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MSHA STANDARD METHOD FOR FIBER IDENTIFICATION BY ELECTRON MICROSCOPY

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ABSTRACT

The EPA Provisional Methodology Manual represents an eclectic compendium of techniques employed in the analysis of airborne particulates for asbestos by electron microscopy. Philosophically, the methods tested and the resulting conclusions have a certain degree of universal applicability. In practice, however, absolute adherence to the method may not be possible due to differences in mandated standards, methods, and techniques.

The Mine Safety and Health Administration has developed a standard method for the analysis of airborne particulates which roughly parallels the EPA method. Variations in the technique include optical microscopy for fiber count, transmission electron microscopy (TEM) for photomicroscopy and selected area electron diffraction (SAED), scanning transmission electron microscopy (STEM) for image analysis, and energy dispersive x-ray spectrometry (EDS) for elemental composition.

Applications of the technique as a routine analytical method will be discussed, with particular emphasis on areas of variation from the EPA method.

Key Words: Electron microscope; energy dispersive x-ray spectrometry; image analysis; scanning transmission; selected area electron diffraction; transmission electron microscope.

1. Introduction

From the outset, it may be advantageous to state basic agreement with what I perceive to be the pervading philosophy contained in the EPA Provisional Methodology. That is, the application of minimal sample manipulation which yields a viable analysis reflective of actual environmental conditions.

However, each phase in the construction of the Provisional Methodology was predicated upon absolute latitude in the selection process, such that the entire analytical procedure has an optimized quality. This degree of freedom in the selection process is not always available, especially at the analytical level, where specific definitions and mandated techniques demand a different approach to the solution of similar problems.

What will be described in this paper is an independently developed method which generally parallels the Provisional Methodology, but evolved in response to the imposition of somewhat different constraints.

2. Overview of the MSHA Method

The methods employed by the Mine Safety and Health Administration in the analysis of airborne particulate samples for asbestos is governed by specifications found in the Code of Federal Regulations [1]¹. Therein, the term "fiber" is defined as any particulate with a three to one or greater length to width aspect ratio, and a length of five micrometers or longer. The term "asbestos" is recognized as generic, applicable to a number of hydrated silicates, but its use is specifically limited to describe the minerals chrysotile, amosite, crocidolite, anthophyllite asbestos, tremolite asbestos, and actinolite asbestos.

An optical microscopy method, generally known as phase contrast [2], is also specified for use in obtaining gross fiber counts for each sample filter. It is assumed that the entire fibrous content of the sample, as perceived by the optical microscopy method, has the potential of classification as asbestos. Therefore, if the fiber count established by optical microscopy exceeds the threshold limit value (TLV) for asbestos [3], the sample must also be examined by electron microscopy for fiber identification.

Electron microscopy is utilized to obtain a physical characterization of the analyte material, and to make an analytical determination of the nature of specific components of the analyte. Physical characterization is made by a computer assisted image analysis system. Analysis of the analyte is achieved by the classification of individual fibers based upon the crystallographic data contained in selected area electron diffraction (SAED) patterns in combination with elemental compositional data derived from energy dispersive x-ray spectrometry (EDS).

Results from the optical and electron microscopy methods are combined to yield the number of asbestos fibers per unit of air. These data are used to establish if a health problem exists in a specific mining operation, and if so, the nature and extent of that problem.

3. Specimen Preparation

The first variation from the EPA Provisional Methodology, as well as a number of subsequent variations, was necessitated by the use of the phase contrast optical microscopy method. This method specified the use of cellulose acetate membrane filters for sample collection instead of the polycarbonate filters recommended in the Provisional Methodology. In turn, the use of cellulose acetate filters precludes an initial carbon deposition, since replication of surface features would obscure the analyte. However, this same quality of surface roughness acts as an effective entrainment media, thereby minimizing analyte losses that would be incurred during subsequent transportation from remote sampling locations.

Specimens of each sample are prepared on 10 nm thick carbon substrates. The carbon substrates are produced by floating a thin film of one percent parlodion (high purity cellulose nitrate) dissolved in amyl acetate on the surface of reagent grade water. Electron microscope grids are laid on the parlodion film, and the film covered grids are removed to the vacuum evaporator for carbon deposition. The parlodion film acts only as a temporary support for the deposition of carbon, and thereafter is removed by dissolution in acetone vapor using a modified Jaffe washer [4].

Two sections of the filter are excised adjacent to, and on opposite side from the wedge removed for optical microscopy. These are laid, filtrate side down, on individual carbon substrate covered electron microscope grids contained in a Jaffe washer. After about twenty-four hours exposure to acetone vapor, the cellulose acetate filter is completely dissolved, and each specimen receives a second carbon coating which effectively sandwiches the analyte between two layers of carbon.

