U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-274 750

Review and Evaluation of Analytical Methods for Environmental Studies of Fibrous Particulate Exposures

National Inst for Occupational Safety & Health, Cincinnati, OH

May 77

BIBLIOGRAPHIC DATA SHEET	1. Report No. NIOSH-77-204	2.	
4. Title and Subtitle REVIEW AND EVALUATION OF ANALYTICAL METHODS FOR ENVIRONMENTAL STUDIES OF FIBROUS PARTICULATE EXPOSURES			May 1977
			6.
7. Author(s) Ralph D. Zumwalde, and John M. Dement			8. Performing Organization Rept. No.
9. Performing Organization Name and Address National Institute for Occupational Safety and Health 4676 Columbia Parkway Cincinnati, Ohio 45226			10. Project/Task/Work Unit No.
			11. Contract/Grant No.
12. Sponsoring Organization Name and Address			13. Type of Report & Period Covered
			14.

15. Supplementary Notes

16. Abstracts

Sampling and analytical methods which may be used to identify and quantify fibrous particulates in environmental samples are reviewed and the electron microscopic methods used by NIOSH are described in detail. The main topics covered include: optical microscopic methods; differential thermal analysis; X-ray diffraction; microchemical techniques; comparison of transmission and scanning electron microscopy; methods for fiber sampling and analysis, including sample collection and preparation, analytical instrumentation, and identification and characterization procedures. Electron photomicrographs of many fibrous minerals are included.

17. Key Words and Document Analysis. 17a. Descriptors

Environmental surveys
Atmosphere contamination control
Dust control
Air pollution
Analyzing
Microscopy
Microanalysis

17b. Identifiers/Open-Ended Terms

Analytical methods

17c. COSATI Field/Group 06/J

18. Availability Statement	19. Security Class (This Report)	
Release unlimited	UNCLASSIFIED	
	20. Security Class (This Page) UNCLASSIFIED	22. Price A04-A01

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REVIEW AND EVALUATION OF ANALYTICAL METHODS FOR ENVIRONMENTAL STUDIES OF FIBROUS PARTICULATE EXPOSURES

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For sale by the Superintendent of Documents U S Government Printing Office Washington, D C 20402

May 1977

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DHEW (NIOSH) Publication No. 77-204

ABSTRACT

This report reviews sampling and analytical methods which may be used to identify and quantify fibrous particulates in environmental samples and describes in detail the electron microscopic methods used by the National Institute for Occupational Safety and Health (NIOSH). Electron photomicrographs of many fibrous minerals are included.

ACKNOWLEDGMENT

The authors express sincere appreciation to Dennis Roberts and Robert Phillips of the Industrial Hygiene Section, Industry-Wide Studies Branch, Division of Surveillance, Hazard Evaluations, and Field Studies, NIOSH, for their assistance in producing the electron photomicrographs and other photographs shown in this paper. Special acknowledgment must also be made for the efforts of Patricia Johnson, who assumed the responsibility for typing and proofreading.

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INTRODUCTION

Concern for potential health hazards associated with occupational exposures to fibrous particulates has spurred much research in this area. In addition to laboratory toxicity studies, the National Institute for Occupational Safety and Health (NIOSH) has underway numerous epidemiological studies of occupational cohorts with exposure to fibrous particulates. A large part of these efforts involve studies to fully characterize occupational exposures.

This characterization of exposure and the identification of microscopic fibrous particulates has become an important priority in recent years due to their presence in the environment and their association with pathogenesis.

Many fibrous particulates are minerals which occur in a fibrous (e.g., acicular, spiny, needle, tabular, etc.) geological state. Some of these minerals are selectively mined whereas the majority are contaminants found in commercial products. Appendix A lists some minerals which are commonly found in a fibrous state.

With the aid of a transmission electron microscope capable of selected area electron diffraction and energy dispersive X-ray analysis, it is possible to identify the various types of fibrous minerals. This identification depends upon the production of accurate microchemical data, fiber morphology and structural characteristics, and interpretation of selected area electron diffraction patterns. A transmission electron microscope fitted with these analytical accessories is more versatile than other combinations of electron, optical, and X-ray analytical equipment. It can display the microchemical X-ray spectra obtained from the particulate and simultaneously obtain crystal structure data with selected area electron diffraction.

For studies to assess the potential adverse health effect of these particulates, an analytical design has been developed that will definitively identify fibrous particulates and allow concentration determinations in environmental samples. Particular attention is given to differentiating asbestiform and non-asbestiform minerals.

Although a variety of analytical methods have been proposed and used to identify and quantify fibrous minerals, each has limitations. Available analytical methods are presented and discussed in this paper along with a detailed description of a method now being used within NIOSH.

REVIEW OF ANALYTICAL TECHNIQUES FOR FIBERS

Many analytical techniques have been proposed to identify and quantify fibrous minerals. These techniques include optical and electron microscopy, microchemical analysis, X-ray diffraction, and differential thermal analysis. All these have instrumental limitations which depend upon the quantity of material present, morphology, orientation, and chemical composition of the sample. Hence, identifying and quantifying any fibrous particulate in air, water, or tissue are difficult for a variety of reasons:

1) Asbestos and other fibrous minerals are generally present in low mass quantities even though fiber number concentrations may be high.
2) Many analytical techniques cannot differentiate between fibrous and nonfibrous mineralogical polymorphs.
3) Many fibrous minerals present in both water and air samples generally have physical dimensions below the resolution limits of optical microscopy.
4) In some instances, environmental conditions to which the fibers have been subjected and/or the different elemental compositions of geologic formations may alter elemental composition ratios, making positive identification by chemical techniques impractical.
5) Identification by morphology is extremely difficult and impractical for many asbestos and nonasbestos fibrous minerals.
6) The analytical methodology of selected area electron diffraction (SAED) offers some identification capabilities, but only under ideal conditions. Difficulties in identification are often due to the improper orientation of fibers, lack of appropriate mineral standards, and dissemination of other elements within the fibers.

In addition to the identification and quantification of fibrous minerals in air, water, and tissue, other factors such as particle size and morphology should also be determined. The following paragraphs describe analytical methods which have been used to quantify fibrous minerals in environmental, tissue, and bulk samples. Although most of this discussion centers on the asbestos minerals, similar arguments apply to each of the fibrous minerals listed in Appendix A.

OPTICAL MICROSCOPIC METHODS

Several optical microscopic techniques have been used to identify and quantify asbestos fibers. In the United States, an optical microscopic technique for quantitative determinations of asbestos fibers in air is used to determine compliance with the occupational exposure standards.[1] The method consists of collecting breathing zone samples over 15-minute to 4-hour periods on membrane filters (Millipore AA). Samples are analyzed by first dissolving the membrane filter to make it optically transparent and then counting the fibers at 400-450X magnification by phase contrast optical microscopy. Asbestos fibers are defined as those particulates with lengths greater than 5 micrometers (μm) and a length-to-diameter ratio of 3-to-1 or greater. This technique is not specific for asbestos fibers or any other fiber type. In addition it cannot detect fibers less than approximately 0.2 μm in diameter.

Petrographic microscopic techniques may be used to identify fibers greater than approximately 0.2 to 0.3 μm in diameter. Various optical crystallographic measurements such as refractive index, extinction angles, and sign of elongation may be measured with the polarizing microscope, and compared with data reported for standard asbestos reference samples. Typical optical data for selected asbestos minerals are shown in Table 1.[2]

Table 1

TYPICAL OPTICAL DATA FOR ASBESTOS

Asbestos Type	Crystal System	Refractive Indices	Extinction Angles	Sign Of Elongation
Chrysotile	monoclinic	1.49-1.57	$\gamma AL^* = 0^o$	+
Anthophyllite	orthorhombic	1.60-1.66	$\gamma AL = 0^o$	+
Amosite	monoclinic	1.66-1.70	$\gamma AL = 14\text{-}21^o$	+
Crocidolite	monoclinic	1.69-1.71	$\gamma AL = 3\text{-}15^o$	-
Tremolite	monoclinic	1.60-1.65	$\gamma AL = 10\text{-}21^o$	+
Actinolite	monoclinic	1.62-1.68	$\gamma AL = 10\text{-}15^o$	+

*L = long direction of fiber

Dispersion staining used with plane polarized light may also be used to differentiate between asbestos and other fibers.[2,3] With this technique, the fibers are immersed in a mounting liquid with a similar refractive index but a steeper dispersion curve than that of the fibers. A central or annular stop is used in the back focal plane of the objective lens to allow for appropriate dispersion colors. When plane polarized light is used, asbestos fibers show two characteristic dispersion staining colors, one for the light vibration parallel to the fiber length and another perpendicular to the fiber length. The dispersion colors depend on the refractive index of the medium in which the fibers are mounted, as shown in Table 2. Dispersion staining colors may change slightly depending on the geographic area from which the asbestos is mined. Fibers less than 0.5 μm in diameter may not be identified by this technique due to difficulties in distinguishing colors.

DIFFERENTIAL THERMAL ANALYSIS

Differential thermal analysis has been used to a limited extent in determining asbestos fiber levels in talc samples.[4] Chrysotile (serpentine minerals) shows a dehydroxylation endotherm at approximately 650°C and an exotherm at approximately 820°C associated with the formation of forsterite. These peaks may be used for quantitative analysis. When a 140-mg sample holder with an exposed loop differential thermocouple and a 10°C/minute heating rate is used, a 1% level of chrysotile can be detected in pharmaceutical grade talc.[4] With this method, a dynamic helium atmosphere is maintained to expel gaseous mineral decomposition products and to prevent oxidation.

Table 2

DISPERSION STAINING COLORS FOR ASBESTOS
USING A CENTRAL STOP AND
PLANE POLARIZED LIGHT

Asbestos Type	Refractive Index Liquid	Dispersion Staining Colors	
		Parallel	Perpendicular
Chrysotile	1.560	light blue	magenta
Anthophyllite	1.610	blue-green	golden yellow
Amosite	1.670	red magenta	golden yellow
Crocidolite	1.700	magenta	blue magenta

Differential thermal analysis has not been used for environmental samples because the lower limits of mass detection are extremely poor. In addition, differential thermal analysis is not capable of differentiating between fibrous and nonfibrous mineralogical polymorphs.

X-RAY DIFFRACTION

X-ray powder diffractometry is a standard mineralogical technique used in the analysis of solid crystalline phases. It is also widely used to identify and quantify asbestos fibers in bulk materials such as talc[5,6] and other industrial materials,[7,8,9] and to study amphibole asbestos contamination of water.[10]

X-ray diffraction is generally considered more sensitive for asbestos than light microscopy but less sensitive than some analytical methods using electron microscopy.[10] Diffraction lines and relative intensities for each of the asbestos minerals, as well as other fibrous minerals, have been published and catalogued in the ASTM Powder Diffraction File. Variations in fiber chemical composition, especially for the amphiboles, may result in slight peak shifts from reported X-ray diffraction data.

Quantitative determinations of asbestos and nonasbestos fiber levels in bulk samples requires that average particle size be 0.1 to 10 μm. A number of techniques have been used to minimize preferred orientation including binder and slurry mounting methods and backloading of dry powders. Rohl and Langer have developed a method for reducing preferred orientation by filtering an aqueous slurry through Millipore filters using a filtration adapter attached to a hypodermic syringe.[6] Other investigators have used the backloading technique with multiple X-ray diffraction scans.

Using conventional X-ray diffraction scan rates (0.5 to 1 degree 2 theta per minute), the lower limits of detection for the asbestos minerals are approximately 5 percent.[7,8] Automated step scanning procedures, by which diagnostic reflections are slowly scanned and integrated counts recorded, have been reported to significantly increase detectable limits. Using the automated step scanning procedure, the sensitivity for asbestos in talc has been increased to detect as low as 0.1 percent asbestos when using external dilution standards for calibration.[6] Similar lower detectable levels have been reported by other investigators.[5]

X-ray diffraction has limited application for routine analysis of environmental samples for asbestos fibers. Birks et al.[11] studied the quantitative analysis of airborne asbestos by X-ray diffraction. They used a specially-designed diffraction apparatus housing two X-ray detectors. Their technique involved alignment of the asbestos fibers in an electrostatic field to enhance diffraction intensity. A lower detection limit of 0.4 to 0.5 micrograms for chrysotile was reported. However, this technique has not been applied to actual environmental samples. NIOSH is presently evaluating this technique with chrysotile collected on silver membrane filters. Preliminary results suggest a lower limit of detection of 15 micrograms.

Amphibole and cummingtonite-grunerite mass concentrations in water samples have been semiquantitatively determined using X-ray diffraction with step scanning.[10] This technique requires filtering the water through 0.45 micrometer Millipore filters followed by step scanning a major amphibole diffraction peak (110) and a peak specific to cummingtonite-grunerite (310). The integrated peak count above background is recorded with mass concentrations determined using external dilution standards.

The proper selection of diagnostic reflections to maximize detection sensitivity and minimize interference due to other mineral phases is necessary for the best use of X-ray diffraction. It must also be recognized that X-ray diffraction methods, like differential thermal analysis, cannot distinguish between fibrous and nonfibrous mineralogical polymorphs.

ELECTRON MICROSCOPY

Both transmission and scanning electron microscopy have been used to identify and quantify particulates. Data from morphological observation, analytical data from selected area electron diffraction, and microchemical analytical techniques may be used to identify particulates.

