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Techniques for the Detection, Identification and Analysis of Fibers

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Applicability and limitations of the techniques used for the detection, identification, and analysis of mineral fibers are discussed with particular emphasis as to the problem of evaluating health hazards resulting from exposure to fibers and the discovery of the mechanisms of the diseases involved.

BIOLOGICALLY ACTIVE FIBERS have long been an important public health problem. As early as 1900, asbestos fibers were identified as the causative agent in a chronic pneumoconiosis (asbestosis) occurring among asbestos textile workers. Later investigators noted an excess of cancer occurring among workers exposed to asbestos fibers. A relationship between the known excess of cancer in urban environments and asbestos has been suggested by the presence of "asbestos" or "ferruginous" bodies in the lungs of a high proportion of urban dwellers.

Fibrous glass has been given considerable attention as a possible hazard of the general type of asbestos, although current manufacturing practices produce few fibers capable of penetrating into the pulmonary air spaces. A trend toward the production of smaller glass fibers and the emergence of small, respirable, graphite, silicon carbide, and other man-made fibers suggests that the problem of the health effects of fibers will increase. These effects are of concern, not only to the industrial population, but also to urban dwellers who may breathe fibers from many sources: occupants of buildings with fibrous glass-lined ducts and others who may unknowingly become exposed.

To protect the health of the population and avoid penalizing a valuable economic material which is causing no harm, it is important to learn as much as possible about fibers in materials, in the air, and in the body. Fibers occurring in the environment must be detected and identified so they can be traced to their source. Quantitative estimates of fiber concentration in industrial and community air are needed to establish any relationship between disease patterns and to determine the effectiveness of control measures. The trace chemical constituents of these fibers are of importance in uncovering the mechanisms of the diseases resulting from exposure.

Sample Types

The methods of analysis used and the results that may be expected depend to a great extent on the type of sample available. In general, the choice may be either a large sample which is remote from the site of activity and which yields analytical data of doubtful significance or a minute sample too small for useful analysis found at the site of the biological event. The solution to this quandary may lie in the selective use of information regarding the suspect material from point of origin to final deposition. For example, it is

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not necessary to identify each fiber seen in a dust sample collected in an asbestos textile plant if the plant uses only chrysotile asbestos; and the ferruginous body seen in the lung of a known asbestos worker may safely be called an asbestos body although this would not be true of a fiber in the lung of a non-occupationally exposed individual without adequate supporting analytical data. Certain trace metals and polynuclear aromatic hydrocarbons in the raw material which can be traced to laboratory prepared respirable fractions and possibly to airborne dust may also be entering the exposed individual.

Bulk Samples

Large samples of fibrous raw materials, products made from fibers, and settled dust in factories are usually readily available. High volume samples of airborne dust weighing several grams are also easy to obtain and may be regarded as bulk samples. These samples generally also contain materials other than the fibers being considered and in some cases the fibers constitute such a small percentage that the sample should be treated as an airborne dust exposure sample. Generally, all methods of analysis are appropriate to these samples; the results are difficult to interpret by themselves, since it is not readily apparent which of the many materials present are likely to become airborne and penetrate into the deep lung.

Airborne Dust Exposure Samples

Any study of the effect of inhaled fibers must include a consideration of the fibrous dust the worker breathes. Since the airborne dust may vary considerably with location, the air sample should be taken in the worker's breathing zone, even though the practical instrumentation available for the task permits the taking of only small (several milligrams) samples. When only dust capable of penetrating the pulmonary air spaces is to be considered, a size selective presampler that passes only the "respirable" dust should be used. This reduces the weight of material collected. The fiber-containing sample will again be a mixture of fibers, materials associated naturally with the fibers, other process materials, and airborne dusts having nothing to do with the fibers.

Lung Fiber Samples

Single fibers may be isolated from lung samples on autopsy. These may be free fibers, more or less in their natural states, or they may be coated with an iron-protein layer and considerably altered. The presence of fibers in the lung suggests that they may have been the cause of an existing disease, although other respirable fibers later removed by some clearance mechanism, may have been responsible for the damage. A typical airborne asbestos fiber in a textile plant weighs about 10^{-10} gram.[1] Since most of the fibers deposited in the pulmonary air spaces are smaller than this average size, the most sensitive methods are necessary for identification and gross analysis of fibers found in the lung.

