Spectroscopic Techniques in Industrial Hygiene

Jin Wang, Paul D. Siegel, Daniel M. Lewis, Evanly Vo, William E. Wallace, Kevin Ashley, and Lloyd E. Stettler

in
Encyclopedia of Analytical Chemistry
R.A. Meyers (Ed.)
pp. 4783–4824

Spectroscopic Techniques in Industrial Hygiene

Jin Wang, Paul D. Siegel, Daniel M. Lewis, Evanly Vo, and William E. Wallace
US Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Morgantown, USA

Kevin Ashley and Lloyd E. Stettler
US Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, USA

Over the past few decades the pace of change in spectroscopic techniques has been remarkable. Spectroscopic techniques are emerging as important, powerful, and versatile tools in determining exposure levels of hazards generated in working environments. Occupational safety and health studies employ spectroscopic techniques to analyze hazardous chemicals, biomarkers, and particulate matters of exposure. In comparison with many traditional detection techniques such as gravimetric methods, spectrometric techniques are much more sensitive, selective and accurate. The major spectroscopic techniques used in industrial hygiene include mass spectrometry (MS), scanning electron microscopy (SEM), X-ray microanalysis (XM), atomic spectrometry (AS), ultraviolet/visible (UV/VIS) photometry, fluorescent spectrometry (FS), Fourier transform infrared (FTIR) spectroscopy, and Raman spectroscopy (RS). Interest in using MS in industrial hygiene is driven by its value in understanding basic physical, chemical, and biological processes related to workers' exposure to occupational hazards, and in devising new methodologies to monitor exposures. SEM has become particularly useful in the study of pneumoconioses and workplace environmental particles since being complemented with energy dispersive X-ray (EDX) analysis and automated image analysis capabilities. SEM and EDX have been used extensively to characterize particles found in lung tissues. Atomic spectrometric methods are used widely for occupational health evaluation of inorganic

Encyclopedia of Analytical Chemistry
R.A. Meyers (Ed.)

metals. The development of inductively coupled plasma atomic emission spectrometry (ICPAES) techniques has become increasingly attractive, and has been applicable to analysis of nearly all the elements. FTIR and Raman spectroscopies are employed to detect highly toxic gas and vapor mixtures. Additionally, field-portable methods for monitoring airborne workplace contaminants and toxins have received increasing attention. To date, highly specific, selective, and sensitive spectroscopic technologies have allowed for the development of novel methodologies and new indicators for exposure characterization. Assessment of actual body burden of chemicals, which are more directly related to potential adverse occupational health effects, can be accomplished. The major spectroscopic techniques and their applications to industrial hygiene are described in this article.

1 INTRODUCTION

The pace of change in spectroscopic techniques has been remarkable. They are emerging as important, powerful, and versatile tools, and have increasing applications in the workplace. For occupational safety and health studies, spectroscopic techniques are primarily used to detect and analyze hazardous chemicals, biomarkers (e.g. metabolites, DNA adducts, protein conjugates, and allergens), and to investigate pneumoconioses and workplace environmental particles. They play an important role in determining exposure levels of hazards generated in the working environment, and in finding the adverse effects of exposures and their mechanisms of action. In comparison with many traditional detection techniques such as gravimetric methods, spectrometric or spectrophotometric techniques are much more sensitive, selective and powerful. The major spectroscopic techniques used in industrial hygiene studies include MS, SEM, XM, AS, UV/VIS photometry, FS, FTIR, and RS. In this article, the basic principles of these major spectroscopic techniques and their applications to industrial hygiene are described.

The interest in using MS by scientists in studies pertinent to industrial hygiene is driven by the need to understand the basic physical, chemical, and biological processes related to workers' exposure to occupational hazards and to devise new methodologies to monitor exposures in the work environment. MS is capable of looking at the details of exposures, and as the most sensitive tool, to analyze small molecules and macromolecules in biological systems. With tandem mass spectrometry (MS/MS) techniques, MS brought special capabilities to providing specific characterization of molecular structures and to detecting target analytes at trace levels. The development of new ionization methods such as electrospray ionization, atmospheric pressure chemical ionization (APCI), and matrix-assisted laser desorption ionization (MALDI) have contributed to great strides forward in the study of biological macromolecules. The ability to ionize polar, labile and involatile species has been the fundamental basis upon which MS extends its applications. It is important for us to recognize the breakthroughs in ionization techniques of the 1980s and 1990s. These advances have allowed application of MS to progress and develop rapidly. Additionally, combining gas chromatography (GC) or liquid chromatography (LC) with MS offers the possibility of taking advantage of both chromatography as a powerful separation technique and MS as a powerful and sensitive detection and identification technique. Furthermore, Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS), and a new generation of high performance time-of-flight mass spectrometry (TOFMS), which includes nanoflow electrospray hybrid quadrupole time-of-flight mass spectrometry (QTOFMS) and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOFMS) technologies, are just beginning to add much needed analytical power to our arsenal for macromolecular identification and characterization. These developments will bring important methodology to scientists and thus accelerate the integration of MS into work-related research strategies. The range of applications in environmental health and industrial hygiene studies includes development of qualitative and quantitative analytical procedures, structural determination of aberrant proteins, investigation of biomarkers for exposure to hazards, characterization of allergens related to occupational asthma, and identification of microbial products in complex samples. MS and its related techniques and applications are described in section 2.

SEM is introduced in section 3. It is ideally suited to study pneumoconioses and workplace environmental particles when it is equipped with an EDX analysis system and an image analyzer (IA). Particle matter has been recognized as a cause of various lung diseases for many years. Specific pneumoconioses such as coalworker's pneumoconiosis, asbestosis, and silicosis may result from inhalation of particles in the workplaces. SEM and EDX have been used extensively to characterize particles found in lung tissues. While it is not possible to compare a measured lung dust burden to the actual exposure due to the lack of exposure data and to particle clearance, the measured lung particulate burden does represent retained particle dose. The SEM and EDX analysis may be used for a number of applications in occupational safety and health including the analysis of particles collected on air filter samples taken from the working environment, analysis of individual particles found in bulk dusts, and in the study of pneumoconioses.

Bulk compositional analyses of respirable particulate material, and even particle-by-particle compositional analyses, may not always be sufficient to predict the level of biological activity of respirable particles or the health risks of exposure to them. Toxicants may be located on the particle surface or may be more heavily concentrated there, resulting in heightened biological availability and expression of toxic activity. Or, in some cases, surface coating materials may have a prophylactic effect on expression of the toxicity of the underlying bulk of the particle. An example of the latter effect is the modified activity of the quartz component of some mixed composition mineral dust exposures for causing mixed dust pneumoconioses. SEM with EDX or wavelength dispersive X-ray spectroscopy can provide some information about the elemental composition with depth into a particle, by acquiring X-ray spectra at two or more electron beam accelerating voltages. This method has been used to detect thin submicrometer aluminosilicate coatings or clay "surface occlusion" on respirable quartz particles. Applications of SEM to the study of pneumoconioses, including determination of lung particulate burden, particle chemistry, and respirable particle surface characterization, are described in section 3 of this article.

Atomic spectrometric methods continue to be used widely for occupational health evaluation of inorganic metals and are documented briefly in section 4. The development of ICPAES techniques has been increasing, and has been applied to elemental analysis of nearly all the elements. ICPAES offers a simultaneous or rapid sequential multielement determination capability at the major, minor, and certain trace concentration levels. It has become established as a widely accepted methods for the analysis of metallic aerosols, powders of metals, dusts, and fly ashes. A vast amount of published atomic spectrometric methodology is available, covering a wide range of application areas. This is because trace elements released into the atmosphere in industrial processes have aroused great interest. Major advantages include specificity, speed, and ease of use. However, for ultratrace or trace level analysis atomic spectrometric methods have been increasingly replaced by inductively coupled plasma mass spectrometry (ICPMS). AS and its related techniques and applications are described in section 4.

Spectrophotometric detectors coupled to chromatographic separation techniques are often used to characterize the workplace environment and are discussed briefly in section 5. These techniques are employed to confirm the accuracy of direct reading instruments or when direct reading instruments are not available for the environmental contaminant, when the workplace environment is complex and contains multiple chemicals that need to be measured, for regulatory documentation of exposure levels, and for biological monitoring. Spectrophotometric detectors that are coupled to high-performance liquid chromatography (HPLC) or GC include UV/VIS, fluorescent, light scattering, refractive index, diode array, MS, infrared (IR), radioactivity, and luminescence. The choice of detector is dependent on the analyte's spectral properties, and the required sensitivity and selectivity. Both the sensitivity and selectivity of spectroscopic detectors are dependent, in part, on wavelength(s) employed. In addition, it is desirable to choose a detector with a wide linear working range to be able to assess both major and minor chemical components in the workplace environment. In monitoring the workplace environment, the most commonly employed HPLC detectors are the UV/VIS and fluorescent photometers, whereas, the most commonly used GC detectors are the flame photometric detector (FPD) and the mass spectral detector (MSD).

With the increased use of highly toxic gas and vapor mixtures in science and industry, FTIR and Raman spectroscopies have played an important role in industrial hygiene monitoring. Although FTIR and Raman spectroscopies are similar in that both techniques provide information on vibrational frequencies, there are many differences between the two techniques. For example, some vibrations are only Raman active while others are only IR active; the vibration of a heteropolar diatomic molecule is IR active, whereas that of a homopolar diatomic molecule is not IR active. The method for determining quartz content in respirable coal dust is often based on dispersive IR or FTIR spectroscopy. Like IR, RS is a powerful technique and has a variety of applications. They are complementary, and both are utilized whenever possible. IR and Raman spectroscopes and their related techniques and applications are discussed briefly in section 6.

Finally, field-portable methods, which have received increasing attention, are summarized in section 7. Field-portable methods for monitoring airborne workplace contaminants and toxins have received increasing attention. A number of portable monitors for airborne contaminants have been commercially available for many years, but new developments may provide for on-site compliance monitoring, which has heretofore been more the exception than the rule. The ability to conduct measurements on-site in the occupational setting offers significant advantages. Field-portable methods are often desired so that decisions regarding worker protection, engineering controls, and so on can be made quickly. The capability for rapid decision-making offered by on-site monitoring can help to save costs, and also offers a means to assess, and thereby prevent, worker overexposures to toxic substances in a timely manner. Field-based monitoring is especially useful for applications in the construction industry, in agriculture, and in other situations where

jobs may be short-term and the workforce is transient. On-site techniques can also be beneficial in instances where short-term monitoring is desired. In section 7, field-portable spectrometric techniques are covered, and some applications are presented.

To date, highly specific, selective, and sensitive spectroscopic technologies have allowed for the development of novel methodologies and new indicators of exposures. Therefore, the assessment of the actual body burden of chemicals, which is directly related to potential adverse occupational health effects, can be accomplished.

2 MASS SPECTROMETRY

2.1 Introduction

The mass spectrometer is an instrument capable of producing a beam of ions by converting neutral molecules into gaseous ions, and then separating these ions according to their mass-to-charge ratio and recording the relative abundances of the separated ion species as a mass spectrum.[1] Today MS has brought special capabilities to a wide variety of scientific research by providing specific analyses of substances, their metabolites, and biological macromolecules, often with structural information. The range of applications includes qualitative and quantitative analytical procedures employed in environmental health and industrial hygiene.

In instrumentation, the mass detector has been developed in a variety of types, shapes, and sizes.[2–4] The selection of the detector is based on the needs of the user and its functions:[1] the quadrupole analyzer is employed to provide an electron ionization (EI) or chemical ionization (CI);[2] the ion trap spectrometer, which is a highly geometrically modified quadrupole analyzer, is adequate for low-energy collision;[3] the ion cyclotron resonance spectrometer, which is the basis for Fourier transform MS, has high resolution;[4] the time-of-flight (TOF) spectrometer, which separates ions in time rather than space and has an almost unlimited mass range, is used for detection of macromolecules; and[5] the magnetic sector instrument, in which ion separation is achieved spatially by the application of a magnetic field (sometimes coupled with an electrostatic field), is suited to conduct collisionally activated dissociation at high energy. Furthermore, by coupling of two or more types of the above-mentioned mass analyzers, an MS/MS or an ion trap mass spectrometry (MS^n) technique is achieved. MS/MS is a key instrumental development in analytical and bioanalytical chemistry. It is widely applied in the characterization of molecular structures and in the trace analysis of targeted analytes. The most widely used activation method is called collisionally induced dissociation, in which precursor ions are selected in the first MS of multistage MS for repulsive collisions with inert gases such as helium or argon. In addition, the coupling of MS with separation techniques and the development of new ionization methods such as electrospray ionization, APCI, and MALDI have contributed to great strides forward in this field. The ability to ionize polar, labile and involatile species has been the fundamental basis upon which MS extends its applications. It is important for us to recognize the breakthroughs in ionization techniques of the 1980s and 1990s. These advances have allowed application of MS to progress and develop rapidly.

The interest in using MS by scientists in studies pertinent to industrial hygiene is driven by the need to understand the basic physical, chemical, and biological processes related to workers' exposure to hazards and to devise new methodologies to control exposures in the work environment. MS is capable of looking at the details of exposure, and is a highly sensitive tool for structural determination of aberrant proteins, development of biomarkers for exposures, identification of microbial products in complex samples, and characterization of hazards in occupational and environmental health evaluations.

2.2 Gas Chromatography/Mass Spectrometry

2.2.1 Principles and Instrumentation

The coupling of GC with MS was first achieved in 1957.[5] It is a combination of two microanalytical techniques: a separation technique, GC, and an identification technique, MS. The gas chromatography/mass spectrometry (GC/MS) combination overcomes certain deficiencies or limitations caused by using each technique individually, and gives a two-dimensional identification consisting of both a GC retention time and a mass spectrum for each component of the mixture. This combination has several advantages. First, it can separate components of a complex mixture so that mass spectra of individual compounds can be obtained for qualitative purposes; second, it can provide quantitative information on these same compounds. GC/MS can provide a complete mass spectrum from as little as 1 pmol of an analyte, which gives direct evidence for the molecular weight and a characteristic fragmentation pattern or chemical fingerprint that can be used as the basis for identification. Although the direct GC/MS method is limited to the analysis of those compounds that can be made volatile without thermal decomposition, many compounds that are nonvolatile can be handled successfully after chemical derivatization. The instrumentation of GC/MS consists essentially of three components: the gas chromatograph, the mass spectrometer and a data system. GC/MS has developed

into one of the most sensitive and selective analytical techniques for the separation, identification and quantification of components of complex mixtures.

The GC/MS technique has been utilized in a diverse range of applications such as toxicology, environmental monitoring, molecular biology, clinical health, and industrial hygiene, as well as many others. In these applications, the analyte is often present in a complex matrix consisting of a great number of compounds which may mask its presence or otherwise inhibit its detection. One of the most common ways in which GC/MS is used in industrial hygiene is as a diagnostic tool, which is particularly useful in the analysis of complex mixtures where the analytes are present in low quantities. GC/MS is also widely used in determining hazardous materials, derived metabolites, and protein/DNA adducts in biological fluids (e.g. blood or urine) from workers exposed to specific hazards.

2.2.2 Methodology

2.2.2.1 Sample Preparation Sample preparation is important for successful analyses by GC/MS. Industrial airborne hazards include gases, vapors, liquids, and particulates. Air sampling is of course a crucial step within the total scheme of air analysis. There are a variety of methods available for the collection of airborne particles. Generally, a method selected will often depend on the purpose for which the sample is being taken, and also depend on the type of compounds to be analyzed. A few preprocessing steps may be needed to manipulate the sample into a form ready for analysis. In addition, the physical state of sample material will affect the method to be used for the introduction of sample into the spectrometer. A wide variety of techniques are available for processing gaseous, liquid and solid samples. In industrial hygiene studies, one prevalent strategy in dealing with air samples consists of filter collection of particles, followed by an appropriate second stage adsorption of organic chemical vapor or fumes onto XAD-2 sorbent [treated with 2-(hydroxymethyl)piperidine] or charcoal tubes, and then solvent desorption and GC/MS analysis. There have been considerable improvements in air sampling and GC/MS detection.[6,7]

Another commonly used strategy of GC/MS analysis is the derivative method. Derivatization is an approach for increasing volatility of target analytes which is particularly useful for biological samples. In those cases where compounds are too polar or thermally unstable to be amenable to GC analysis, the situation can be improved by the formation of a suitable derivative with characteristics that render it more amenable to GC. In addition, derivatization can enhance sensitivity and selectivity by altering the fragmentation mechanism of the molecule. A further beneficial effect of derivatization is that it normally results in a compound possessing characteristic ions at mass-to-charge ratios. In tissue analysis, the sample usually requires dialysis after homogenization to remove free monomeric sugars and amino acids. Sample pretreatment of microorganisms for GC/MS analysis generally involves extraction of the class of compounds of interest (e.g. lipids, proteins, or carbohydrates) followed by derivatization.

2.2.2.2 Electron Ionization and Chemical Ionization EI is one of the main ionization methods employed in GC/MS system,[8] while CI has also been employed occasionally. In EI, energy sufficient for ionization and fragmentation of the analyte molecules is acquired by interaction with electrons (ca. 70 eV) from a hot filament. Some structural features of the analyte molecule can be deduced from the fragmentation pattern of the molecular ion. During the ionization process, in addition to the production of positive ions, a small number of molecules undergo addition of one or more electrons to form negative ions. At the operating ionizing energy of the analytical mass spectrometer (60–100 eV) the sensitivity of negative ion formation is several orders of magnitude less than that for positive ion production. Compared with EI, CI is a soft ionization technique. It achieves ionization of the analyte by collision with reagent ions (usually proton-rich ions), but without transferring excessive energy to the nascent analyte ions. The result is the formation of abundant adduct ions, often protonated molecules, that contain the intact molecular species of the analyte. In this way, CI and EI are complementary. Because of the soft ionization process, the even-electron molecular adduct ions undergo little fragmentation compared with that of the odd-electron molecular ion produced during EI. Often, CI spectra of individual analytes are sufficiently simple to allow the direct analysis of mixtures, and therefore can be used to provide structural information that is not available from an EI spectrum.

2.2.2.3 Quantitative Analysis and Selected Ion Monitoring The quantitative applications of MS are based on comparison between the ion current obtained from the analyte in the sample matrix and the ion current from another compound chosen as an internal standard, or the ion current obtained from analyses of standard aliquots of the pure analyte. The method of recording the ion current is usually by selected ion monitoring (SIM), although the technique of repetitive scanning over a narrow mass range is sometimes employed. The technique of SIM is one of the most versatile and commonly used spectrometric methods. It allows a mass spectrometer to record simultaneously the intensities of a limited number of chosen ions only. This allows the instrument to dwell for a greater proportion of the analysis time on those mass-to-charge ratios of

greatest significance in the mass spectrum of the analyte of interest, resulting in an increase in sensitivity. A major application of this technique is to provide quantitative analysis of compounds at low concentrations.[9]

2.2.3 *Applications*

In the 1990s, the application of GC/MS and related techniques for the characterization and quantitation of organic compounds and biomarkers has grown spectacularly in environmental health and occupational exposure studies. One such example is the use of GC/MS in the analysis of the health effects of the environmental aromatic hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, and some of them are potentially carcinogenic substances, to which humans are exposed in the environment and in certain workplaces. Estimation of the resulting health risk is therefore of great occupational health importance. The determination of PAHs and their metabolites or protein/DNA adducts as biomarkers is the most suitable way to assess the exposure and to estimate this risk.

Sturaro et al.[10] have reported a GC/MS investigation of polycyclic aromatic compounds (PACs) in the manufacture of rubber tubes, using a two-stage air sampler and a GC/MS method to monitor eight PACs. With this method, detection limits of 8–15 ng mL^{-1}, corresponding to a level of 12–23 ng m^{-3} in the workplace, were achieved. Workers exposed to airborne aromatic amines in another rubber manufacturing process were evaluated by Menichini et al.[11] Samples were collected on a glass fiber filter, processed through a silica gel tube, and analyzed by GC/MS/SIM. Their work outlines a procedure for the determination of occupational exposure to airborne aromatic amines in the rubber industry and the application of this method in a tire manufacturing plant. In related work, Menichini et al.[12] determined PAHs in mineral oils and oil aerosols in glass manufacturing. PAHs were analyzed by GC/MS in graphited mineral oils used for mold lubrication and in aerosols emitted during their application in two plants. High boiling PAHs were detected in oils but generally not in air. Volatile PAHs were found in oil samples and to a lesser extent in air near the emission source. Additionally, Bundt et al.[13] have investigated structure-type separation of diesel fuels by solid-phase extraction (SPE) and identification of the two- and three-ring aromatics. Commercially available standards were used for identification. Sulfur-containing PAHs in diesel fuel are mainly represented by methyl-substituted dibenzothiophenes. Cooper[14] has developed a GC/MS method to confirm the presence of *N*-nitrosamines in workplace air samples. Detection limits of the three procedures and retention time precision of both SIM techniques are good. Typical examples of the use of these techniques for confirmation of *N*-nitrosamines are described by Cooper.[14] More applications are summarized in Table 1.

Urinary naphthols (1- and 2-naphthol) have been suggested as route-specific biomarkers for exposure to airborne PAHs.[27] The application of urinary naphthol levels as biomarkers in 119 Japanese male workers was reported. The urinary 1- and 2-naphthol levels were observed three and sevenfold higher, respectively,

Table 1 Typical applications based on GC/MS methods

No.	Chemical hazards	Sample type	Ref.
1	PAHs (glass manufacturing)	Mineral oils and oil aerosols	12
2	PAHs in diesel fuel	Petroleum	13
3	PAHs (cooking plant and foundries)	Workplace air, and dust	21
4	PACs (energy laboratory)	Coal combustion product	16
5	PACs (manufacture of rubber tubes)	Workplace air	10
6	Perchloroethylene (five dry cleaning firms)	Workplace air, and blood of exposed workers	19
7	Airborne aromatic amines (rubber manufacturing)	Workplace air	11
8	*N*-Nitrosamines	Workplace air	14
9	VOCs	Indoor air	18
10	Polychlorinated biphenyls (electrical workers)	Workroom surfaces, tools, palms, and blood	22
11	Polybrominated dioxins, dibenzofurans, octachlorostyrene	Combustion, and flame retardants	20
12	Glycol ethers (newspaper printing plant)	Workplace air	17
13	Organic vapor pollutants	Ambient atmospheres	15
14	Resin acid compounds	Emitted from rosin in soldering flux	23
15	Semivolatile organic compounds	Workplace atmospheres	24
16	Airborne chemical agents	Air	25
17	Coal tar pitch volatiles	Cold tar pitch fume	26

VOCs, volatile organic compounds.

among smokers than among nonsmokers. Also the ratios of urinary 2-naphthol to 1-naphthol were significantly higher among smokers than nonsmokers. In another study, a method for the simultaneous determination of urinary phenanthrene, fluoranthene, pyrene, chrysene and benzo[*a*]pyrene metabolites has been developed for individual risk assessment at a PAH-burdened workplace.[28] The method allows the determination of 25 different components. The PAH exposure of coke plant workers during several consecutive days resulted in fairly constant individual urinary metabolite profiles.[29] It was also demonstrated that in the case of coke plant workers there is a correlation between inhaled PAHs and metabolites excreted. Mass relationships between inhaled PAHs and metabolites excreted were found to differ from one individual to another.

The exposures of agricultural workers to organochlorine pesticides were studied by Guardino et al.[30] The chlorinated pesticides and their metabolites in whole blood samples from 30 farmers and 24 nonoccupationally exposed workers were determined by GC/MS. The potential that sawmill workers might be exposed to chlorophenols was investigated by Kontsas et al.[31] A GC/MS procedure for the determination of chlorophenols in urine was developed. The concentrations of urinary chlorophenols in previously exposed workers were of the same magnitude as those found in nonexposed controls and in the general population. The feasibility of using plasma, blood and hemoglobin (Hb) adducts for monitoring occupational exposure to the suspected human carcinogen 4,4′-methylenebis(2-chloroaniline) (MOCA) was investigated by Vaughan et al.[32] The levels of MOCA in the blood and urine of five individuals who were exposed to MOCA during the manufacture were determined by the GC/MS method. It was found that the use of blood samples for monitoring exposure to MOCA offers advantages over the currently used urinary MOCA measurements.