Selected Area Electron Diffraction

Since all crystalline materials scatter electrons in regular patterns relative to their crystal structure, a transmission electron microscope (TEM) with selected area electron diffraction (SAED) may be used to form a diffraction image on the electron microscope viewing screen. The diffraction image of the scattered electrons can be predicted by Bragg's Law. Observation of single fiber (single crystal) electron diffraction patterns may be used to differentiate chrysotile fibers from amphibole fibers.[12,13] The SAED pattern for any chrysotile fiber tends to be analogous to a rotating or oscillating crystal X-ray diffraction pattern in which the long dimension of the fiber tends to lie nearly parallel to the supporting membrane and therefore perpendicular to the incident beam. Chrysotile fibers usually produce streaked diffraction patterns (due to lattice defects) with the streaks or layer lines perpendicular to the fiber length. The spacing between the layer lines denotes the fiber "a" axis of approximately 5.3 angstroms.[10] The reflections along

the layer lines are usually very streaked and Debye-Scherrer rings may be observed when clumps of randomly oriented fibers are presented. Progressive electron-beam bombardment may, however, alter the diffraction pattern due to fiber damage.[10]

Chrysotile fibers can appear as bundles of fibrils or round single fibrils. Often, the fibrils can be distinguished by their tubular appearance. This tubular appearance is characteristic of chrysotile, but is not always discernible due to beam damage[12] or the attachment of amorphous material.[14] Chrysotile and other fibrous minerals, such as halloysite, have hollow centers.

The amphibole minerals are generally straighter in physical appearance than chrysotile fibers. Observation of amphiboles using transmission electron microscopy often reveals light and dark banding (diffraction images) which may cross the fiber at right angles.[12] Since the selected area electron diffraction patterns for all the amphibole asbestos minerals are similar, observation of these patterns may only identify the fiber as being a fibrous amphibole.[10,12] Amphibole electron diffraction patterns show layers and sometimes streaks perpendicular to the fiber length with the spacing between the layer lines or streaks representing the fiber "c" axis of approximately 5.3 angstroms. There is less streaking along the layer lines, in contrast to chrysotile, with the spot repeat along the lines representing one of the two remaining lattice spacings ("b" or "a"), depending on fiber orientation relative to the electron beam.

In addition to observation of electron diffraction patterns for fiber identification, photomicrographs can be made of the diffraction patterns and crystal "d"

spacings measured from the plate and calculated, using the instrument camera constant.[13] Both "spot" and polycrystalline patterns may be measured; however, these intensities may not be the same as those observed for X-ray powder patterns. Additional reflections may be present, and measurements of "d" spacings are less accurate when electron diffraction is used than when X-ray diffraction is used.

Microchemical (Microprobe) Techniques

Electron beam microchemical analysis may sometimes be used to distinguish asbestos fibers from other fibrous particles.[15,16,17,18] The most common system in use is the energy dispersive X-ray detector in combination with either a scanning or transmission electron microscope. Wavelength X-ray analyzers and the conventional electron microprobe have been used; however, their routine application is limited due to longer data acquisition times.[18] Data acquisition times with energy dispersive analyzers, however, are far less, ranging from 20 to 80 seconds per analysis depending on fiber density, size, and desired statistical confidence.

Semiquantitative microchemical analysis with the electron microscope is performed with a beam of high energy electrons incident upon a fiber which generates X-rays characteristic of the elements in that fiber. These X-rays are detected by a lithium-drifted silicon crystal detector placed in the electron microscope column close to the specimen.

The energy of the X-ray photon is converted to a voltage pulse which is amplified, digitized, stored in a multichannel analyzer or a minicomputer, and can be displayed on a cathode ray tube. Using the energy dispersive detector, all elements with atomic numbers of sodium (Z=11) or higher may be analyzed.

Each of the asbestos minerals has a characteristic X-ray spectrum, which, when combined with fiber morphology, allows for its identification.[15,16,19] Observation of the semiquantitative fiber X-ray spectrum is usually sufficient for asbestos fiber identification; however, three component diagrams have been used after subtracting the continuous background from the semiquantitative X-ray spectrum.[15] For asbestos fiber analysis, matrix corrections are rarely used. Typically iron, magnesium, and silicon are plotted on the three component diagram and compositional boundaries for the asbestos minerals established. Unfortunately, this technique suffers from the inability to use all compositional data obtained, such as the presence or absence of sodium, calcium, aluminum, and manganese which aid in the identification.[12]

With energy dispersive X-ray techniques, the comparison of only elemental intensities may not be sufficient for positive identification between asbestos and nonasbestos fibrous minerals which show similar elemental intensities.[14] For example, chrysotile, anthophyllite, and fibrous talc, all of which have similar elemental compositions, may be difficult to differentiate.[15,19] However, these materials may easily be distinguished when elemental data is supplemented with selected area electron diffraction.

COMPARISON OF TRANSMISSION AND SCANNING ELECTRON MICROSCOPY

Both transmission and scanning electron microscopy offer certain advantages. Scanning electron microscopy, using either secondary or backscattered electron images, offers better observation of surface topography whereas transmission electron microscopy offers far superior image resolution. Fiber identification by scanning electron microscopy is limited since electron diffraction studies are not possible. Scanning electron microscopy combined with microchemical

analysis is sufficient for fiber identification only when the mineralogy of the fiber source is well known.[14] A new application of scanning transmission electron microscopy (STEM) is now being used on a limited scale. Electron diffraction studies may be possible with some of these instruments; however, diffraction patterns are much more difficult to achieve than conventional SAED patterns.

A transmission electron microscope is now available which is equipped with an energy dispersive X-ray detector, allowing simultaneous observation of morphology, crystal structure, and elemental composition. This method has been used to study asbestos fibers in environmental and material samples.[10,19] This combination of analytical instrumentation greatly increases the probability of definitive particle or fiber identification. Many researchers regard this combination as the "state-of-the-art" with regard to particulate and fiber studies.

SAMPLE PREPARATION FOR ELECTRON MICROSCOPY

Particulate concentrations in environmental and tissue samples have been analyzed using a variety of electron microscopy sample preparation techniques. Environmental samples (air and water) are generally collected on cellulose ester (Millipore, Gelman, etc.) or polycarbonate (Nuclepore) filters by concentrating the sample by filtration, centrifuging, etc.[10,20]

For SEM, Nuclepore filters are most often used due to their smooth surface which may be directly coated with an appropriate metal (gold, platinum, etc.) and analyzed. Millipore filters tend to have a rough surface texture and are not generally suitable for direct coating for SEM. Also, small fibers or particles below the filter surface may escape detection.[20]

For TEM, the filter substrate must be removed and the particles mounted on suitable electron microscope grids. A wide variety of mounting techniques have been used. The two most commonly used methods are the Jaffe Wick[21,22] and the condensation washing[23] techniques. These techniques offer simplicity and maintain most of the original particle size distribution on the sample. However, some investigators have reported particle losses up to 60% with Millipore filters when using the condensation washing method with rapid filter dissolution. Losses with the Jaffe Wick method have been reported to be considerable less (<10%).[24] Particle loss decreases in the condensation washing method when much slower filter dissolution is used. A modification of the Jaffe Wick method has been reported to reduce particle loss.[25] The filter is coated with silicon monoxide or carbon by vacuum evaporation prior to dissolving the Millipore filter. Likewise, several investigators have reported minimal particle loss with Nuclepore filters when the filter is coated with carbon prior to dissolving the filter substrate.[10,17]

In addition to the "direct clearing/mounting" techniques mentioned above, a variety of other techniques have also been used for preparing environmental samples. Selikoff et al.[26] have used a "rub-out" technique, in which the Millipore filter is ashed in a low temperature asher to remove organic or carbonaceous material. The residue is then dispersed on a microscope slide using a solution of 1% nitrocellulose in amyl acetate. After grinding with the surface of a watch glass to liberate individual fibers, the sample is dispersed evenly between two microscope slides forming a thin film which is transferred to standard electron microscope grids. With this technique particle losses averaging 50% and increases in the number of fibers were reported.

For examining asbestos fibers in biological tissue samples, a variety of preparation techniques may be employed.[26,27,28,29,30,31] Pooley[27] has used a direct transfer method for formalin-fixed tissue. With this method, diced tissue is first dissolved in a 40% solution of potassium hydroxide, and a drop of the digested lung residue is then transferred directly to electron microscope grids prepared with an appropriate support film. Pooley[27] has also developed a technique for preparing standard histological sections in paraffin. With this technique, a section is deposited on a glass microscope slide and washed with xylene and alcohol to remove the paraffin. The sections are then ashed in a muffle furnace at 450° to 500°C for approximately 15 minutes. The tissue ash plus any associated minerals are then removed from the glass slide by a replication process using polyvinyl alcohol. After carbon coating, the plastic film is removed by a warm distilled water bath and transferred to an appropriate electron microscope grid.

Langer et al.[26] have reported a qualitative method for preparing tissue samples for electron microscopy. The tissue is dissolved in a 40% solution of potassium hydroxide and separated in a centrifuge. The residue is then dispersed in distilled water and pipetted onto formvar coated 200 mesh electron microscope grids. Similar techniques have been used by Pontefract and Cummingham.[32]

Bouffant[31] has also reported a quantitative method for determining asbestos fiber concentrations in biological materials. This technique first incinerates the biological material in activated oxygen at 150°C and then attacks the ash with 1N HCl for 18 hours. The residue is filtered through a membrane filter which has previously been coated with a carbon film. The membrane is then dissolved, depositing the fibers on the electron microscope grid substrate.

Asbestos fiber levels in environmental samples and biological tissue are usually expressed as asbestos fibers per unit volume of sample (fibers/m^3, fibers/liter, fibers/cc, fibers/gm dry lung, etc.). These concentrations are determined by counting fibers within calibrated areas on the electron microscope viewing screen or by counting fibers from photomicrographs. Asbestos fiber concentrations in water samples determined by laboratories using the same mounting techniques have been reported to vary by a factor of 2 to 3.[10] Much larger variations have been reported between laboratories using different techniques.

Asbestos (chrysotile) mass concentrations in environmental samples have also been determined using electron microscopy. This is accomplished by measuring the length and diameter (volume) of each fiber and calculating mass using the appropriate density.[26] The accuracy of this technique has not been studied in detail.

METHODS FOR FIBER SAMPLING AND ANALYSIS FOR ELECTRON MICROSCOPIC STUDIES CURRENTLY USED FOR NIOSH FIELD STUDIES

SAMPLE COLLECTION

Within any environmental parameter (i.e. ambient, industrial, water, etc.) the collection of a suitable sample which most closely characterizes an environment is of utmost importance. This sample must retain the contaminant without changing its physical or chemical characteristics. Sample collection is as important as sample preparation and analysis.

As previously discussed, there are basically two sampling filters which have been used in the collection of particulates, the Millipore (cellulose ester) and the Nuclepore (polycarbonate). Each of these filters is available in a variety of pore sizes and filter diameters which make them applicable to the collection of almost any particulate. The surface properties differ between the two filters due to their composition. The Millipore surface consists of a matted network of cellulose ester, creating a circuitous air path through the filter. The collection efficiency is extremely good due to the surface topography and the tendency for particles to be collected by impact and interception. Unlike the Millipore, the Nuclepore has a smooth collection surface, making it advantageous for electron microscopical evaluation since the collected particulates all lie in one plane. Unfortunately, as the particulate load increases on the filter surface, the pressure drop increases proportionately. This makes it difficult, during field collection, to obtain a sample of sufficient size for electron microscopy. In addition, redistribution

and particle loss from the surface of the Nuclepore often occurs during handling of the filter. Because of the disadvantages in sample retention with the Nuclepore, the Millipore is preferred by NIOSH for sample collection.

SAMPLE PREPARATION

The analytical procedure requires a sample preparation that is suitable for TEM. Like all preparation methods for TEM, the filter substrate must be removed and the sampled material deposited on a suitable electron microscope grid. This procedure must be gentle so as not to cause redistribution, change in physical or chemical nature, or loss of the sample. The two most commonly used preparation methods reported in the literature for both Millipore and Nuclepore have been the Jaffe Wick[21,22] and the condensation washing[23] techniques. However, these methods do offer some difficulty in the complete dissolution of the filter as well as substantial particle loss.

The sample preparation method currently being used by NIOSH is a modification of a particle transfer technique developed by Ortiz and Isom.[25] The technique is designed to utilize various Millipore filters (Millipore AA, HA, GS, and VM) with different pore size diameters. With this technique, observed particle losses have not been size dependent, with spherical particle losses not exceeding 10% and fibrous particle loss less than 5%.[25]

Sample preparation requires cutting a section of the membrane filter either with a cork bore (8mm diameter) or a scalpel. The section is removed and placed

sample side up on a clean microscope slide. If the circular section from the cork bore is used, the edges of the section can be fastened to the slide with a gummed binder ring. Likewise, if a section is removed with a scalpel, it can be fastened to the slide using narrow strips of transparent tape (Figure 1). The slide assembly is placed in a petri dish on top of four Whatman filters which have been saturated with acetone and covered (Figure 2). The acetone vapors destroy the microporous structure of the filter by slow dissolution and produce a fused, microscopically smooth surface on the sampled side of the membrane filter. The extent of filter exposure to the vapor is important and is controlled by limiting the time that the filter is kept in the vapor bath. Specific filter fusion and dissolution times vary with the types of collected particles. Normally 2 to 15 minutes is sufficient to produce a smooth matrix. If the filter exposure time is too long, the fusing membrane may flow around and over the collected particulates and encapsulate them. These encapsulated particles may later be washed away by the solvent during the subsequent dissolution step. If the vapor exposure is too short, the surface fusion of the membrane is incomplete and the final preparation will contain a coarse, grainy background which results from replicating the residual porous filter structure. A 10-minute fusion time has been found to be generally acceptable when Millipore AA filters are used.