Sample Preparation

Several recent developments in methods of sample preparation enable the analyst to separate the fibers from associated materials and to deposit the material in a suitable receptacle for analysis.

Microsieving

A fraction of a sample containing the respirable fibers may be prepared from a bulk sample by microsieving. The bulk sample material is dispersed in 95% ethyl-alcohol and placed in a 10-micron sieve in an ultrasonic bath. At 15-minute intervals, sieved portions are removed from the bath and more alcohol is added to the sieve. For one gram of the bulk material, the separation is complete in about four hours. X-ray diffraction analysis of sieved chrysotile asbestos has shown no change in the pattern of the sieved fraction as compared with that of the bulk sample. This technique has been used to demonstrate that there is a greater concentration of nickel and chromium in the finer particle fraction.[2]

Low-Temperature Dry Ashing

A commercial instrument is now available for the dry ashing of organic matter without volatilizing trace elements. This technique allows the oxidative reactions to proceed in a very gentle manner, avoiding any disturbance of the structure of crystalline substances or

sintering of ultrafine particles. The technique appears to be promising for the isolation, without alteration, of mineral substances deposited in body tissues. Excited oxygen species produced when molecular oxygen is passed through a high-frequency electromagnetic field attack the sample which is maintained at about 1 mm Hg pressure in an oxidation chamber from which the exhaust gases are removed by a mechanical vacuum pump (Figure 1).

The glow region in the RF discharge of oxygen contains 10% to 20% oxygen atoms and about an equivalent amount of electronically excited molecular oxygen.[3] The rest of the plasma consists of normal ground-state oxygen molecules, some ionized species, and a free electron concentration of 1×10^{11} cm.$^{-3}$ A few millimeters away from the glow region, most of the ionic species have disappeared but the "active" species persist and are carried downstream before they deactivate reactively or by collision.

The concentration of oxygen atoms is higher than predicted from the rates of the directly contributing electron-molecule reactions, molecule ion-electron reactions and electronically excited molecular-oxygen dissociation. This high concentration of atomic oxygen is largely due to catalytic effects of such foreign gases as hydrogen, nitrogen, or water vapor, which provide the kinetic pathways to atomic oxygen production.

This low-temperature (approximately 150C), dry-ashing method has been used experimentally to oxidize the organic matrix of lung and other biological tissues without changing the crystal lattice of deposited fibers and particulates.

Dissolution of Trace Metals

A 20- to 30-minute digestion of chrysotile asbestos fibers with 5*N* hydrochloric or nitric acid is more than adequate to dissolve the metallic constituents of bulk and airborne samples containing this mineral. However, neither of these acids attacks amosite, crocidolite or glass completely, and it is necessary to use hydrofluoric acid (HF) for the digestion of these types of fibers prior to analysis of the metallic elements. An evaluation of HCl and HF digestion methods is presented in Table I.

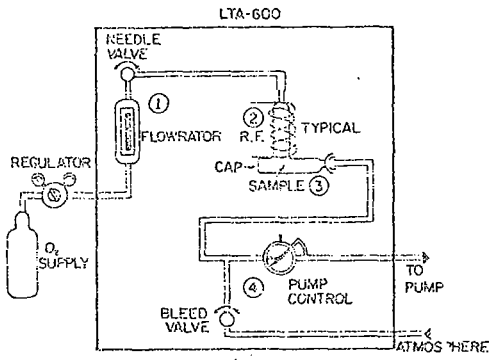


FIGURE 1. Low-temperature dry ashing apparatus.

TABLE I
Evaluation of Acid Digestion Methods for 0.5-Gram Portions of Asbestos Minerals

Type of Asbestos	Element	Digestion Procedure	
		HCl	HF
		(μg element/gm sample)	
Chrysotile	Ni	365	380
	Cr	197	220
	Co	37	30
	Mn	270	270
Amosite	Ni	None	50
	Cr	20	35
	Co	5	16
	Mn	910	4,450
Crocidolite	Ni	None	20
	Cr	10	25
	Co	None	10
	Mn	55	180

In view of the findings shown in Table I, all samples for heavy metal analyses should be digested with HF in platinum dishes to ensure a more complete solubilization of the metallic elements contained within the crystal lattice or between the fibrils of the asbestos materials. The routine application of this digestion technique ensures a more complete dissolution of all samples regardless of what is known or not known about their mineral composition.