Occupational exposure to toluene diisocyanate (TDI) among workers in a polyurethane foam factory[33] was studied during 48-h periods and biological samples from nine subjects. Five workers were found to show high average urinary elimination rates of TDI. The elimination rate curves for all of the subjects studied had a linear relationship with exposure to TDI. The study indicates that it is possible to monitor exposure to TDI by monitoring urinary concentrations of TDI by GC/MS. A study of exposure to benzidrine was reported by Hsu et al.[34] Exposure to benzidine, which is subsequently acetylated to *N*-acetylbenzidine and *N*,*N*′-diacetylbenzidine, has been implicated in the development of bladder cancer in humans. In this study, an isotope dilution GC/negative ion CI MS method was developed to quantitate urine concentrations of benzidine and its acetylated metabolites. The method is applicable to the measurement of other aromatic amines and their acetylated metabolites. Worker exposure to sawing fumes from pine was investigated by Eriksson et al.[35] Three metabolites from α-pinene have been identified in human urine after occupational exposure to it. Urine was enzymatically hydrolyzed, and metabolites were identified by GC/MS using EI and CI with isobutane as the reagent gas. The use of Hb and serum–protein adducts of hazard reagents as biomarkers for occupational and environmental exposure assessment has received increasing interest. The environmental pollutant 2,4,6-trinitrotoluene (TNT) is an important occupational health hazard, and is taken up through the skin and by inhalation. It is therefore essential to have fast and reliable methods to monitor human exposure. In a related work, a GC/MS method, which quantifies Hb adducts of TNT for 50 workers and controls from a Chinese munition factory, was reported by Sabbioni et al.[36] The Hb adduct levels ranged from 3.7 to 522 ng. However, in control samples no adducts could be found. In another study,[37] alachlor–protein adducts were examined as potential biomarkers of alachlor exposure, a genotoxic and carcinogenic herbicide. The method developed was based on the observation that cleavage of *S*-cysteinyl alachlor–protein adducts by methanesulfonic acid gave the rearrangement product.

Hb samples from ethylene oxide-exposed workers and nonexposed referents were analyzed by Farmer et al.[38] GC/MS was used to determine an Hb adduct as its methyl ester heptafluorobutyryl derivative, after hydrolysis of the protein and isolation of the alkylated amino acid. Ranasinghe et al.[39] have reported an application of GC/electron capture negative CI high-resolution MS for characterization and quantitation of DNA and protein adducts. The method has adequate sensitivity and specificity to measure accurately DNA and protein adducts as low as endogenous concentrations in rodent and human tissues. Additional applications are listed in Table 2.

2.3 Liquid Chromatography/Mass Spectrometry, Liquid Chromatography Tandem Mass Spectrometry and Liquid Chromatography Ion Trap Mass Spectrometry

2.3.1 Principles and Instrumentation

The history of liquid chromatography/mass spectrometry (LC/MS) starts in the early 1970s,[62] and since then the technique has been developed rapidly. There are several general reviews[63–66] and books published on this subject.[67–70] The combination of LC and MS offers the possibility of taking advantage of both LC as a powerful separation technique and MS as a selective and sensitive detector. A considerable number of LC/MS

Table 2 Typical biomonitoring based on GC/MS methods

No.	Biomarkers	Sample type	Ref.
1	Alachlor–protein adducts (alachlor exposure)	In vitro and in vivo	37
2	Hb adducts (ethylene oxide exposure)	Blood	38
3	Hb adducts (methyl bromide exposure)	In vitro erythrocytes	40
4	Trinitrotoluene and metabolites	Urine	42
5	Hb adducts (3,3-dichlorobenzidine exposure)	Rat erythrocytes	43
6	PAHs and metabolites (PAH-exposed workers)	Urine	28
7	DNA adducts (2,3-epoxy-4-hydroxynonanal exposure)	Calf thymus DNA	44
8	Metabolites (monoterpenes α-pinene and β-pinene exposure)	Urine	35
9	Hb adducts (TNT)	Blood	36
10	Benzidine and metabolites (benzidine exposure)	Urine	34
11	Hb [(4,4-methylenebis(2-chloroaniline)]	Blood, plasma, and urine	32
12	Hb adducts and metabolites (MDI exposure)	Urine	45
13	PAHs (at various workplaces)	Urine	29
14	DDT and related compounds (agricultural workers)	Whole blood	30
15	Chlorophenols (sawmill workers)	Urine	31
16	VOCs	Whole blood	41
17	MDA and metabolites (MDA exposure)	Urine and blood plasma	46
18	Hb adduct	Rat erythrocytes	47
19	Pesticide metabolites (pesticides exposure)	Urine	48
20	*N*-Phenylvaline (benzene exposure)	Blood	49
21	*S*-Benzyl-*N*-acetyl-L-cysteine (toluene exposure)	Urine	50
22	DNA damage (benzo[*a*]pyrene exposure)	Human lymphocytes	51
23	Toluenediamine (TDI)	Urine and plasma	33
24	Polyaromatic carcinogen–DNA adducts	Review (humans)	52
25	Hb adducts (PAH exposure)	Blood	53
26	Phenol and metabolites (phenol exposure)	Urine and plasma	54
27	Hb adducts (ethylene oxide exposure)	Blood	55
28	*S*-(2-Carboxyethyl)cysteine (acrylamide exposure)	Blood	56
29	DNA–protein adducts (PAH exposure)	Review	57
30	DNA adducts (trace organic exposure)	Review	58
31	PAH–DNA adducts (PAH exposure)	Review	59
32	DNA adducts (exposure assessment)	Review	60
33	DNA adducts (chemical carcinogenesis)	Review	61

DDT, 1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane; MDI, methylenediphenyl diisocyanate; MDA, methylenebisaniline.

interfaces have been developed. Widely used interfaces included particle beam, thermospray, continuous-flow fast atom bombardment (FAB), electrospray and APCI. Electrospray ionization was one of the most important ionization methods in the 1990s, and is extensively used in LC/MS systems. Electrospray is the result of charging a liquid at a needle tip by applying a high potential. With the increase in the potential, the droplet size is reduced and the droplets begin to have a horizontal component in their movement as well as a higher speed. Nowadays most types of atmospheric pressure ion sources can be used in combination of an electrospray and APCI interface, which is the most widely adapted interface to LC/MS systems.

As a hyphenated technique, LC coupling to MS results in a powerful and versatile analytical tool. Furthermore, LC coupling to a tandem or an ion trap mass spectrometer (LC/MS/MS or LC/MS^n) plays another important role in the development of new analytical strategies. MS/MS, generally, includes a collision cell in which deliberate fragmentation of parent ions can be achieved. Collision-induced fragmentation provides daughter ions by collisional energy transfer between parent ions and a collision gas, normally helium, nitrogen or argon, at an elevated pressure. The most widely used MS/MS configuration is the triple quadrupole instrument, where mass analysis is performed in the first and third quadrupole while the second quadrupole is used as a collision cell. It offers a major advantage for structural characterization of components in mixtures and trace analysis. Successful interfacing of this technique has created an effective methodology in the analysis of nonvolatile, labile, and macromolecular compounds. A wide variety of important applications include determination of large portions of protein sequences with either on-line or off-line enzymatic hydrolysis, and development of molecular biomarkers, which represent a more accurate determinant of potential risk of exposure to hazards or carcinogens than those which only assess external exposure.

2.3.2 Methodology

2.3.2.1 Sample Preparation Both on-line and off-line sample pretreatment by SPE are commonly employed in LC/MS, LC/MS/MS, and LC/MS^n methods. In quantitative studies of metabolites, and protein–DNA adducts, off-line sample pretreatment appears to be preferred. This is partly due to the composition of biological samples, where the presence of proteins may cause clogging of the SPE columns or cartridges used. For example, a typical Hb and DNA adducts assay involves extraction of DNA or Hb from its biological matrix (e.g. blood or tissue), cleavage of the macromolecule to smaller components, and the use of enrichment techniques, such as derivatization and/or SPE preconcentration. To speed up a complete procedure, the sample pretreatment can be performed on an automated batch-scale SPE. Sometimes, improving the speed and/or performance of the sample pretreatment prior to LC/MS analysis is a matter of considerable research.

After determination of the molecular mass and the elemental composition, the next step in the qualitative analysis is the interpretation of fragment ion peaks in the mass spectrum to achieve structure elucidation. A powerful tool in structure elucidation is the use of MS/MS. The information from a measured spectrum is reduced to a small number of the most significant peaks and then compared with the library spectra. Computerized library searching is very useful as it provides ideas on which direction to search when a completely unknown analyte must be identified, or provides adequate confirmation when the presence of a compound is to be confirmed.

2.3.2.2 Interface Technology In the 1970s, 1980s and 1990s, LC/MS development has resulted in a considerable number of different interfaces. A major effort for this research was to improve the instrument capability to ionize analytes including highly polar, labile and biomacromolecules directly from the liquid phase. The most widely used interfaces are electrospray, APCI, thermospray, particle beam, and continuous-flow FAB. Detailed interfacing strategies are available from a book by Niessen.[62] The electrospray interface provides one of the most promising interfaces for LC/MS in that it disposes of the mobile phase during spray generation and offers detection limits into the femtomole range. It has the following advantages: (1) direct ionization from solution; (2) production of multiply charged ions which extend the effective mass range of the mass analyzer; (3) introduction of methods to aid in desolvation of the analyte; and (4) low background from the ionization process. It is remarkable that we can obtain the mass spectrum of a protein with a large molecular mass, and consume an amount of sample of the order of picomoles to femtomoles. APCI is also used for continuous monitoring of an HPLC column. The vaporized eluate from the HPLC is forced through the APCI source with a slow heated stream of nitrogen gas.

2.3.3 Applications

Application of LC/MS in the analysis of workplace and environmental samples has been increasing, especially in the development of exposure indicators. For example, direct analysis of DNA adducts by using LC combined with electrospray ionization MS can obviate those problems arising from the employment of chemical derivatization needed for GC/MS. That is because DNA adducts are usually very polar, and the derivatization is often difficult and less successful. A number of studies have demonstrated that protein–DNA adducts in biological samples can be quantified precisely and accurately by using LC coupling to MS or MS/MS.[71–75] Workers are occupationally exposed to a wide array of chemical compounds. The compounds or their metabolites can interact with biological macromolecules such as proteins, RNA, and DNA. These interactions can result in covalent bonding between the chemicals and macromolecules, leading to DNA damage and the formation of DNA adducts. If these damages are not enzymatically repaired, they can be the cause of mutations and might lead to chemically induced carcinogenesis. The structural elucidation of these DNA adducts is an important research topic in cancer prevention. The use of DNA adducts as biological markers for risk assessment and occupational and environmental monitoring has generated great interest because they represent direct indications of primary damage to genetic material by chemicals. Therefore, protein–DNA adducts may prove to be more accurate and reliable than measurement of external exposure. To accomplish these goals, a sensitive and specific LC/MS method is capable of detecting low picomole quantities of adduct in relatively small complex biological samples, and is specific enough to confirm the structure of the adducts.

The coupling of LC to MS and MS/MS pertinent to industrial hygiene studies has been reported by a number of researchers. Some of them are summarized in Table 3. In one study,[76] sensitive and specific isotope dilution LC/MS and LC/MS/MS methods were developed for the detection and quantitation of DNA adducts formed upon exposure of animals to carcinogenic 1,2-dihaloethanes, 1,2-dichloroethane and 1,2-dibromoethane. These are important industrial chemicals used as additives in gasoline, as intermediates in the production of vinyl chloride, vinyl bromide, and other halogenated organics, as components of grain or soil fumigants, and as solvents for cleaners and other industrial products. In another study, an HPLC/electrospray MS method was developed for the analysis of 7-(2-hydroxyethyl)guanine, the major

Table 3 Typical applications based on LC/MS, LC/MS/MS, and LC/MS^n methods

No.	Chemical hazards and/or biomarkers	Methods	Ref.
1	DNA adduct (1,2-dichloroethane and 1,2-dibromoethane exposure)	LC/MS/MS	76
2	Benzene metabolites (benzene exposure)	LC/MS/MS	82
3	Aromatic sulfonates (textile industry or construction)	LC/MS	71
4	CP and IF (health care personnel occupationally exposure)	LC/MS/MS	81
5	DNA adducts (1,3-butadiene exposure)	LC/MS/MS	80
6	Hb adducts (methyl bromide exposure)	LC/MS/MS	83
7	DNA adducts (treated with *N*-nitrosodiethylamine)	LC/MS	79
8	PAH–DNA adducts (in vitro reaction with PAHs)	LC/MS	72
9	DNA adducts (in vitro reaction with bisphenol A diglycidyl ether)	LC/MS	78
10	Ethylene oxide–DNA adduct (ethylene oxide exposure)	LC/MS	73
11	DNA adducts (vinyl chloride exposure)	LC/MS	77
12	PAH metabolites (PAH exposure)	Review	74
13	DNA adducts (in vitro reaction with bisphenol A diglycidyl ether)	LC/MS/MS	75
14	Hazardous industrial chemicals	LC/MS	85
15	Aliphatic isocyanates	LC/MS	86
16	Polymeric MDI and other isocyanates	LC/MS	87
17	TDI	LC/MS	88

CP, cyclophosphamide; IF, ifosfamide; MDI, methylenediphenyl diisocyanate.

DNA adduct formed after exposure to ethylene oxide. The method is based on DNA neutral thermal hydrolysis, adduct microconcentration, and final characterization and quantification by HPLC coupled to single-ion monitoring electrospray MS. The method was found to be selective, sensitive, and easy to handle with no need for enzymatic digestion or previous sample derivatization. Yen et al.[77] have developed a method to quantify *N*,3-ethenoguanine, a promutagenic DNA adduct of vinyl chloride exposure. The applicability of the method was established by determining DNA adduct in rats treated with chloroethylene oxide and an unexposed human liver. It was observed that the concentration of DNA adduct in the rat livers increased with increasing dose, but was inversely related to the time after exposure. This trend suggests rapid DNA repair and that adducts reduce in rat livers.

Vanhoutte et al.[78] developed a nanoscale LC/electrospray MS methodology for the detection and identification of DNA adducts by in vitro reaction mixture resulting from the interaction of calf thymus DNA with bisphenol A diglycidyl ether. In other work, Singh et al.[79] developed an HPLC/electrospray MS method to detect and characterize two major ethylated DNA adducts for monitoring exposure to genotoxic ethylating agents. This approach was shown to be capable of detecting the DNA adduct in liver tissue from mice treated intraperitoneally with *N*-nitrosodiethylamine. A major chemical in rubber and plastics manufacture, 1,3-butadiene, inducing DNA adducts in vivo and in vitro, was investigated by Tretyakova et al.[80] The LC/electrospray MS/MS methods developed in this work provide the means to study accumulation, repair and dose–response relationships of 1,3-butadiene–DNA adducts in vivo.

Occupational exposure to cyclophosphamide (CP) and ifosfamide (IF) was investigated by Minoia et al.[81] An LC/MS/MS system was employed to monitor CP and IF exposure of 24 workers. The extent of exposure was assessed by the analysis of air samples, wipe samples, dermal pads, and urinary excretion at the beginning and at the end of the work shift. The results of this investigation demonstrate that higher risk may be caused by incorrectly using airflow hoods.

Benzene is an important industrial chemical and ubiquitous environmental pollutant. It is used in the manufacturing of a wide variety of consumer products. Melikian et al.[82] developed a sensitive and specific LC/MS/MS assay for determination of urinary benzene metabolites. The objective of this study was to investigate how various levels of exposure affect the metabolic activation pathways of benzene in humans and to examine the relationship between urinary metabolites and other biological markers. Ferranti et al.[83] used LC/MS/MS for the structural study of adducts formed in human Hb by in vitro exposure of erythrocytes to the alkylating agent methyl bromide (MeBr). MeBr is a highly toxic gas widely used as a fumigant of field soil for control of a wide spectrum of pests and diseases. Peptide mapping by this method allowed location of methylated amino acids within the protein sequence. The results demonstrated the usefulness of the analytical approach for the characterization of Hb adducts with methyl bromide or similar compounds, which can constitute the basis for biomonitoring of human exposure.

The LC/MS, LC/MS/MS, or LC/MS^n techniques have been developed for characterization and quantitation of pesticides, herbicides, and insecticides.[84] For example,

carbamate pesticides are used in large quantities. Since their thermal liability prohibits GC analysis, the analysis of these compounds and their metabolites is usually performed by LC/MS. They have been analyzed using most commercially available interfaces. Positive ion detection with a soft ionization technique is the method of choice. Phenylurea herbicide is one of a group of herbicides which are frequently analyzed by LC/MS or LC/MS/MS.[84] Chlorinated phenoxy acid herbicides have been extensively analyzed by means of particle-beam, thermospray, and electrospray LC/MS.[84]

2.4 Inductively Coupled Plasma Mass Spectrometry

2.4.1 Principles and Instrumentation

The inductively coupled plasma (ICP) was first utilized as an ion source for analytical MS by Montaser.[89] The degree of ionization of most elements is 90% or greater. Since its introduction, ICPMS has exhibited a large number of special attributes. The most important and unique characteristics of the argon ICP are as a viable source for simultaneous multielement analysis with high sensitivity. In ICPMS, the test sample is typically converted to an aerosol and transported into the plasma where the desolvation–vaporization–atomization–excitation–ionization processes occur. In comparison with classical combustion flames, the argon ICP exhibits a high gas temperature (4500–8000 K) and a high electron temperature (9800–10 000 K). Such conditions coupled with the relatively long plasma–sample interaction times lead to nearly complete vaporization–atomization of sample aerosol, and also reduce the chemical and physical interferences in the plasma.

In general the ICP source coupled with a quadrupole MS allows a multiple-elemental, multiple-isotope analysis to be performed. Increasingly, the argon ICPMS is replacing established atomic emission and atomic absorption spectrometries for trace or ultratrace element research. ICPMS offers simultaneous or rapid sequential multielement determination capability at major, minor, trace, and ultratrace concentration levels. Indeed, it is the most powerful means for determination of trace inorganic metals.

2.4.2 Methodology

2.4.2.1 Sample Preparation The sample preparation for ICPMS measurement is not substantially different from that encountered in other atomic spectrometric methods. Dissolution of a heterogeneous sample which provides homogeneity at the molecular level is usually required. Elemental analysis can be performed in nearly all kinds of matrices. The development of sample decomposition for ICPMS is an important step in sample preparation. It relies on sample type, specific analyte species of interest, and analyte molecular interactions. Most biological samples are prepared by decomposing the sample using thermal or chemical means, followed by dissolution of the ash residue and dilution to a specific volume prior to analysis. Microwave digestion is often used. Another common technique is a hot-plate dissolution procedure, but there are several primary limitations, such as long dissolution times and the potential loss of volatile elements. In a microwave digestion technique, closed vessels are utilized to decompose samples and minimize loss of volatile elements during the digestion process. During sample preparation, the elements being determined, their analysis requirements, and specific interferences that might be encountered for their determination dictate whether separation and preconcentration steps might be required. Sometimes, sample preparation may be a lengthy and complex process, depending on the form of the sample and the specific elements being determined.

2.4.2.2 Quantitative Analysis Quantitative analysis by ICPMS can be achieved by the use of a precise peak-hopping/signal integration procedure. Two modes of operation typically are used: rapid spectral scanning and peak hopping. The most commonly used mode, the rapid scanning method, covers the entire mass range. By closely matching the bulk chemical composition of the calibration standards to the known matrix of the samples, improved accuracy can be obtained. The use of internal standards is highly recommended to achieve maximum precision and accuracy. The calibration curves for selected elements, when plotted on logarithmic axes, demonstrate linearity over a wide dynamic range. Most modern ICPMS instruments offer a wide dynamic range. The ICPMS technique also provides sufficient isotope ratio precision and sensitivity to enable isotope dilution quantitation at trace concentration levels. Additionally, flow injection analysis (FIA) has been successfully used to improve the quantitative determination of trace elements in samples with high dissolved solids. Chemical modification of the sample can be performed by the addition of reagents to the transport line. Another significant advantage of the flow injection technique is the ability to make measurements on microsized samples, especially when high efficiency nebulizers are used.

2.4.2.3 Laser Ablation Laser ablation was first reported for sample introduction into ICPMS by Gray.[90] With this method, samples can be analyzed with minimal sample preparation. Usually specimens of metal alloys or similar materials are prepared by grinding or polishing a flat surface. Similar to other solid sampling techniques, it provides viable analytical results when suitable solid standards are available. Laser ablation ICPMS also gives important spatial resolution, which is particularly useful for the determination of the chemical composition

of grain boundaries or mineral inclusions. It is a very powerful method for the analysis of particulates or solid samples.

2.4.3 Applications

ICPMS has been utilized effectively in industrial work environments. It has been effectively used for trace analysis with high sensitivity, especially for the traditionally "difficult-to-excite" refractory elements such as molybdenum (Mo), vanadium (V) and zirconium (Zr), and with multielement detection capability. The applications include the determination of trace elements in air, exhaust, liquid, and dust in various working environments.

Monitoring occupational exposure to heavy metals with the ICPMS technique had been reported by several researchers, although atomic absorption spectrometry has until now been used most extensively in occupational and environmental health. In one study, Schramel et al.[91] established an ICPMS analytical method to determine the concentration of antimony (Sb), bismuth (Bi), lead (Pb), cadmium (Cd), mercury (Hg), palladium (Pd), platinum (Pt), tellurium (Te), tin (Sn), thallium (Ti) and tungsten (W) in urine. The aim of this work was to develop a method which is equally suitable for the determination of occupationally as well as environmentally caused metal excretion. In another study, Apostoli et al.[92] evaluated multiple exposure to metals in eight types of metal welding, such as manual metal arc for mild and stainless steel, continuous wire, submerged arc, and brazing. Environmental monitoring was carried out in eight different occupational situations and the ICPMS technique was adopted in order to characterize exposure to several elements simultaneously and with high accuracy. The results showed that up to 23 elements could be measured. The highest concentration was found for aluminum (Al), manganese (Mn), iron (Fe), nickel (Ni), chromium (Cr), copper (Cu) and zinc (Zn). Karpas et al.[93] presented a simple method, based on ICPMS, for determination of uranium in urine at levels that indicate occupational exposure. Sample preparation involves a 50-fold dilution of the urine by nitric acid (2% HNO_3) and no other chemical treatment or separation. The analytical procedure is fully automated so that over 100 analyses may be performed per day. Measurement by ICPMS of lead in plasma and whole blood of lead workers and controls was reported by Schutz et al.[94] The levels of lead in blood plasma and whole blood were measured by ICPMS in 43 male lead smelter workers and seven controls. The samples were handled under routine laboratory conditions. By a simple dilution procedure, lead in plasma may be determined accurately and with good precision down to the concentrations present in controls. It suggested that lead in blood plasma should be considered as a complement to current indicators of lead exposure and risk. Application of ICPMS to monitor radionuclide was reported by Vita and Mayfield.[95] An ICPMS method was developed to detect U-235 and U-238 in urine. The strong nitric and hydrochloric acid digestion of the urine and the application of the anion exchange resin for the uranium separation provided dependable recovery. In addition, multiple exposure to arsenic (As), antimony (Sb), and other elements in art glass manufacture was studied by Apostoli et al.[96] The results confirmed that arsenic, which is the main hazard in glass production, reaches a high air concentration.

2.5 Time-of-flight Mass Spectrometry, Fourier Transform Ion Cyclotron Resonance Mass Spectrometry and Future Trends

2.5.1 Introduction

TOFMS was introduced commercially in the late 1950s.[97] However, it was only in the 1990s that the high mass range and the high sensitivity multichannel recording capabilities were realized, which make this type of spectrometry an attractive instrument for contemporary research. A typical TOFMS system contains three main components: ion source, ion drift region, and detection system. A new generation of high-performance TOFMS instrumentation includes nanoflow electrospray hybrid quadrupole TOFMS and MALDI/TOFMS. Modern TOF instruments have many intrinsic advantages. They are ideally suited for pulsed ion sources because they have a sufficiently narrow pulse width, and a fast response. They can provide macromolecular measurements of proteins with considerably higher accuracy than gel electrophoresis, or can be used to map enzymatic digests, reveal post-translational modifications, determine the positions of disulfide bonds, assess carbohydrate heterogeneity in glycopeptides, or provide amino acid sequences.

FTICRMS is now an established viable analytical technique.[4] FTICRMS allows in principle an unlimited mass range to be reached with an extremely high resolution. As a technique based on Fourier transform, the resolution depends on the observation time, which is linked to the disappearance of the detected signal. In order to achieve high resolution, a very high cell vacuum is necessary, which is a major limitation of this technique.

The advancing technologies of hybrid QTOFMS, MALDI/TOFMS and FTICRMS are just beginning to add much needed analytical power to our arsenal for macromolecular identification and characterization. An application of more efficient collision-induced dissociation and product-ion detection is using a hybrid of quadrupole and TOF analyses in QTOFMS. These developments will bring important methodology to scientists and thus accelerate the integration of MS into

work-related research strategies. Research will include structural determination of aberrant proteins, development of biomarkers for specific hazard to exposed workers, characterization of protein allergens related to occupational asthma, and identification of microbial products in complex environmental samples.