Once the filter section has been fused, the slide assembly is placed in a vacuum evaporator on a rotary stage, where the sampled side of the filter is then coated with carbon by conventional techniques. The rotating filter

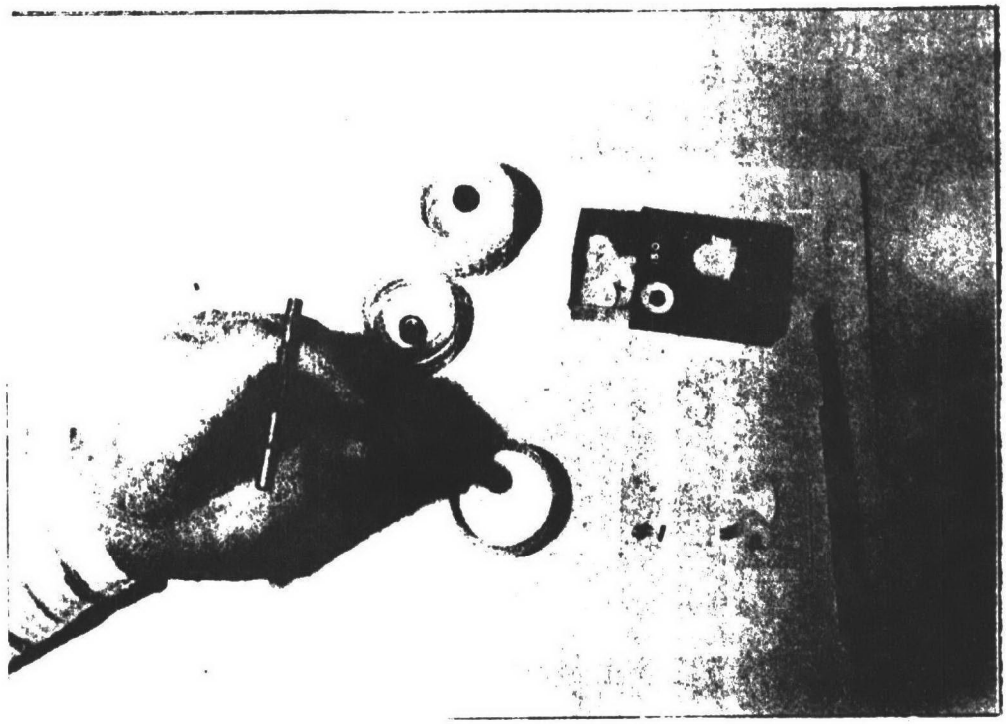


Figure 1. Cutting and removing Millipore filter section for TEM sample preparation

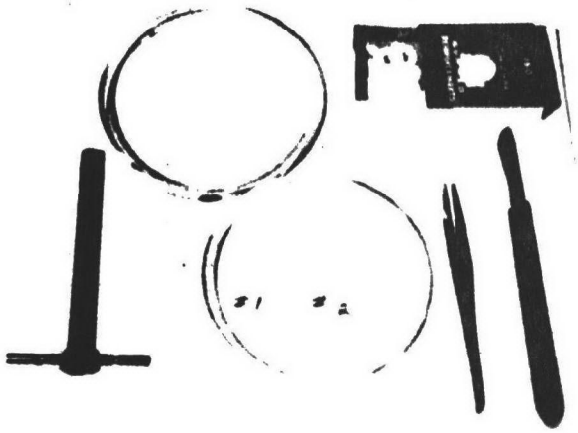


Figure 2. Petri dish with
Whatman filters used for
fusing Millipore filters

is coated heavily with carbon using a carbon tip which measures 5 mm in length by 1 mm in diameter. The tip of the carbon rod should be perpendicular, approximately 10 cm from the center of the filter section. The continuous carbon film produced adheres directly to the fused membrane surface and to the collected particulates (Figure 3).

Next, using the petri dish as a reservior, four Whatman filters are placed in the base and saturated with acetone. Several blank 200-mesh formvar/carbon-coated electron microscope (EM) grids are placed right side up on the Whatman filters. Sections, somewhat larger in size than the grids, are cut from the coated filter and placed, sample side down, on the EM grid. The sections can be readily cut out using an appropriate cork bore or scalpel (Figure 4). Care must be taken to prevent the coated filter section from curling or shriveling when it is placed on the EM grid. This can be achieved by holding down the edges of the filter section with tweezers until the acetone solution has made contact.

The acetone saturated Whatman filters provide a porous platform for the gentle chemical dissolution of the original membrane filter. The filter matrix is dissolved, leaving the particulates adhering to the carbon film, which is, in turn, supported by the EM grids.

Saturation of the Whatman filters should be maintained for 8 to 16 hours to complete total dissolution of the filter. Acetone may have to be added periodically due to evaporation. The acetone solution should never permeate above the surface of the top Whatman filter.

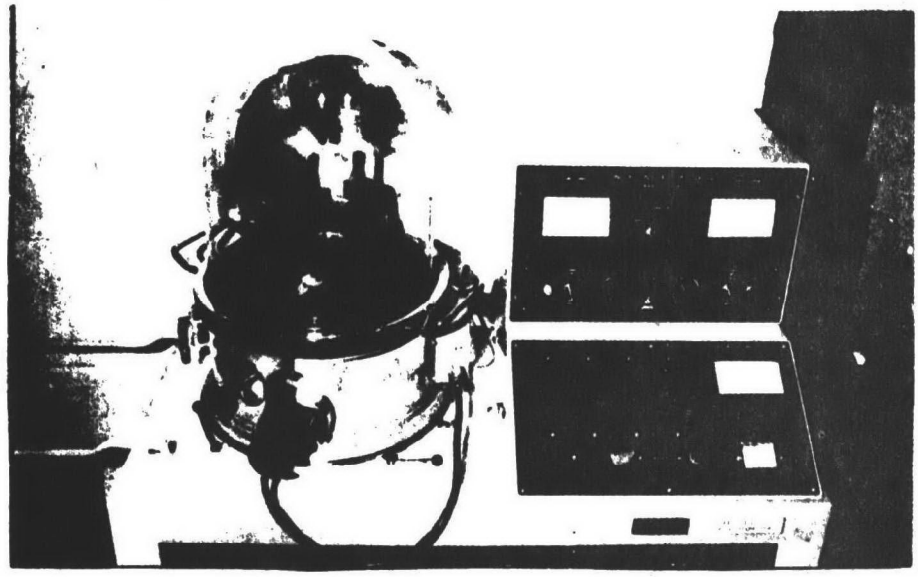


Figure 3. Vacuum evaporator assembly for carbon coating fused Millipore filters

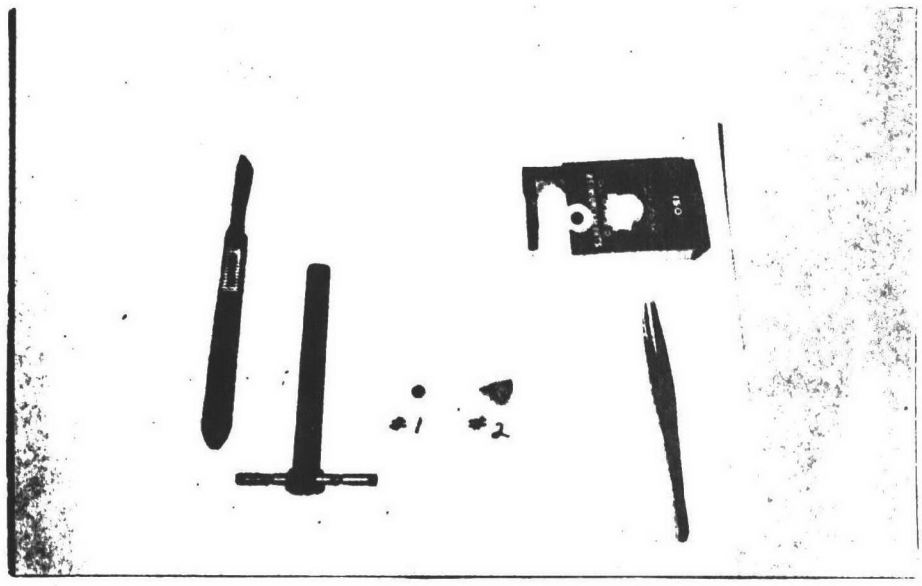
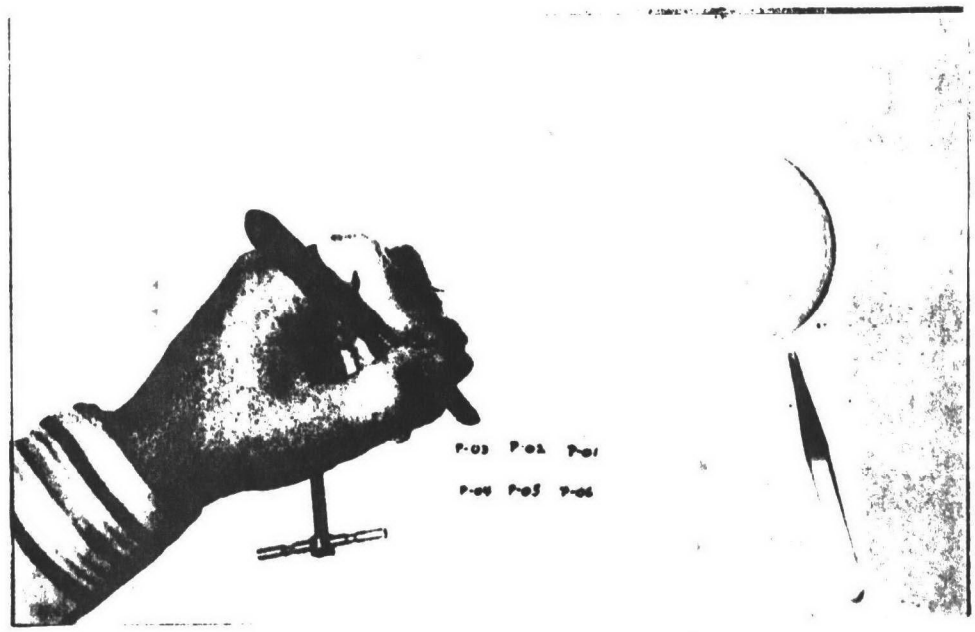


Figure 3. Carbon coated Millipore filter sections



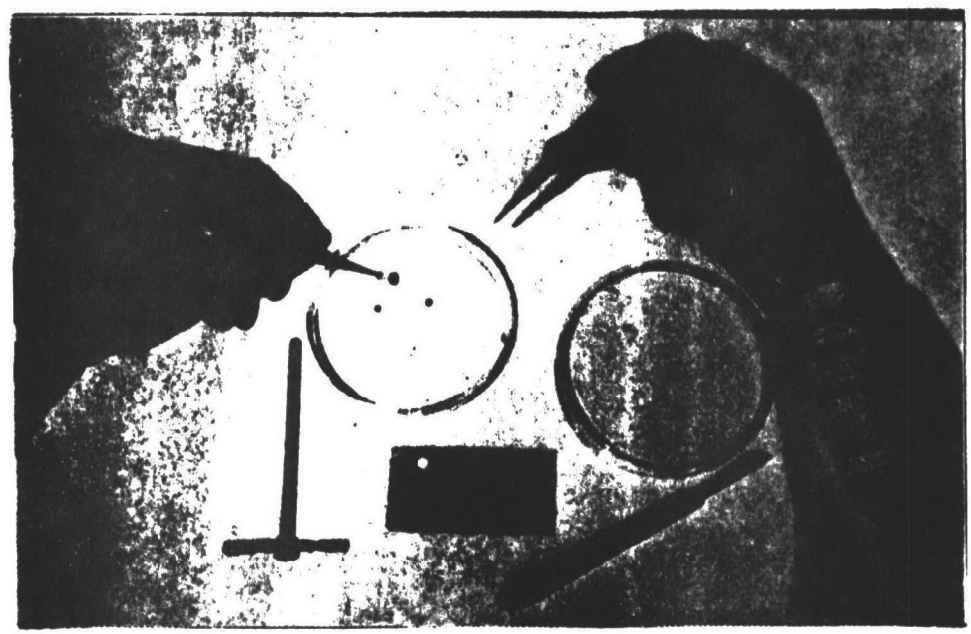


Figure 4. The removing and mounting of a carbon coated sample preparation onto a TEM grid