Polynuclear Aromatic Hydrocarbon Extraction

Cyclohexane extracts of large samples of airborne fibers and dusts, or of bulk asbestos-containing materials are prepared by the method of Monkman.[4] The extraction is con-

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ducted overnight in a Soxhlet apparatus with a spectrofluorometric grade reagent. The 400 ml extract is concentrated to 1 ml and transferred to a silica gel column. (The silica gel is kept activated by storage in an oven at 150°C.) The aliphatic components are eluted with 400 ml of iso-octane. The aromatic fraction is then eluted with 100 ml of benzene. The polycyclic hydrocarbons present in the latter eluate are then separated by a column of activated 100 to 200 mesh alumina prepared by drying the alumina at 150°C, followed by adding water to provide 1.8% H_2O in final alumina bed. For example, if a high activity column of 1.4% H_2O is used, the elution time is very long, and if more than 2% H_2O is present, the elution is poor. For this separation, the benzene extract is evaporated to dryness with filtered air, and the residue is dissolved in a small volume of cyclohexane which is then transferred to the alumina column which is 10 cm high by 1.0 cm diameter. After this column is capped with 0.5 cm of deactivated alumina, the hydrocarbons are eluted in turn with cyclohexane. The separate eluates are monitored for individual polycyclic hydrocarbons with a recording UV spectrophotometer. Addition of 1% ether after the decline of the eluted chrysene aids the subsequent elutions of benzo(e)pyrene, benzo(a)pyrene, and benzo(k)fluoranthene

Mounting of X-ray Diffraction Samples

The technique of mounting representative portions of dust samples on molecular membrane filters was described by Talvitie and Brewer.[5] A known weight of the finely comminuted sample is suspended in a 250 ml MCA volumetric flask with the aid of a wetting agent. This mixture is then dispersed with a small ultrasonic bath, and an aliquot of the thoroughly agitated suspension containing a known fraction of the sample is pipetted out and filtered through a molecular membrane filter. This technique provides a sample of known weight on the filter. Asbestos-containing dust samples are amenable to this procedure after a preliminary dry screening of gross particles through a 200-mesh sieve, followed by grinding fibers to lengths of <3.5 microns. The membrane filter containing the sample aliquot is mounted in the x-ray diffractometer.

Airborne dusts and fibers collected on a membrane filter are moved by placing the filter in a test tube containing a few drops of a suitable wetting agent and sufficient water to cover it. Immersion of the test tube in the ultrasonic bath for five minutes promotes the removal of the dust and fiber deposit from the filter. After the original filter is removed from the test tube, the resuspended fibrous dust sample is deposited on a second membrane filter which is mounted in the x-ray diffractometer.

Sample Examination

Since fibers are defined by their shape and, generally, some other materials mixed with the fiber have the same chemical and crystallographic characteristics as the fiber, the only certain way of detecting fibers is by visual examinaion. Furthermore, the only way to insure obtaining an analysis of a single fiber from a mixture of other materials is to visually present the fiber to the analytical instrument. It is also possible to identify some fibers if sufficient fine detail can be resolved.

Light Microscopy

In the early days of counting dust particles obtained in impinger samples in asbestos textile factories, few fibers were seen, and as a result all particles were counted to give an estimate of overall dustiness. The lack of visible fibers in these samples was due in part to the inefficiency of the impinger in the collection of particles of such small aerodynamic size and in part to the low resolving power of the 10× objective used. As a result of recent studies of the asbestos industry, techniques were evolved for detecting many of the airborne fibers previously missed.[6,7] Membrane filters with a pore size of 0.8 micron are known to have surface collection efficiencies close to 100% for particles down to a few hundredths of a micron. Airborne dust samples collected on these filters may be viewed by high resolution transmitted light microscopy if the filter can be rendered transparent or "cleared." Acetone, glyceral triacetate, immersion oil, and other materials have been used to clear membrane filters. How-

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ever, the fiber being sought frequently has an index of refraction very near the index of the cleared filter. Thus, under bright field illumination, the fiber may be invisible. Phase contrast illumination, which utilizes the interference patterns produced by the combination of two out-of-phase columns of light, is capable of revealing objects imbedded in media with an index of refraction only slightly different from that of the object. The sensitivity of this method to index differences necessitates the use of glyceral triacetate or a diethyl oxalate-dimethyl phthalate mixture to clear the filter perfectly. Clearance of the filter with either of the liquids alone may cause displacement of the particles by too rapid solution of the filter. To prevent this, the particles are either fixed with perspex in chloroform before clearing with glyceral triacetate (British practice) or pure membrane filter material is added to the diethyl oxalate-dimethyl phthalate mixture before it is used to clear a filter to slow the rate of filter solution (USPHS method). With these techniques, fibers whose thickness is only a few tenths of a micron can be seen.

