (SAED), energy dispersive X-ray spectroscopy (EDS), nanoparticles, forensic science, trace evidence, morphology, carbon nanotubes

ABSTRACT

Transmission electron microscopy (TEM) is rarely used as an analytical tool in forensic science, and consequently there is no standard method on how to conduct trace evidence analysis using TEM. This article will introduce a newly developed method specifically for forensic trace evidence analysis done by TEM. This article will also explain the ongoing development of a TEM database containing known particles of various types. The purpose of the particle database is to be used as a reference for particle identification when conducting forensic trace evidence analyses via TEM.

INTRODUCTION

The TEM uses a high-energy electron beam to image samples that are thin enough to be partially electron transparent. The electron "shadow" of a sample can be viewed and recorded. Transmission electron microscopy can be very useful in forensic trace evidence analyses because of its magnification capabilities, the ability to gather elemental composition information on nanoparticles, and the ability to determine the internal structure of these particles. All of these tasks may be accomplished using the three major analytical functions of the TEM: morphological analysis, selected area electron diffraction (SAED), and energy dispersive X-ray spectroscopy (EDS).

Morphology is the size and shape of a particle. One of the TEM's advantages over light microscopy is its ability to magnify a particle up to 1 million times with point-to-point resolution better than 2 nm, which allows for easy observation of the morphology of nano-sized particles. Figures 1-3 are examples of diesel soot shown at three different magnifications. Figure 1 has been magnified 1,400X. This magnification is comparable to the maximum useful magnification of the light microscope. Figure 2 is the same image magnified 125,000X to show how the TEM allows one to see the characteristic morphology of the individual nanoparticles. Figure 3 is a high resolution TEM image of diesel soot magnified at approximately 450,000X, showing the turbostratic layering within a single soot particle. The layer spacing is approximately 0.34 nm.

TEM allows one to determine whether a particle is crystalline or amorphous by using SAED, which occurs when a very thin particle is subjected to a parallel beam of high-energy electrons. If the particle is crystalline, a series of spots, streaks or concentric circles will be displayed on the TEM screen. Each spot corresponds to a satisfied diffraction condition of the particle's crystal structure. If the particle is non-crystalline, there will be no spots, streaks or concentric circles displayed. See Figure 4 for an example of a SAED pattern.

Different crystalline materials will display an arrangement of spots and/or streaks that are somewhat

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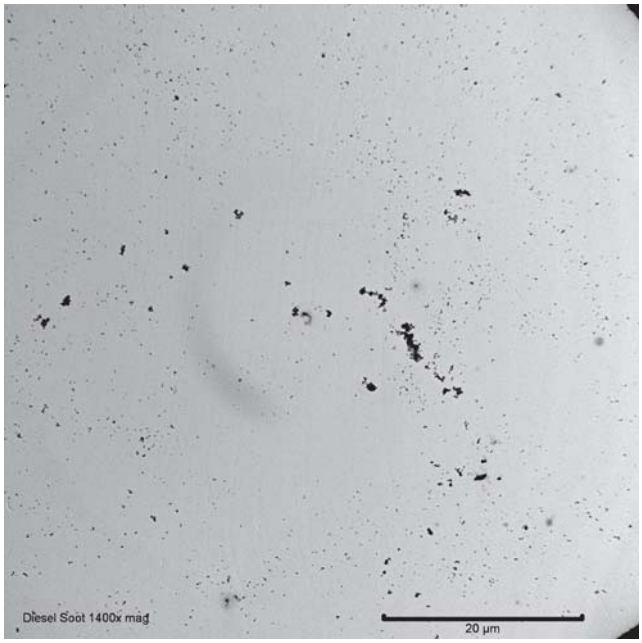

Figure 1. Diesel soot, TEM, magnified 1,400X.