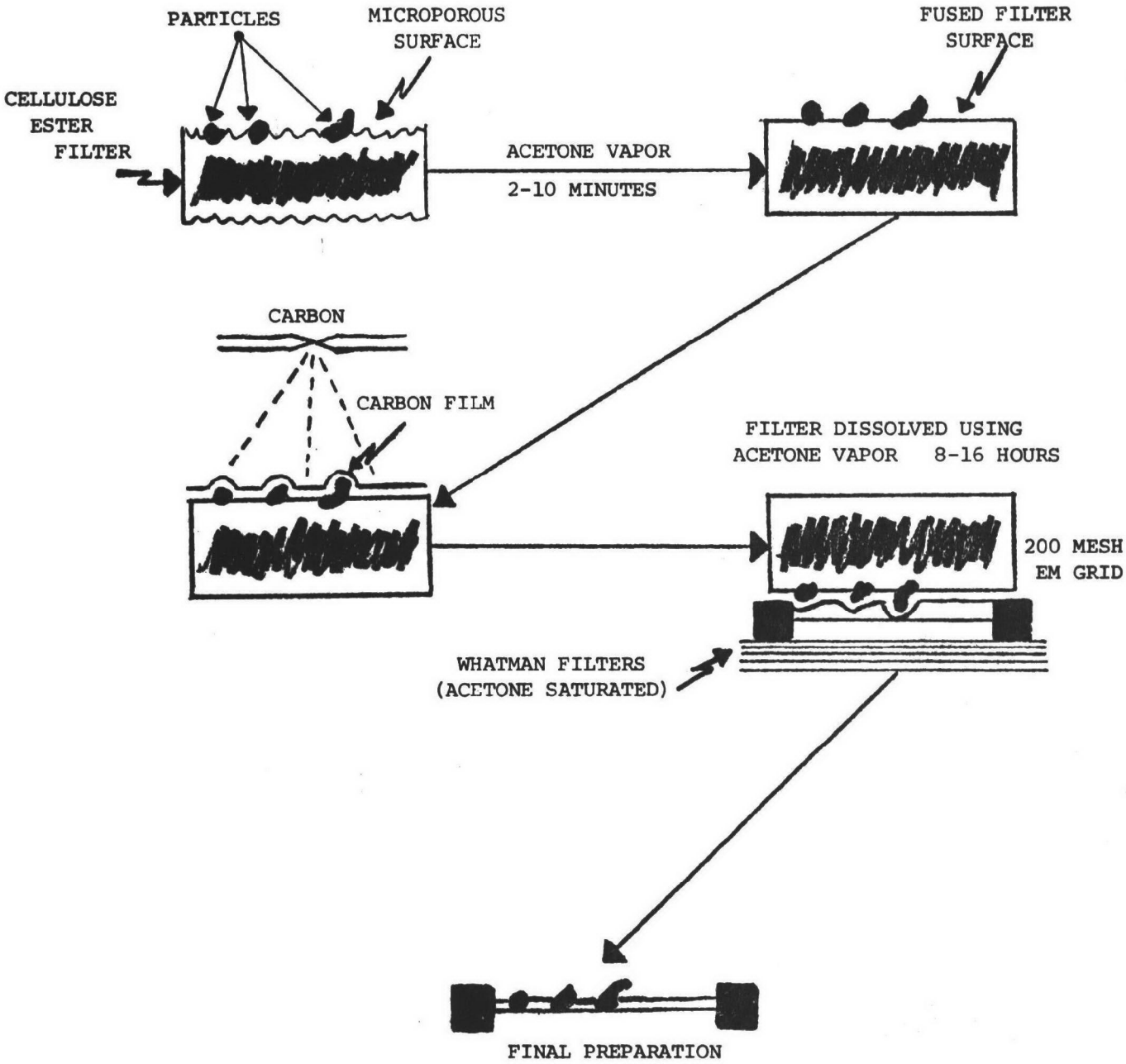


Figure 5. Modified Ortiz and Isom mounting technique

ANALYTICAL INSTRUMENTATION

The analytical system consists of a JEOL, JEM 100B transmission/scanning electron microscope equipped with an EDAX energy-dispersive X-ray spectrometer. The TEM has a side entry specimen stage that can be tilted $\pm 60^{\circ}$ or the specimen rotated 360°. The energy-dispersive X-ray detector (lithium-drifted silicon crystal) is fitted through a port in the TEM column parallel to the specimen holder. The specimen-to-detector distance is approximately 10 mm with the specimen holder tilted 39° degrees toward the detector for optimum X-ray collection. The X-ray energy from the specimen is converted to a voltage pulse which is amplified, digitized, and stored in the multichannel analyzer. The energy-dispersive detector (EDS) being used is capable of detecting elements with atomic numbers of 11 (sodium) or higher and has an actual energy resolution of less than 170 electron volts. The X-ray detector is collimated such that a spatial resolution for microchemical analysis of better than 0.5 micrometers is realized.

This combination of analytical instrumentation (TEM plus EDS) permits visual characterization of particulate morphology, simultaneous observation of the single-fiber SAED pattern, and fiber chemical composition by X-ray microchemical analysis. The analytical instrumentation is pictured in Figure 6.

IDENTIFICATION AND CHARACTERIZATION PROCEDURES

Samples prepared by the method previously described are placed in the TEM where three basic pieces of data are gathered to identify and

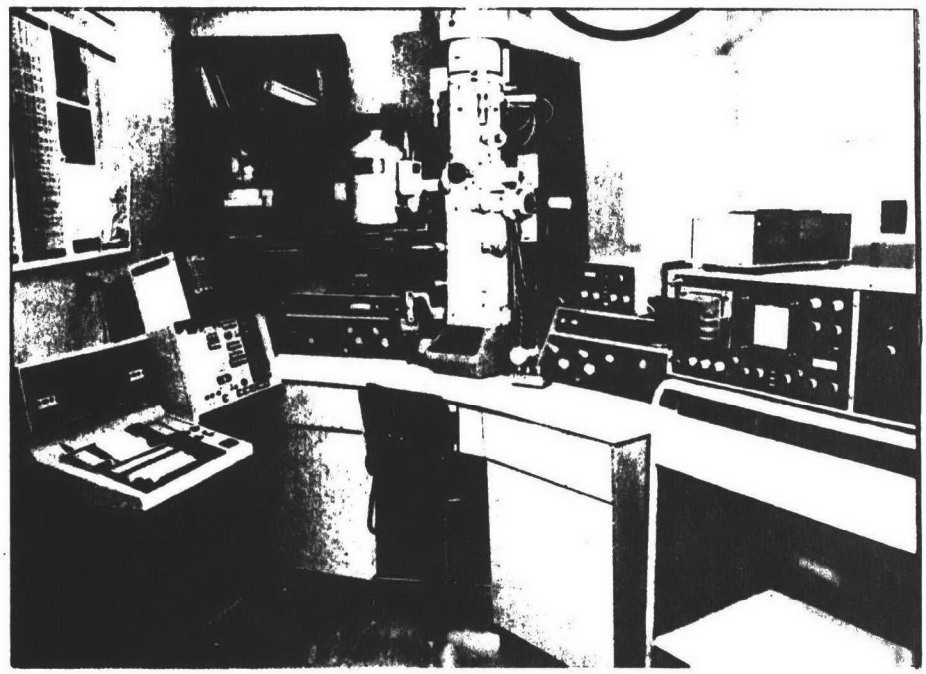


Figure 6. Analytical instrumentation used for fiber studies

characterize all fibrous (3 to 1 aspect ratio) particulates. These include: (1) visual identification of single-fiber electron diffraction patterns, (2) visual identification of semiquantitative elemental analysis spectra using X-ray microchemical techniques, and (3) determination of fiber length and diameter.

To aid in interpretation of collected data and to allow for comparison with fiber standards, several operational parameters of the TEM are maintained constant. These include (1) sample tilt (39°), (2) accelerating voltage (100,000 electron volts), (3) beam current (100 microamps), (4) energy-dispersive x-ray detector to specimen distance (10mm), and (5) the electron diffraction camera constant, which is periodically calibrated. Other data recorded with each analysis includes screen magnification, average area of the sample grid opening, and the number of grids and grid openings analyzed.

Prior to the identification and characterization of the sample, the prepared grid is first scanned to insure even distribution of particulates and to ascertain the quality and suitability of the sample preparation. A magnification is then selected that will allow observation of most fibers within the sample and will permit their size determination. Grid openings for analysis are randomly selected and all fibrous particulates identified in the following manner.

1. particulates must have a length-to-width ratio $\geq$ 3 to 1 to be identified as a fiber.
2. SAED is performed on the fiber which is classified as follows by visual observation:

a. Positive amphibole diffraction pattern.

b. Positive chrysotile diffraction pattern.

c. Nonasbestos diffraction pattern (positive for another mineral).

d. Ambiguous diffraction pattern (does not allow positive identification as amphibole, chrysotile, or nonasbestos mineral, and includes fibers whose SAED is obscured by debris or overlap).

e. No SAED pattern (includes amorphous fibers and fibers too thin to give an observable SAED).

3. Microchemical analysis is performed on the fiber displaying the spectrum on the video display. Background X-ray counts are subtracted, the elemental ratios compared, and an identification made by visual comparison with known standards.

4. The identified fiber is sized with calibrated circles or millimeter markings etched on the TEM viewing screen.

5. Selected pictures of microchemical analysis and/or SAED patterns are taken and stored for reference.

The format used to record the above information is shown in Figure 7.

Fiber concentrations are then calculated using the average grid opening area as the counting field area. Average grid opening areas are determined by optical microscopy by measuring randomly selected grid openings. Measurements are performed periodically to assure continuity of grid opening areas.

To optimize statistical accuracy of the analysis while keeping analysis time to acceptable limits, 10 grid openings or 50 fibers are analyzed

Sample Data

Sample #: _____

Study: _____

Date: _____

Analyst: _____

Filter Type: _____

Mounting Tech: _____

Operating Conditions

Mode: _____

Beam Current μA: _____

Sample Tilt°: _____

Magnification: _____

Av. Grid Area, mm^2: _____

No. of Grids Counted: _____

Fiber #	Diffraction Pattern					Possible EDXRA ID	Fiber Size, mm		Picture Taken?
	Positive Amphibole	Positive Chrysotile	Non Asbestos	Ambiguous Pattern	No SAED Pattern		Dia.	Length	
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
Total									

Comments:

Figure 7. Data sheet used for fiber analyses

from each sample, with a minimum of 5 grid openings analyzed. Typical analysis times average 90 minutes to 2 hours per sample.

In order to be as definitive as possible in both the identification of the SAED and the simultaneous microchemical analysis, bulk quantities of the sampled minerals, preferably from the same geologic source, should be obtained and also characterized. These bulk minerals can be utilized as reference standards for both SAED and microchemical analysis. These reference minerals are often more reliable than UICC standards, since chemical composition ratios differ for like minerals due to the association of other mineral fragments and the leaching of chemical elements during initial formation.

DISCUSSION

The addition of X-ray analytical equipment with a standard TEM, it is possible to obtain semiquantitative chemical analysis of single fibers, even though they may be submicroscopic in size. The ability to accumulate microchemical data from a particulate is often dependent upon the size, angle of detection, and density of the given particulate. The microchemical analysis of these particulates can be enhanced by operating at 100 kilovolts, reducing the size of the beam spot for increased spatial resolution, and adjusting the beam stigmator to elongate the beam over the fiber. These procedures allow fibers with diameters as small as 200 angstroms to be effectively analyzed.

Most environmental samples containing fibrous minerals will readily allow microchemical analysis for 90% to 95% of the observed fibers. The identification of fibrous minerals, however, should not be based entirely upon a visual examination of the X-ray spectra, unless a bulk quantity of the observed mineral has been characterized for a chemical composition reference and a substantial number of the fibrous minerals present have been simultaneously identified with SAED. This is necessary when characterizing any sample of unknown and/or mixed fibrous minerals because of observed similarities in chemical compositions of many asbestos and nonasbestos minerals. Fibrous minerals with similar chemical compositions are illustrated in Appendix B.

Most researchers agree that the SAED of a fibrous mineral may often be sufficient for a positive identification as long as measurements of crystal spacings are performed and compared with a reference. Identification by

this methodology may be difficult due to the preferred orientation of the fiber to the beam and the length of time required to identify a single fiber. In addition, the identification of observed fibers by SAED very seldom exceeds 50% to 60% when the sample is collected from water, ambient air, or industrial environments.

A rate of identification of fibers greater than 50% to 60% can be obtained if microchemical analysis is utilized along with the SAED. Positive identification of additional fibers is possible when both SAED and microchemical analysis are used. Those fibers which do not exhibit a SAED pattern can be distinguished if the chemical composition determined by microchemical analysis for that fiber is identical to the composition of those fibers which previously have been identified by both SAED and microchemical methods. By utilizing both of these analytical methods, a more definitive, qualitative, and quantitative analysis can be achieved.

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Appendix A

Examples of Minerals Which May Occur in a Fibrous State

Examples of Minerals Which May Occur in a Fibrous State

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrence b) Associated Minerals c) Similar Minerals
Serpentine (Chrysotile) (Antigorite)	$Mg_6(OH)_8Si_4O_{10}$	Mg-Si	a) hydrothermally decomposed olivine; proxene, amphibole b) olivine, tremolite, talc, opal, pyrope garnierite c) ----------
Talc (Steatite)	$Mg_3(OH)_2Si_4O_{10}$	Mg-Si	a) alteration of serpentine; anthophyllite b) chlorite, serpentine, magnetite, pyrite, dolomite c) pyrophyllite, kaolinite
Amosite (Cummingtonite) (Grunerite)	$(MgFe)_7[OHSi_4O_{11}]_2$	Mg-Si-Fe	a) variety of cummingtonite b) ---------- c) chrysotile asbestos
Riebeckite (Crocidolite)	$Na_2Fe_3Fe_2$ $[(OH,F)Si_4O_{11}]_2$	Na-Si-Fe	a) in crystalline schists, yellow tiger eye b) ---------- c) ----------
Tremolite	$Ca_2Mg_5(OH,F)_2$ $[Si_4O_{11}]_2$	Mg-Si-Ca	a) in metamorphic limestone & dolomite, in talc schists b) ---------- c) chrysotile, pectolite, wollastonite
Actinolite	$Ca_2(MgFe)_5Si_8O_{22}(OH)_2$	Mg-Si-Ca-Fe	a) in impure limestone or dolomite b) ---------- c) pyroxenes

Examples of Minerals Which May
Occur in a Fibrous State
(Cont.)

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrence b) Associated Minerals c) Similar Minerals
Byssolite	$Ca_2Mg_5(OH,F)_2[Si_4O_{11}]_2$	Mg-Si-Ca	a) in metamorphic limestone and dolomites, in alpine cracks b) ---------- c) ----------
Anthophyllite	$(MgFe)_7[OHSi_4O_{11}]_2$	Mg-Si ± Fe	a) in crystalline schists, mica schists, in metamorphic rock b) ---------- c) chrysotile
Hornblende	$CaNa(MgFe)(AlFeTi)_3Si_6O_{22}(O,OH)_2$	Na-Mg-Al-Si-Ca-Ti-Fe	a) in metamorphic & igneous rocks, in crystalline schists b) biotite, garnet, epidote, magnetite c) augite, tourmaline
Epsomite (Bitter Salt)	$Mg[SO_4]7H_2O$	Mg-S	a) weathering product in ore deposits, efflorescent crusts, alteration product of kieserite b) ---------- c) kieserite
Wollastonite (Table Spar)	$Ca_3[Si_3O_9]$	Si-Ca	a) in contact metamorphic limestone, in crystalline schists b) quartz, garnet, vesuvianite, pyroxene c) pectolite, tremolite

NOTE: (±) may or may not be present

Examples of Minerals Which May
Occur in a Fibrous State
(Cont.)