Larger fibers (over 1 micron thick) may sometimes be identified by dispersion staining.[8] If a transparent particle is immersed in a liquid with a steeper dispersion curve, that is, large variation in index of refraction with wavelength, and if the index of the particle matches the index of the liquid at some wavelength in the visible range, a dispersion color will be produced. When the objective lens is equipped with a central stop in the back focal plane of the lens, the primary image will be eliminated and only the light deviated by the dispersion will be seen. The color of the particle thus observed will depend on the wavelength of light at which the index of the particle matches the index of the liquid. A wide variety of high dispersion liquids covering the index of refraction range from 1.550 to 2.11 are available. With the proper liquid, transparent isotropic particles, such as glass fibers, may be easily identified. For anisotropic minerals, such as asbestos, a mixture of colors is produced and identification becomes more difficult. The addition of a polarizer results in the production of the single dispersion color corresponding to the index of refraction of the fiber along the axis observed. By rotating the polarizer and noting the "highest" (most red) color observed, the various indices along different axes may be measured and the particle identity determined. In this manner asbestos may be differentiated from other minerals although determination of the type of asbestos is more difficult due to a variation in the index of refraction with composition. By using an objective with a centered stop (centered screening or dark field dispersion), isotropic particles of about 1 micron may be identified.

Electron Microscopy

The morphology of certain fibers is sufficiently distinctive to permit positive identification with enough resolution to reveal fine structures when the fiber is observed. For example, the hollow tube down the middle of a chrysotile asbestos fiber distinguishes it from other forms of asbestos and is clearly visible in a 10,000× electron micrograph. Bulk materials may be prepared for electron microscopy in a variety of ways, but the mounting of airborne dust or lung samples is more difficult. An electrostatic precipitator has been developed which is capable of depositing the dust on the flat surface of a copper grid previously coated with formvar.[9] The efficiency of particle collection by this instrument is not size dependent, and the particles are essentially undisturbed by the collection process. However, this device is not suitable for breathing zone samples, and a long sample period is often necessary to obtain a heavy enough deposit. Dust collected on membrane filters used in personal breathing zone samples may be transferred directly to coated grids. A small square, slightly larger than the grid is cut from the filter and placed dust side down on a coated grid resting on a support screen in a spot plate. Acetone is added to the plate cavity and allowed to contact the grid and filter from underneath by capillary action. The filter dissolves through the formvar, leaving the dust lying on the grid. Several treatments with clean acetone are necessary to completely dissolve the filter.

Fibers in lungs must first be located before they can be seen by electron microscopy.

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Since the fibers are very sparse, especially in the lungs of people without occupational exposures, and since the electron microscope field area is so small, direct mounting of a lung specimen is generally not practical. A better method involves concentrating the lung residue by ashing or digestion and locating a fiber by means of a light microscope. The fiber is encased in a collodion film and then transferred to the electron microscope grid.

Analytical Method

Methods are available to determine either the chemical, or elemental composition or the crystal structure of mixtures of materials containing fibers, pure fiber bundles or single fibers. Their limitations are determined by the amount of sample required and their ability to distinguish the fiber from the associated material.

X-Ray Diffraction

Milligram quantities of chrysotile, amosite and crocidolite asbestos may be determined by x-ray diffraction. Both external[10] and internal[11,12] standard methods for the analysis of these minerals have been developed. In the latter case the samples are ground dry in a mixer mill to an average fiber length of <3.5 microns and passed through a 325-mesh U. S. Standard sieve prior to being mixed with a prescribed quantity of the internal standard. Standard samples of the pure asbestos mineral and the internal standard are prepared in a series of known weight ratios and used to calibrate the method. Quartz serves as the internal standard for amosite and crocidolite; and aquamarine, for chrysotile. The ratios of the intensities of the quartz 3.34 Å peak to the crocidolite 3.11 Å peak (or amosite 3.08 Å) are plotted against the weight ratios to obtain the standard curve. With aquamarine the 3.28 Å peak is used as the internal standard.