2.5.2 *Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry*

A rapidly advancing area in which TOFMS plays a major role is in MALDI/TOFMS. It has developed into a powerful tool for characterizing biological macromolecules. In a MALDI/TOFMS experiment, the analyte of interest is mixed with an appropriate matrix material which facilitates the desorption and ionization of intact analyte molecules with masses up to several hundred thousand m/z. This absorbs strongly at the wavelength of the incident laser light. Many very large biomolecules have been mass analyzed by this technique. MALDI/TOFMS is able accurately to sequence single strands from DNA without the need for labels or primers,[98] and to identify single base mutations within a polymerase chain reaction (PCR) product. Although the effective range of sequencing may not be as wide as with conventional methods, MALDI/TOFMS can sequence short DNA strands in less time with the ability to identify all of the bases in the strand. Single strands and PCR products of up to 500 bases have also been detected, but it is not clear whether the mass accuracy and resolution will be sufficient for sequencing long strands. Peptide mapping by MALDI/TOFMS is gradually reaching a confidence level owing to improved mass accuracy and sample preparation methods and the availability of complete genomic information for a number of organisms.

MALDI/TOFMS has been applied and established as a valuable analytical technique for the detection of PAHs with high spatial resolution in the work environment. Bezabeh et al.[99] investigated nitrated PAH pollutants generated by incomplete combustion using negative ion laser desorption ionization TOFMS. In related work, spatially resolved laser desorption/laser ionization TOFMS has been used for the detection of PAH–picrate complexes,[100] and PAHs in individual micrometer-sized diesel particulate.[101]

2.5.3 *Hybrid Quadrupole Time-of-flight Mass Spectrometry*

The development of a hybrid QTOFMS instrument overcomes the mass range and precursor ion resolution limitations of a triple-quadrupole mass spectrometer.[102] A particular advantage that this hybrid has over the triple-quadrupole instrument is that it can provide high precursor ion resolution by virtue of its double focusing properties and it may be used to obtain MS/MS information from precursor ions of the same nominal mass. The sequencing of larger proteins by enzymatic cleavage and subsequent MS/MS analysis of the resulting peptides by QTOFMS is well documented. With the advantage of electrospray, also enabling an on-line nanoflow LC coupling to QTOFMS, this approach has found a few applications.[103] For example, Deforce et al.[104] characterize DNA oligonucleotides by coupling of capillary zone electrophoresis to electrospray ionization QTOFMS. A procedure for fast and precise molecular weight, purity, and base composition determination of oligonucleotides was described. This method has been useful not only for determination of the purity and the length of bases in oligonucleotides, but also for confirmation of the expected base composition, making this technique an extremely useful tool for quality control in the field of oligonucleotide research. Hybrid QTOFMS has just started to be applied to occupational health studies.

3 SCANNING ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS

3.1 Introduction

The scanning electron microscope, when equipped with an EDX analysis system and an IA, is ideally suited to study pneumoconioses and workplace environmental particles. The salient features of this instrumentation which make it valuable in occupational safety and health applications are discussed very briefly below. For a comprehensive description of the instrumentation and theory, the reader is referred to the texts by Goldstein et al.[105] and Lee.[106] An excellent short description of SEM and X-ray analysis principles and instrumentation is given by Ingram et al.[107]

3.1.1 *Instrumentation*

In SEM, a focused beam of electrons is scanned in a raster pattern across a specimen of interest. Numerous complex events occur at each point where the electron beam impinges on the atoms of the specimen. Some, but by no means all, of the signals produced by the electron beam–specimen interaction are illustrated in Figure 1.

By utilizing an appropriate detector for a specific signal, an image of the specimen can be constructed on a point by point basis. For instance, the detection of secondary electrons, which are very low energy electrons arising from very near the surface of the specimen, is used to produce the secondary electron image (SEI). The SEI is the three-dimensional-like image

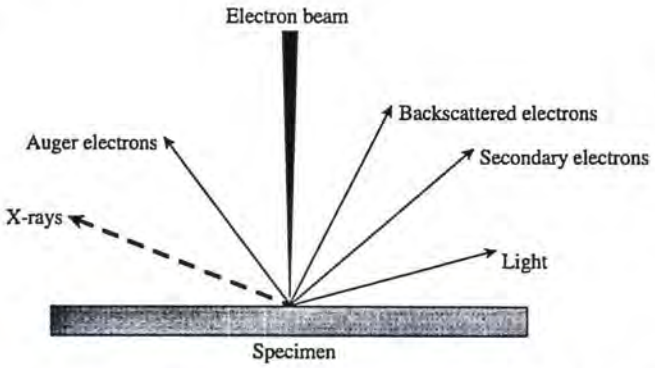

Figure 1 Some signals resulting from the interaction of the electron beam with a specimen.

Figure 2 BEI of a 5-μm thick section of lung tissue from a subject with silicosis showing numerous particles (brighter areas). Marker = 10 μm.

typically associated with SEM. A second image which is particularly valuable in the study of pneumoconioses is the backscattered electron image (BEI). Backscattered electrons result when the original beam electrons are elastically scattered from the nuclei of atoms within the substrate. Hence, the backscattered electron signal is affected by atomic number. Furthermore, the BEI may provide some information about composition beneath the specimen surface. In our laboratory, we generally use the BEI for our pneumoconiosis studies since the exogenous particles generally are of higher average atomic number than the surrounding lung tissue and are easily identified. Figure 2 shows a BEI image from a 5-μm thick section of lung tissue from a subject with silicosis.

X-rays are generated by the inelastic interaction of the electron beam and atoms of the specimen. Two types of X-ray are produced: (1) white radiation or bremsstrahlung and (2) characteristic X-rays. The bremsstrahlung results from the interaction of the beam electrons with the nucleus of the specimen atoms, and forms a continuous background over which characteristic X-ray signals are superimposed. Characteristic X-rays result from the ejection of inner shell electrons of the specimen atoms by the electron beam. The generation of characteristic X-rays forms the basis for chemical analysis of the specimen since both the wavelength and the energy of the characteristic X-rays can provide definitive information about the atoms from which they emanate. In SEM, X-rays may be measured through the use of either a wavelength dispersive spectrometer (WDS) or an energy dispersive spectrometer (EDS). With the WDS, the wavelengths of X-rays are determined using crystal diffraction and Bragg's law. The EDS detects the energies of X-rays using a lithium-drifted Si crystal.The WDS has better spectral resolution compared with the EDS, and is capable of light element detection. However, only one element at a time can be determined with a WDS. The characteristic peaks of all elements with an atomic number of 9 (fluorine) or greater can be detected simultaneously with the standard EDS. Light element detection is also possible with EDS systems that have windowless detectors or detectors with ultrathin windows. The texts by Goldstein et al.,[105] Lee,[106] Heinrich,[108] and Friel[109] contain extensive discussions of X-ray analysis and instrumentation.

Another extremely valuable attachment for SEM is an IA. A computer-based IA permits rapid, real-time automated characterization of SEM image features, including size measurements and chemical analyses when used in conjunction with an EDS. The system currently used in our laboratory is PC-based (personal computers) and controls the scanning of the electron beam across the specimen. A regular grid point spacing pattern is used with a preset point density to locate features. At each point, the image signal (we use the backscattered electron signal) is compared to an adjustable threshold to determine whether the beam is on a feature of interest. Once a feature is found, a narrower grid point spacing pattern is used to determine physical parameters, and subsequently an X-ray analysis of the feature is performed. For more information on IAs, the reader is referred to the review paper by Lee and Kelley,[110] and the text by Friel.[109]

3.1.2 Listing of Occupational Safety and Health Applications

SEM and EDX analysis may be used for a number of applications in occupational safety and health including the analysis of particles collected on air filter samples taken from the working environment, analysis of individual particles found in bulk dusts, and in the study of pneumoconioses. Applications of SEM to the study of pneumoconioses, including determination of lung particulate burden, particle chemistry, and respirable particle surface characterization, will be described in the subsequent sections.

3.2 Sample Preparation

There are two ways to study particles found in lung tissue. One examines the particles in situ, i.e. particles may be located by SEM (usually using the BEI) and an EDX analysis performed while the particles are still in the tissue. An alternative procedure is to extract the particles from the tissue matrix followed by EDX analysis. Sample preparation procedures and the advantages and disadvantages of these two techniques are described briefly below. A complete discussion of SEM preparation techniques is given by Shelburne et al.[111] The paper by DeNee[112] also describes various preparation techniques.

3.2.1 Tissue Preparation for In Situ Particle Analysis

The key advantage of SEM in situ analysis in the study of pneumoconioses is the opportunity to correlate the location, types, and concentration of particles found with any tissue pathology seen by light microscopy. On the other hand, in situ analysis is very time-consuming because particles must be identified and analyzed manually. The preparation of lung (or other types of) tissue for light microscopic and SEM examination generally involves formalin fixation, followed by paraffin embedding. Serial sections (5-μm thick) are cut from the paraffin block both for light microscopy and SEM analysis. The sections for SEM are mounted on a carbon substrate (we use 1-inch, or 2.5-cm, diameter carbon planchets), de-paraffinized using xylene, and air dried. The final specimens generally do not need coating for conductivity. However, if specimen charging is a problem, the specimen may be coated with carbon using a vacuum evaporator. Metal coatings should be avoided because they may interfere with subsequent EDX analyses. As can be seen in Figure 2, particles are easily located in the 5-μm section; however, they may occur in aggregates, making individual particle analyses a challenge.

3.2.2 Tissue Particle Isolation Techniques

The key advantage of isolating particles from the tissue matrix is the speed with which subsequent analyses may be performed. Once the tissue matrix is removed, a filter containing the residual particles may be prepared, and the particles analyzed using an automated SEM/EDX/IA procedure. The analysis procedure used in our laboratory is described in section 3.3.1. The primary disadvantage of the particle isolation technique is that the opportunity to relate tissue pathology to particle type, location, and concentration is lost. Two procedures most often used to extract particles from the tissue matrix, low-temperature ashing and chemical digestion, are described below.

3.2.2.1 Low-temperature Ashing In the low-temperature ashing process, an oxygen plasma is used to oxidize the organic components of the tissue matrix. A review of the application of low-temperature ashing in preparing samples for SEM analysis is given by Thomas and Hollahan.[113] The procedure we use for lung tissue consists of the following steps: 1–2 g of fresh tissue is selected and diced into 1–2-mm cubes, and freeze dried to constant weight. Approximately 0.1–0.2 g of the dry tissue is accurately weighed, placed in a clean glass vial, and ashed in a low-temperature asher at 90 W for 7 h at an oxygen pressure of 2 Torr. The ash is suspended in 50 mL of a solution of 0.05% Aerosol OT® in filtered, deionized water, and then placed in an ultrasonic bath for 15 min. One milliliter of glacial acetic acid is added to the suspension which is then made up to a final volume of 100 mL with filtered, deionized water and allowed to stand overnight. Since the particulate burdens of lungs vary considerably, preparation of samples with the proper particle loading for automated analysis is accomplished by filtering varying aliquots of the final suspension onto 25-mm diameter, 0.1-μm pore size polycarbonate (Nuclepore®) filters. The filters are attached to carbon planchettes with colloidal graphite, and may be examined uncoated under SEM. Filters with proper loading have minimal particle overlap and generally contain approximately 50–100 particles per field of view at a magnification of 1000X. It should be noted that some particles present originally as aggregates in the tissue may not be broken apart during the low-temperature ashing procedure.[114]

3.2.2.2 Chemical Digestion Biological tissues may be chemically digested using a number of different agents including oxidizing agents (sodium hypochlorite), strong alkaline solutions (sodium or potassium hydroxide), and proteolytic enzymes (ficin). Digestion with each of these agents is reviewed briefly in the paper by Mastin et al.[115] In our laboratory, we have used an adaptation of the sodium hypochlorite digestion procedure described by

Coin et al.[116] to prepare lung tissue for particle analysis. Approximately 0.1–0.5 g of wet, formalin-fixed tissue is accurately weighed and placed in a clean glass vial. A second piece of wet tissue is also weighed and then dried to constant weight to determine the wet-to-dry weight ratio. Ten milliliters of triple-filtered sodium hypochlorite are added to the glass vial containing the wet tissue, and the vial is then shaken gently for 30–45 min. The digestate is filtered onto a 0.2-μm pore size polycarbonate filter and washed with 10 mL of deionized water. Lipids and other undigested debris are then extracted from the filtrate by two treatments with the following sequence of triple-filtered reagents: 10 mL of isopropanol, 10 mL of 7% oxalic acid, 10 mL of Clorox®, and 10 mL of deionized water. The filter may now be dried and processed as before for SEM analysis. We have found that this digestion procedure takes less time to prepare samples than low-temperature ashing. There are a couple of negatives, however. The washing steps may lead to an uneven redistribution of the particles on the filter. In addition, achieving the proper particle loading on the filter may require some experimentation with the initial sample wet weight or separation of the initial digestate into aliquots of varying volumes.

3.3 Lung Particle Analysis

Particulate matter has been recognized as a cause of various lung diseases, the pneumoconioses, for many years. Specific pneumoconioses such as coalworker's pneumoconiosis, asbestosis, and silicosis may result from inhalation of particles at the worksite. Numerous methods, as reviewed by Mastin et al.,[115] have been used to analyze particulate matter deposited in human lungs. SEM and EDX have been used extensively to characterize particles found in lung tissue (see reviews by Shelburne et al.[111] and Baker et al.[117]). While it is not possible to compare a measured lung dust burden to the actual exposure because of the lack of exposure data and to particle clearance, the measured lung particulate burden does represent retained particle dose.

Everyone has a background lung particulate burden resulting from inhalation of respirable particles present in the ambient environment. Hence, a good database for the particle contents of the lungs of subjects with no pneumoconioses and/or history of occupational exposures, i.e. "normal" lungs, is needed so that the particle analysis data from diseased lungs can be properly interpreted. The application of SEM and EDX to the determination of inorganic particulate burdens for normal and diseased lungs, as well as respirable particle surface characterization, will be discussed in the next sections.

3.3.1 Normal Lung Inorganic Particulate Burdens

We have used an automated SEM/EDX/IA method to determine the lung nonfibrous, inorganic particulate burdens for a large set of subjects with no overt pneumoconioses. This procedure is not suitable for fiber analyses because mineral fibers usually have very small diameters and do not produce a BEI with enough contrast for automated analysis. In addition, organic particles, such as coal, are destroyed in the low-temperature ashing process. Complete details of the automated method for nonfibrous particles are given in the paper by Stettler et al.[118] Briefly, a small portion of freeze-dried lung homogenate from each subject was ashed in a low-temperature asher and filters containing the residue prepared as described earlier in this report. The filter samples were analyzed in the electron miscroscope which was equipped with an EDS and an IA using the BEI image. After a field of view is selected, particles in the field are automatically detected by the IA, sized, and then analyzed for 31 elements using an X-ray spectrum acquire time of 5 s. In our procedure, a minimum of 1000 exogenous particles in a minimum of 20 randomly selected fields of view at a magnification of 1000X are analyzed. The IA grid point spacing density used in the procedure is set to find particles 0.2 μm in diameter and larger. After X-ray analysis, the particles are classified by the IA using a chemistry definition file which defines particle types by their major elemental components and net fractional X-ray intensities. The list of elements analyzed and a description of the chemistry definition file are given in the book chapter by Stettler et al.[119] More than 145 000 individual particles were analyzed in this study. The average (±standard deviation) exogenous particle concentration found in 87 lungs was $476 \pm 380 \times 10^6$ particles per gram (ppg) of dry lung with a range of $71–1860 \times 10^6$ ppg. On average, various aluminum silicates accounted for 38.1% and particles classified as silica accounted for 21.0% of the particles in the 87 lungs. Large numbers of various metal-containing particles, primarily titanium and iron occurring singly or in combination with Si, were also found in the lungs. The particle levels seen in the study by Stettler et al.[119] were similar to those seen in other studies of normal lungs by Churg and Wiggs[120,121] and by Paoletti et al.[122] Analytical transmission electron microscopy methods were used in the other studies with average particle concentrations in the range $180–465 \times 10^6$ ppg.

3.3.2 Inorganic Particulate Burdens of Lungs with Pneumoconioses

Although the automated SEM/EDX/IA method described above has been used to determine the particulate burdens in lungs with suspected occupational exposures

by Stettler et al.,[114] many studies have involved manual in situ analyses using 5-μm thick sections of lung tissue. Of particular note is the work of Abraham et al.[123-125] who have developed and used an in situ procedure to determine the nonfibrous, inorganic particulate burdens of over 400 lungs. Their procedure uses 5-μm thick sections mounted on carbon. Complete descriptions of their SEM analysis procedure are given elsewhere.[124,125] Briefly, a morphometric point counting approach using both the SEI and BEI at a magnification of 6000X is used to locate particles in randomly selected fields. The number of particles in 100 consecutive fields are counted. If fewer than 100 particles are counted, additional fields are analyzed. The particles are sized and analyzed using a 20-s X-ray analysis. Size and elemental data in the form of net X-ray counts per second for each element observed are recorded. EDX data are used to sort exogenous particles into three major classes: silica (showing only a silicon peak), silicates (showing silicon and other cations such as Al, Mg, K, Ca, Fe), and other (mostly metals, either singly or in combinations). Particle concentration data are reported in terms of particles per cubic centimeter. The average total exogenous particle concentration for 433 cases by this technique was $473 \pm 113 \times 10^6$ particles cm^{-3} with concentrations in the range $1-33\,450 \times 10^6$ particles cm^{-3}.[123] The major types of particles found included silica, aluminum silicates, metals, and talc.

3.3.3 Respirable Particle Surface Characterization

Bulk compositional analyses of respirable particulate material, and even particle-by-particle compositional analyses, may not always be sufficient to predict the level of biological activity of respirable particles or the health risks of exposure to them. Toxicants may be located on the particle surface or may be more heavily concentrated there, resulting in heightened biological availability and expression of toxic activity. Or, in some cases, surface coating materials may have a prophylactic effect on expression of the toxicity of the underlying bulk of the particle. An example of the latter effect is the modified activity of the quartz component of some mixed composition mineral dust exposures for causing mixed dust pneumoconioses by LeBouffant et al.,[126] Kriegseis and Scharmann,[127] and Harrison et al.[128]

3.3.3.1 Multiple-voltage Scanning Electron Microscopy with X-ray Spectroscopy: Experimental SEM with EDS or WDS X-ray spectroscopy can provide some information on the elemental composition with depth into a particle, by acquiring X-ray spectra at two or more electron beam accelerating voltages.[129] The depth of penetration of electrons and of the excitation of characteristic X-rays is dependent on the incident electron energy and on the density of the particle target. For densities in the range of most respirable particulate minerals, a 20–30 keV electron beam will excite spectra to a depth of the order of one to a few micrometers. This range is a function of electron voltage such that a 5-keV electron will excite spectra to a depth of the order of 0.1–0.01 μm in such materials. Thus, comparing spectra acquired at 20 keV and at 5 keV can provide some indication of the homogeneity or heterogeneity of elemental distribution with depth into a particle. This method has been used to detect thin submicrometer aluminosilicate coatings or clay "surface occlusion" on respirable quartz particles.[130] To control for correction factors, comparison is not made of the absolute values of elemental spectral line intensities; instead, the ratio of spectral line intensities of different elements in a particle is measured at one voltage and compared with the ratio measured at another excitation voltage. For example, the ratio of the silicon line intensity to the aluminum line intensity of a particle is measured at 20-keV excitation and then is measured at 5-keV excitation, or is measured at a series of voltages, e.g. 20, 11, 9, 7, 5 keV. The beam generally must be relocated on the particle of interest after a voltage change. This can be done in an automated mode with modern SEM/EDS computer-controlled systems. Using such systems, several fields of particles can be analyzed for several hundreds of particles at one voltage, and the same fields can be reanalyzed at a second voltage. Typically, some smaller particles identified at one voltage in computer-controlled data acquisition will be missed at the other voltage. But visual editing of the resultant files can easily assure proper matching of particles for comparison of the ratio of elemental line intensities between the two voltages. Obviously, the electron beam voltage must be greater than the energy (frequency) of the X-ray lines used in the analysis. This can be a problem for analyses at the low electron beam accelerating voltages: 5-keV electrons will not excite the K series lines for heavier elements. In some cases this can be circumvented by using the higher series lines (L, M, ...) which occur below 2 keV.

3.3.3.2 Multiple-voltage Scanning Electron Microscopy with X-ray Spectroscopy: Interpretation Change in measured spectral line intensity ratios versus electron beam accelerating voltage can be predicted as a function of particle density and size and structure with an analytical formula.[130] Diminution of the electron beam intensity with depth of penetration into a material can be modeled in one dimension as a Beer's law process, i.e. differential loss of intensity is proportional to intensity in a differential thickness of material.[131] Empirical relationships are available for this proportionality constant as a function

of electron voltage.[132] In the differential thickness of material the electron beam will stimulate characteristic X-rays. An X-ray line intensity is a function of the intensity of the electron beam with adequate energy to stimulate the given X-ray line, of the concentration of target atoms, of the cross-section for ionization of the target atom shell by those electrons, and of the fluorescence yield or probability that the electron-excited atom will decay by the specific X-ray emission. A simple empirically determined function provides a model for the effective scattering ionization cross-section versus incident electron energy and X-ray spectral line frequency.[133] Tabulated values of fluorescence yield are available.[134] These together can be used to describe the generation of X-rays with depth by the electron beam. Then a Beer's law model can describe extinction of the generated X-ray as it leaves the particle.[134] Together these models of electron stimulation of X-rays and of X-ray emission provide a differential equation for X-ray line intensity as a function of material thickness, material density, electron beam voltage, X-ray spectral line frequency, and fluorescence yields. This differential equation can be solved across the particle boundaries for alternative models of particle structure with depth, e.g. for a homogeneous mixed composition particle versus a heterogeneously structured particle. This provides a prediction of the change in measured elemental composition ratios versus electron beam accelerating voltage. These predictions then can be used to interpret measurements of X-ray elemental spectral line intensity ratios of a particle versus electron beam accelerating voltage in terms of particle compositional structure with depth. An example in the literature details application of the model to aluminosilicate-contaminated silica particles.[135] Predictive models for a silica particle homogeneously contaminated with aluminum and for a silica core particle with an aluminosilicate clay coating were compared with experimental data of silicon to aluminum line intensity ratios measured at 20–5-keV electron beam accelerating voltages. As an example of "occluded" particle behavior, for a 2-μm thick particle of 97% Si/(Si + Al) overall composition, the measured fraction of silicon drops to below 80% at 5 keV, in concert with the prediction for a clay-coated particle.[136]

3.3.3.3 Scanning Electron Microscopy with Scanning Auger Spectroscopy for Particle Surface Analyses

Scanning Auger spectroscopy uses electron imaging and excitation of particulate samples much as SEM does. However, electron-excited emission of Auger electrons rather than characteristic X-rays provides the mechanism for elemental analysis. The shallow escape depth of these Auger electrons results in information being obtained only from the depth of a few atomic layers into the sample.[137] This provides a very near surface analysis compared to that provided by SEM/EDS, essentially measuring elemental composition in a nanometer thickness compared to a micrometer thickness. Scanning Auger spectroscopy also provides a more shallow depth of analysis by one to two orders of magnitude than is reasonably achievable by multiple-voltage SEM/EDS, that is, 1 nm depth by Auger versus 10–100 nm minimum depth by multiple-voltage SEM/EDS. To some extent, sample preparation and purity constraints are alleviated in the multiple-voltage SEM method: incidental surface contamination on samples may need to be removed prior to Auger analysis by argon ion bombardment of the sample. For analysis of particles in lung tissue thin sections, scanning Auger spectroscopy is not appropriate, while multiple-voltage SEM/EDS can provide some identification of surface occluded mineral particles in 5-μm thick tissue sections. For dust samples, a combination of conventional (20–30-keV) SEM/EDS, of multiple-voltage SEM/EDS, and of scanning Auger spectroscopy provides the possibility of analyzing respirable particle samples for elemental composition from their bulk to their surface. Performing these joint analyses on explicit particles is challenging but possible.[138] Modern automated methods provide the capability for performance of that suite of analyses on a large population of respirable-sized particles with the same specific particles analyzed in all three regimens. This has been demonstrated for the combination of SEM/EDS and scanning Auger spectroscopy for cobalt, chromium, and tungsten analyses of respirable hard metal particles.[139]

4 ATOMIC SPECTROMETRY

4.1 Introduction

Atomic spectrometric techniques are the most widely used analytical techniques for quantitative analysis of inorganic metals. The techniques have been applicable to nearly all the elements, including most of the metals and semimetals.[140] The development of ICPAES techniques has been increasing. ICPAES offers a simultaneous or rapid sequential multielement determination capability at the major, minor, and certain trace concentration levels. It has become established as a widely accepted method for the analysis of metallic aerosols, powders of metals, dusts, and fly ashes. To date, a vast number of published atomic spectrometric methods are available, covering a wide range of applications. Atomic spectrometric methods are widely employed for occupational health evaluation, owing to the presence of trace elements in the atmosphere released from various industrial processes.