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrence b) Associated Minerals c) Similar Minerals
Pectolite	$Ca_2NaH[Si_3O_9]$	Na-Si-Ca	a) in fissures in igneous rocks b) zeolite, calcite c) tremolite, wollastonite
Zeolite (Natrolite)	$Na_2Al_2Si_3O_{10} \cdot 2H_2O$	Na-Al-Si	a) in cavities in igneous rocks, in fissurey in granites & crystalline schists b) other zeolites, calcite, apophyllite c) aragonite scolezite, thomsonite, mesolite, wavellite
Pyrophyllite	$Al_2[(OH)_2Si_4O_{10}]$	Al-Si	a) in quartz veins & ore veins, in slate clays b) ---------- c) talc, kaolinite
Stilpnomelan	$(K,H_2O)(Fe,Mg,Al)_3[(OH)_2Si_4O_{10}](H_2O)_2$	Mg-Al-Si-K-Fe	a) in ore veins b) pyrite, siderite, limonite sphalerite, quartz c) ----------
Anhydrite	$Ca[SO_4]$	Ca-S	a) in ore veins, in salt deposits b) halite, gypsum, dolomite c) cryolite, gypsum, barytes, calcite
Sillimanite (Fibrolite)	$Al_2[OSiO_4]$	Al-Si	a) in crystalline schists, granulites eclogites, in contact-metamorphic rocks b) ---------- c) cyanite

Examples of Minerals Which May
Occur in a Fibrous State
(Cont.)

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrence b) Associated Minerals c) Similar Minerals
Zoisite & Clino-zoisite	$Ca_2Al_2[OOHSiO_4Si_2O_7]$	Al-Si-Ca	a) in crystalline schists & metamorphic rocks b) amphibole, garnet, vesuvianite epidote, quartz c) tremolite
Epidote & Pistacite	$Ca_2(FeAl)Al_2[OHSiO_4Si_2O_7]$	Al-Si-Ca-Fe	a) in fissures & vesicles of basic igneous rocks & crystalline schists b) zeolite, calcite, axinite garnet, cooper, vesuvianite c) hemimorphite, aragonite, staffelite; tourmaline, actinolite.
Zeolite (Thomsonite)	$NaCa_2[Al_2(AlSi)Si_2O_{10}]_2 \cdot 6H\ O$	Na-Al-Si-Ca	a) in vesicles in basic igneous rocks, in vesuvianite lavas b) other zeolites, analcite, calcite c) natrolite, prehnite
Palygorskite (Attapulgite)	$(MgAl)_2[OHSi_4O_{10}] \cdot 4H_2O$	Mg-Al-Si	a) weathering product of serpentine b) chalcedony, opal, chlorite, magnesite c) ----------
Sepiolite (Meerschaum)	$Mg_4[(OH)_2Si_6O_{15}] \cdot 2H_2O + 4H_2O$	Mg-Si	a) weathering product of serpentine b) opal, chalcedony, magnesite, chlorite c) ----------

Examples of Minerals Which May
Occur in a Fibrous State
(Cont.)

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive	a) Occurrence b) Associated Minerals c) Similar Minerals
Halloysite	$Al_2Si_2O_5(OH)_4$	Al-Si	a) weathering product of kaolinite b) feldspars, other clays c) ----------
Brucite (Nemolite)	$Mg(OH)_2$	Mg	a) low temperature in serpentine or dolomite metamorphic rocks b) periclase c) ----------
Magnesite	$MgCO_3$	Mg	a) metasomatic deposits replacing limestone & dolomite, in serpentine in talc schists b) ---------- c) ankerite, calcite, dolomite
Zeolite (Laumontite)	$Ca[AlSi_2O_6]_2 \cdot 4H_2O$	Al-Si-Ca	a) in ore veins, in cavities & fissures in eruptive rocks b) other zeolites, calcite, chlorite c) feldspars
Aragonite & Calcite	$CaCO_3$	Ca	a) in rock-fissures, in ore deposits embedded in sulfur as sinter formation b) ---------- c) calcite, barytes, coelestine, strontianite, natrolite, topaz, dolomite

Examples of Minerals Which May
Occur in a Fibrous State
(Cont.)

Mineral Name(s)	Formula	Dectectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrence b) Associated Minerals c) Similar Minerals
Apjohnite	$MnAl_2[SO_4]_4 \cdot 22H_2O$	Mn-Al-S	a) in rock as weathering product of sulphides b) ---------- c) alunogen
Gypsum & Selenite	$CaSO_4 \cdot 2H_2O$	S-Ca	a) rocks in salt deposits, weathering product of sulphides in sedimentary rocks, in ore deposits b) anhydrite, aragonite, sulphur c) mica, talc, kaolinite
Valentinite (Antimony Bloom)	Sb_2O_3	Sb	a) weathering product of antimony ores b) antimonite, galena c) cerussite
Arsenopyrite	FeAsS	S-Fe-As	a) in ore veins b) galena, silver c) lollingite, chloanthite, skutterudite
Lollingite (Leucopyrite)	$FeAs_2$	Fe-As	a) in ore veins b) arsenopyrite c) ----------

Examples of Minerals Which May
Occur in a Fibrous State
(Cont.)

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrance b) Associated Minerals c) Similar Minerals
Gedrite	$(MgFe)_6Al_2[OH(AlSi)Si_3O_{11}]_2$	Mg-Al-Si-Fe	a) in metamorphic rocks, in crystalline schists, in granites, in ore veins b) ---------- c) bronzite
<u>Pyroxene Family</u>			
1) Diopside	$CaMg[Si_2O_6]$	Mg-Si-Ca	a) in magnetite lodes, in fissures in metamorphic rocks b) chlorite, hessonite, magnetite, apatite, biotite c) clinochlore, augite
2) Violane	$CaMg[Si_2O_6] \pm$ Mn, Fe	Mg-Si-Ca $\pm$ Mn, Fe	a) ---------- b) ---------- c) ----------
3) Enstatite	$Mg_2[Si_2O_6]$	Mg-Si	a) rock constituent in serpentine, in pegmatic apatite veins b) apatite, phlogopite, olivine, bronzite c) hypersthene

NOTE: ($\pm$) may or may not be present

Examples of Minerals Which May Occur in a Fibrous State (Cont.)

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrence b) Associated Minerals c) Similar Minerals
4) Augite	$(Ca,Mg,Fe_2,Fe_3,Ti,Al)[(SiAl)_2O_6]$	Mg-Al-Si-Ca-Ti-Fe	a) rock constituent in basic rocks, in tuffs, lavas & volcanic ejecta b) ---------- c) amphibole
5) Hedenbergite	$CaFe[Si_2O_6]$	Si-Ca-Fe	a) in metamorphic & metasomatic rocks b) magnetite, pyrite c) ----------
6) Acmite-Aegirite	$NaFeSi_2O_6$	Na-Si-Fe	a) common in high-soda, low-silica rocks b) ---------- c) nepheline, leucite
Alunogen	$Al_2[SO_4]_3 \cdot 18H_2O$	Al-S	a) in ore veins, in coal piles, in clays b) pyrite melanterite c) alunite
Halotrichite	$FeAl_2[SO_4]_4 \cdot 22H_2O$	Al-S-Fe	a) weathering product of pyrites in ore deposits, in lignites b) ---------- c) apjohnite

Examples of Minerals Which May Occur in a Fibrous State (Cont.)

Mineral Name(s)	Formula	Detectable Elements By Energy-Dispersive X-Ray Analysis	a) Occurrence b) Associated Minerals c) Similar Minerals
Celestite	$SrSO_4$	S-Sr	a) in sedimentary rocks, in sandstone or limestone b) fluorite, calcite, gypsum, dolomite, galena, sphalerite c) ----------

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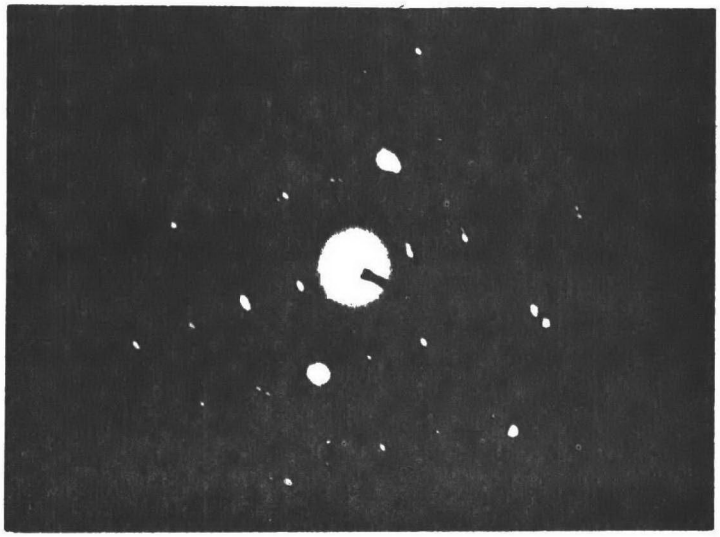
Appendix B

Electron Photomicrographs, Selected Area Electron Diffraction Patterns and Typical Energy Dispersive X-Ray Spectra for Selected Fibrous Minerals