This method has been used for the quantitative determination of each of the three asbestos minerals in bulk or settled dust samples. Recoveries over the 1 mg to 10 mg range extends from 85% to 103%. With respirable dust samples of very small particle size, some variability in recovery has been noted, and the method is not as reliable.

Atomic Absorption Spectrophotometry

Atomic absorption spectrophotometry is a method for the elemental analysis of samples in solution in an aqueous or organic solvent. A portion of this solution is aspirated into the flame (Figure 2) where the vaporized sample constituents are irradiated by a lamp containing a cathode made from the analysis element(s). The ground-state atoms of the element in the vaporized sample absorb the resonance-line radiation emitted by this same element in the hollow-cathode lamp, thereby

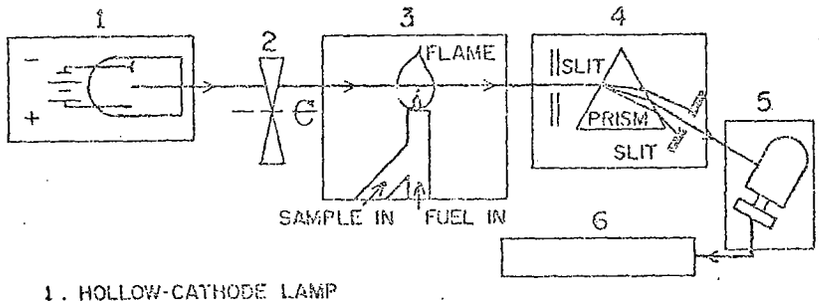


FIGURE 2. Atomic absorption spectrometer light path.

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effecting a decrease in the intensity of the light passing through the instrument's monochromator to the photodetector. The percentage of this absorption by the atomic vapor of the analysis element is a measure of the element's concentration in the sample. The advantages of the atomic absorption method are two-fold:

(1) It provides a high degree of sensitivity, for a direct-reading method, because it detects elements at their ground-state energy levels. At temperatures of 3,000 K, and below, at which flames operate, more than 99.99% of the atoms of many elements are in their ground state.

(2) It is highly specific, as it eliminates optical interference by operating on the very narrow resonance lines of the analysis element. (The resonance line is the transition involved in the drop of an excited atom to its ground state in a single jump.)

Atomic absorption has been used in the analysis of certain metallic elements present in samples of airborne asbestos fibers and of bulk asbestos minerals.[23]

The magnesium in the airborne dust collected in personal breathing zone samples for workers exposed to chrysotile ($3\ MgO \cdot 2\ SiO_2 \cdot 2H_2O$) may be measured. The filter samples are transferred to separate borosilicate beakers, digested with 5*N* HCl for 30 minutes, diluted to volume and analyzed directly. The magnesium 2852 Å resonance line provides a sensitivity (1% absorption) of 0.01 μg Mg per ml of final solution. This sensitivity permits magnesium analysis of filter halves with deposits so small that light field counts of particles may be made on the other half of the same filter. In the case of samples where the use of this line requires too great a dilution, the magnesium 2026 Å line is used. In either event, the estimated magnesium concentration is converted to the quantity of chrysotile in the total filter sample.

Comparison of this method with the x-ray diffraction procedure has shown a variation of results no greater than ±10% to 15% for a limited number of samples containing a sufficiently large dust deposit for the x-ray technique. The atomic absorption method for magnesium has permitted us to run thousands of personal samples, including many whose total dust weighed less than 0.1 mg. The application, of course, is limited to exposures containing only one source material of the analysis element; chrysotile asbestos in this case. However, where only one etiologic agent containing the analysis element is present in an airborne dust, the technique offers the recognized advantages of high sensitivity, accuracy, specificity, and speed common to all atomic absorption methods. The error of the method is $< \pm 5\%$ as determined from the analysis of known samples in our laboratory.