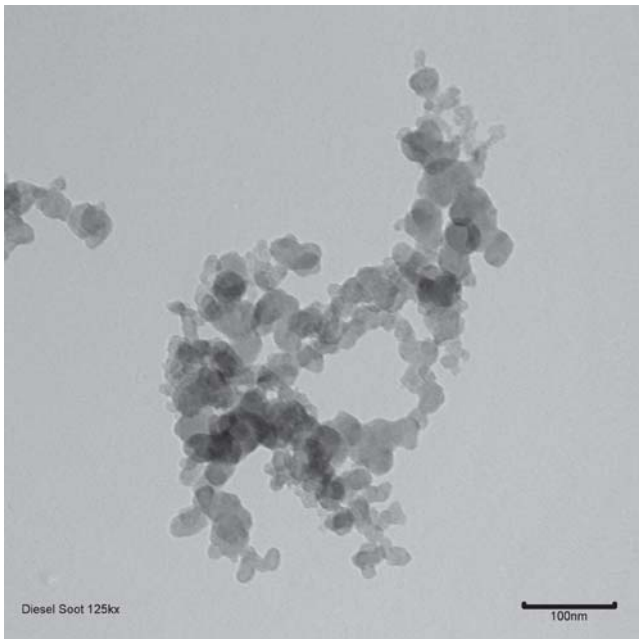

Figure 2. Diesel soot, TEM, magnified 125,000X.

unique to that particular compound. Diffraction spots represent planes of symmetry from the atoms in a crystal. Measurements of distances between dots that form a linear pattern and comparison with measurements obtained for known spacings of reference materials (i.e., gold) allows the analyst to determine the interval (d-spacing) between parallel planes of atoms in a crystal. The measurement of angles between linear spot arrangements (planes of atoms) also provides useful crystallographic information. Concentric circles usually indicate that a particle is microcrystalline with a discordant arrangement of substituent microcrystals. The results of electron diffraction on the TEM can be interpreted using the JCPDS-ICDD database of powder diffraction patterns, which includes the d-spacings and relative intensities of observable diffraction peaks (1).

One can determine the chemical composition of very small particles using another analytical function available on the transmission electron microscope called energy dispersive X-ray spectroscopy (EDS). Bombardment of a particle by the electron beam causes the particle to emit X-rays, some having energies characteristic of the elements comprising the particle. These X-rays are collected, sorted and counted to characterize the elemental composition of the particle, and the result is presented as an EDS spectrum in which the presence of peaks indicates the presence of the associated element (Figure 5). To a first approximation, the height of the peaks can be related to the concentration of the element within the particle. Quantitative elemental analysis is possible by applying ZAF (Z=atomic number, A=absorption, F=fluorescence) correction factors.

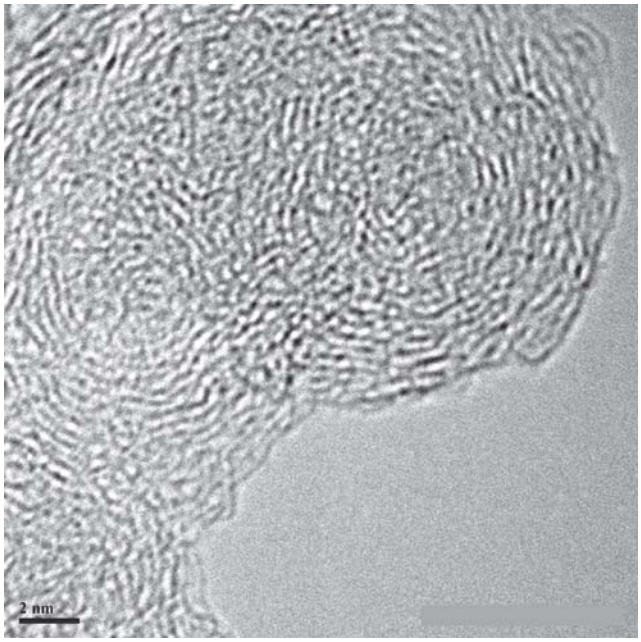

Figure 3. Diesel soot, high-resolution TEM, magnified approximately 450,000X.