4.2 Flame Atomic Absorption Spectrometry

4.2.1 Principles and Instrumentation

Flame atomic absorption spectrometry (FAAS) has been used for the determination of about 65 elements with detection limits ranging from a few parts per billion to a few parts per million.[141] Instruments are reliable, robust and simple to use. It is a single channel instrument, but operating conditions can be changed rapidly so that several elements can be determined in one sample in an automated sequential run. Two types of flame mixtures are commonly used: air–acetylene and nitrous oxide–acetylene. The latter flame is significantly hotter and is needed to atomize some elements such as aluminum, which form refractory oxides. The sample solution is pneumatically nebulized into a spray chamber where a sample mist is formed and mixed with a fuel gas. Then, the sample aerosol enters a flame where dissociation and atomization occur. During the rather limited residence time in the flame, the droplets are dried and the resulting salt particles vaporized. The resulting molecular species may be atomized by thermolysis or by chemical reaction with reducing species such as carbon and carbon monoxide. Generally, FAAS is selective, rapid, and amenable to automation with adequate sensitivity.

4.2.2 Methodology

4.2.2.1 Sample Preparation In a typical FAAS assay, an analyte is dissolved into a solution. The sample preparation procedure depends strongly on the properties of both the target elements and the solvent matrices. The principal objective of sample preparation is to dissolve target analytes, and to remove interferences. The method of liquid–liquid extraction is a convenient way of preparing some samples in which the target analytes are easily dissolved in the solution. It is relatively simple, rapid, and favorable to FAAS. Organic solvent can also be used to enhance detection sensitivity. Methyl isobutyl ketone is the most popular solvent because of its extraction and nebulization efficiency as well as combustibility. Other solvents such as ketones or esters can be used as well. In the pulse-nebulization mode of FAAS, a number of elements can be successively determined in an extract. Another alternative sample preparation is to use microwave heating as a source of intense energy for rapid mineralization of liquid and solid samples. The reduction in the digestion time and the higher reaction speed may be due to the fact that the energy transfer is improved, and the microwave field has a specific chemical influence on organic molecules in acidic media. Generally, a few sources of systematic error should be considered. These include contamination, losses of trace elements, volatilization, and physical/chemical transformations of the samples.

4.2.2.2 Flow Injection Analysis FIA has nowadays become a powerful analytical tool for sample preparation and introduction before measurement. It offers a convenient and fast approach to enhance and automate preliminary steps for atomic spectrometric detectors. Moreover, flow manifolds can ease the well-known problem of sample introduction to atomizers or even expand the classical scope of atomic/elemental information. Flow injection strategies with atomic spectrometric detectors are used in research and analytical laboratories. For detailed information, the reader is referred to a book by Sanz-Medel.[142]

4.2.2.3 Interferences In FAAS assay, minor ionization interferences may be encountered with Rb and Li in an air–acetylene flame and additional elements such as Al, Ba, Be, Ga, and Si in a nitrous oxide–acetylene flame.[143] The interferences can be suppressed by adding ionization buffers. Background absorption is not a particular problem and is compensated for by using a deuterium background corrector. Sometimes, specific solute–volatization interferences are observed in the determination of B, Ba, Cr, Mo, Pt, and Sn. They can be overcome by adding suitable spectrochemical buffers. Transport interferences are encountered with viscous sample solutions. Various nonspecific matrix effects are observed with nebulizing solutions of high salt and/or acid content. In such cases, acid-matched calibration and standard addition checks are advisable.

4.2.3 Applications

FAAS is a rather selective instrument for many metals. It has been used in a number of studies associated with occupational and environmental health. In a field investigation carried out at a North American nickel alloy production facility, the levels of worker exposures to inhalable and total nickel-containing aerosol during nickel alloy production were studied by Tsai et al.[144] Worker exposures in a range of workplaces throughout the facility were assessed. The results showed that inhalable aerosol exposure levels for both overall aerosol and for total nickel were consistently and significantly higher than the corresponding total aerosol levels. In related work, Torjussen et al.[145] investigated the concentration and distribution of heavy metals in nickel-exposed workers and of controls. Biopsy specimens from 30 nickel-exposed individuals and six controls were analyzed by FAAS to determine the content of nickel, copper, cobalt, zinc and iron. Some differences in epithelial types between specimens from the nickel-exposed group and the control group were seen.

Pilger and Broder[146] reported a method that is suitable for assessing exposure to toxic metals in occupational indoor environments. The method has been evaluated for 21 metals including antimony, cadmium, chromium, cobalt, copper, lead, iron, zinc, and so on. The method is element specific. In another study, Bellido-Milla et al.[147] detected hygiene hazards involved in naval industry welding processes. The metal contents of welding fumes produced at the shipyard were investigated to assess the hygiene hazards. Personal and environmental samples were collected on cellulose or polyvinylchloride filters. Samples were analyzed to encompass every possible working condition. Quantitative metal determinations were carried out by FAAS. The results obtained for metals and particles were compared and conclusions were drawn according to the type of welding procedure, sampling place, and use of fume extractor.

Burguera et al.[148] reported the determination of lead in hair of exposed gas station workers and in unexposed adults by microwave-aided dissolution of samples and flow injection atomic absorption spectrometry (FIAAS). Lead content in head hair of 53 gas station workers together with an equal number of normal controls was determined. Samples of hair were washed with ethanol and water and were subject to microwave digestion prior to the determination of lead by FIAAS. The lead content in hair of the gas station workers ($48.7 \pm 17.5\,\mu g\,g^{-1}$) was significantly higher than that of the normal controls ($17.2 \pm 8.1\,\mu g\,g^{-1}$). The effects of washing and sample digestion procedures, head sampling site, hair color, age, smoking habits and duration of exposure to the metals were discussed. In related work, Othmān[149] had reported a preliminary investigation of the lead level in whole blood of normal and occupationally exposed populations in Damascus City. Tsalev et al.[150] studied the manganese in whole blood of exposed workers and unexposed individuals in a manganese alloy plant. The purpose of this work was to elucidate the state of health of workers employed in a manganese industry.

4.3 Electrothermal-, Hydride Generation- and Cold Vapor Atomic Absorption Spectrometry

4.3.1 Principles and Instrumentation

An electrothermal atomic absorption spectrometry (ETAAS) system is equipped with a graphite tube, which is aligned in the spectrophotometer optical path, and is enclosed in an inert gas, usually argon atmosphere. The graphite tube furnace is electrically heated to preset "dry", "ash", "atomize", and "clean-out" temperatures, so that it sequentially removes the solvent, organic matter/volatile matrix constituents, volatizes/atomizes the analyte, and eventually expels matrix/analyte residues. A transient peak signal is thus produced. Although graphite tube furnaces are most popular, there are also some other atomization devices such as carbon cups, Ta ribbon, Mo microtube, and so on. Graphite furnace atomic absorption spectrometry systems provide a 50-fold to 500-fold improvement in sensitivity relative to FAAS. Generally, an important ETAAS technique is to apply it to trace element analysis of biological samples with low limit of detections (LODs), and small size of samples. A typical ETAAS analysis is at nanogram per milliliter levels.[140,141]

Hydride generation atomic absorption spectrometry (HGAAS) involves the generation of a volatile hydride of an analyte by means of a reducing agent added to a reaction vessel containing an acidic solution of the sample. The generated hydrides are transported to a heated atomizer cell, which can be a heated quartz tube or graphite tube. Sodium borohydride is most commonly used as a reducing agent. The thermally decomposed hydride produces atomic vapors that can be measured quantitatively. Since the hydride is separated from the matrix, advantages include high sensitivity and reduced interferences. However, this technique is only applicable to a limited number of elements. Those elements that form volatile hydrides include As, Se, Bi, Sb, and Te.[140,141]

Cold vapor atomic absorption spectrometry (CVAAS) analysis is used for specific determination of mercury, which can exist in an atomic state at ambient temperature owing to its high vapor pressure. In a manner similar to HGAAS, a reducing agent is added to a reaction vessel containing a sample with trace levels of ionic mercury. Stannous chloride and sodium borohydride are the most commonly used reducing agents. Other constructions as well as automated continuous flow devices have been applied to biological samples.[140,141]

4.3.2 Methodology

4.3.2.1 Sample Preparation The sample preparation for ETAAS and HGAAS measurements is not substantially different from that encountered in other atomic absorption spectrometry methods. Liquid–liquid extraction is highly suitable as a manual pretreatment procedure for HGAAS and speciation. The implementation of liquid–liquid extraction in a continuous fashion enables on-line coupling to AS instruments and contributes advantages inherent in automatic methods of analysis. A detailed technical description of continuous liquid–liquid extraction processes can be found in several books.[140–143] Most biological samples are prepared by decomposing the sample using thermal or chemical means. The microwave approach for sample preparation is becoming a powerful tool. Calibration with standard

addition and verification of modified procedures by means of certified reference materials are often used.

4.3.2.2 Interferences ETAAS and HGAAS are methods of choice for the majority of elements. However, they have some drawbacks and limitations. Matrix effects in ETAAS are common, pronounced and complex. Therefore, background correction must always be provided. An adequate calibration is then required to compensate for residual discrepancies. Matrix/analyte modification techniques are commonly used and are aimed at either decreasing the relative volatility of the analyte or increasing the volatility of the matrix, or both. HGAAS has been applied to the determination of several volatile elements such as As, Se, Sn, Cd, Cu, and so on, which are not easily determined by flame and ETAAS. This technique is relatively selective and amenable to automation. In operation, the organic matter should be completely oxidized and the analyte should be in an oxidation state. Some potential interferences may be expected owing to acids, oxidants, and ions of noble metals. Their nature and extent depend on many factors such as acidity or pH, and the presence of oxidation states of both the analyte and the interferent. Generally, the important interferences and adverse effects can be reduced or eliminated by properly optimizing the experimental procedures.

4.3.3 Applications

Numerous papers have appeared that described application of ETAAS, HGAAS, and CVAAS methods to workplace occupational exposure studies. Rollin and Nogueira[151] reported identification of aluminum fractions in serum by Zeeman atomic absorption spectrometry in order to ascertain the distribution of aluminum (Al) in normal and occupationally exposed sera. It was found that the relative distribution of Al between high molecular mass and low molecular mass fractions was statistically significantly different. This suggests that at high concentrations of total Al in serum, the percentage of the Al bound to the low molecular mass is lower, but the absolute quantity of Al circulating as the low molecular mass complex is increased. This low molecular mass Al complex is thought to play an important role in intracellular accumulation of Al. In related work, Gitelman et al.[152] measured serum Al and urinary Al/creatinine ratios in 235 Al workers and 44 controls in the Al industry to examine the association between occupational exposure to airborne Al and Al absorption. Serum and urine samples were taken before and after 3–5-day work shifts. Occupational exposure was estimated from Al measurements of respirable and total particulates in air. Median exposure values were 25 and 100 $\mu g\,m^{-3}$, respectively. These results are consistent with the systemic absorption of Al from occupational exposure, and suggest the presence of a sensitive uptake process for airway Al. In other related work, Rollin et al.[153] investigated the effect of exposure to Al on concentrations of essential metals in serum of foundry workers. The concentrations of Al in serum and urine of 33 volunteers exposed to inhalation of Al_2O_3 dust were measured. These were compared with results from 20 normal subjects not exposed. The Al concentration in serum was significantly raised in the subjects exposed to dust, but urine showed no significant difference from controls. This redistribution was selective, as the serum concentration of Cu was decreased whereas the serum concentration of Zn was increased. The serum concentration of Fe did not change significantly. Biological monitoring of occupational Al powder exposure was reported by Letzel et al.[154] Fifty-four workers from the exposed group were studied.

The measurement of salivary cadmium by ETAAS and its use as a biological indicator of occupational exposure has been reported by White et al.[155] The method has been developed and employed to measure cadmium levels in saliva samples collected by two different methods from a group of ex-workers previously exposed to cadmium, two groups of currently exposed workers, and an unexposed population as a control. Salivary cadmium levels were significantly raised in both of the groups of currently exposed individuals and in past workers with previous long-term exposure when compared with an unexposed population. In related work, Abernathy et al.[156] developed a method for measuring cellular Cd and DNA-bound Cd following micromolar exposures to cadmium dichloride. Following low-level exposure to cadmium dichloride, atomic absorption spectrometry with Zeeman background correction was used to measure total cell-associated Cd in wet-ashed cells. The lower LODs were determined to be 100 pg of Cd per 10^6 cells. This method is sensitive and reproducible, and is suitable for the detection of Cd in biological matrixes after low levels of Cd exposure. The determination of silver in whole blood and its application to biological monitoring of occupationally exposed groups were studied by Armitage et al.[157] Blood silver levels were determined in 98 occupationally exposed workers involved in bullion production, cutlery manufacture, chemical manufacture, jewellery production and silver reclamation. Other occupational applications of the ETAAS methods included study of urinary excretion of nickel in nickel–chromium electroplaters.[158]

HGAAS methods have been used in several studies of interest in occupational and environmental health. In a field investigation, Jensen et al.[159] reported the sum of concentrations of inorganic arsenic, methylarsonic acid and dimethylarsinic acid in urine from adults and children living in an unpolluted area. The results

from the unpolluted area were compared with the corresponding sum from adults and children living in an area polluted with arsenic, and the corresponding sum from persons occupationally exposed to arsenic. The median values for 22 adults and 10 children aged 3–10 years living in the unpolluted area were 9.3 and 19.8 nmol As $mmol^{-1}$ creatinine, respectively. The corresponding ranges were 3.2–27.9 and 7.7–57.8 nmol As $mmol^{-1}$ creatinine, respectively. The arsenic level in urine from adult workers handling arsenic-treated wood was approximately four-fold higher than controls. The arsenic levels in urine from two glass workers were nine- and two-fold higher, respectively. In another work, Blas et al.[160] developed a method for determination and speciation of arsenic in human urine by HGAAS. This method is applicable to urine samples in studies relating to arsenic exposure and its monitoring.

Another atomic spectrometric technique, the CVAAS method, has been used in mercury analyses. Martin et al.[161] reported a study of using spot urine samples for low-level occupational mercury exposure assessment, and demonstrated a relationship of Hg exposure with porphyrin and creatinine excretion rates. Hg and porphyrin levels in single void urine spot samples were compared with calculated 24-h urine levels in 35 practicing dentists who had been occupationally exposed to low levels of elemental Hg. The study aimed to determine the individual variability for Hg and porphyrin concentrations in spot samples over a 24-h period, and determine the time of day at which a spot sample would give an Hg concentration closest to the 24-h average concentration. The results confirmed previous reports of a first-order diurnal pattern with a mid-morning peak for Hg concentration. In other studies, the CVAAS method was also used in the determination and speciation of mercury, methylmercury, ethylmercury and phenylmercury concentrations in urine samples taken from students and staff of a dental workplace.[162]

4.4 Inductively Coupled Plasma Atomic Emission Spectrometry

4.4.1 Principles and Instrumentation

The ICPAES technique has been available commercially since the mid-1970s.[163] The technique is an emission spectroscopic method in which the sample is dissociated into its atomic form and excited to high energy levels including the ionic form by introducing the sample into the center of a gaseous plasma sustained inside an induction coil energized with a high frequency alternating current. The excited species then emit characteristic radiation as they relax back to the atomic and ionic ground states. Principally, in ICPMS analytes are atomized, excited, and ionized, and then identified by their optical spectrum.

Today ICPAES has become widely accepted, and has been applied to elemental analysis of nearly all the elements. It offers simultaneous or rapid sequential multielement determination capability at the major, minor, and trace concentration levels. Because of its simplicity of use, wide linear dynamic range, and accuracy of analysis, it has become established as the accepted method for the analysis of metallic aerosols, powders of metals, dusts, and fly ashes.

4.4.2 Methodology

4.4.2.1 Sample Preparation In multielement analysis by ICPAES, the dissolution of a sample can be quite complex. Like most classical chemical analysis and dissolution methods for atomic absorption spectrometry, the sample preparations are designed to bring the analytes into solution, without loss or gain. In general, there is no universal solvent or universal dissolution method. Knowledge of a wide range of sample types and dissolution procedures is needed to make a choice. When a large range of elements is under consideration, high temperatures can cause the loss of volatile elements, while low temperatures may result in the incomplete dissolution of refractory elements. Dry ashing methods can be used for large batches of samples, but there can be problems involving the loss of certain volatile elements. Acid digestion with either hot or cold is widely applicable to the analysis of a majority of elements. There are two groups of acids. One group includes oxidizing acids such as nitric, sulfuric and perchloric acids. Another group is nonoxidizing acids including hydrochloric, hydrobromic and hydrofluoric acids, and so on. Both groups can be used to dissolve metals, oxides, and carbonates. Microwave extraction or digestion is a safer, faster, cheaper procedure, and causes less contamination of sample prepared for trace analysis. It is increasingly replacing conventional techniques such as hotplate acid digestion. For a particular sample type, a specific method should be developed through experiments in which the acid mixture, the microwave power setting, and the heating period are varied to determine which combination gives the best results. Because ICPAES can tolerate high levels of organic material, the complete destruction of organic material is not necessary.

4.4.3 Applications

Many papers have been published that deal with the measurement of metals of interest in workplace air, or dust, and in biological monitoring by ICPAES. Lo and Arai[164] developed a rapid method for the simultaneous determination of 11 metals (As, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb and Zn) in urine by

ICPAES. Acidification of the urine was the only sample preparation required. This procedure has been applied for routine screening of workers for occupational exposure to toxic metals. Biological exposure evaluation and hair analysis in workers handling chromium compounds were reported by Kudo et al.[165] The hair of 40 workers exposed to chromium compounds was analyzed for 18 elements by ICPAES; 21 subjects worked in a factory that manufactured chromate pigments, 11 in a painting factory and 8 in a Cr plating factory. It was found that simultaneous measurements of Cr in the hair and urine were useful for determining the extent of exposure to Cr. Determination of multiple elements should be the best means of estimating the degree of exposure to Cr compounds in which they are found.

Olson et al.[166] described trace element analysis of As, Be, Cd, Cr, Cu, Fe, Pb, V, and Zn in airborne particulates and in human urine by ICPAES. The principle of the method, range and sensitivity, interferences, accuracy and precision, advantages and disadvantages are detailed in their paper. Franzlau[167] used ICPAES in screening for trace metal exposures in an industrial population. A heterogeneous group of asymptomatic industrial workers were examined and had hair and blood samples analyzed for 10 metals via the ICPAES technique. Hull[168] employed ICPAES for the multielement analysis of industrial hygiene samples. Experiments were conducted to define the lower limit of quantitative determination and the analytical range of each element for which an OSHA (occupational safety and health administration) personal exposure limit exists. The effects of varying solution matrices and interelement effects were investigated. Cadmium emissions from fumes were studied during processing of Cd-containing thermoplastics processing. Air sampling volumes of 120–388 L were collected in the door area, at the machine nozzle and mold vent, and 1 m away from the machine approximating the position of the operator, and analyzed for Cd by ICPAES.

5 CHROMATOGRAPHIC SPECTROPHOTOMETRIC DETECTORS

5.1 Introduction

Spectrophotometric detectors coupled to chromatographic separation techniques are often used to characterize the workplace environment. These techniques are employed[169] to confirm the accuracy of direct reading instruments or when direct reading instruments are not available or when the workplace environment is complex and contains multiple chemicals that need to be measured for regulatory documentation of exposure levels[170] and for biological monitoring.[171] Standardized methods are published by a variety of government agencies and societies, including the National Institute of Occupational Safety and Health (NIOSH), OSHA, Mine Safety and Health Administration (MSHA), Environmental Protection Administration (EPA), and American Society for Testing and Materials (ASTM). Many of these methods can now be obtained through the Internet. The NIOSH Manual of Analytical Methods can be found at www.cdc.gov/niosh/nmam/nmampub.html. This site also contains links to the other organizations mentioned. Harper et al.[169] have reviewed the literature published in the field of industrial hygiene. Many references to specific air monitoring and biological techniques are sited within this review. The following is a short overview of chromatographic spectrometric detectors, emphasizing UV/VIS and fluorescence detectors.

5.2 Spectrophotometric Detectors for High-performance Liquid Chromatography

Spectrophotometric detectors that can be coupled to HPLC include UV/VIS, fluorescent, light scattering, refractive index, diode array, MSD, IR detectors, radioactivity and luminescence. The choice of detector is dependent on the analyte's spectral properties, and required sensitivity and selectivity. Both the sensitivity and selectivity of spectroscopic detectors are dependent, in part, on wavelength(s) employed. The wavelength where maximum absorption occurs for the chemical of interest may not necessarily be used to quantify levels in a particular occupational environment if chromatographically coeluting contaminants absorb at that wavelength. The settings may be changed slightly or moved to a secondary absorbance peak of the analyte to eliminate the interference. Wavelength selection can also be limited by mobile-phase requirements for chromatographic separation of the environmental components of interest. Water, methanol and acetonitrile are mobile phases used in reversed-phase HPLC with ultraviolet cutoff points of $\leq$210 nm. Choice of detector can also be influenced by the linearity and dynamic range (concentration versus detector response) requirements. It is desirable to choose a detector with a wide linear working range to be able to assess both major and minor chemical components in the workplace environment. Comparison of detector sensitivities for a particular analyte can be made by using the LOD, which is reported as the amount of analyte that provides a signal that is two to three times that of background noise.

The most commonly employed HPLC detectors in monitoring the workplace environment are the UV/VIS photometric and fluorescent detectors. UV/VIS detectors are available as fixed, variable and scanning-wavelength detectors. Diode array detectors, which

Table 4 NIOSH Manual of Analytical Methods: HPLC–spectroscopic method[a,b]

Method number	Chemical/chemical class	Detector	Wavelength(s) (nm)	LOD/sample
3507	Acetaldehyde	UV	254	0.1 mg
2514	Anisidine	UV	254	0.35 μg
5031	Aspartame	UV	220	2 μg
5509	Benzidine, 3′,3′-dichlorobenicidine	UV	254	0.05 μg
5509	Benzoyl peroxide	UV	254	10 μg
5510	Bromoxynil, bromoxynil octanoate	UV	254	0.6, 0.3 μg
2014	*p*-Chlorophenol	UV	280	2.5 μg
5001	2,4-D and 2,4,5-T	UV	284	15 μg
5013	Dyes (*o*-dianisidine, *o*-tolidine, benzidine)	UV	280	3 μg
2540	Ethylenediamine	UV	254	0.9 μg
	Diethylenetriamine			0.16 μg
	Triethylenetetramine			0.3 μg
5700	Formaldehyde	UV	365	0.08 μg
2532	Glutaraldehyde	UV	365	0.3 μg
5004	Hydroquinone	UV	290	10 μg
5522	Isocyanate	Fluor	275/320	0.1–0.3 μg
3512	Maleic anhydride	UV	254	15 μg
5029	4,4′-Methylenedianiline	UV	254	0.12 μg
5033	*p*-Nitroaniline	UV	375	20 μg
5003	Paraquat	UV	254	10 μg
5512	Pentachlorophenol	UV	254	8 μg
5032	Pentamidine isethionate	Fluor	270/430	18 ng
5506	Polyaromatic hydrocarbons	UV/Fluor	254; 340/425	0.1–0.8 μg
5008	Pyrethium	UV	225	10 μg
5007	Rotenone	UV	290	4 μg
5016	Strychnine	UV	254	0.8 μg
5005	Thiram	UV	254	5 μg
5516	2,4- and 2,6-Toluene diamine	UV	229	0.1 μg
2535	Toluene-2,4-diisocyanate	UV	254	0.1 μg
5018	2,4,7-Trinitrofluoren-9-one	UV	280	0.04 μg
5002	Warfarin	UV	280	2.5 μg

[a] UV, ultraviolet; fluor, fluorescent; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid.
[b] Fluorescent wavelengths are given for excitation/emission.

provide simultaneous spectral data on each peak eluted from the column, are increasingly being employed. Sensitivity of UV/VIS detectors is limited by the detector flow cell path with respect to volume restrictions. In general, the detector cell volume for standard column HPLC should be no greater than 8 $\mu L\,cm^{-1}$ of the optical path length. Sensitivity for an analyte can be enhanced by conjugation of chemical functional groups to specific chromophores.