All substrate and specimen preparation, exclusive of carbon deposition, is performed in separate class 100 environmental chambers, both for the safety of personnel involved and to prevent specimen contamination. Sheets of clean room quality polyurethane are used in preference to fibrous filter papers in the Jaffe washer, and for all cleaning application

¹Figures in brackets refer to the literature references at the end of this paper.

within the environmental chambers. Blank specimens are prepared in conjunction with each group of specimens to test the integrity of the system.

A number of grid materials and mesh sizes have been explored for applicability to analytical electron microscopy, carbon coated composites, beryllium, and gold in size ranges from 75 to 400 mesh. The carbon composite grids proved unusable due to an inherent dimensional instability which caused them to curl during substrate preparation. Both beryllium and gold grids have been successfully applied in the production and analysis of asbestos mineral specimens. However, the openings of the beryllium grids are poorly defined; they are difficult and expensive to obtain in large quantities, and their use poses some very significant health hazards. For these reasons, gold grids appear to be the best selection for large scale routine asbestos analysis.

With respect to grid mesh size, grids with large openings provide a significantly higher percentage of open area for analysis. An example can be found by contrasting a 100 mesh grid with a 400 mesh grid, where the 100 mesh grid will have nearly twice as much open area. The increase in open area, however, means a loss in specimen support, with a resulting increase in specimen substrate failure. Also, each grid opening contains more than one field of view, and therefore care must be exercised that a small degree of overlap is maintained in each field of view as a grid opening is examined. These disadvantages notwithstanding, the increased efficiency realized by the use of large mesh size grids makes their selection advisable.

4. Analytical Procedures

Specimens of the sample are systematically examined in a scanning transmission electron microscope (STEM) at an acceleration voltage of 100 keV and a magnification of 3700 diameters. Each fiber encountered during the examination is photographed at 6000 diameters, and an SAED pattern is obtained and photographically recorded at zero degree tilt angle.

The STEM unit is then switched to the scanning mode at 20 keV, and computer aided image analysis is utilized to obtain individual fiber measurements and statistical data relevant to those measurements. The accumulated statistical data generated by the image analysis system, and printed at the end of each field of view, is used to determine when adequate data for the physical characterization of the analyte has been achieved.

A deadtime corrected EDS spectra is accumulated for 200 seconds from each fiber at a rate of 300 counts per second. A computer program compares this spectra to an internally stored linear regression and quadratic plots of intensity versus concentration for 10 key elements (Na, Mg, Al, Si, K, Ca, Ti, Cr, Mn, and Fe), and calculates elemental composition for the unknown spectra. The internal plots utilized in this calculation were generated by actual analysis of chrysotile and the five specified asbestiform amphibole minerals obtained from at least two different sources, and represented by at least 10 different spectra from each mineral species.

At the end of analysis all SAED patterns are intensely examined, and each fiber is classified as either chrysotile, asbestiform amphibole, not asbestos, or ambiguous. The classification of an SAED pattern produced by chrysotile is relatively straight forward, since these patterns are composed of uniquely streaked diffraction spots arranged in well defined arrays known as layer lines, which approximately correspond to a 0.53 nm periodicity [5,6]. Other minerals can also exhibit a 0.53 nm layer line periodicity; however, none of these display the prominent streaking so evident in the case of chrysotile.

Differentiation between asbestiform amphibole minerals and associated nonasbestos minerals can also be made by careful and critical inspection of the SAED pattern. The characteristic patterns produced by the asbestiform amphibole minerals will have prominent layer lines, numerous reasonably well defined diffraction spots that are closely spaced on each layer line, a 0.53 nm layer line periodicity, and low angle diffraction spots. However, individual members of the mineral group cannot be readily distinguished by visual analysis of the SAED pattern.

Species identification of the asbestiform amphibole minerals is achieved by confirmation that the SAED pattern is typical of this group, and that the computed elemental

composition derived from the EDS spectra is within the published compositional range [7-11] of one of these minerals for all 10 key elements. If either of these two identification criterion of positive SAED pattern classification as asbestiform amphibole and a fit of the EDS spectra to the published compositional data are not achieved, then the fiber cannot be identified as asbestos.

Fibers which produce SAED patterns that cannot be positively classified as chrysotile or asbestiform amphibole, are classified as not asbestos. Typically, such an SAED pattern would fail to have a definite layer line morphology (i.e., quasi-hexagonal array), a layer line periodicity not equal to 0.53 nm, an inadequate number of diffraction spots, or a combination of the above conditions. Fibers which do not yield SAED patterns, or which produce patterns with insufficient crystallographic data are classified as ambiguous.

5. Summary

The methods outlined in this paper are the result of both practical experience and carefully designed experimentation. It is neither the intent nor the essence of this presentation to advocate a system, method, or technique simply for the purpose of "marching to the beat of a different drummer".

Instead, what has been described is a viable analytical method evolved in response to a specific set of circumstances other than those imposed in the development of the EPA Provisional Methodology. Although this violates, so to speak, the letter of the law, the spirit remains intact.

References

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