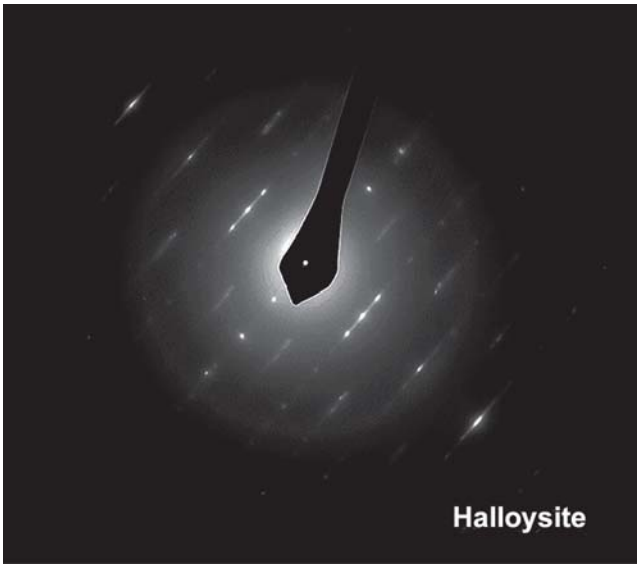

Figure 4. SAED pattern of Halloysite clay.

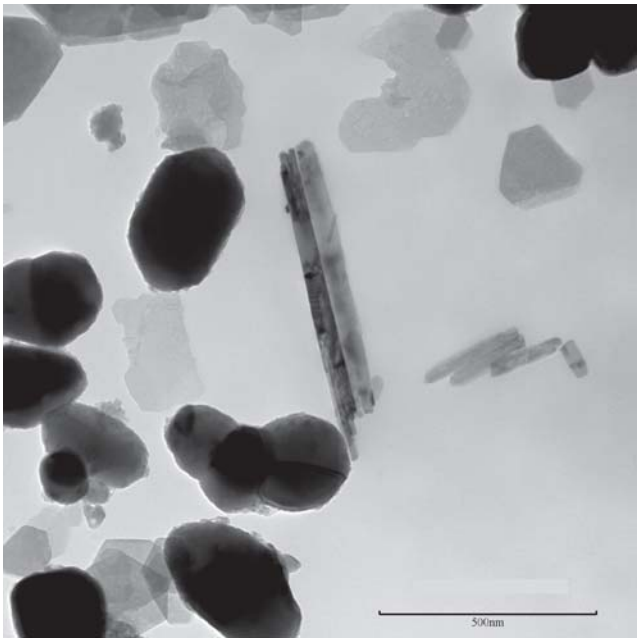

Figure 6. TEM image of paint pigments (Fe oxide fibers, TiO_2 and kaolinite clay particles) found in an architectural paint during a forensic analysis.

METHOD FOR FORENSIC TRACE EVIDENCE ANALYSIS WITH TEM

Sample preparation for TEM is crucial because the sample must be very thin or made very thin in order for the electron beam to penetrate the sample.

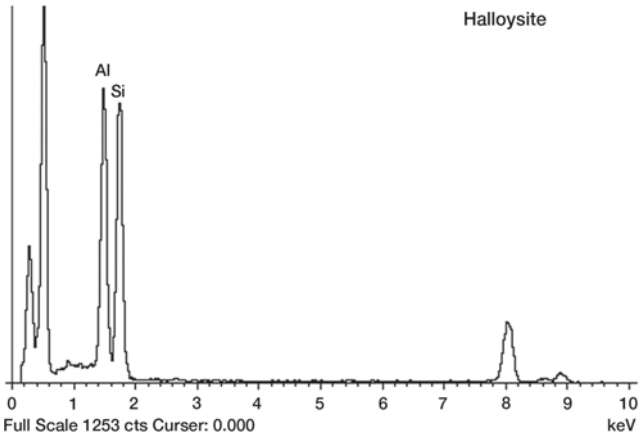

Figure 5. EDS of Halloysite clay.

Sample Collection

Begin each TEM analysis by collecting the finest portion of the sample. The collection of this material can be done by centrifuge or sedimentation.