Fluorimetric detectors are considered, in general, to provide better selectivity and sensitivity than UV/VIS detectors, although both UV/VIS and fluorimetric detectors are available for HPLC detection. The emitted light is usually measured at right angles to the excitation source. The enhanced sensitivity in fluorescence techniques over absorption detectors is mainly due to the reduction in noise caused by background light. Flow cells for fluorimetric detectors must optimize excitation and emission collection efficiency for the small volume of the cell. This requires use of a high-intensity excitation light that is usually provided by mercury or xenon arc lamps. Lasers are becoming more commonly employed as the excitation source. They can focus a high intensity beam onto a small capillary cell and the intensity of the source does not decay with age as seen with traditional excitation lamps. Lasers that emit light in the lower ultraviolet ranges, at present, are very costly, which has limited the spectrum of analytes and conjugates that have been employed with this technique. Secondary excitation/emission wavelengths can be used if interferences are encountered and derivatizing agents can be conjugated to a nonfluorescent analyte to allow for detection. This can improve the LOD for an analyte from 1 to 3 orders of magnitude over UV/VIS detection.

Table 4 is a list of HPLC/ultraviolet and fluorescent methods from the NIOSH Manual of Analytical Methods (4th edition) for chemical analysis of air samples. The majority of the methods take advantage of UV and/or fluorescent spectral properties of the analyte. The 254-nm wavelength is standard on fixed wavelenth detectors, because of the broad range of chemicals that absorb at that wavelength. Many methods in the past

have been developed using 254 nm as the detection wavelength because of this. Derivatization to stabilize and/or enhance spectral properties is used in several of the methods. Naphthylisothiourea derivatives are employed for the measurement of ethylenediamine, diethylenetriamine, and triethylenetetramine (method number 2540), and 2-4-dinitrophenyldrazone formaldehyde conjugation in method number 5700. Isocyanates are derivatized to a stable form for analysis by either ultraviolet or fluorescent detectors. Newer reagents have been developed for trapping and detection of isocyanates, such as 1-(9-anthracenylmethyl)piperazine, 9-(methylaminomethyl)anthracene, 1-(2-methoxyphenyl)-piperazine and dibutylamine, because of the potential exposure to multiple polymeric forms. These reagents employ UV, fluorescent and/or MSDs to give a fingerprint of the multiple polymer isocyanate exposure.

5.3 Spectrophotometric Detectors for Gas Chromatography

Spectrophotometric detectors for GC include MSD, IR, FPDs, and chemiluminescence–redox detectors (CRDs). The majority of the GC applications for industrial hygiene are based on flame ionization detectors, however, the MSD is becoming increasingly common for both air and biological marker analysis. Flame photometric detectors (FPDs) are highly selective for both phosphorus and sulfur. Phosphorus-containing compounds are detected at 510 and 526 nm. Sulfur species emits light at around 394 nm. Applications for the FPD include analysis of pesticides and sulfur-containing gases and fuels.

FTIR is often used to complement the MSD and can provide functional group data and an IR fingerprint to help identify unknown components in the workplace environment or a chemical metabolite following exposure. The traditional light pipe FTIR instrument is relatively insensitive. This has limited its application to mainly qualitative analysis, e.g. identification of trichothecene mycotoxins in bulk samples. One commercially available FTIR instrument has employed direct deposition of the analyte onto a moving ZnSe window to improve detectability into the picogram (of dodecane) range. This increase in sensitivity should allow for the development of applications for qualitative and quantitative analysis of the workplace environment through both air and biological sampling.

CRDs are useful for compounds that are poorly detected by FPDs. The CRD detects the resultant chemiluminescence following specific redox reactions of compounds such as ammonia, sulfur dioxide, and thiols. A variety of other speciality spectroscopic detectors are available, including pyrochemiluminescent nitrogen and pyrofluorescent sulfur detectors that have been used for specific types of analyses. These detectors convert nitrogen-containing compounds to nitric oxide (NO) and sulfur-containing compounds to sulfur dioxide (SO_2). The NO is reacted with ozone to form the excited state of NO_2^*, which releases a photon of light.

6 INFRARED AND RAMAN SPECTROSCOPIES

6.1 Introduction

Emissions of hazardous chemical pollutants are concerns in both the environment and the workplace for safety reasons.[172,173] Identification of chemical pollutants in these places can be accomplished by various techniques.[174] IR spectroscopy, especially FTIR, and RS are the techniques of choice for the identification of the majority of chemical pollutants. Many pollutants have chemical groups of toxicological importance that can be identified and quantified by IR or RS, which measures the vibrational excitation of atoms around the bonds that connect them.

Although IR and Raman spectroscopies are similar in that both techniques provide information on vibrational frequencies, there are many differences between the two techniques. Some vibrations are only Raman active while others are only IR active; the vibration of a heteropolar diatomic molecule is IR active, whereas that of a homopolar diatomic molecule is not IR active. In molecules having a center of symmetry, a vibration is IR active, Raman active, or active in both; however, totally symmetric vibrations are always Raman active.

In IR and Raman, the spectra range from 4000 to 50 cm^{-1}. In both vibrational spectroscopies, the small quantity of samples needed, the speed with which a spectrum can be obtained, and the wide applicability of the methods combine to make IR and Raman spectroscopies two of the most useful tools available to the chemist, industrial hygiene chemist, and industrial hygienist. Both spectroscopies have a great potential because of their applicability to many different fields, such as in structural chemistry,[175,176] biology,[177] biochemistry,[178,179] medicine,[180] and industry.[181] In the following, some specific applications of FTIR and Raman spectroscopies in occupational and environmental hygiene chemistry are presented.

6.2 Infrared Spectroscopy

6.2.1 Fourier Transform Infrared Spectroscopy for Monitoring Airborne Gas and Vapor Contaminants

With the increased use of highly toxic gas and vapor mixtures in science and industry, the need has come for more

sensitive and versatile air monitoring technologies. Of the instruments that are appropriate for industrial hygiene monitoring of gases and vapors cited herein,[182–184] only FTIR spectroscopy is presented in the following discussion.

In the 1990s, a number of IR instruments have been developed, including filter, optical null and ratio recording grating, Fourier transform, and tune laser diode spectrophotometers.[185] FTIR has been shown to be particularly valuable in the monitoring of airborne gas and vapor contaminants.[186] The earliest applications of IR spectroscopy for the identification and quantification of pollutants in air were made using a long-path gas cell. Stephens et al. employed a prism spectrometer (with thermocouple detectors) to measure atmospheric chemistry related to smog pollution.[187] In later studies with an FTIR long-path system, Tuazon et al. studied trace pollutants in ambient air and synthetic atmosphere.[188] Herget and Levine[189] used an FTIR spectrometer with a 20-m gas cell as a near real-time monitoring method for semiconductor process gas emissions. Some other research has been carried out using FTIR for measurement of mobile source emissions,[190] polluted urban air,[191] and reactivity of hydrocarbons.[192] Many studies have utilized remote sensing FTIR to provide reliable air pollution information.[193,194] In remote systems, the gas cells are replaced by telescopes that are used to collimate, send, and receive IR light. With an interferometer system, which employed telescopic optics, the remote sensing FTIR has been used to measure the concentration of gaseous pollutants ranging from 10 ppm to 1 ppb over a 1-km path length.[195]

An open-path FTIR system has been developed to monitor gas and vapor contaminants quantitatively in the workplace.[196] This system was tested in the workplace to monitor numerous organic vapors, such as ammonia, methanol, methylene chloride, and sulfur hexafluoride.[197] An open-path FTIR system has also been applied to air pollution monitoring of trace gases in ambient air.[198] The ability of open-path FTIR instruments to monitor multiple compounds in real time makes it an ideal industrial air monitoring instrument.

6.2.2 *On-filter Analysis of Quartz in Respirable Coal Dust Using Fourier Transform Infrared*

The method for determining quartz content in respirable coal dust is based on dispersive IR spectroscopy.[199] This method has been adequate for many years with a working range capable of measuring 25–250 μg of quartz. A development of the FTIR method is that 10 μg of quartz can be detected in coal dust samples.[200] FTIR employs an interferometer to obtain information about the transmission of IR energy of all wavelengths emitted by the source and passing through the sample. Since there are no entrance or exit slits in FTIR, a greater amount of energy reaches the detector, resulting in increased sensitivity. The interferometer in FTIR contains a fixed mirror and a moving mirror, and the laser tracking of the moving mirror results in greater precision of the wavelength measurement. This permits multiple scans to be averaged, so the signal-to-noise ratio of the FTIR spectrum increases. The quantitative FTIR analysis of quartz in respirable coal dust samples collected on filters would provide the analyst with many benefits, such as speed, convenience, and productivity.[201]

Min-U-Sil 5 (US Silica Co.) quartz dust (pure quartz particles, 5 μm or smaller) is used as the standard for FTIR analysis.[199,202] Polyvinyl chloride or acrylonitrile copolymer filters used for this study have a pore size of 5 μm or less. The filter diameter must be appropriate to the sampler used. The dust samples are collected on the filters. After ashing the membrane filter, the dust samples are dispersed into isopropyl alcohol. Details of the sample treatments and filtration procedure are described by Tuchman.[200] Calibration samples, using Standard Reference Material,[203] are prepared over the range of 2–20 μg.[200] The filter samples are placed in the filter holder to be analyzed by FTIR. The routines for determining quartz using FTIR are the same as described by Tuchman.[200] The FTIR bands of the quartz spectrum have been reported at 1087, 799, 780, 695, 524, and 467 cm^{-1}.[200] These band intensities as shown in the quartz absorption spectrum[200] occur closer to the far-IR region and are not obscured by random noise. However, the absorption band at 1087 cm^{-1} is strong but very broad while the 695 cm^{-1} is weak; therefore, these two bands are problematic for quantifying quartz. The remaining four bands at 799, 780, 524, and 467 cm^{-1} are suitable for quantifying quartz.

6.2.3 *Studies on Structural Changes of Collagen in Silicosis Using Fourier Transform Infrared*

Silicosis is a well-known occupational disease that continues to spread even after exposure to dust stops, and there is no effective treatment. Therefore, prevention of exposure to dust pollutants and early detection in the work environment are required. Although studies on structural changes of collagen in silicosis can be accomplished by several techniques,[204,205] only studies on structural changes of silicotic collagen using FTIR are presented as follows. Test samples in the lung, lymph node, and other various tissues of normal and silicosis-affected humans or animals are used for FTIR studies.[206] The tissues are fixed in KBr as described by Yurui et al.[206] The wavenumber of FTIR spectroscopy

ranges from 4000 to 400 cm^{-1}. The bands of the normal spectrum have been reported at 3400–3200, 2900–1200, and 1200–1000 cm^{-1}.[206] These band intensities as shown in the normal spectrum[206] are broad, and their intensities are medium. The band positions and relative intensities of the differential spectrum between normal and silicosis spectra are compared to those of the normal spectrum. It has been found that there are higher band intensities in the differential spectrum at 3400–3200, 2900–1200, and 1200–1000 cm^{-1}.[206] The higher band intensities at 3400–3200 and 1200–1000 cm^{-1} correspond to increases in –OH and –Si–O–R groups,[207] respectively, while the higher band intensity at 2900–1200 cm^{-1} indicates the shortening of the –C–C– backbone.[206] The increase in –Si–O–R groups indicates that silica forms linking bridges between collagen which may be the cause of progressive enlargement of nodules.

6.3 Raman Spectroscopy

Like IR, RS is a powerful technique and has a variety of applications. IR and Raman spectroscopies are complementary, and both are utilized whenever possible. Since experimental techniques and applications of RS have been reviewed extensively, only microscopic inclusions caused by inhaled particles (including talc, rutile, α-quartz, and calcite) which have been identified in situ by Raman microspectroscopy are covered. Other experimental techniques and their applications should be referred to: nonlinear RS,[208] time-resolved RS,[209] matrix-isolation RS,[210] high-pressure RS,[211] Fourier transform RS,[212] surface-enhanced RS,[213] and Raman spectroelectrochemistry.[214]

6.3.1 Experimental

The sample being studied is placed on the stage of the microscope and illuminated by light from the transmission illuminator. The sample is focused and adjusted by viewing from the optical viewpoint. Then, the illuminator lamp is switched off and the laser beam is passed through filtering optics. The scattered light from the sample is collected by the objective and sent into the spectrometer. A cooled photomultiplier detector is used for detection.

6.3.2 Identification of Inhaled Particles, Including Talc, Rutile, α-Quartz, and Calcite

6.3.2.1 Identification of Talc A pure talc particle (talcum powder), used as a reference, and test particle samples in the foreign bodies in various tissues[215] that contain a talc particle (both are about the same size, 5–10 μm) are used for RS. The tissue samples are fixed in formaldehyde, dehydrated, and embedded in Paraplast™ as described by Mul et al.[216] Sections of paraffin-embedded material are placed on the stage of the microscope for Raman measurements and control purposes. The wavenumber for RS ranges from 4000 to 50 cm^{-1}. The Raman peaks of the reference spectrum have been reported at 115, 197, 366, 679, and 1049 cm^{-1}.[217] The 197 and 679 cm^{-1} bands as shown in the reference spectrum[217] are very strong, the 115 and 366 cm^{-1} bands are strong, and the 1049 cm^{-1} band is medium. A direct comparison between the test sample spectrum and the reference spectrum (the peak positions and their relative intensities) is sufficient to make an assignment for the talc particle.

6.3.2.2 Identification of Rutile (TiO_2) A pure rutile particle, used as a reference, and test particle samples in the foreign bodies in various tissues[215] that contain a rutile particle (both are about the same size, 5–10 μm) are used for RS. The tissue samples are fixed and placed on the stage of the microscope as described above. The wavenumber of RS ranges from 4000 to 50 cm^{-1}. The Raman peaks of the pure rutile spectrum have been reported at 240, 440, and 610 cm^{-1}.[218] The 240, 440, and 610 cm^{-1} bands as shown in the pure rutile spectrum[218] are broad, and their intensities are medium. A direct comparison between the test sample spectrum and the pure rutile spectrum (the frequencies, relative intensities, and linewidths) is sufficient to make an assignment for rutile in test particle samples.

6.3.2.3 Identification of α-Quartz (SiO_2) A reference particle and test sample particles in the foreign bodies in various tissues[215] that contain α-quartz particles (both are about the same size, 5–10 μm) are used for RS. The tissue samples are fixed and placed on the stage of the microscope as described above. The typical peaks of a reference spectrum have been reported at 128, 206, and 466 cm^{-1} band.[219] The 128 and 466 cm^{-1} bands as shown in the reference spectrum[219] are sharp, and the 206 cm^{-1} band is broad. A direct comparison between the test sample spectrum and the reference spectrum (the peak positions, shape, and their relative intensities) is sufficient to make an assignment for α-quartz samples.

6.3.2.4 Identification of Calcite Test particle samples and a reference particle in the foreign bodies in various tissues[215] that contain a calcite particle (both are about the same size, 5–10 μm) are used for RS. The tissue samples are fixed and placed on the stage of the microscope as described above. The Raman peaks of a reference spectrum have been reported at 156, 285, 715, and 1088 cm^{-1}.[220] A direct comparison between the test sample spectrum and the reference spectrum (the spectral positions and line shape) is sufficient to make an assignment for the calcite particle.

Table 5 Portable spectrometric techniques for monitoring gases and vapors

Instrumental technique	Applicable analyte(s)	Comments
IR photometers	CO, CO_2, NO_x, N_2O, SO_2, hydrocarbons, fluorocarbons, etc.	Detection limits sub-ppm to few percent range. Single-species or multigas devices
Colorimetric detection	Formaldehyde, TDI, HCN, Cl_2, H_2S, SO_2, NO_x, etc.	Sub-ppm detection limits for most species. Specific for certain target analytes
UV/VIS photometers	Hg vapor, O_3, SO_2, NO_x, NH_3, organic vapors, etc.	Sub-ppm to ppm detection limits. Analyte specific
Chemiluminescent detectors	Ozone, NO_x	Highly sensitive and selective. Detection limits ~ 10 ppb
Photometric analyzers (includes devices based on flame photometry, fluorescence, other)	CO, SO_2, sulfur compounds., halogenated hydrocarbons, phosphorus compounds, etc.	Measurement in ppb to ppm range, depending on type. Single- and multispecies devices

7 FIELD-PORTABLE SPECTROSCOPY

7.1 Introduction

Field-portable methods for monitoring airborne workplace contaminants and toxins have received increasing attention. A number of portable monitors for airborne contaminants have been commercially available for many years, but new developments may provide for on-site compliance monitoring, which has heretofore been more the exception than the rule. The ability to conduct measurements on-site in the occupational setting offers significant advantages. Field-portable methods are often desired so that decisions regarding worker protection, engineering controls, and so on can be made quickly. The capability for rapid decision-making offered by on-site monitoring can help to save costs, and also offers a means to assess, and thereby provide timely prevention of, worker overexposures to toxic substances. Field-based monitoring is especially useful for applications in the construction industry, in agriculture, and in other situations where jobs may be short term and the workforce is transient. On-site techniques can also be beneficial in instances where short-term monitoring is desired. In this section, field-portable spectrometric techniques are covered, and some applications are presented. Because of limited space here, a general overview is presented; more specific information is available by consulting the literature referenced herein.

7.2 Portable Gas and Vapor Analyzers

While many commonly used portable gas and vapor analyzers are based on electrochemical or electrical measurement,[221-223] some rely on spectrometric means. Direct-reading instruments which rely on the use of IR, fluorescence, luminescence, or colorimetry have proven to be most popular.[222-225] Other spectrometries have also been used for on-site real-time gas and vapor monitoring. Some portable instruments, e.g. IR, allow for multigas detection, while others are designed for the measurement of single species of interest, such as carbon monoxide or ozone. Table 5 summarizes direct-reading spectrometric devices that are commonly used in the industrial hygiene field for monitoring gases and vapors. A wide variety of commercial instruments are available.

Most portable IR gas analyzers are nondispersive IR instruments that ordinarily require a plug-in power source.[222,223,226] Some dispersive instruments have also been introduced.[222] Battery-powered instruments have been produced, but these are generally species-specific; for instance, an IR photometer for monitoring carbon dioxide over a wide concentration range relies on Ni–Cd batteries as an optional power source. Portable multigas IR analyzers are becoming more popular as their applicability is enhanced through interfacing of the instrument with spectral libraries via computer. A wide variety of organic and inorganic gases can be monitored semiquantitatively or in some cases quantitatively. For example, portable IR monitors for CO are able to measure quantitatively this dangerous compound in the parts per million range, where such concentration levels are potentially hazardous to life and/or health.

Field-portable FTIR instruments are now available,[227,228] and a national voluntary consensus standard has been published which describes a portable FTIR method for determining gaseous compounds.[229] IR or FTIR monitoring allows for real-time or near real-time measurement of numerous toxic gases and vapors, and has applications in many occupational settings. An advantage of IR or FTIR monitoring is that sample preparation is minimal, and gases and vapors may be monitored following sampling by using a suitable sampler; or alternatively no samples may be needed at all (depending on the application). Open-path FTIR for real-time in situ monitoring of airborne gaseous pollutants has become popular for remote sensing, and also offers promise for applications in occupational settings.[230,231] A new IR spectral database standard, National Institute of Standards and Technology (NIST) SRM 79, has been made available recently.[232]

This NIST IR database contains absorption coefficient data for 21 hazardous air pollutants, and provides for quality assurance for quantitative FTIR open-path in situ measurements of these species.

Colorimetric and UV/VIS spectrophotometric monitors for gases and vapors are widely used in the industrial hygiene field (Table 5). Most of these monitoring devices, e.g. for ozone, mercury vapor, NO_x, ammonia, and sulfur dioxide, function by means of ultraviolet absorption.[222,233] Colorimetric monitors ordinarily employ a reaction between a selective reagent and the analyte of interest in order to form a colored complex which can be measured in the visible spectral range. Most commercially available colorimetric and UV/VIS photometers require an external power source for their operation. However, there are examples of such portable devices that can be operated using battery power, e.g. some Hg and SO_2 monitors. Portable, battery-powered devices are useful for personal monitoring, while monitors requiring an external power source are limited to use as area (static) monitors. UV/VIS and colorimetric instruments are able to detect most species of interest in the parts per million range, and many are equipped with alarms if readings are high. Many of the UV/VIS direct-reading instruments offer continuous monitoring capability, with response times of ~1 s for measurements in the parts per million range.

Direct-reading chemiluminescent detectors offer a means for measuring ozone and oxides of nitrogen, with excellent specificity and high selectivity (Table 5). The operation of chemiluminescent detection involves excitation of O_3 molecules via chemical reaction and the subsequent detection of photon emission from the excited state species, which may either be an intermediate or a product of the reaction.[234] Ozone may function as a reagent for NO_x analysis or, of course, as the analyte of interest.[235] Luminol has also been used for field-portable monitoring of NO_2,[236] and ethylene has been employed as a reactant for field-portable monitoring of ozone.[237] Owing to high power needs, field chemiluminescence instruments generally require an external power source.[222,228]

Photometric analyzers are used widely in the industrial hygiene field for the on-site, real-time monitoring of numerous gas and vapor species (Table 5). The operation of the detector is via measurement of light emission from a high-temperature H_2 flame.[238] FPDs are useful for selective measurement of gaseous sulfur or phosphorus compounds, with detection limits in the parts per billion range for these species. The high power required for operation of these devices necessitates the use of an external power source.

Portable photoionization detectors (PIDs) for organic vapors are commercially available.[239] These require less power than the aformentioned photometric analyzers, and simple hand-held, battery-powered devices can be used to monitor volatile organic compounds (VOCs) in real time. However, for enhanced performance (such as minimizing interferences from more abundant hydrocarbons), PIDs can be used as detectors for portable GC instruments.[240]

Fluorescence analyzers are available for monitoring CO and SO_2 (Table 5). An Xe or Hg arc lamp is used to excite the analyte species, which give rise to sufficient fluorescence intensity so that detection limits in the few parts per billion range can be achieved.[222] Photomultiplier tubes are used for detection. Like photometric analyzers, there is a need for high power in order to facilitate source excitation and operation of photomultiplier detectors, and thus an external power source is required. The performance of the portable fluorimetric SO_2 analyzer has been shown to be equivalent to that of a colorimetric reference method promulgated by the EPA.[228]

Other types of photometric analyzer are commercially available, whereby alternative chemical strategies may be used to produce a spectral signal or color change.[222] Spectral intensity analyzers, for example, can be used for general, nonspecific monitoring of halogenated hydrocarbons. Other types of photometer allow for automated sampling by use of media which undergo a color change upon reaction with target analytes (e.g. like that already mentioned for monitoring SO_2). Reflectance may be used for the measurement of a variety of species such as ammonia, phosgene, HCN, and arsine, with detection limits in the parts per million range. In a few cases, field portability and on-site monitoring applications are enhanced by the use of battery power instead of an external power source.

New developments in spectrometric gas and vapor monitors have provided for better detection limits and other attributes. Fiber optic chemical sensors for continuous monitoring have been an area of wide interest,[241] and applications in the measurement of gases and vapors are widespread. Fiber optics have proven to be useful for the design of field-portable devices for optical,[242,243] fluorimetric,[244] and IR[245] monitoring of such species as VOCs, Hg, explosive agents, and so on. The use of optical sensor arrays for multispecies monitoring is also an area of significant promise.[246] Miniaturization of TOF mass spectrometric devices has allowed for the on-site monitoring of gaseous analytes, with excellent prospects for multispecies monitoring.[247] Hand-held ion mobility spectrometry (IMS) instruments have found applications in industrial hygiene and military applications.[248] A hand-held IMS device was used to monitor VOCs on-site in the workplace.[249] The

performance of IMS is enhanced when used as a detection scheme following GC separation.[250,251]

7.3 Portable Aerosol Monitors

Direct-reading portable aerosol monitors which are used for industrial hygiene purposes are often based on light-scattering or light-attenuation properties (Table 6). The most widely used are light-scattering devices known as aerosol photometers or nephelometers.[223] Light-attenuating photometers are also available, as are other real-time aerosol monitors that are not based on optical techniques.[252] Portable instruments have been developed which are applicable over different aerosol size ranges (Table 6) and each device has its own benefits and limitations.

Aerosol photometers operate by directing polychromatic light toward an aerosol as it is passed through an optical chamber, and by measuring the light which is scattered at a chosen scattering angle with respect to the incident light beam. Optical aerosol particle counters use a monochromatic light source such as a laser, photodiode or tungsten filament lamp to illuminate the aerosol sample. For both photometers and optical particle counters, photomultiplier tubes or photodiodes are generally used for detection of the scattered light. Smaller scattering angles are best for the detection of large particles, while a scattering angle of 90° offers maximum sensitivity for small particles.[223] Many factors contribute to the light scattering profile, e.g. wavelength of the incident light beam, size and shape of the particles, refractive index of the particle, density and concentration of the aerosol, and size distribution of the aerosol.[253] Thus it can be seen that these devices may suffer from numerous limitations which can restrict their applicability for quantitative monitoring, and therefore are generally not used for compliance monitoring purposes. Nevertheless, they are very useful for applications as on-site screening instruments and semiquantitative measurement of aerosol concentrations.