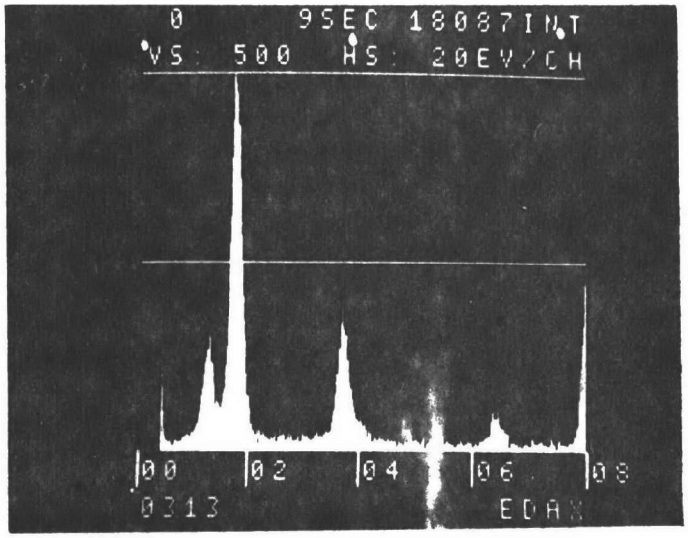
ACTINOLITE

Photomicrograph

1 micrometer

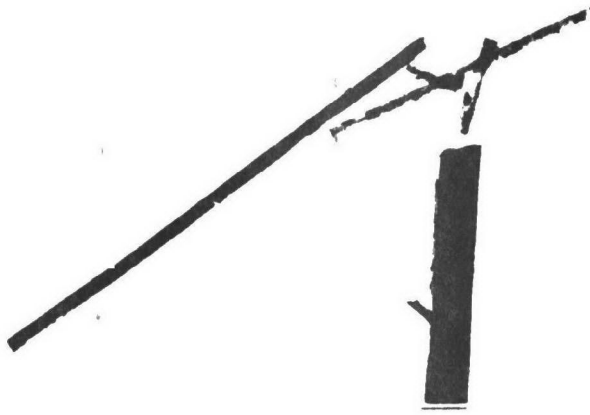


Selected Area Electron Diffraction



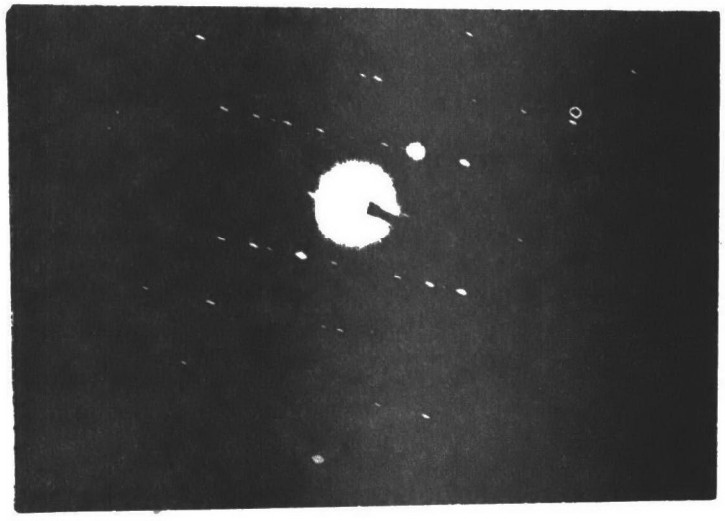
X-Ray Spectrum
Mg-Si-Ca-Fe

AMOSITE

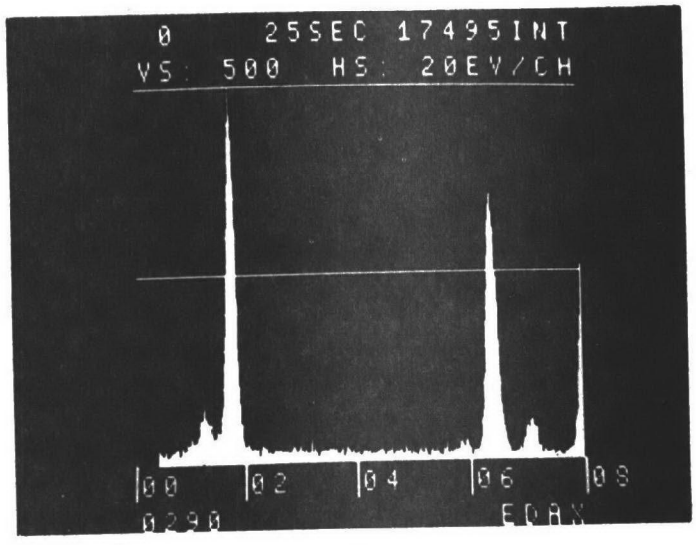


Photomicrograph

1 micrometer



Selected Area Electron Diffraction

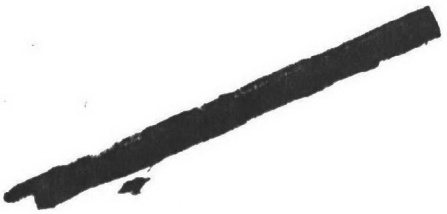


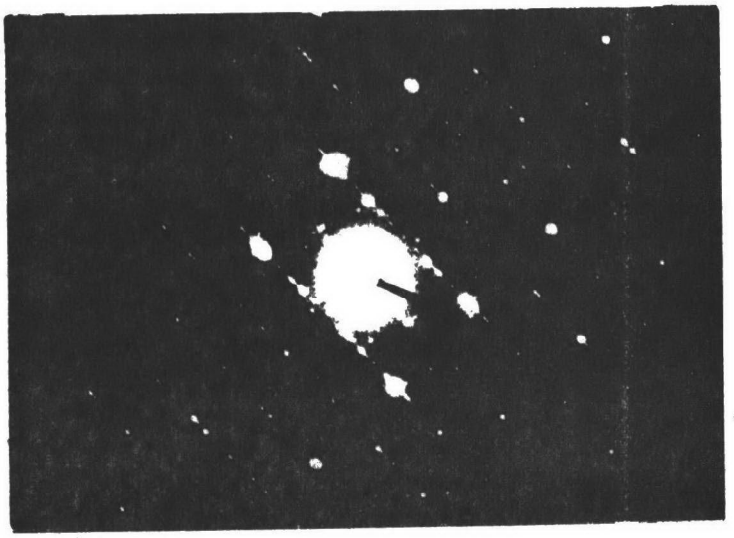
X-Ray Spectrum
Mg-Si-Fe

ANTHOPHYLLITE

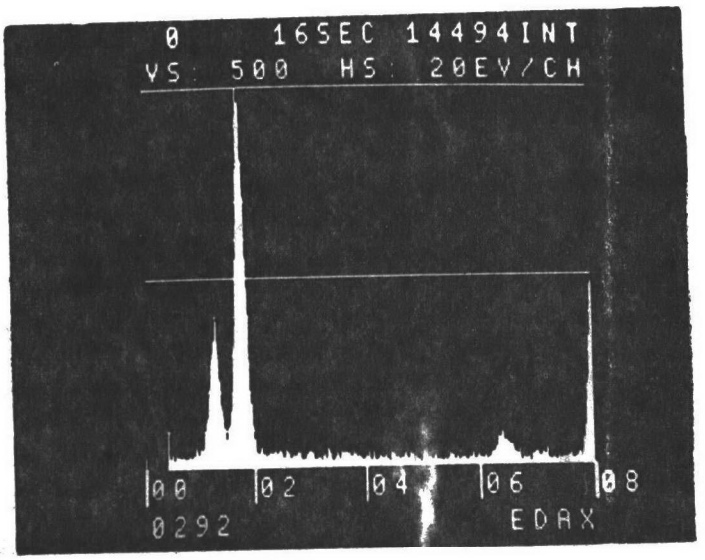
Photomicrograph

1 micrometer





Selected Area Electron Diffraction

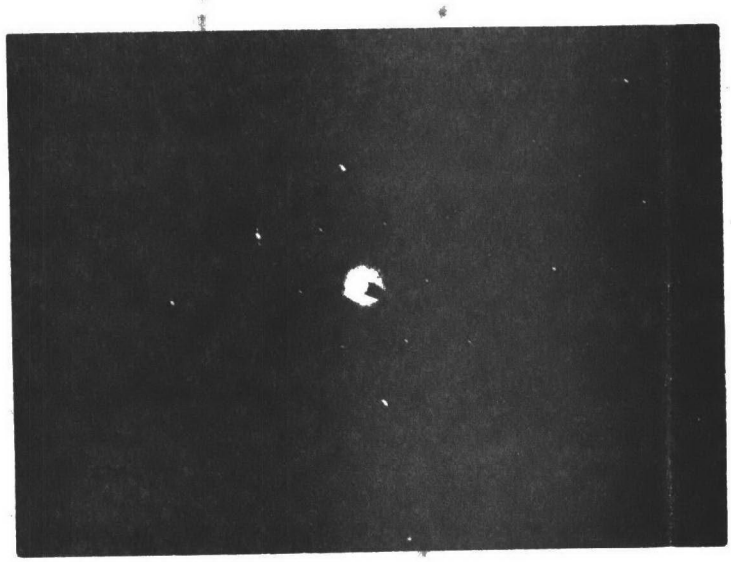


X-Ray Spectrum
Mg-Si trace Fe

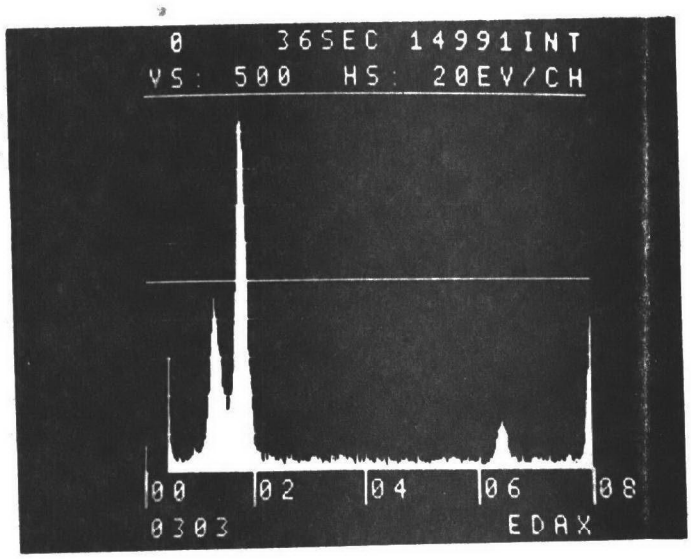
ANTIGORITE

Photomicrograph

1 micrometer



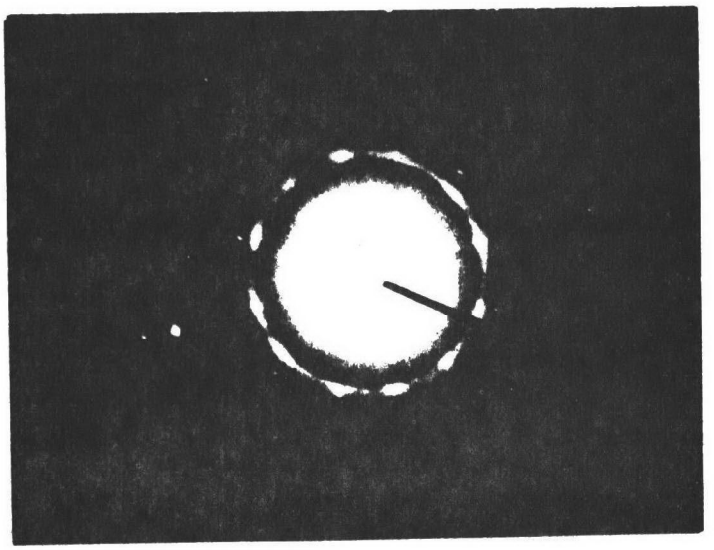
Selected Area Electron Diffraction



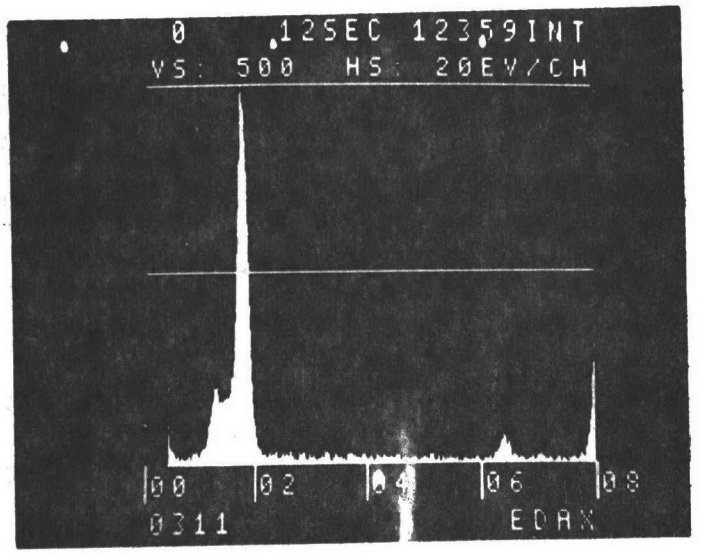
X-Ray Spectrum
Mg-Si trace Fe

Photomicrograph

1 micrometer



Selected Area Electron Diffraction

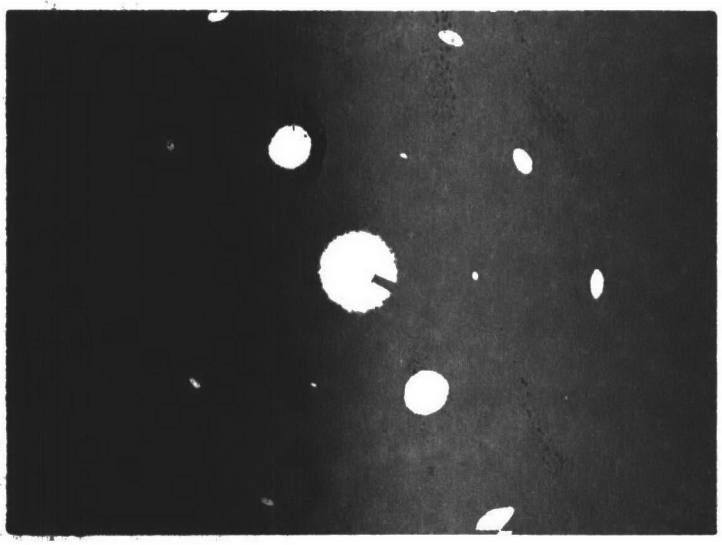


X-Ray Spectrum
Mg-Al-Si trace Fe

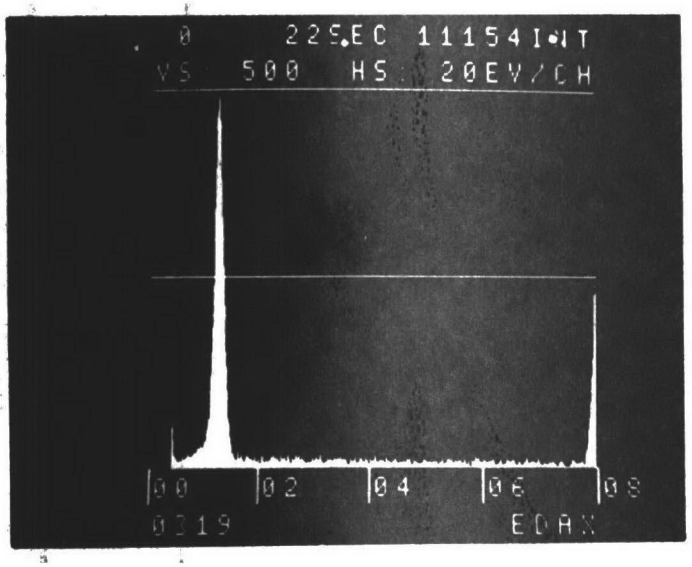
BRUCITE

Photomicrograph