Sample Preparation

There are two different methods in which samples can be prepared for forensic trace evidence analysis via TEM. In the first method, the fine portion of the sample is prepared by ultrasonic dispersion as described in ASTM D6602 (2). Droplets of each suspension are then deposited onto carbon film substrates on 200 mesh copper grids. A modification of ASTM D6602 for the trace evidence method for TEM involves the use of Spectragrade acetone, instead of chloroform, in the ultrasonic dispersion. In the second method, the sample is prepared by filtration. The sample is filtered using a polycarbonate (PC) 0.1-0.2 μm pore size filter with a 5.0 μm backing filter. The standard diameter of the filter used in this preparation procedure is 47 mm, but it may vary depending on the preferred concentration of material on the filter. The filter is then prepared according to ASTM D5755 (3). A portion of the prepared filter is then excised and placed onto a copper grid.

For this research, sample grids were analyzed using a Philips CM 120 transmission electron microscope operated at 100 kV and equipped with a bottom mount 4-megapixel digital camera. Electron micrographs were generated of the sample material using various instrument magnifications. The microscope is calibrated at each magnification using a standard reference diffraction grating replica with 2,160 lines per millimeter, prior to the analysis. Each image was annotated with a scale bar having a pixel length determined by the grating replica calibration.

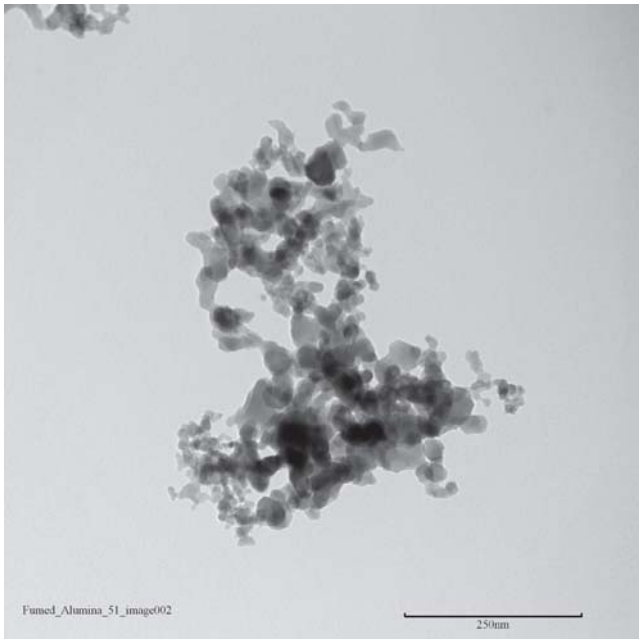

Figure 7. TEM image of fumed alumina.

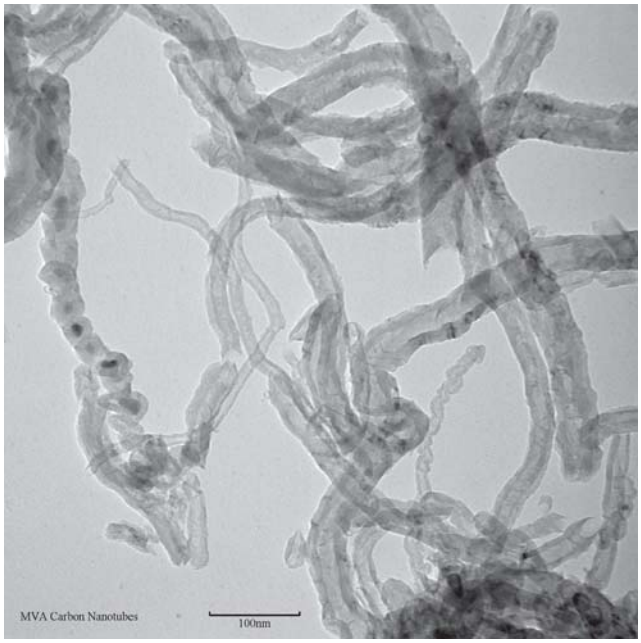

Figure 8. TEM image of multiwalled carbon nanotubes.