Atomic absorption has also been used to determine several heavy metals in bulk samples of chrysotile, amosite, and crocidolite, the asbestos minerals most used in this country. For this purpose, 0.25 gm of a bulk material is digested with hydrofluoric acid in a platinum dish for three to four hours, a procedure which involves a double evaporation to dryness. The final residue is dissolved in 1:1 redistilled hydrochloric acid and double distilled water and then filtered through a Whatman No. 42 paper. After the filter is washed, the solution is concentrated and then finally diluted to 25 ml. A one-half portion of this solution is reserved; the remainder is diluted, as required, for the individual analyses. Ammonium chloride is added to provide a concentration of 20 mg/ml to reduce the interference of iron with chromium. Iron suppresses the chromium response; for example, 200 μg Fe will suppress the response of 5 μg Cr to that of < 3 μg Cr. Some elements found in asbestos samples are listed in Table II along with their sensitivities (amounts giving 1% absorption) at the stated resonance lines.

Neutron Activation Analysis

This technique is based on the interaction of neutrons with the stable nuclei of chemical elements. These nuclear reactions are similar to ordinary chemical reactions in that they exhibit a mass change and an energy of activation and they proceed at definite reaction rates which depend upon the experimental conditions. There are several sources of neutron particles available, but research reactors,

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TABLE II
Elements Determined by Atomic Absorption Spectrophotometry Applied to HF Digests of Asbestos Minerals

Element	Resonance Line, A	Sensitivity,* μg/ml (1% Absorption) 1 X Scale	3 X Scale
Co	2407	—	0.2
Cr	3579	0.4	0.15
Fe	3720	1.0	—
Fe	2483	0.15	—
Mn	2795	0.1	—
Ni	2320	0.4	0.15

*Determined in authors' laboratory.

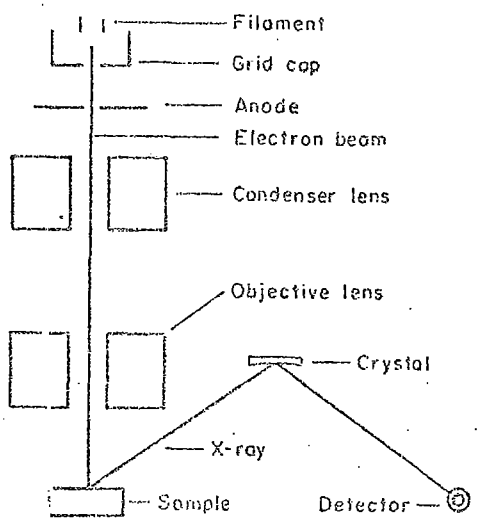


FIGURE 3. Electron microprobe schematic.

with their high thermal neutron flux, are generally used to irradiate samples for trace element analyses. A comparison of the results of neutron activation analysis with those obtained by atomic absorption spectrophotometry as applied to duplicate portions of a chrysotile sample is given in Table III.

TABLE III
Micrograms Element/Gram Asbestos

Element	Atomic Absorption Method	Neutron Activation Analysis Method
Ni	1690	1400
Cr	1090	1060
Co	78	80
Mn	423*	1100
	538*	

*Separate acid leachings.

Electron Microprobe

The electron microprobe (Figure 3) is an instrument for spot chemical analysis using a finely-focused electron beam to excite characteristic x-radiation from a small volume at the surface of a solid specimen (Figure 4). The wavelength and intensity of the characteristic x-ray lines are then used to identify the elements present at the target site and to determine their relative mass concentrations. At the present time, analyses can be performed for all elements of atomic number 5 and greater.

The commercial electron microprobe usually consists of up to three curved crystal, x-ray monochromators that are used to analyze the characteristic x-ray lines, which have been excited by the finely-focused electron beam, impinging upon a selected spot on the surface of the sample material (Figure 5). The sample composition is determined from the wavelength and the intensity of these characteristic x-ray lines. The specimen may be moved under the electron beam for analysis of specific areas located with the aid of an optical microscopic viewing system.

The instrument is capable of producing an electron focal spot of the order of 0.5 micron in diameter depending on the beam power and the sample being analyzed. The effective spot obtained with the electron beam is smaller when a material of high atomic number is analyzed than when a material of low atomic number is analyzed because of the additional penetration of the beam into the low-atomic sample. The use of a low intensity beam will permit the analysis of biological and asbestos-type samples.