Table 6 Direct-reading optical aerosol monitors

Type of monitor	Applicable aerosol size range (μm)	Comments
Aerosol photometer or nephelometer	0.1–1.0	Integral light scattering; aerosols with same size distribution
Optical particle counter	~0.1–>10	Monochromatic or polychromatic source; light scattering
CNC	<0.01–1.0	Particles enlarged for photometric measurement

CNC, condensation nucleus counter.

Several popular instruments for estimation of aerosol concentrations in real-time are based on light-scattering methods, and a variety of techniques have been employed to improve their performance.[223,254] Techniques for sampling and analysis have been developed for both "extracted" samples and for in situ analysis.[252] Several devices utilize monochromatic IR or near-IR radiation to illuminate the sample, and some employ size-selective devices in order to isolate the aerosol range of interest. These devices are generally applicable for monitoring aerosols which are about 1.0 μm in diameter or greater, and are therefore not useful for the detection of small diameter aerosols. However, some have been claimed to give accurate aerosol concentration measurements over the range of 0.01–100 mg m^{-3} for aerosols of 0.1–20 μm in diameter.[223]

For measurement of very small aerosols, condensation nucleus counters (CNCs) are usually employed (Table 6). The CNC functions by actually enlarging the aerosol particles to a size which can be measured photometrically.[223,252] This is usually accomplished by subjecting the ultrafine aerosol to a vapor, and then cooling the mixture to cause supersaturation. In this manner the aerosol particles operate as condensation nuclei upon which the supersaturated vapor can nucleate and cause the aerosol particles to grow in diameter. In so doing, the intensity of scattered light can be used to measure the concentration of the enlarged particles. CNCs are widely used for testing high-efficiency particulate air filters in respirator fit-testing.[255]

With some exceptions, real-time aerosol monitors are survey instruments which can only be used to measure total concentrations of airborne particulates. Furthermore, the size ranges of aerosols which real-time aerosol monitors can measure tend to be limited.[256] Direct-reading, real-time aerosol monitoring instruments generally cannot give any information on the identities of airborne contaminants which may be present in the test aerosol. However, in recent instrument developments, efforts have been made to obtain more species-specific information using real-time optical monitoring. For example, a real-time monitor for respirable particles based on laser light scattering was said to be capable of detecting 0.1 fibers cm^{-3} with very short sampling times.[257] The instrument uses a diode array to sense scattered light and then assigns a particle to a particular class depending on its scattering characteristics. In most direct-reading instrumental applications, knowledge about the specific makeup of the aerosol being monitored is necessary before a survey instrument is employed.

Portable methods for measuring chemical species in captured aerosols ordinarily require that an aerosol sample be prepared and analyzed on-site in the field. In some cases sample preparation may be minimal, while

in other instances considerable sample treatment may be needed prior to on-site analysis. The option of using a field-portable analytical method depends on the needs of the user. If it is desirable to have an analytical result quickly, then it may be necessary to perform on-site analysis. Another possible reason for conducting analysis on-site has to do with the reactivity of target analytes which can exist in the sampled aerosol.[258] Reactive compounds may need to be analyzed quickly in the field using field-portable instrumentation, because the chemical form(s) of the analyte(s) of interest may change if samples are stored for too long a time period (e.g. for subsequent fixed-site laboratory analysis).

Various field-portable spectrometric techniques for on-site determination of heavy metals in collected aerosol samples have been evaluated. For instance, portable X-ray fluorescence (XRF) has been used to measure metal species in air filter samples.[259,260] In one study, portable XRF was used to determine metals in filters which were prepared from aerosolized metal oxides.[259] Excellent quantitative results for up to 18 metals were obtained, although detection limits for Pb and Cd were somewhat high compared to the action levels of interest. Very good correlations were obtained between portable XRF data and results obtained using a laboratory XRF instrument. Thus portable XRF offers the potential for on-site multielement monitoring of aerosol filter samples collected in the field. Since the method is nondestructive, the samples can be analyzed subsequently for metals content using a confirmatory technique such as ICPMS.

In related studies, modern portable XRF devices have been used to determine lead in filter samples, with a view toward obtaining lower detection limits for this metal. In one investigation, air filter samples were collected from construction sites where lead paint removal activities were undertaken.[261] The lead loading range of the data set was 0.1–1500 μg of lead per sample. Portable XRF measurements were conducted on the filter samples using a protocol which accounted for the variability in the density of the aerosol which was deposited on the filters. A NIOSH reference technique, graphite furnace atomic absorption spectrometry, was used for confirmatory analysis and method evaluation purposes.[262] For the portable XRF method, a lower detection limit of ~6 μg Pb/filter was determined, and the portable XRF method accuracy was ±16.4%. The performance of the portable XRF instrument indicated that the device can be used for the quantitative analysis of lead air filter samples over a wide concentration range. The practicing industrial hygienist can use portable XRF to produce a rapid on-site determination of lead exposure and immediately communicate to workers and help identify appropriate levels of personal protection.

Some other techniques that offer promise for on-site multimetals spectrochemical analysis include laser-induced breakdown spectroscopy (LIBS)[263] and spark-induced breakdown spectroscopy.[264] While neither instrument has been commercialized, prototypes of both have been evaluated for their ability to determine a number of heavy metals in air samples. For metals such as lead, LIBS may offer lower detection limits than portable XRF devices.[265] Efforts to make prototype LIBS devices more easily field portable have focused on the use of fiber optics.[265–267]

Spectrometric methods for the on-site analysis of species present in aerosols following a sample dissolution step have been published. For example, a field-portable method for the determination of airborne hexavalent chromium, Cr(VI), was developed and evaluated.[268] The procedure employed ultrasonic extraction[269,270] in order to solubilize Cr(VI) in test samples. Subsequently SPE using strong anion exchange was employed to separate Cr(VI), which is anionic, from Cr(III) and other metal cations. Following elution of the isolated Cr(VI), trace concentrations of Cr(VI) were measured using the diphenylcarbazide method[271,272] by means of a field-portable battery-powered spectrophotometer. It is expected that additional field-portable spectrometric measurement methods for more analytes will become more widely used in the future for industrial hygiene monitoring.

ABBREVIATIONS AND ACRONYMS

APCI	Atmospheric Pressure Chemical Ionization
AS	Atomic Spectrometry
ASTM	American Society for Testing and Materials
BEI	Backscattered Electron Image
CI	Chemical Ionization
CNC	Condensation Nucleus Counter
CP	Cyclophosphamide
CRD	Chemiluminescence–Redox Detector
CVAAS	Cold Vapor Atomic Absorption Spectrometry
EDS	Energy Dispersive Spectrometer
EDX	Energy Dispersive X-ray
EI	Electron Ionization
EPA	Environmental Protection Administration
ETAAS	Electrothermal Atomic Absorption Spectrometry
FAAS	Flame Atomic Absorption Spectrometry

FAB	Fast Atom Bombardment
FIA	Flow Injection Analysis
FIAAS	Flow Injection Atomic Absorption Spectrometry
FPD	Flame Photometric Detector
FS	Fluorescent Spectrometry
FTICRMS	Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
Hb	Hemoglobin
HGAAS	Hydride Generation Atomic Absorption Spectrometry
HPLC	High-performance Liquid Chromatography
IA	Image Analyzer
ICP	Inductively Coupled Plasma
ICPAES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICPMS	Inductively Coupled Plasma Mass Spectrometry
IF	Ifosfamide
IMS	Ion Mobility Spectrometry
IR	Infrared
LC	Liquid Chromatography
LC/MS	Liquid Chromatography/Mass Spectrometry
LIBS	Laser-induced Breakdown Spectroscopy
LOD	Limit of Detection
MALDI	Matrix-assisted Laser Desorption Ionization
MALDI/TOFMS	Matrix-assisted Laser Desorption Ionization/Time-of-flight Mass Spectrometry
MOCA	4,4′-Methylenebis-(2-chloroaniline)
MS	Mass Spectrometry
MS^n	Ion Trap Mass Spectrometry
MSD	Mass Spectral Detector
MSHA	Mine Safety and Health Administration
MS/MS	Tandem Mass Spectrometry
NIOSH	National Institute of Occupational Safety and Health
NIST	National Institute of Standards and Technology
OSHA	Occupational Safety and Health Administration
PAC	Polycyclic Aromatic Compound
PAH	Polycyclic Aromatic Hydrocarbon
PCR	Polymerase Chain Reaction
PID	Photoionization Detector
QTOFMS	Quadrupole Time-of-flight Mass Spectrometry
RS	Raman Spectroscopy
SEI	Secondary Electron Image
SEM	Scanning Electron Microscopy
SIM	Selected Ion Monitoring
SPE	Solid-phase Extraction
TDI	Toluene Diisocyanate
TNT	2,4,6-Trinitrotoluene
TOF	Time-of-flight
TOFMS	Time-of-flight Mass Spectrometry
UV/VIS	Ultraviolet/Visible
VOC	Volatile Organic Compound
WDS	Wavelength Dispersive Spectrometer
XAD-2	2-(Hydroxymethyl)piperidine
XM	X-ray Microanalysis
XRF	X-ray Fluorescence

RELATED ARTICLES

Environment: Trace Gas Monitoring **(Volume 3)**
Fourier Transform Infrared Spectrometry in Atmospheric and Trace Gas Analysis

Environment: Water and Waste **(Volume 3)**
Inductively Coupled Plasma Mass Spectrometry in Environmental Analysis • Infrared Spectroscopy in Environmental Analysis

Industrial Hygiene **(Volume 5)**
Chromatographic Techniques in Industrial Hygiene • Dust, Measurement of Trace Elements in

Industrial Hygiene cont'd **(Volume 6)**
Metals in Blood and Urine: Biological Monitoring for Worker Exposure

REFERENCES

1. J.T. Watson, *Introduction to Mass Spectrometry*, 3rd edition, Lippincott-Raven, New York, 1997.
2. M. Harper, C.R. Glowacki, P.R. Michael, 'Industrial Hygiene', *Anal. Chem.*, **69**, 307R–327R (1997).
3. J.E. Adkins, N.W. Henry, 'Industrial Hygiene', *Anal. Chem.*, **67**, 349R–376R (1995).
4. E.D. Hoffmann, J. Charette, V. Stroobant, *Mass Spectrometry – Principles and Applications*, John Wiley & Sons, New York, 1996.
5. M. McMaster, C. McMaster, *GC/MS – A Practical User's Guide*, Wiley-VCH, New York, 1998.

6. J.P. Lodge, Jr, *Methods of Air Sampling and Analysis*, 3rd edition, Lewis Publishers, Chelsea, MI, 1989.
7. D.R. Booker, W.D. Griffiths, D.M. Lyons, S.L. Upton, *Aerosol Sampling Guidelines*, AEA, UK, 1998.
8. A.L. Burlingame, R.K. Boyd, S.J. Gaskell, 'Mass Spectrometry', *Anal. Chem.*, **70**, 747R–716R (1998).
9. E. Constantin, A. Schnell, *Mass Spectrometry*, Paramount Publishing, Amsterdam, 1991.
10. A. Sturaro, G. Parvoli, L. Doretti, G. Gori, G.B. Bartolucci, 'GC-MS Investigation of Polycyclic Aromatic Compounds in the Manufacture of Rubber Tubes', *Ann. Occup. Hyg.*, **37**(3), 327–334 (1993).
11. E. Menichini, L. Boniforti, S.D. Marzio, 'Occupational Exposure to Airborne Aromatic Amines in Rubber Manufacturing: Determination by GC-MS', *Toxicol. Environ. Chem.*, **22**, 9–16 (1989).
12. E. Menichini, L. Bonanni, F. Merli, 'Determination of Polycyclic Aromatic Hydrocarbons in Mineral Oils and Oil Aerosols in Glass Manufacturing', *Toxicol. Environ. Chem.*, **28**, 37–51 (1990).
13. J. Bundt, W. Herbel, H. Steinhart, 'Structure-type Separation of Diesel Fuels by Solid Phase Extraction and Identification of the Two- and Three-ring Aromatics by Capillary GC-MS', *J. High Resolut. Chromatogr.*, **14**(2), 91–98 (1991).
14. C.V. Cooper, 'GC-MS Analysis of Extracts of Workplace Air Samples for Nitrosamines', *Am. Ind. Hyg. Assoc. J.*, **48**(3), 265–270 (1987).
15. E.D. Pellizzari, J.E. Bunch, R.E. Berkley, J. McRae, 'Collection and Analysis of Trace Organic Vapor Pollutants in Ambient Atmospheres', *Anal. Lett.*, **9**(1), 45–63 (1976).
16. K.S. Chiu, K. Biemann, 'Structural Characterization of Polycyclic Aromatic Compounds by Combined GC-MS and GC-FTIR', *Anal. Chem.*, **56**, 1610–1615 (1984).
17. E.R. Dennedy, P.F. O'Connor, 'Application of Multidimensional GC-MS to the Determination of Glycol Ethers in Air', *J. Chromatogr.*, **522**, 303–313 (1990).
18. S. Dai, L. Zhang, Z. Bai, 'The Analysis of VOCs by GC/MS with Whole Column Cold Trapping on a Fused Silica Capillary Column in Indoor Environment', *Anal. Sci. Technol.*, **8**(4), 829–834 (1995).
19. R. Podeu, M. Einhaus, G. Rezniczek, 'Identification Von Inhalation Noxen am Arbeitsplatz mit Hilfe Von GC/MS', *Atemw-lungenkrkh*, **21**, S67–S69 (1995).
20. E.S. Lahaniatis, W. Bergheim, C. Rainer, 'Hazardous Halogenated Substances Formed During Combustion Processes', *Toxicol. Environ. Chem.*, **20–21**, 501–506 (1989).
21. E. Yrjanheikki, L. Pyy, E. Hakala, T. Lapinlampi, A. Lisko, K. Vahakangas, 'Exposure to Polycyclic Aromatic Hydrocarbons in New Cooking Plant', *Am. Ind. Hyg. Assoc. J.*, **56**, 782–787 (1995).
22. M. Maronj, A. Colombi, S. Cantoni, E. Ferioli, V. Foa, 'Occupational Exposure to Polychlorinated Biphenyls in Electrical Workers', *Br. J. Ind. Med.*, **38**, 49–54 (1981).
23. P.A. Smith, D.A. Gardner, D.B. Drown, G. Downs, W.W. Jederberg, W.W. Still, *Am. Ind. Hyg. Assoc. J.*, **58**, 868–875 (1997).
24. F. Mangani, L. Lattanzi, M. Maione, *Chromatographia*, **47**, 57–62 (1998).
25. G. Radolovich, D. Stewart, J. Pieri, M.J. Gooden, M. Chatfield, 'Development of a GCZ-MS/FPD System for the Simultaneous Analysis of VX, GB and HD', *Proc. ERDC Sci. Conf. Chem. Biol. Def. Res.*, ed. D.A. Berg, National Technical Information Service, Springfield, VA, 165–173, 1996.
26. E. Scobbie, D.W. Dabil, J.A. Groves, *Ann. Occup. Hyg.*, **42**, 45–59 (1998).
27. M. Yang, M. Koga, T. Katoh, T. Kawamoto, 'A Study for the Application of Urinary Naphthols, New Biomarkers for Airborne PAHs', *Arch. Environ. Toxicol.*, **36**, 99–108 (1999).
28. G. Grimmer, J. Jacob, J. Dettbarn, K.W. Naujack, 'Determination of Urinary Metabolites of Polycyclic Aromatic Hydrocarbons (PAH) for the Risk Assessment of PAH-exposed Workers', *Int. Arch. Occup. Environ. Health*, **69**, 231–239 (1997).
29. J.P. Buchet, J.P. Gennart, F. Mercado-Calderon, J.P. Delavignette, L. Cupers, R. Lauwerys, 'Evaluation of Exposure to Polycyclic Aromatic Hydrocarbons in a Coke Production and a Graphite Electrode Manufacturing Plant', *Br. J. Ind. Med.*, **49**, 761–768 (1992).
30. X. Guardino, C. Serra, J. Obiols, M.G. Rosell, M.J. Berenguer, F. Lopez, J. Brosa, 'Determination of DDT and Related Compounds in Blood Samples from Agricultural Workers', *J. Chromatogr. A*, **719**, 141–147 (1996).
31. H. Kontsas, C. Rosenberg, P. Pfaffli, P. Jappinen, 'GC-MS Determination of Chlorophenols in the Urine of Sawmill Workers with Past Use of Chlorophenol-containing Anti-stain Agents', *Analyst*, **120**(6), 1745–1749 (1995).
32. G.T. Vaughan, R.S. Kenyon, 'Monitoring for Occupational Exposure to 4,4′-Methylenebis(2-chloroaniline) by GC-MS Analysis of Hemoglobin Adducts, Blood, Plasma, and Urine', *J. Chromatogr. B*, **678**, 197–204 (1996).
33. P. Persson, M. Dalene, G. Skarping, M. Adamsson, L. Hagmar, 'Biological Monitoring of Occupational Exposure to Toluene Diisocyanate', *Br. J. Ind. Med.*, **50**, 1111–1118 (1993).
34. F.F. Hsu, V. Lakshmi, N. Rothman, V.D. Bhatnager, R.B. Hayes, R. Kashyap, D.J. Parikh, S.K. Kashyap, J. Turk, T. Zenser, B. Davis, 'Determination of Benzedrine, *N*-Acetylbenzidine, and *N,N′*-Diacetylbenzidine in Human Urine by Capillary GC/NICI-MS', *Anal. Biochem.*, **234**, 183–189 (1996).
35. K. Eriksson, J.-O. Levin, 'GC-MS Identification of Metabolites from α-Pinene in Human Urine after Occupational Exposure to Sawing Fumes', *J. Chromatogr. B*, **677**, 85–98 (1996).

36. G. Sabbioni, J. Wei, Y.-Y. Liu, 'Determination of Hemoglobin Adducts in Workers Exposed to 2,4,6-Trinitrotoluene', *J. Chromatogr. B*, **682**, 243–248 (1996).
37. G.R. Lambert, W.T. Padgett, M.H. George, K.T. Kitchin, S. Nesnow, 'Quantitative Analysis of Alachlor Protein Adducts by GC-MS', *Anal. Biochem.*, **268**, 289–296 (1999).
38. P.B. Farmer, E. Bailey, S.M. Gorf, M. Tornqvist, S. Osterman-Golkar, A. Kautiainen, D.P. Lewis-Enright, 'Monitoring Human Exposure to Ethylene Oxide by the Determination of Hemoglobin Adducts using GC-MS', *Carcinogenesis*, **7**(4), 637–640 (1986).
39. A. Ranasinghe, N. Scheller, K.Y. Wu, P.B. Upton, J.A. Swenberg, 'Application of GC/ECNCI-HRMS for Analysis of DNA and Protein Adducts', *Chem. Res. Toxicol.*, **11**, 520–526 (1998).
40. P. Ferranti, N. Sannolo, G. Mamone, I. Fiume, V. Carbone, M. Tornqvist, A. Bergman, A. Malorni, 'Structural Characterization by MS of Hemoglobin Adducts formed After In Vitro Exposure to Methyl Bromide', *Carcinogenesis*, **17**(12), 2661–2671 (1996).
41. D.L. Ashley, M.A. Bonin, F.L. Cardinali, J.M. McCraw, J.S. Holler, L.L. Needham, D.G. Patterson, 'Determining Volatile Organic Compounds in Human Blood from a Large Sample Population by using Purge and Trap GC/MS', *Anal. Chem.*, **64**, 1021–1029 (1992).
42. M. Coombs, V. Schillack, 'Determination of Trinitrotoluene and Metabolites in Urine by Means of GC-MS', *Int. Arch. Occup. Environ. Health*, **71**, S22–S25 (1998).
43. R. Joppich-Kuhn, R. Hanggi, P. Sagelsdorff, A.E. Smith, H.J. Weideli, M. Joppich, 'Determination of Dichlorobenzidine–Hemoglobin Adducts by GC/MS-NCI', *Int. Arch. Occup. Environ. Health*, **69**, 240–246 (1997).
44. H.-J.C. Chen, L. Zhang, J. Cox, J.A. Cunningham, F.-L. Chung, 'DNA Adducts of 2,3-Epoxy-4-hydroxynonanal: Detection of 7-(1′,2′-Dihydroxyheptyl)-3*H*-imidazo[2,1-*I*] Purine and 1,*N*-Ethenoadenine by GC/NICI-MS', *Chem. Res. Toxicol.*, **11**, 1474–1480 (1998).
45. D. Schutze, O. Sepai, J. Lewalter, L. Miksche, D. Henschler, G. Sabbioni, 'Biomonitoring of Workers Exposed to 4,4′-Methylenedianiline or 4,4′-Methylenediphenyl Diisocyanate', *Carcinogenesis*, **16**(3), 573–582 (1995).
46. P. Brunmark, M. Kalene, G. Skarping, 'GC/NICI-MS of Hydrolysed Human Urine and Blood Plasma for the Biomonitoring of Occupational Exposure to 4,4′-Methylenebisaniline', *Analyst*, **120**, 41–45 (1995).
47. M.W. Patrick, S. Shane, J.R. Froines, 'Determination of Hemoglobin Adduct Levels of the Carcinogen 2,4-Diaminotoluene using GC/EIPI-MS', *J. Chromatogr. B*, **667**, 166–172 (1995).
48. R.H. Hill, D.B. Shealy, S.L. Head, C.C. Williams, S.L. Bailey, M. Gregg, S.E. Baker, L.L. Needham, 'Determination of Pesticide Metabolites in Human Urine using an Isotope Dilution Technique and Tandem MS', *J. Anal. Toxicol.*, **19**, 323–329 (1995).
49. M. Bader, G. Lehnert, J. Angerer, 'GC/MS Determination of *N*-phenylvaline, a Possible Biomarker for Benzene Exposure in Human Hemoglobin by the *N*-alkyl Edman Method', *Int. Arch. Occup. Environ. Health*, **65**, 411–414 (1994).
50. S. Takahashi, M. Dagawa, K. Shiwaku, K. Matsubara, 'Determination of *S*-benzyl-*N*-acetyl-L-cysteine by GC/MS as a New Marker of Toluene Exposure', *J. Anal. Toxicol.*, **18**, 78–80 (1994).
51. K. Allam, S. Abdel-Baky, R.W. Giese, 'KO2 Chemical Transformation/MS Detection of Covalent Damage to the DNA of Cultured Human Lymphocytes Exposed to Benzo[*a*]pyrene', *Anal. Chem.*, **65**, 1723–1727 (1993).
52. G. Talaska, J.H. Roh, '32P-Postlabelling and MS Methods for Analysis of Bulky Polyaromatic Carcinogen-DNA Adducts in Humans', *J. Chromatogr.*, **580**, 293–323 (1992).
53. B.W. Day, S. Naylor, L.-S. Gan, Y. Sahali, T.T. Nguyen, P.L. Skipper, J.S. Wishnok, S.R. Tannenbaum, 'Molecular Dosimetry of Polycyclic Aromatic Hydrocarbon Epoxides and Diol Epoxides via Hemoglobin Adducts', *Cancer Res.*, **50**, 4611–4618 (1990).
54. W.M. Pierce, D.E. Nerland, 'Qualitative and Quantitative Analyses of Phenol Phenylglucuronide, and Phenylsulfate in Urine and Plasma by GC/MS', *J. Anal. Toxicol.*, **12**, 344–347 (1988).
55. P.B. Farmer, E. Bailey, S.M. Gorf, M. Tornqvist, S. Osterman-Golkar, A. Kautiainen, D.P. Lewis-Enright, 'Monitoring Human Exposure to Ethylene Oxide by the Determination of Hemoglobin Adducts using GC/MS', *Carcinogenesis*, **7**(4), 637–640 (1986).
56. E. Bailey, P.B. Farmer, I. Bird, J.H. Lamb, J.A. Peal, 'Monitoring Exposure to Acrylamide by the Determination of *S*-(2-Carboxyethyl) Cysteine in Hydrolyzed Hemoglobin by GC/MS', *Anal. Biochem.*, **157**, 241–248 (1986).
57. J. Angerer, C. Mannschreck, J. Gundel, 'Biological Monitoring and Biochemical Effect Monitoring of Exposure to Polycyclic Aromatic Hydrocarbons', *Int. Arch. Occup. Environ. Health*, **70**, 365–377 (1997).
58. R.W. Giese, M. Saha, S. Abdel-baky, K. Allam, 'Measuring DNA Adducts by GC/EC-MS Trace Organic Analysis', *Mass Spectrom.*, **271**, 505–523 (1997).
59. R.W. Giese, P. Vouros, 'Methods Development Toward the Measurement of Polyaromatic Hydrocarbon-DNA Adducts by MS', *Health Effects Institute Research Report*, Number 61, 1–36, 1993.
60. D.D. La, J.A. Swenberg, 'DNA Adducts: Biological Markers of Exposure and Potential Applications to Risk Assessment', *Mutat. Res.*, **365**, 129–146 (1996).
61. E. Kriek, M. Rojas, K. Alexandra, H. Bartsch, 'Polycyclic Aromatic Hydrocarbon–DNA Adducts in Humans: Relevance as Biomarkers for Exposure and Cancer Risk', *Mutat. Res.*, **400**, 215–231 (1998).