1 micrometer



Selected Area Electron Diffraction



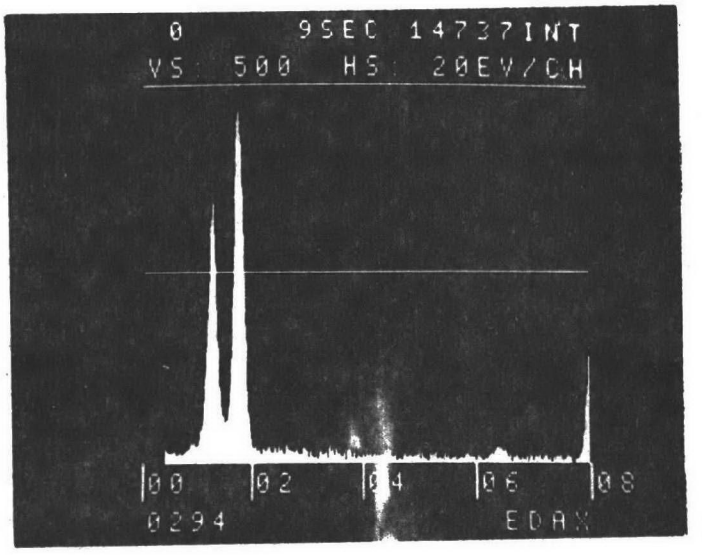
X-Ray Spectrum
Mg

CHRYSOTILE

Photomicrograph

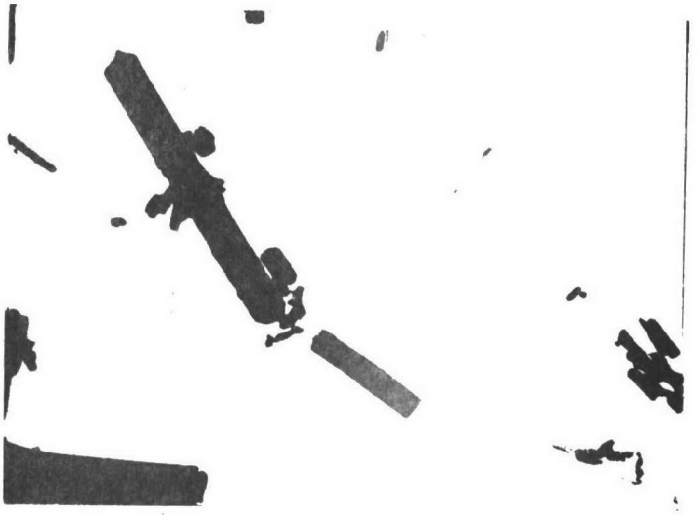
1 micrometer

Selected Area Electron Diffraction



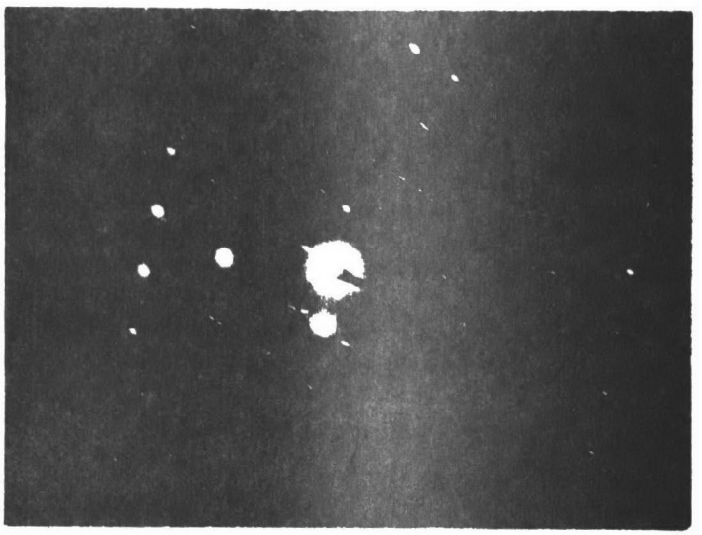
X-Ray Spectrum
Mg-Si

CROCIDOLITE

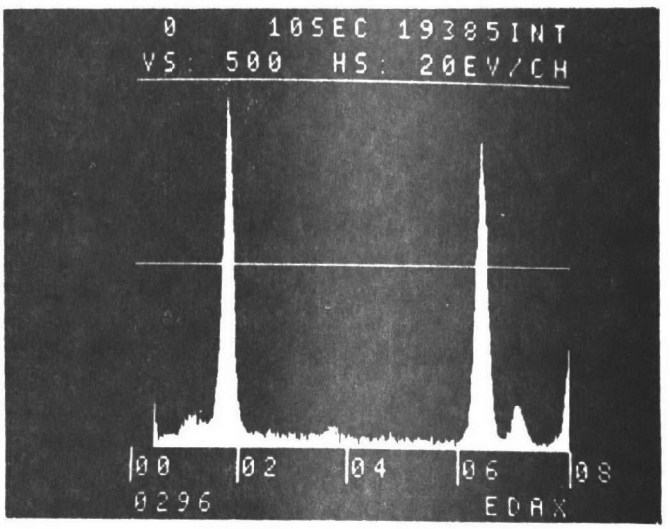


Photomicrograph

1 micrometer

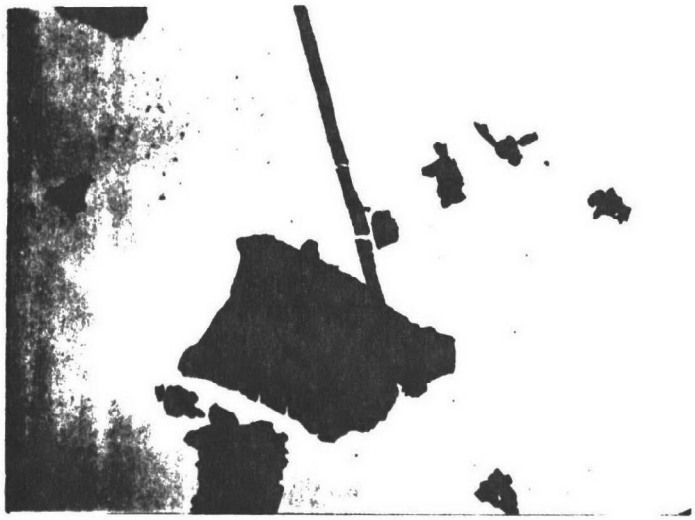


Selected Area Electron Diffraction



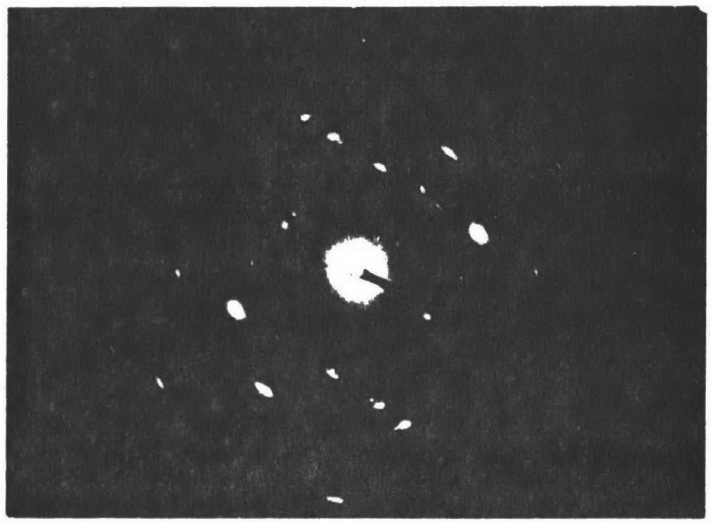
X-Ray Spectrum
Na-Si-Fe

GYPSUM

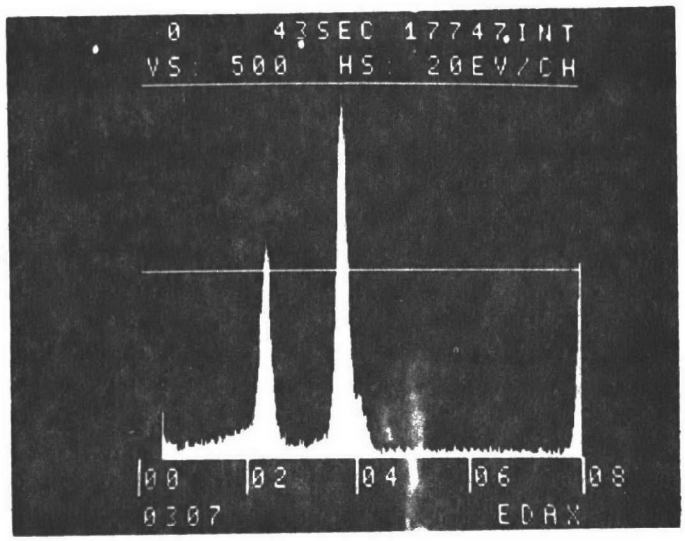


Photomicrograph

1 micrometer



Selected Area Electron Diffraction

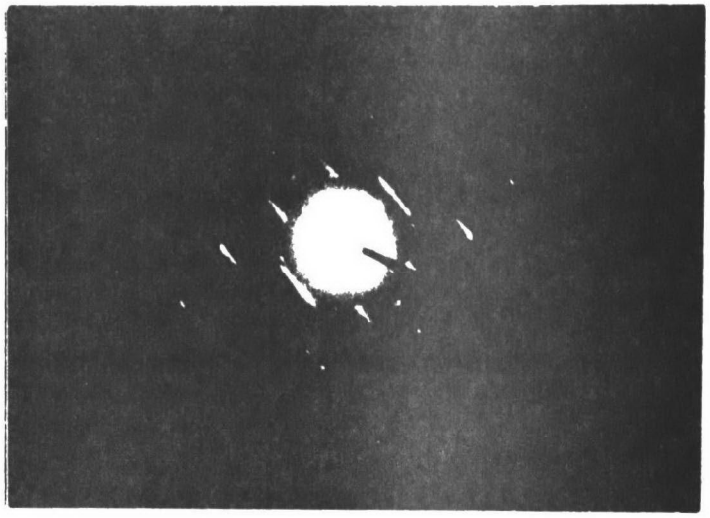


X-Ray Spectrum
S-Ca

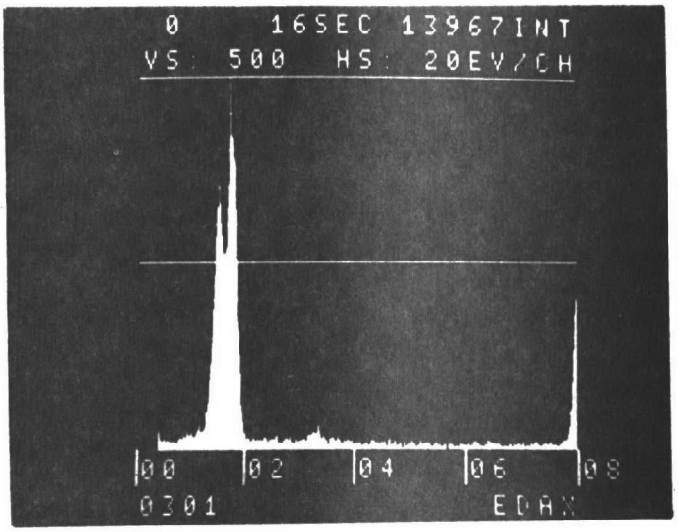
HALLOYSITE

Photomicrograph

1 micrometer



Selected Area Electron Diffraction

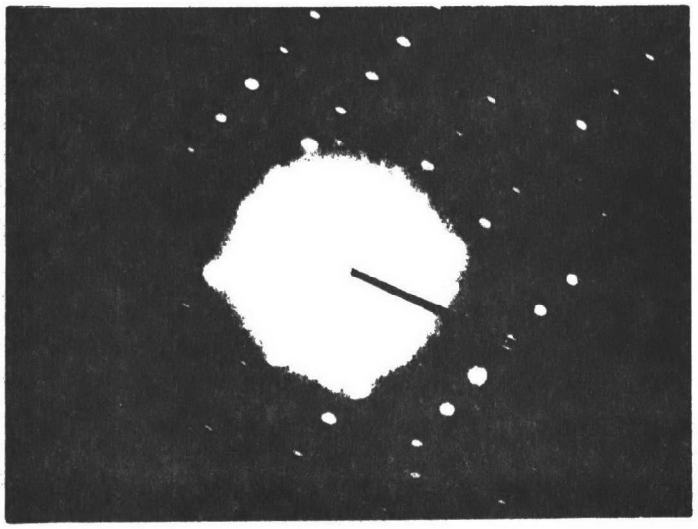


X-Ray Spectrum
Al-Si

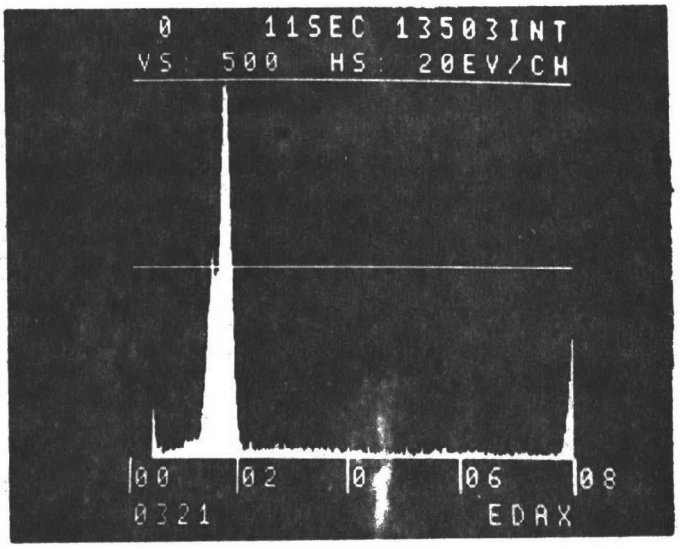
NATROLITE

Photomicrograph

1 micrometer



Selected Area Electron Diffraction

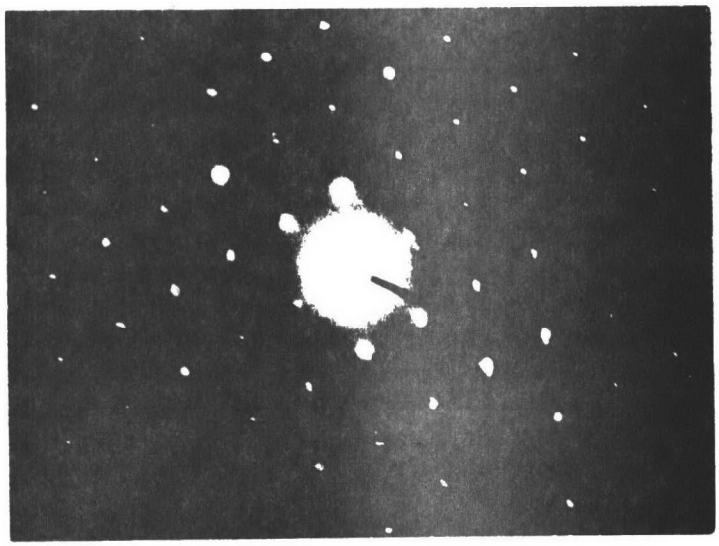


X-Ray Spectrum
trace Na-Al-Si

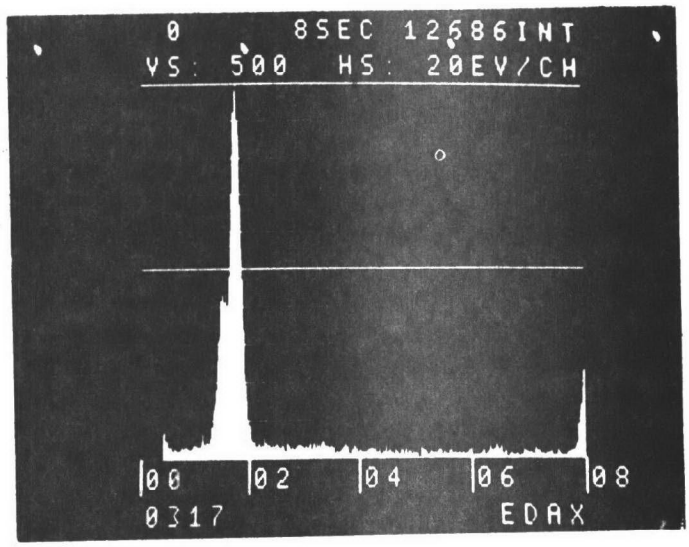