The electron microprobe can be used to identify metallic impurities located on the surfaces of, or associated within, the lattice of asbestos fibers. When the electron microprobe is applied to fibers obtained from lung samples, it has been possible to demonstrate the existence of an amosite asbestos fiber at the core of an asbestos body taken from the lung of an asbestos worker.[14] A pseudo-asbestos body, not otherwise distinguishable from a true asbestos body, in the lung of a foundry

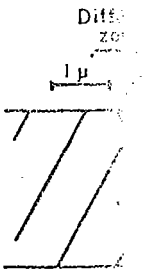


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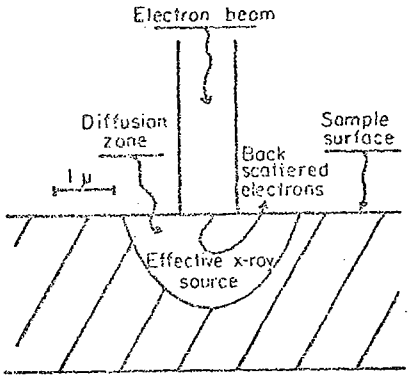


FIGURE 4. Electron microprobe effective x-ray source region.

worker was found to have no asbestos fiber at the core.

A ternary-system phase-diagram may be used to characterize the ratios of the elements Fe, Si, and Mg analyzed by the electron microprobes. Pure asbestos specimens all lie in certain clearly defined zones on this diagram. An untreated asbestos body, however, will shift toward the iron end due to the iron-protein coating on the fiber. When this coating is removed, the bare fiber is found in the same location as a pure fiber.

The theoretical sensitivities of this technique for the determination of trace elements in particles are given in Table IV. As can be seen, an element such as a metal distributed throughout an asbestos fiber in a concentration of a few parts per million would not be detectable. However, concentrated particles of a metal compound contained in, or on, a fiber bundle could be identified.

Emission Spectroscopy

Emission spectroscopy is the study of the radiations emitted by atoms, ions, and a few molecular species after suitable excitation by a thermal or electrical source of energy. These radiations lie in the near infrared, the visible, and the ultraviolet regions of the electromagnetic spectrum. Spectrochemical methods of analysis make practical use of these radiations for qualitative and quantitative analytical purposes. The emission spectrograph may be used to analyze more than 70 metallic or

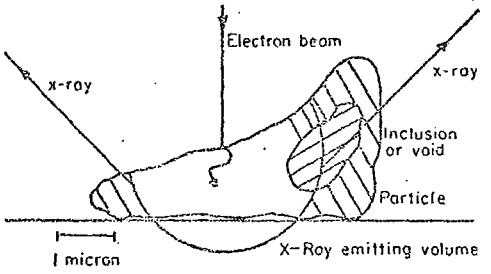


FIGURE 5. Electron microprobe analysis of a single particle.

TABLE IV
Sensitivity of Electron Microprobe Analyzer

Element	Min. Detect. Mass. gm	Min. Detect. % in 1μ Particle	Spectrometer
Be	1×10^{-14}	20.0	Grating
Ca	8×10^{-16}	0.1	ADP-LiF
Co	4×10^{-16}	0.03	LiF
Cr	5×10^{-16}	0.03	LiF
Fe	4×10^{-16}	0.03	LiF
Mn	5×10^{-16}	0.03	LiF
Ni	2×10^{-16}	0.04	LiF
Pb	5×10^{-14}	0.01	LiF-ADP

metal-like elements in any type of solid or liquid sample.

Most spectrographic analyses in the field of industrial hygiene require the sample to be burned in spectroscopically pure graphite electrodes in a D. C. arc. This source of excitation provides the greatest sensitivity for the detection and determination of trace quantities of the metallic elements. Quantitative methods developed for specific determinations are based upon the employment of optimal excitation conditions and the use of spectroscopic buffer salts which minimize the production of the background or "continuum" and, at the same time, increase the intensity of atomic spectra.