62. W.M.A. Niessen, *Liquid Chromatography–Mass Spectrometry*, 2nd edition (revised and expanded), Marcel Dekker, New York, 1999.
63. J.F. Garcia, D. Bacelo, 'LC-MS Interfacing Systems', *J. High Resolut. Chromatogr.*, **16**, 633–641 (1993).
64. P.J. Arpino, 'LC-MS, a Review', in *MS in the Biological Sciences*, ed. M.L. Gross, Kluwer Academic, The Netherlands, 253–267, 1992.
65. K. Tomer, C. Parker, 'Biochemical Application of LC-MS, *J. Chromatogr.*, **492**, 189–221 (1989).
66. D. Barcelo, 'Applications of LC-MS in Environmental Chemistry', *J. Chromatogr.*, **59**, 543 (1996).
67. A.L. Yergey, C.G. Edmonds, L.A.S. Lewis, M.L. Vestal, *LC/MS Techniques and Applications*, Plenum Press, London, 1990.
68. D.F. Parriott, *A Practical Guide to HPLC Detection*, Academic Press, San Diego, 1992.
69. G. Patonay, *HPLC Detection: Newer Methods*, VCH, Weinheim, 1992.
70. M.A. Brown, *LC-MS-Applications in Agricultural, Pharmaceutical, and Environmental Chemistry*, American Chemical Society, Washington, DC, 1990.
71. M.J. Suter, S. Riediker, W. Giger, 'Selective Determination of Aromatic Sulfonates in Landfill Leachates and Groundwater using Microbore LC Coupled with MS', *Anal. Chem.*, **71**, 897–904 (1999).
72. J. Ding, P. Vouros, 'Capillary Electrochromatography and Capillary Electrochromatography–MS for the Analysis of DNA Adduct Mixtures', *Anal. Chem.*, **69**, 379–384 (1997).
73. L. Leclercq, C. Laurent, E.D. Pauw, 'High-performance LC/Electrospray MS for the Analysis of Modified Bases in DNA', *Anal. Chem.*, **69**, 1952–1955 (1997).
74. P. Strickland, D. Kang, P. Sithisarankul, 'Polycyclic Aromatic Hydrocarbon Metabolites in Urine as Biomarkers of Exposure and Effect', *Environ. Health Perspect*, **104**, 927–932b (1996).
75. K. Vanhoutte, W.V. Dongen, E. Esmans, 'A Strategy for the Identification of 2′-Deoxynucleoside and 2′-Deoxynucleotide Adducts using Electrospray Tandem MS', *Eru. Mass Spectrom.*, **2**, 181–192 (1996).
76. H. Huan, J. Ahmedin, D. Connie, A. Steven, S.A. Barker, D.H. Swenson, J.C. Means, 'Analysis of DNA Adducts, *S*-[2-(*N*-guanyl)ethyl]glutathione by LC/MS and LC/Tandem MS', *Anal. Biochem.*, **265**, 139–150 (1998).
77. T.-Y. Yen, N.I. Christova-Gueoguieva, N. Scheller, S. Holt, J.A. Swenberg, M.J. Charles, 'Quantitative Analysis of the DNA Adduct *N*,-3-Ethenoguanine using LC/Electrospray Ionization MS', *J. Mass Spectrom.*, **31**, 1271–1276 (1996).
78. K. Vanhoutte, W.V. Dongen, H.I. Lemiere, E.L. Esmans, H.V. Onckelen, V. Eeckhout, R.E. Soest, A.J. Hudson, 'Development of a Nanoscale LC/Electrospray MS Methodology for the Detection and Identification of DNA Adducts', *Anal. Chem.*, **69**, 3161–3168 (1997).
79. R. Singh, G.M. Sweetman, P.B. Farmer, D.E. Shuker, K. Rich, 'Detection and Characterization of Two Major Ethylated Deoxyguanosine Adducts by HPLC/Electrospray MS, and ^{32}P-Postlabeling', *Chem. Res. Toxicol.*, **10**, 70–77 (1997).
80. N.Y. Tretyakova, S.-Y. Chiang, V. Walker, J.A. Swenber, 'Quantitative Analysis of 1,3-Butadiene-induced DNA Adducts In Vivo and In Vitro using LC/Electrospray Ionization Tandem MS', *J. Mass Spectrom.*, **33**, 363–376 (1998).
81. C. Minoia, R. Turci, C. Sottani, A. Schiavi, L. Perbellini, S. Angeleri, F. Draicchio, P. Apostoli, 'Application of HPLC/Tandem MS in Environmental and Biological Monitoring of Health Care Personnel Occupationally Exposed to Cyclophosphamide and Ifosfamide', *Rapid Commun. Mass Spectrom.*, **12**, 1485–1493 (1998).
82. A.A. Melikian, R. O'Connor, A.K. Prahalad, P. Hu, H. Li, M. Kagan, S. Thompson, 'Determination of the Urinary Benzene Metabolites *S*-phenylmercapturic Acid and *trans,trans*-Muconic Acid by LC/Tandem MS', *Carcinogenesis*, **20**(4), 719–726 (1999).
83. P. Ferranti, N. Sannolo, G. Mamone, F. Immacolata, V. Carbone, M. Tornqvist, A. Bergman, A. Malorni, 'Structural Characterization by MS of Hemoglobin Adducts Formed after In Vitro Exposure to Methyl Bromide', *Carcinogenesis*, **17**(12), 2661–2671 (1996).
84. J.D. Rosen, *Applications of New Mass Spectrometry Techniques in Pesticide Chemistry*, John Wiley & Sons, New York, 1987.
85. J. White, R.H. Brown, M.R. Clench, 'Particle Beam Liquid Chromatography/Mass Spectrometry Analysis of Hazardous Agricultural and Industrial Chemicals', *Rapid Commun. Mass Spectrom.*, **11**, 618–623 (1997).
86. D. Karlsson, 'Determination of Complex Mixtures of Airborne Isocyanates and Amines. Part 5: Determination of Low Molecular Weight Aliphatic Isocyanates as Dibutylamine Derivatives', *Analyst*, **123**, 1507–1512 (1998).
87. H. Tinnerber, M. Spanne, M. Dalene, G. Skarping, 'Determination of Complex Mixtures of Airborne Isocyanates and Amines. Part 3: Methylenediphenyl Diisocyanate, Methylenediphenylamino Isocyanate and Methylenediphenyl-diamine and Structural Analogs after Thermal Degradation of Polyurethane', *Analyst*, **122**, 275–278 (1997).
88. H. Tinnerberg, D. Karlsson, D. Dalene, M. Skarping, *J. Liq. Chromatogr. Relat. Technol.*, **20**, 2207–2219 (1997).
89. A. Montaser, *Inductively Coupled Plasma MS*, Wiley-VCH, New York, 1998.
90. A.L. Gray, 'Solid Sample Introduction by Laser Ablation for ICP Source MS', *Analyst*, **110**, 551–556 (1985).
91. P. Schramel, I. Wendler, J. Angerer, 'The Determination of Metals (Antimony, Bismuth, Lead, Cadmium, Mercury, Palladium, Platinum, Tellurium, Thallium, Tin and Tungsten) in Urine Samples by ICP-MS', *Int. Arch. Occup. Environ. Health*, **69**, 219–223 (1997).

92. P. Apostoli, P.E. Brunelli, L. Alessio, 'Multiple Exposure to Metals in Eight Types of Welding', *G. Ital. Med. Lav. Frg.*, **19**(2), 8–14 (1997).
93. Z. Karpas, L. Halicz, J. Roiz, R. Marko, E. Katorza, A. Lorber, Z. Goldbart, 'ICP-MS as a Simple, Rapid and Inexpensive Method for Determination of Uranium in Urine and Fresh Water', *Health Phys.*, **71**(6), 879–885 (1996).
94. A. Schutz, I.A. Bergdahl, A. Ekholm, S. Skerfving, 'Measurement of ICP-MS of Lead in Plasma and Whole Blood of Lead Workers and Controls', *Occup. Environ. Med.*, **53**, 736–740 (1996).
95. O.A. Vita, K.C. Mayfield, 'Analysis of Urine for U-235 and U-238 by ICP-MS', in *Application of ICP-MS to Radionuclide Determinations*, ed. R.W. Morrow, J.S. Crain, ASTM Special Technical Publication (PCN, 04-012910-35), West Constohocken, PA, 1995.
96. P. Apostoli, S. Giusti, D. Bartoli, A. Perico, P. Bavazzano, L. Alessio, 'Multiple Exposure to Arsenic, Antimony, and Other Elements in Art Glass Manufacturing', *Am. J. Ind. Med.*, **34**, 65–72 (1998).
97. R.J. Cotter, *Time-of-Flight Mass Spectrometry Instrumentation and Applications in Biological Research*, Am. Chem. Soc., Washington, DC, 1997.
98. P.L. Ross, P.A. Davis, P. Belgrader, 'Analysis of DNA Fragments from Conventional and Microfabricated PCR Devices using Delayed Extraction MALDI/TOF-MS', *Anal. Chem.*, **70**, 2067–2078 (1998).
99. D.Z. Bezabeh, T.M. Allen, E.M. McCauley, P.B. Kelly, 'Negative Ion Laser Desorption Ionization TOF-MS of Nitrated PAH', *J. Am. Soc. Mass Spectrom.*, **8**, 630–636 (1997).
100. S.M. Hankin, P. John, G. Smith, 'Laser TOF-MS of PAH–Picrate Complexes, *Anal. Chem.*, **69**, 2927–2930 (1997).
101. S. Hankin, P. John, 'Laser TOF-MS Analysis of PAHs on Single Diesel Particulates', *Anal. Chem.*, **71**, 1100–1104 (1997).
102. M.J. Lee, S. Monte, J. Sanderson, N.J. Haskins, 'A Preliminary Study using Fast Gradient LC Coupled to a Q-TOF/MS', *Rapid Commun. Mass Spectrom.*, **13**, 216–221 (1999).
103. F.G. Hanisch, B.N. Green, R. Bateman, J. Peter-Katalinic, 'Localization of *o*-Glycosylation Sites of MUC1 Tendem Repeats by Q-TOF ESI Mass Spectrometry', *J. Mass Spectrom.*, **33**, 358–362 (1998).
104. D.L. Deforce, J. Raymackers, L. Meheus, V. Wijnendaele, A.D. Leenheer, V.D. Eeckhout, 'Characterization of DNA Oligonucleotides by Coupling of Capillary Zone Electrophoresis to Electrospray Ionization Q-TOF-MS', *Anal. Chem.*, **70**, 3060–3068 (1998).
105. J.I. Goldstein, D.E. Newbury, P. Echlin, D.C. Joy, A.D. Romig, C.E. Lyman, C. Fiori, E. Lifshin, *Scanning Electron Microscopy and X-ray Microanalysis*, Plenum, New York, 1–820, 1992.
106. R.E. Lee, *Scanning Electron Microscopy and X-ray Microanalysis*, PTR Prentice Hall, Englewood Cliffs, NJ, 1–458, 1993.
107. P. Ingram, J.D. Shelburne, A. LeFurgey, 'Principles and Instrumentation', in *Microprobe Analysis in Medicine*, eds. P. Ingram, J.D. Shelburne, V.L. Roggli, Hemisphere Publishing, New York, 1–34, 1989.
108. K.F.J. Heinrich, *Electron Beam X-Ray Microanalysis*, Van Nostrand Reinhold, New York, 1–578, 1981.
109. J.J. Friel, *X-Ray and Image Analysis in Electron Microscopy*, Princeton-Gamma-Tech, Princeton, NJ, 1–97, 1998.
110. R.J. Lee, J.F. Kelley, 'Overview of SEM-based Automated Image Analysis', *Scanning Electron Microsc.*, **I**, 303–310 (1980).
111. J.D. Shelburne, P. Ingram, A. LeFurgey, R. Herlong, 'Preparatory Techniques', in *Microprobe Analysis in Medicine*, eds. P. Ingram, J.D. Shelburne, V.L. Roggli, Hemisphere Publishing, New York, 35–53, 1989.
112. P.B. DeNee, 'Identification and Analysis of Particles in Biological Tissue using SEM and Related Techniques', *Scanning Electron Microsc.*, **I**, 461–468 (1976).
113. R.S. Thomas, J.R. Hollahan, 'Use of Chemically-reactive Gas Plasmas in Preparing Specimens for Scanning Electron Microscopy and Electron Probe Microanalysis', *Scanning Electron Microsc.*, **I**, 83–92 (1974).
114. L.E. Stettler, D.H. Groth, S.F. Platek, 'Automated Characterization of Particles Extracted from Human Lungs: Three Cases of Tungsten Carbide Exposure', *Scanning Electron Miscrosc., I*, 439–448 (1983).
115. J.P. Mastin, L.E. Stettler, J.D. Shelburne, 'Quantitative Analysis of Particulate Burden in Lung Tissue', *Scanning Microsc.*, **2**(3), 1613–1629 (1988).
116. P.G. Coin, V.L. Roggli, A.R. Brody, 'Deposition, Clearance, and Translocation of Chrysotile Asbestos from Peripheral and Central Regions of the Rat Lung', *Environ. Res.*, **58**, 97–116 (1992).
117. D. Baker, K.G. Kupke, P. Ingram, V.L. Roggli, J.D. Shelburne, 'Microprobe Analysis in Human Pathology', *Scanning Electron Microsc.*, **II**, 659–680 (1985).
118. L.E. Stettler, S.F. Platek, R.D. Riley, J.P. Mastin, S.D. Simon, 'Lung Particulate Burdens of Subjects from the Cincinnati, Ohio Urban Area', *Scanning Microsc.*, **5**(1), 85–94 (1991).
119. L.E. Stettler, D.H. Groth, S.F. Platek, J.R. Burg, 'Particulate Concentrations in Urban Lungs', in *Microprobe Analysis in Medicine*, eds. P. Ingram, J.D. Shelburne, V.L. Roggli, Hemisphere Publishing, New York, 133–146, 1989.
120. A. Churg, B. Wiggs, 'Mineral Particles, Mineral Fibers, and Lung Cancer', *Environ Res.*, **37**, 364–372 (1985).
121. A. Churg, B. Wiggs, 'Types, Numbers, Sizes, and Distribution of Mineral Particles in the Lungs of Urban Male Cigarette Smokers', *Environ. Res.*, **42**, 121–129 (1987).
122. L. Paoletti, D. Batisti, S. Caiazza, M.G. Petrelli, F. Taggi, L. De Zorzi, M.A. Dina, G. Donelli, 'Mineral Particles

in the Lungs of Subjects Resident in the Rome Area and Not Occupationally Exposed to Mineral Dust', *Environ. Res.*, **44**, 18–28 (1987).

123. J.L. Abraham, B.R. Burnett, A.L. Hunt, 'Development and use of a Pneumoconiosis Database of Human Pulmonary Inorganic Particulate Burden in over 400 Lungs', *Scanning Microsc.*, **5**(1), 95–108 (1991).
124. J.L. Abraham, B.R. Burnett, 'Quantitative In Situ Analysis of Inorganic Particulate Burden in Tissue Sections–an Update', in *Microprobe Analysis in Medicine*, eds. P. Ingram, J.D. Shelburne, V.L. Roggli, Hemisphere Publishing, New York, 111–131, 1989.
125. J.L. Abraham, B.R. Burnett, 'Quantitative Analysis of Inorganic Particulate Burden In Situ in Tissue Sections', *Scanning Electron Microsc.*, **II**, 681–696 (1983).
126. L. LeBouffant, H. Daniel, J.C. Martin, S. Bruyere, 'Effects of Impurities and Associated Minerals on Quartz Toxicity', *Ann. Occup. Hyg.*, **26**, 625–634 (1982).
127. W. Kriegseis, A. Scharmann, 'Specific Harmfulness of Respirable Dusts from West German Coal Mines V: Influence of Mineral Surface Properties', *Ann. Occup. Hyg.*, **26**, 511–525 (1982).
128. J.C. Harrison, P.S. Brower, M.D. Attfield, C.B. Doak, M.J. Keane, R.L. Grayson, W.E. Wallace, 'Surface Composition of Respirable Silica Particles in a Set of US Anthracite and Bituminous Coal Mine Dusts', *J. Aerosol Sci.*, **28**, 689–696 (1997).
129. W.E. Wallace, M.J. Keane, 'Differential Surface Composition Analysis by Multiple-voltage Electron Beam X-ray Spectroscopy', US Patent 5210414, US Government Printing Office, Washington, DC, 1993.
130. W.E. Wallace, J. Harrison, M.J. Keane, P. Bolsaitis, D. Eppelsheimer, J. Poston, S. Page, 'Clay Occlusion of Respirable Quartz Particles Detected by Low Voltage Scanning Electron Microscopy–X-ray Analysis', *Ann. Occup. Hyg.*, **34**, 195–204 (1990).
131. V.E. Cosslett, R.N. Thomas, 'Multiple Scattering of 5–30 keV Electrons in Evaporated Metal Films', *Br. J. Appl. Phys.*, **15**, 883–907 (1944).
132. J.I. Goldstein, H. Yakowitz, *Practical Scanning Electron Microscopy, Electron and Ion Microprobe Analysis*, Plenum Press, New York, 83, 1975.
133. K.F.J. Heinrich, *Electron Beam X-Ray Microanalysis*, Van Nostrand Reinhold, Scarborough, CA, 240, 1981.
134. W.J. Veigele, 'Attenuation Cross-sections in cm^2/g at Selected Wavelengths for 94 Elements', in *Handbook of Spectroscopy*, ed. J.W. Robinson, CRC Press, Boca Raton, FL, Vol. 1, 1974.
135. W.E. Wallace, M.J. Keane, J.C. Harrison, J.W. Stephens, P.S. Brower, R.L. Grayson, M.D. Attfield, 'Surface Properties of Silica in Mixed Dusts', in *Silica and Silica-Induced Lung Diseases*, eds. V. Castranova, V. Vallyathan, W.E. Wallace, CRC Press, Boca Raton, FL, 1996.
136. W.E. Wallace, J.C. Harrison, R.L. Grayson, M.J. Keane, P. Bolsaitis, R.D. Kennedy, Q.A. Wearden, M.D. Attfield, 'Aluminosilicate Surface Contamination of Respirable Quartz Particles from Coal Mine Dusts and from Clay Works Dusts', *Ann. Occup. Hyg.*, **38**, 439–445 (1994).
137. K.D. Childs, D.L.A. LaVanier, P.M. Lindley, B.W. Scheuler, G. Mulholland, A.C. Diebold, 'Comparison of Submicrometer Particle Analysis by Auger Electron Microscopy, Time-of-Flight Secondary Ion Mass Spectrometry, and Secondary Electron Microscopy with Energy-dispersive X-ray Spectroscopy', *J. Vac. Sci. Technol., A*, **14**, 2392–2404 (1996).
138. A. Grekula, E. Ristolainen, V.P. Tanninen, H.K. Hyvarinen, P.L. Kalliomaki, 'Surface and Bulk Chemical Analysis on Metal Aerosols Generated by Manual Metal Arc Welding of Stainless Steel', *J. Aerosol Sci.*, **17**, 1–9 (1995).
139. J.W. Stephens, J.C. Harrison, W.E. Wallace, 'Correlating Auger Electron Spectroscopy with Scanning Electron Microscopy–Energy Dispersive Spectroscopy for the Analysis of Respirable Particles', *Scanning*, **20**, 302–310 (1998).
140. D.L. Tsalev, Z.K. Zaprianov, *Atomic Absorption Spectrometry in Occupational and Environmental Health Practice*, CRC Press, Florida, Vols I & II, 1984.
141. S.J. Haswell, *Atomic Absorption Spectrometry-Theory, Design and Applications*, Oxford, New York, 1991.
142. A. Sanz-Medel, *Flow Analysis with Atomic Spectrometric Detectors*, Lausanne, New York, 1999.
143. D.L. Tsalev, *Atomic Absorption Spectrometry in Occupational and Environmental Health Practice*, CRC Press, New York, Vol. III, 1995.
144. P.J. Tsai, J.H. Vincent, G.A. Wahl, G. Maldonado, 'Worker Exposures to Inhalable and Total Aerosol During Nickel Alloy Production', *Ann. Occup. Hyg.*, **40**(6), 651–659 (1996).
145. W. Torjussen, F.-M.S. Haug, I. Andersen, 'Concentration and Distribution of Heavy Metals in Nasal Mucosa of Nickel-exposed Workers and of Controls', *Acta Otolaryngol.*, **86**, 449–463 (1978).
146. C.W. Pilger, I. Broder, Determination of Metals on Airborne Particulates by AAS, Adapted from Method P & CAM 173, *NIOSH Manual of Analytical Methods*, Vol. I, 1977, and Method ID-121, OSHA, 1985.
147. D. Bellido-Milla, M.P. Hernandez-Artiga, J.L. Hidalgo-Hidalgo, J.A. Murioz-Leyva, 'Analytical Study of Hygiene Hazards Involved in Naval Industry Welding Processes', *Appl. Occup. Environ. Hyg.*, **10**(11), 921–925 (1995).
148. J.L. Burguera, M. Burguera, C.E. Rondon, C. Rivas, J.A. Burguera, O.M. Alarcon, 'Determination of Lead in Hair of Exposed Gas Station Workers and in Unexposed Adults by Microwave-aided Dissolution of Samples and FI/AAS', *J. Trace Elem. Electrolytes Health Dis.*, **1**, 21–26 (1987).
149. A. Othman, 'Preliminary Investigation of the Lead Level in Whole Blood of Normal and Occupationally Exposed