PYROPHYLLITE

Photomicrograph

1 micrometer



Selected Area Electron Diffraction

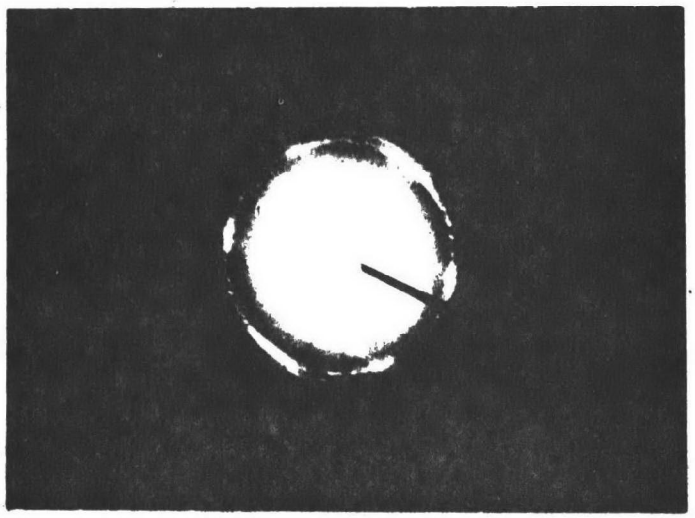


X-Ray Spectrum
Al-Si

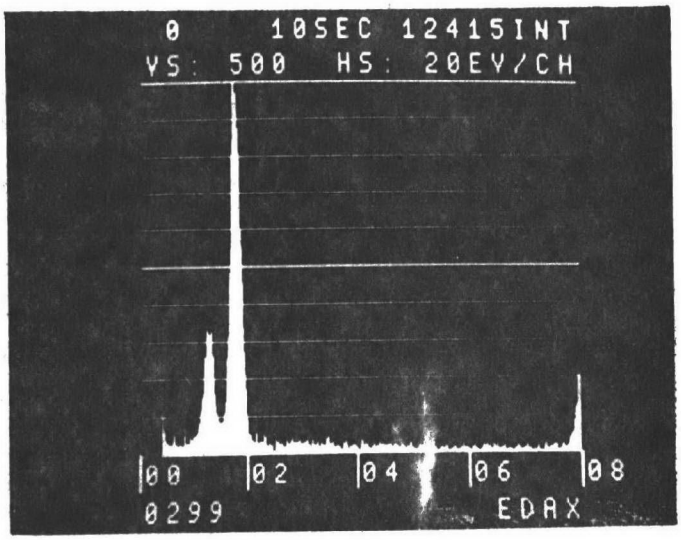
SEPIOLITE

Photomicrograph

1 micrometer



Selected Area Electron Diffraction

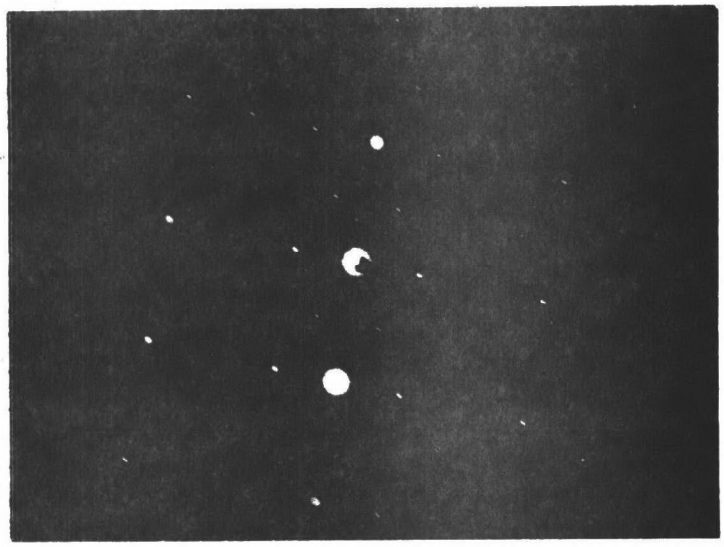


X-Ray Spectrum
Mg-Si

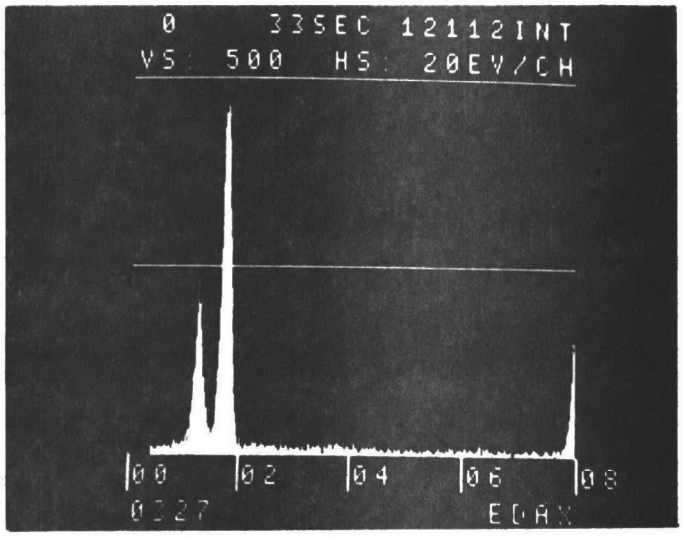
TALC

Photomicrograph

1 micrometer



Selected Area Electron Diffraction

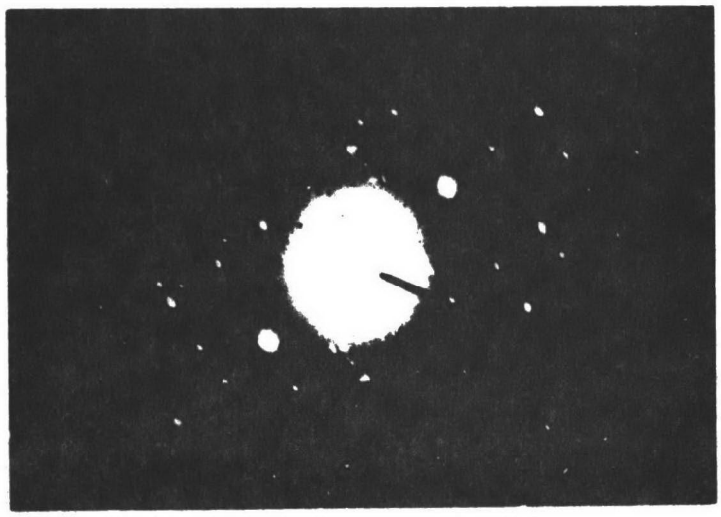


X-Ray Spectrum
Mg-Si

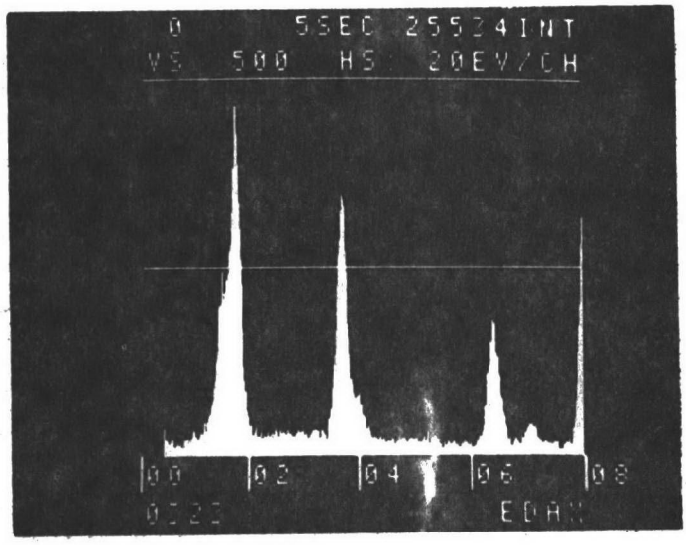
THOMSONITE

Photomicrograph

1 micrometer



Selected Area Electron Diffraction

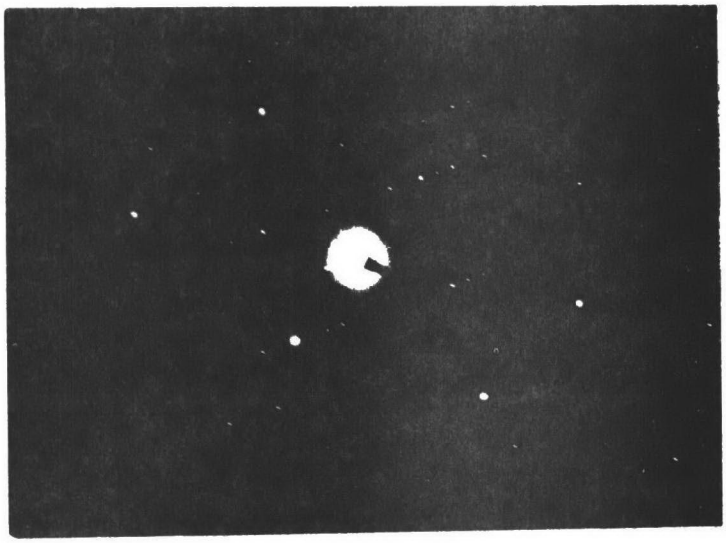


X-Ray Spectrum
Al-Si-Ca trace Fe

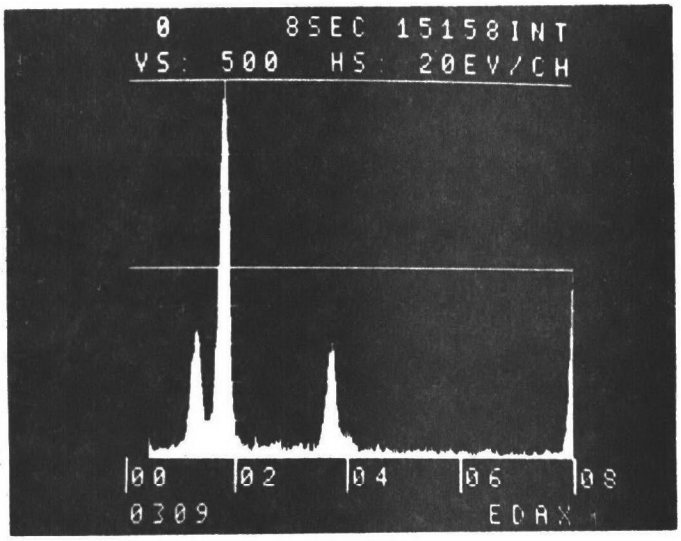
TREMOLITE

Photomicrograph

1 micrometer

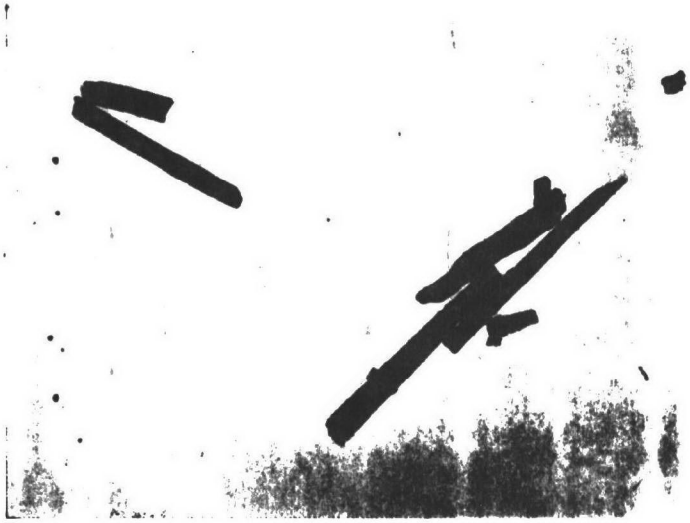


Selected Area Electron Diffraction



X-Ray Spectrum
Mg-Si-Ca

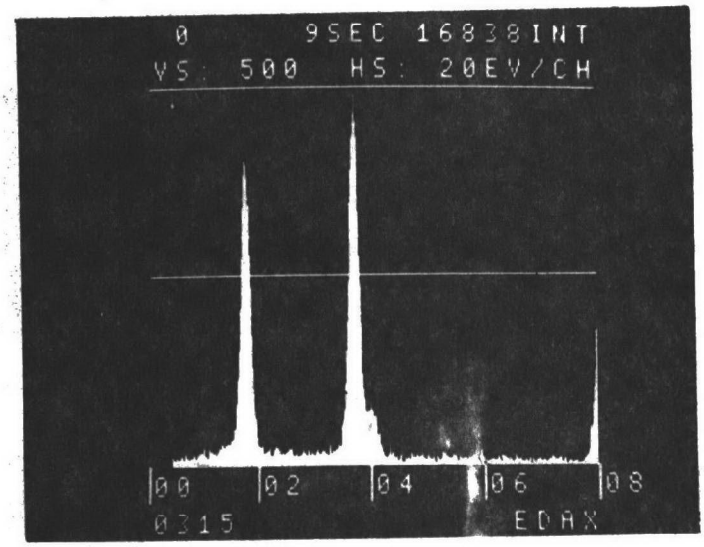
WOLLASTONITE



Photomicrograph

1 micrometer

Selected Area Electron Diffraction



X-Ray Spectrum
Si-Ca

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