Mineral substances may be ground and then analyzed directly by spectrographic procedures. However, some of the metallic impurities in such minerals as chrysotile, amosite or crocidolite types of asbestos may be quite low in concentration or may not be homogeneously dispersed throughout the fibrous matrix. In such cases, the analyst will be required to digest the mineral by means of the hydrofluoric acid procedure to reduce the

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TABLE V
Summary of Methods and Their Application

Method	Application	Characteristic Determined	Type Sample	Comments
Light Microscope				
Light Field (100×)	Detection and Enumeration	Total dust	Impinger	Traditional standard method for evaluating asbestos exposure.
Phase Contrast (400×)	Detection and Enumeration	Fibers by shape	Membrane filter and lung residue	New method for tentative asbestos TLV. Do not see fibers smaller than about 0.1 μ diameter.
Dispersion Staining	Identification	Refractive Index	Bulk or airborne dust	Distinguish asbestos from non-asbestos fibers.
Electron Microscope	Detection and Identification	Fiber by shape Fine structure	Bulk, airborne dust and lung	May identify fibers with unique fine structure.
X-ray Diffraction	Identification and Analysis	Crystal structure	Bulk, airborne dust (>1.0 mg)	Cannot distinguish fibrous from non-fibrous minerals of same crystal structure.
Atomic Absorption	Analysis	Elemental composition	Bulk and airborne dust	Sensitive enough for trace elements in mg samples and gross composition of count samples.
Emission Spectroscopy	Analysis	Elemental composition	Bulk	Gross and trace constituents.
Electron Microprobe	Detection, Identification and Analysis	Elemental composition	Bulk, airborne dust and lung	May determine gross composition of selected fibers and grains.

bulk of the mineral diluent and to provide a solution of the silica-free residue. The resulting sample solution is diluted or concentrated, as required, for the analysis of the elements of interest. Sample solutions are measured precisely into the craters of graphite electrodes with micropipets. After the addition of an internal standard solution, the electrode is dried at 105C, cooled and burned in the D. C. arc—just like other solid samples.

The technique is sensitive to approximately 0.01 microgram of cobalt, nickel, vanadium, chromium, and other elements of similar volatility contained in a 2-milligram charge of the sample residue introduced into the electrode crater.

Application of Methods

A summary of the methods discussed and their use is given in Table V. The light microscope is the easiest and most direct method of fiber detection and identification, but only fibers with diameters greater than about 0.1 micron can be seen in this way. It is used in the standard methods for the counting of dust and fibers to determine compliance with the Threshold Limit Values (TLV's). It may also be used to count fibers found in lung residues although lung samples examined should not be assumed to be representative since dust deposits are not uniformly distributed throughout the lung. If the entire lung were ashed or digested, a sample taken of a uniform dispersion of the residue, and an adequate number of fibers observed, the analyst might have an accurate estimate of the number of fibers in the lung.

The electron microscope may be used to see fibers smaller than those visible under the light microscope and to identify some types of fibers. Counting fibers is difficult since the field area observed is very small and many plates must be examined to find enough fibers for an accurate count. Lung fibers may also be examined although it is generally necessary to concentrate the lung dust and may be desirable to first locate the fibers by light microscope.

X-ray diffraction will identify the presence of certain minerals, except where strong interferences from associated materials are present. This method cannot distinguish between a fibrous mineral and the parent non-fibrous mineral with the same crystal structure. For example, in analyzing for asbestos, the result will include the non-fibrous serpentine with the fibrous chrysotile. This method also appears to be somewhat dependent on particle size and has a sensitivity of about 1 mg of the mineral in question, which is not generally

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low enough for airborne dust exposure samples.

Both the atomic absorption and emission spectroscopy methods determine the elemental composition of the total material analyzed. Atomic absorption is preferred wherever it is applicable since it is easier and has more freedom from interferences. Atomic absorption has been used for the analysis of magnesium in airborne dust samples of chrysotile asbestos with deposits so small that the samples may also be used for counting. As with x-ray diffraction, however, the total of both chrysotile and serpentine is obtained.

The electron microprobe shows promise of being one of the most sensitive and versatile analytical instruments. It is the only instrument that permits the selection and elemental analysis of a single bundle of fibers. At present it is not sensitive enough to permit analysis of trace elements uniformly distributed throughout a fiber, although improvements in design and technique may make this possible.

Conclusion

Virtually all available methods applicable to the detection, identification, and analysis of fibers have been brought to bear on the problem of evaluating health hazards resulting from exposure to fibers and the discovery of the mechanisms of the diseases involved. Frequently, due to limitations in sample size and the necessity of dealing with samples of fibers mixed with other materials, indirect sources of data have had to be combined to obtain an overall characterization of the fiber. Work is proceeding, however, in several laboratories to develop new and improved methods.

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