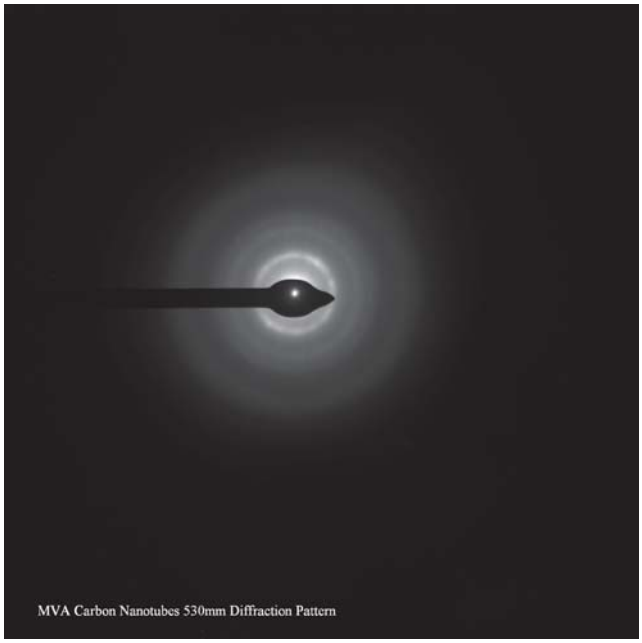

Figure 9. SAED pattern obtained for carbon nanotubes.

Sample Analysis Process

Begin by analyzing 25 random particles less than 5 μm in size, taking an image, EDS and an SAED of each particle. Briefly give a description of each particle and classify it based on the characteristics portrayed by each particle. Particles may also be characterized by comparison with a TEM database (referred to below). Additional analysis is then done, looking for anthropogenic particles or particles not typical of the natural environmental setting of the sample such as combustion products, fumes, metals, etc. These particles are described and recorded in the same manner as the first 25 particles analyzed.

DISCUSSION

The analysis of the random 25 particles gives a general idea of the type of environment being analyzed. The less-than 5 μm size was chosen to ensure that the examination only includes particles that might not normally be analyzed using other microscopical methods. The search continues for anthropogenic particles such as combustion particles, metals, fumes, etc. to give an indication of any unusual man-made activity possibly taking place in this environment. Examples of particles that may be found during forensic analysis by TEM are shown in Figures 6 and 7.

TEM images of particles found during environmental forensic investigations are also shown in a previously published paper by Millette and others (4).

Nanoparticles

The production and application of nanoparticles continues to increase significantly. Therefore, it is very important to characterize these particles in order to

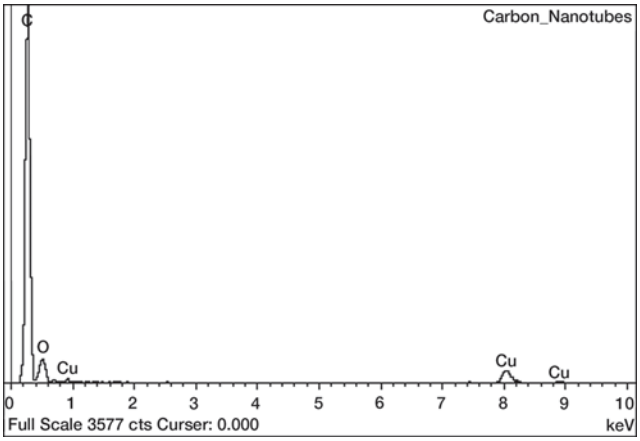

Figure 10. EDS of carbon nanotubes.