Populations in Damascus City', *Sci. Tot. Environ.*, **43**, 141–148 (1985).
150. D.L. Tsalev, F.J. Langmyhr, N. Gunderson, 'Direct Atomic Absorption Spectrometric Determination of Manganese in Whole Blood of Unexposed Individuals and Exposed Workers in a Norwegian Manganese Alloy Plant', *Bull. Environ. Contam. Toxicol.*, **17**(6), 660–666 (1977).
151. H.B. Rollin, C.M.C.A. Nogueira, 'Identification of Aluminium Fractions in Serum using the Techniques of HPLC, Ultrafiltration and Zeeman AAS', *Eur. J. Clin. Chem. Clin. Biochem.*, **35**, 215–222 (1997).
152. H.J. Gitelman, F.R. Alderman, M. Kurs-Lasky, H.E. Rockette, 'Serum and Urinary Aluminium Levels of Workers in the Aluminium Industry', *Am. Occup. Hyg.*, **39**(2), 181–191 (1995).
153. H.B. Rollin, P. Theodorou, T.A. Kilroe-Smith, 'The Effect of Exposure to Aluminium on Concentrations of Essential Metals in Serum of Foundry Workers', *Br. J. Ind. Med.*, **48**, 243–246 (1991).
154. S. Letzel, K.H. Schaller, J. Angerer, H. Drexler, A. Weber, K. Schmid, D. Weltle, 'Biological Monitoring of Occupational Aluminium Powder Exposure', *Occup. Hyg.*, **3**, 271–280 (1996).
155. M.A. White, S.A. O'Hagan, A.L. Wright, H.K. Wilson, 'The Measurement of Salivary Cadmium by Electrothermal AAS and its use as a Biological Indicator of Occupational Exposure', *J. Expos. Anal. Environ. Epid.*, **2**(2), 195–206 (1992).
156. T.V. Abernathy, K.B. Lee, R.J. Parker, E. Reed, 'The Measurement of Cadmium in Biological Materials using Graphite Furnace AAS with Zeeman Background Correction', *Oncol. Rep.*, **6**, 155–159 (1999).
157. S.A. Armitage, M.A. White, H.K. Wilson, 'The Determination of Silver in Whole Blood and its Application to Biological Monitoring of Occupationally Exposed Groups', *Am. Occup. Hyg.*, **40**(3), 331–338 (1996).
158. M.A. White, A.M. Boran, *Urinary Excretion of Nickel in Nickel-Chromium Electroplates, Nickel and Human Health: Current Perspectives*, eds. E. Nieboer, J.O. Nriagu, John Wiley & Sons, New York, 1992.
159. G.E. Jensen, J.M. Christensen, O.M. Poulsen, 'Occupational and Environmental Exposure to Arsenic-increased Urinary Arsenic Level in Children', *Sci. Tot. Environ.*, **107**, 169–177 (1991).
160. O.J. Blas, S.V. Gonzalez, R.S. Rodriguez, J.H. Mendez, *Determination and Speciation of Arsenic in Human Urine by Ion-exchange Chromatography/FIA with Hydride Generation/AAS*, ed. C.E. Van Hall, ASTM, Philadalphia, 302–323, 1994.
161. M.D. Martin, T. Mccann, C. Naleway, J.S. Woods, B.G. Leroux, A.M. Bollen, 'The Validity of Spot Urine Samples for Low-level Occupational Mercury Exposure Assessment and Relationship to Porphyrin and Creatinine Excretion Rates', *J. Pharmacol. Exp. Ther.*, **277**, 239–277 (1996).
162. M.A. Seckin, S. Aygun, Y. Ataman, 'Determination, Speciation of Mercury in a Dental Work Place by Cold Vapor AAS and Gas–Liquid Chromatography', *Int. J. Environ. Anal. Chem.*, **26**, 1–17 (1986).
163. G.L. Moore, *Introduction to ICP-AES-Analytical Spectroscopy Library*, New York, Vol. 3, 1989.
164. F.B. Lo, D.K. Arai, 'Biological Monitoring of Toxic Metals in Urine by Simultaneous ICP-AES', *Am. Ind. Hyg. Asoc. J.*, **50**(5), 245–251 (1989).
165. M. Kudo, Y. Aizawa, T. Takata, 'Biological Exposure Evaluation and Hair Analysis in Workers Handling Chromium Compounds', *Kitasato Lgaku*, **18**(1), 39–47 (1988).
166. K.W. Olson, W.J. Haas, V.A. Fassel, 'Analyses of Airborne Particulates and Human Urine by ICP-AES', *DHEW (NIOSH)*, Publication No. 79–110, NIOSH, Cincinnati, OH, 117, 1978.
167. A. Franzlau, 'Use of ICP-AES in Screening for Trace Metal Exposures in an Industrial Population', *Environ. Res.*, **46**(1), 15–24 (1988).
168. R.D. Hull, 'Multielement Analyses of Industrial Hygiene Samples', *American Industrial Hygiene Association Conference*, May 25–29, 1981.
169. M. Harper, C.R. Glowacki, P.R. Michael, 'Industrial Hygiene', *Anal. Chem.*, **69**, 307R–327R (1997).
170. P.M. Eller, M.E. Cassinelli (eds), *NIOSH Manual of Analytical Methods*, 4th edition, U.S. DHHS, PHS, CDC, NIOSH, DPSE, Cincinnati, OH, 1994.
171. J.A. Dean, *Analytical Chemistry Handbook*, McGraw-Hill, NY, 1995.
172. J.E. Davis, E.R. Stevens, D.C. Staff, *Bull. Environ. Contam. Toxicol.*, **31**, 631 (1983).
173. W.F. Durham, H.R. Wolfe, J.W. Elliott, 'Absorption and Excretion of Parathion by Spraymen', *Arch. Environ. Health*, **24**, 381 (1972).
174. J.E. Peterson, in *Industrial Hygiene Aspects of Plant Operations*, eds. L.J. Cralley, L.V. Cralley, MacMillan, New York, 222–247, Vol. 2, 1984.
175. N.S. Ham, A. Walsh, *Spectrochim. Acta*, **12**, 88 (1958).
176. G.W. King, in *Spectroscopy and Molecular Structure*, ed. R.J.H. Clark, Winston, New York, 1964.
177. M. Tasumi, M. Fujiwara, 'Vibrational Spectra of Chlorophylls', in *Advances in Spectroscopy*, eds. L.J. Cralley, R.E. Hester, John Wiley & Sons, New York, 1987.
178. M. Manfait, T. Theophanides, 'Drug–Nucleic Acid Interactions', in *Advances in Infrared and Raman Spectroscopy*, eds. L.J. Cralley, R.E. Hester, John Wiley, New York, Vol. 13, 1986.
179. W. Levin, 'Vibrational Spectroscopy in Membrane Assemblies', in *Advances in Infrared and Raman Spectroscopy*, eds. L.J. Cralley, R.E. Hester, John Wiley, New York, Vol. 11, 1984.
180. Y. Ozaki, K. Iriyama, 'Potential of Raman Spectroscopy in Medical Science', in *Laser Light Scattering Spectroscopy of Biological Objects*, eds. J. Stepanek, P. Anzenbacher, B. Sedlacek, Elsevier, Amsterdam, 1987.

181. V.A. Maroni, J.R. Ferraro, in *Practical Fourier Transform Infrared and Raman Spectroscopy-Industrial Laboratory Chemical Analysis*, eds. J.R. Ferraro, K. Krishman, Academic Press, San Diego, 1990.
182. National Institute for Occupational Safety and Health, *NIOSH Manual of Analytical Methods*, ed. P. Eller, Cincinnati, Ohio, 1986.
183. C.T. Helmers, 'Industrial Central Atmospheric Monitor', *Sensors*, **2**, 20–25 (1984).
184. B.S. Samimi, 'Calibration of MIRAN Gas Analyzer: Extent of Vapor Loss within a Closed Loop Calibration System', *Am. Ind. Assoc. J.*, **44**, 40–45 (1983).
185. H. Xiao, S.P. Levine, J.B. D'Arcy, G. Kinnes, D. Almaguer, 'Comparison of the Fourier Transform Infrared (FTIR) Spectrophotometer and the Miniature Infrared Analyzer (MIRAN) for the Determination of Trichloroethylene (TCE) in the Presence of Freon-133 in Workplace Air', *Am. Ind. Hyg. Assoc. J.*, **7**, 395–401 (1990).
186. C.R. Strang, S.P. Levine, W.F. Herget, 'Evaluation of the Fourier Transform Infrared (FTIR) Spectrometer as a Quantitative Air Monitor for Semiconductor Manufacturing Process Emissions', *Am. Ind. Hyg. Assoc. J.*, **50**, 70–77 (1989).
187. E.R. Stephens, W.E. Scott, P.L. Hanst, R.C. Doerr, 'Recent Developments in the Study of the Organic Chemistry of the Atmosphere', *J. Air Pollut. Control. Assoc.*, **6**, 159–165 (1956).
188. E.C. Tuazon, R.A. Graham, A.M. Winer, R.R. Easton, J.N. Pitts, P.L. Hanst, 'A Kilometer Pathlength Fourier-transform Infrared System for the Study of Trace Pollutants in Ambient and Synthetic Atmosphere', *Atmos. Environ.*, **12**, 865–875 (1978).
189. W.F. Herget, S.P. Levine, 'Fourier Transform Infrared (FTIR) Spectroscopy for Monitoring Semiconductor Process Gas Emissions', *Appl. Ind. Hyg.*, **1**, 110–112 (1986).
190. J.E. Sigsby, A. MaCarver, R. Snow, *Evaluation of a FTIR Mobile Source Measurement System*, EPA 600/S3/036, EPA, Washington, DC, 1989.
191. P.L. Hanst, W.E. Wilson, R.K. Patterson, B.W. Gay, L.W. Chaney, C.S. Burton, *A Spectroscopic Study of California Smog*, EPA 650/4-75-006, EPA, Washington, DC, 1975.
192. J.M. Heuss, W.A. Glasson, 'Hydrocarbon Reactivity and Eye Irritation', *Environ. Sci. Technol.*, **2**, 1109–1116 (1968).
193. G.M. Russwurm, R.H. Kagann, O.A. Simpson, W.A. MaClenny, W.F. Herget, 'Long-path FTIR Measurements of Volatile Organic Compounds in an Industrial Setting', *J. Air Waste Manag. Assoc.*, **41**, 1062–1066 (1991).
194. G.M. Russwurm, W.A. MaClenny, 'A Comparison of FTIR Open Path Ambient Data with Method TO-14 Canister Data', in *Proceeding of International Conference on Optical Remote Sensing*, Air and Waste Management Association, Pittsburgh, PA, 248–253, 1990.
195. W.F. Herget, J.D. Brasher, 'Remote Measurement of Gaseous Pollutant Concentrations using a Mobile Fourier Transform Interferometer System', *Appl. Opt.*, **18**, 3404–3420 (1979).
196. H. Xiao, S.P. Levine, J.B. D'Arcy, R. Spear, T. Pritchett, 'A Transportable Remote Sensing, Infrared Air-monitoring System', *Am. Ind. Hyg. Assoc. J.*, **52**, 449–457 (1991).
197. O.A. Simpson, R.H. Kagann, 'Measurement of Emissions at a Chemical Wastewater Treatment Site with an Open Path Remote Fourier Transform Interferometer', in *Proceeding of International Conference on Optical Remote Sensing*, Air and Waste Management Association, Pittsburgh, PA, 937–939, 1990.
198. L.T. Tso, W.C. Liao, S.I. Chang, 'Portable Long Open Path FTIR Applied in In Situ Measurement of Trace Gases of Ambient Air Pollution', in *Proceeding of International Conference on Optical Remote Sensing*, Air and Waste Management Association, Pittsburgh, PA, 95–106, 1992.
199. U.S. Mine Safety and Health Administration, 'Infrared Determination of Quartz in Respirable Coal Mine Dust', *Chemical Method No P-7*, 19, 1989.
200. D.P. Tuchman, *Research Toward Effects of Quartz Dust on Filters using FTIR Spectroscopy*, US Bureau of Mines, Washington, DC, 1–17, 1992.
201. C. Lorberau, 'Investigation of the Determination of Respirable Quartz on Filter Media using Fourier Transform Infrared Spectrophotometry', *Appl. Occup. Environ. Hyg.*, **5**, 348–350 (1990).
202. C.C. Anderson, 'Collaborative Tests of Two Methods for Determining Free Silica in Airborne Dust', in *NIOSH-210-79-0059*, Washington, DC, 157, 1983.
203. National Institute for Occupational Safety and Health, 'Development of Respirable Pure Quartz and Cristobalite Calibration Standard Reference Materials with known Particle Size Distribution', in *NIOSH-IA-80-36*, Washington, DC, 1–15, 1984.
204. H.K. Beard, R.R. Brown, H. Muir, 'Immunochemical Localization of Collagen Types and Proteoglycan in Pig Invertebral Discs', *Immunology*, **41**, 491 (1980).
205. R. Ganesh, L.J. Striker, L.D. Hudson, G.E. Striker, 'Extracellular Matrix in Normal and Fibrotic Human Lungs', *Am. Rev. Respir. Dis.*, **131**, 281–289 (1985).
206. L. Yurui, H. Xun, Y. Zhu, 'Studies on Structural Changes of Collagen in Silicosis', *Biomed. Environ. Sci.*, **7**, 302–306 (1994).
207. A. Leesmith, 'Infrared Spectroscopy', in *Practical Handbook of Spectroscopy*, ed. I.W. Robinson, CRC Press, Boca Raton, FL, 579, 1991.
208. J.J. Barrett, *Appl. Spectrosc. Rev.*, **21**, 419 (1985).
209. G.H. Atkinson, in *Advances in Infrared and Raman Spectroscopy*, eds. R.J.H. Clark, R.E. Hester, John Wiley, New York, Vol. 9, 1982.

210. L.M. Proniewicz, I.R. Paeng, K. Nakamoto, *J. Am. Chem. Soc.*, **113**, 3294 (1991).
211. D.M. Dams, P.D. Hatton, A.C. Shaw, T.K. Tan, *J. Chem. Soc. Chem. Commun.*, 226 (1981).
212. B. Schrader, in *Practical Fourier Transform Infrared and Raman Spectroscopy-industrial Laboratory Chemical Analysis*, eds. J.R. Ferraro, K. Krishman, Academic Press, San Diego, 167–202, 1990.
213. R.L. Garrell, 'Surface-enhanced Raman Spectroscopy', *Anal. Chem.*, **61**, 401 (1989).
214. R.P. Cooney, M.R. Mahoney, A.J. McQuillan, in *Advances in Infrared and Raman Spectroscopy*, eds. R.J.H. Clark, R.E. Hester, Heyden, London, Vol. 9, 1982.
215. J.M. Lerner, F. Adar, *Laser Focus World*, Pennwell Publishing, Feb., 1989.
216. F.F.M.D. Mul, H. Buiteveld, J. Lankester, J. Mud, J. Greve, 'Raman Microspectroscopy in Human Pathology', *Hum. Pathol.*, **15**, 1062–1068 (1984).
217. G.L. Rosasco, J.J. Blaha, 'Raman Microprobe Spectra and Vibrational Assignments of Talo', *Appl. Spectrosc.*, **34**, 140 (1980).
218. R.J. Capwell, F. Spagnolo, M.A. Desesa, 'A Rapid Determination of Low Concentrations of Anatase in Rutile TiO_2 Pigments by Raman Spectroscopy', *Appl. Spectrosc.*, **26**, 537 (1972).
219. S.K. Sharma, J.F. Mammone, M.F. Nicol, 'Raman Investigation of Ring Confirmations in Vitreous Silica', *Nature*, **292**, 141 (1981).
220. H. Buiteveld, F.F.M.D. Mul, J. Mud, 'Identification of Inclusions in Lung Tissue with a Raman Microprobe', *Appl. Spectrosc.*, **38**, 304 (1984).
221. K. Ashley, 'Electroanalytical Applications in Occupational and Environmental Health', *Electroanalysis*, **6**, 805 (1994).
222. M.L. Woebkenberg, C.S. McCammon, 'Direct-reading Gas and Vapor Instruments', in *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*, 8th edition, eds. B.S. Cohen, S.V. Hering, American Conference of Governmental Industrial Hygienists (ACGIH), Cincinnati, OH, 1995.
223. L.A. Todd, 'Direct-reading Instrumental Methods for Gases, Vapors, and Aerosols', in *The Occupational Environment – Its Evaluation and Control*, ed. S.R. DiNardi, American Industrial Hygiene Association, Fairfax, VA, 1997.
224. C.R. Strang, S.P. Levine, 'The Limits of Detection for the Monitoring of Semiconductor Manufacturing Gas and Vapor Emissions by Fourier Transform Infrared (FTIR) Spectroscopy', *Am. Ind. Hyg. Assoc. J.*, **50**, 78 (1989).
225. W.M. Draper, K. Ashley, C.R. Glowacki, P.R. Michael, 'Industrial Hygiene Chemistry: Keeping Pace with Rapid Change in the Workplace', *Anal. Chem.*, **71**, 33R (1999).
226. J.E. Yocom, S.M. McCarthy, *Measuring Indoor Air Quality*, Wiley, New York, 1991.
227. C.H. Lindh, B.A.G. Joensson, H.E. Wilander, 'Direct Measurement of Hexahydrophthalic Anhydride in Workplace Air with a Transportable Fourier Transform Infrared Spectrometer', *Am. Ind. Hyg. Assoc. J.*, **57**, 832 (1996).
228. V.D. Makepeace, C.W. Chase, C.T. Chaffin, T.L. Marshall, P.T. Jaakola, R.M. Hoffman, W.G. Fateley, *Mikrochim. Acta, Suppl.*, **14**, 563 (1997).
229. ASTM D6348, 'Standard Test Method for Determination of Gaseous Compounds by Extractive Direct Interface Fourier Transform Infrared (FTIR) Spectroscopy', in *Annual Book of ASTM Standards*, American Society for Testing and Materials (ASTM), West Conshohocken, PA, Vol. 11.03, 1998.
230. M. Simonde, H.K. Xiao, S.P. Levine, 'Optical Remote Sensing for Air Pollutants–Review', *Am. Ind. Hyg. Assoc. J.*, **55**, 953 (1994).
231. L.A. Todd, 'Evaluation of an Open-path Fourier Transform Infrared Spectrophotometer using an Exposure Chamber', *Appl. Occup. Environ. Hyg.*, **11**, 1327 (1996).
232. NIST, *SRM Quarterly: Standard Reference Materials Program*, National Institute of Standards and Technology, Gaithersburg, MD, 1999.
233. J. Janata, 'Optical Sensors', in *Principles of Chemical Sensors*, Plenum, New York, 241–284, 1989.
234. J.A. Hodgeson, 'Chemiluminescent Techniques for Air Pollution Monitoring', *Toxicol. Environ. Chem. Rev.*, **11**, 81 (1974).
235. USEPA, *Compendium of Methods for Determination of Airborne Pollutants in Indoor Air*, US Environmental Protection Agency, Research Triangle Park, NC, 1988.
236. G.J. Wendel, D.H. Stedman, C.A. Cantrell, L. Damrauer, 'Luminol-based Nitrogen Dioxide Detector', *Anal. Chem.*, **55**, 937 (1983).
237. N.L. Nagda, H.E. Rector, M.D. Koontz, *Guidelines for Monitoring Indoor Air Quality*, Hemisphere Publishing, New York, 1987.
238. P.N. Breysse, P.S.J. Lees, 'Analysis of Gases and Vapors', in *The Occupational Environment – Its Evaluation and Control*, ed. S.R. DiNardi, AIHA, Fairfax, VA, 1997.
239. J.M. Daisy, 'Real-time Portable Organic Vapor Sampling', in *Advances in Air Sampling*, Lewis Publishers, Chelsea, MI, 1988.
240. J.B. Barsky, S.S. Que Hee, C.S. Clark, 'An Evaluation of the Response of Some Portable, Direct-reading 10.2 eV and 11.8 eV Photoionization Detectors, and a Flame Ionization Gas Chromatograph for Organic Vapors in High Humidity Atmospheres', *Am. Ind. Hyg. Assoc. J.*, **46**, 9 (1985).
241. D.R. Walt, C. Munkholm, P. Yuan, S. Luo, S. Barnard, 'Design, Preparation, and Applications of Fiber-optic Chemical Sensors for Continuous Monitoring', in *Chemical Sensors and Microinstrumentation*, ACS

Symposium Series No. 403, eds. R.W. Murray, R.E. Dessy, W.R. Heineman, J. Janata, W.R. Seitz, American Chemical Society, Washington, DC, 1989.
242. F.L. Dickert, S. Thierer, 'Molecularly Imprinted Polymers for Optochemical Sensors', *Adv. Mater.*, **8**, 987 (1996).
243. J.D. Miller, 'Fiber Optic Systems in Potentially Explosive Atmospheres', *Adv. Instrum. Control*, **51**, 421 (1996).
244. J. Peck, M.L. Wilson, M. Rhodes, 'Fiber Optic Fugitive Emission Dosimeter', *Proc. SPIE – Int. Soc. Opt. Eng.*, **2836**, 140–146 (1996).
245. D.E. King, J.D. Webb, 'Infrared Waveguide Sensor with Functionalized Monolayer for Detection of Airborne Pollutants', *Proc. SPIE – Int. Soc. Opt. Eng.*, **2836**, 38–49 (1996).
246. T. Oyabu, S. Hirobayashi, H. Kimura, 'Detection of Gaseous Indoor-air Pollution using a Multisensor System', *Sens. Mater.*, **9**, 177–186 (1997).
247. A.J. White, M.G. Blamire, C.A. Corlett, B.W. Griffiths, D.M. Martin, S.B. Spencer, S.J. Mullock, 'Development of a Portable Time-of-Flight Membrane Inlet Mass Spectrometer for Environmental Analysis', *Rev. Sci. Instrum.*, **69**, 565–571 (1998).
248. G.A. Eiceman, Z. Karpas, *Ion Mobility Spectrometry*, CRC Press, Boca Raton, FL, 1994.
249. A. Graeme, *J. Autom. Chem.*, **20**, 1 (1998).
250. D. Young, C.L.P. Thomas, A.H. Brittain, G.A. Eiceman, 'Extending the Concentration and Linear Dynamic Range of Ion Mobility Spectrometry with a Sheath Flow Inlet', *Anal. Chim. Acta*, **381**, 69–83 (1999).
251. J.P. Dworzanski, M.-G. Kim, A.P. Snyder, N.S. Arnold, H.L.C. Meuzelaar, 'Performance Advances in Ion Mobility Spectrometry Through Combination with High Speed Vapor Sampling, Preconcentration and Separation Techniques', *Anal. Chim. Acta*, **293**, 219 (1994).
252. D.Y.H. Pui, D.L. Swift, 'Direct-reading Instruments for Airborne Particles', in *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*, 8th edition, eds. B.S. Cohen, S.V. Hering, ACGIH, Cincinnati, OH, 1995.
253. J. Gebhart, 'Optical Direct-reading Techniques: Light Intensity Systems', in *Aerosol Measurement – Principles, Techniques, and Applications*, eds. K. Willeke, P.A. Baron, Van Nostrand Reinhold, New York, 1993.
254. P.A. Baron, M.K. Mazumder, Y.S. Cheng, 'Direct-reading Optical Techniques using Optical Particle Detection', in *Aerosol Measurement – Principles, Techniques, and Applications*, eds. K. Willeke, P.A. Baron, Van Nostrand Reinhold, New York, 1993.
255. P.A. Baron, 'Modern Real-time Aerosol Samplers', *Appl. Ind. Hyg.*, **3**, 97–103 (1988).
256. P.A. Baron, 'Modern Real-time Aerosol Samplers', in *Advances in Air Sampling*, Lewis Publishers, Chelsea, MI, 1988.
257. P. Kaye, E. Hirst, Z. Wang-Thomas, 'Neural-network-based Spatial Light-scattering Instrument for Hazardous Airborne Fiber Detection', *Appl. Opt.*, **36**, 6149–6156 (1997).
258. E.R. Kennedy, 'Sampling Reactive Aerosols', in *Advances in Air Sampling*, Lewis Publishers, Chelsea, MI, 1988.
259. A.A. Dost, 'Monitoring Surface and Airborne Inorganic Contamination in the Workplace by a Field Portable X-ray Fluorescence Spectrometer', *Ann. Occup. Hyg.*, **40**, 589–610 (1996).
260. M.B. Bernick, P.R. Campagna, 'Application of Field-portable X-ray Fluorescence Spectrometers for Field-screening Air Monitoring Filters for Metals', *J. Haz. Mater.*, **43**, 91–99 (1995).
261. J.C. Morley, C.S. Clark, J.A. Deddens, K. Ashley, S. Roda, 'Evaluation of a Portable X-ray Fluorescence Instrument for the Determination of Lead in Workplace Air Samples', *Appl. Occup. Environ. Hyg.*, **14**, 306–316 (1999).
262. P.M. Eller, M.E. Cassinelli (eds), *NIOSH Manual of Analytical Methods*, 4th edition, Method No. 7105, National Institute for Occupational Safety and Health, Cincinnati, OH, 1994.
263. Y.-I. Lee, Y.-J. Yoo, J. Sneddon, 'Recent Advances in Laser-induced Breakdown Spectrometry', *Spectroscopy*, **13**(7), 14–21 (1998).
264. M.E. Fraser, 'Spark-induced Breakdown Spectroscopy for Metals Analysis', presented at *Pittcon '98*, 1–5 Mar., New Oleans, LA, 1998.
265. K.Y. Yamamoto, D.A. Cremers, M.J. Ferris, L.E. Foster, 'Detection of Metals in the Environment using a Portable Laser-induced Breakdown Spectroscopy Instrument', *Appl. Spectrosc.*, **50**, 222–233 (1996).
266. D.A. Cremers, J.E. Barefield, A.C. Koskelo, 'Remote Elemental Analysis by Laser-induced Breakdown Spectroscopy using a Fiber-optic Cable', *Appl. Spectrosc.*, **49**, 857–860 (1995).
267. B.J. Marquardt, S.R. Goode, S.M. Angel, 'In Situ Determination of Lead in Paint by Laser-induced Breakdown Spectroscopy using a Fiber-optic Probe', *Anal. Chem.*, **68**, 977–981 (1997).
268. J. Wang, K. Ashley, D. Marlow, E.C. England, G. Carlton, 'Field Method for the Determination of Hexavalent Chromium by Ultrasonication and Strong Anion-exchange Solid-phase Extraction', *Anal. Chem.*, **71**, 1027 (1999).
269. J. Wang, K. Ashley, E.R. Kennedy, C. Neumeister, 'Determination of Hexavalent Chromium in Industrial Hygiene Samples using Ultrasonic Extraction and Flow Injection Analysis', *Analyst*, **122**, 1307 (1997).
270. K. Ashley, 'Ultrasonic Extraction of Heavy Metals from Environmental and Industrial Hygiene Samples for their Subsequent Determination', *Trends Anal. Chem.*, **17**, 366–372 (1998).

271. G.J. Williams, N.M. Blaton, O.M. Peeters, C.J. de Ranter, 'The Interaction of Chromium (VI), Chromium (III) and Chromium (II) with Diphenylcarbazide, Diphenylcarbazone and Diphenylcarbadiazone', *Anal. Chim. Acta*, **88**, 345–352 (1977).

272. B.R. James, J.C. Petura, R.J. Vitale, G.R. Mussoline, 'Hexavalent Chromium Extraction for Soils: a Comparison of Five Methods', *Environ. Sci. Technol.*, **29**, 2377–2381 (1995).