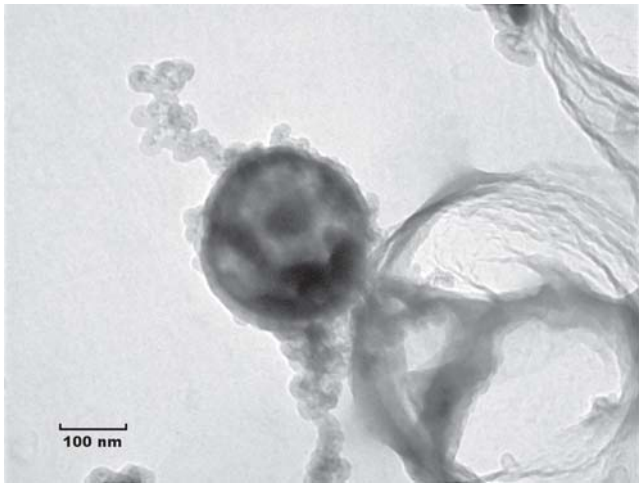

Figure 11. TEM image of gun-shot residue.

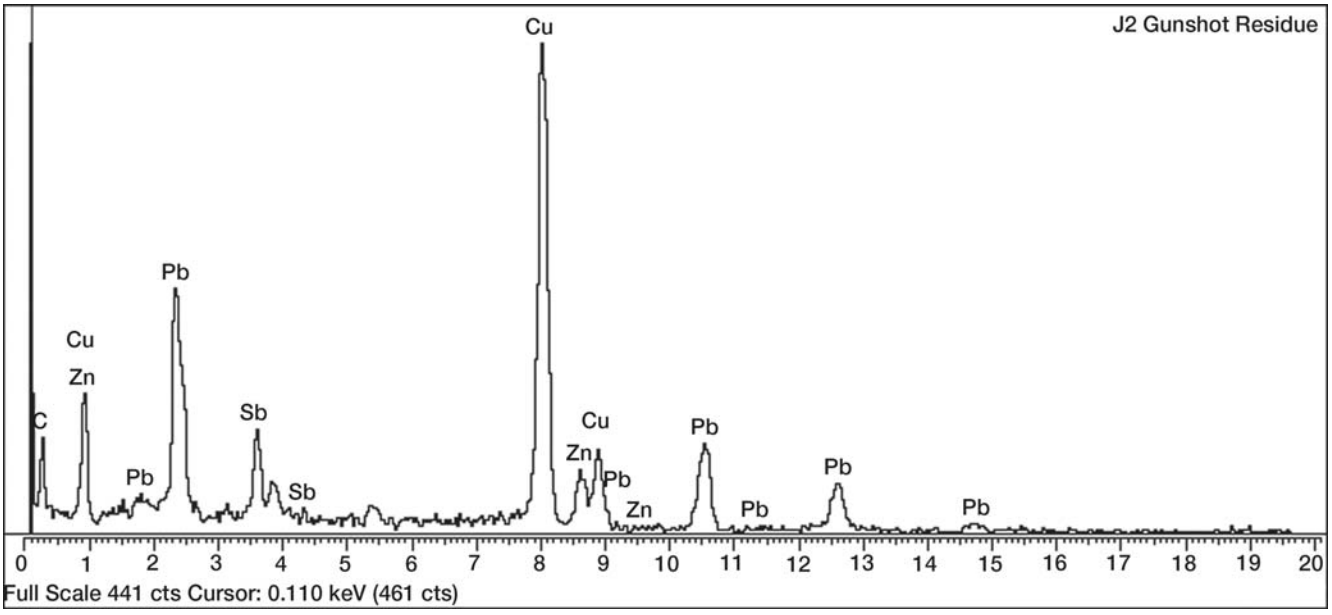

Figure 12. EDS of gun shot residue.

study their impact on trace evidence examinations. MVA is using TEM to develop a particle database that provides a reference atlas of particle types potentially observed during future trace evidence examinations. The database consists of an image, SAED pattern and EDS of several particles of various origins and is constantly being updated. Examples of particles in the database are shown in Figures 8-12. TEM images of specific minerals may also be found in *The Particle Atlas* (5).

Using TEM in forensic science trace evidence examinations can complement other analytical investigations by gathering morphological, elemental and internal structure information on very small particles that might be overlooked or not easily analyzed using other microscopical techniques.

ACKNOWLEDGMENTS

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