Within biological systems, elements may be classified as those essential to the well-being of the organism and those that have no known or demonstrable function and are therefore regarded as nonessential.\(^1\,^2\)

On the basis of the usual concentrations within tissues and body fluids they may also be classified as major or trace elements, trace elements being defined as those that individually contribute no more than 0.01% of the dry body mass.\(^3\) All the major elements and a limited number of trace elements are essential (Table 23.1); all others that may be detected are nonessential. Essential elements are required for various biological functions,\(^1\) e.g.,

- Enzyme structure and function
- Hormone structure and function
- Vitamin structure and function (e.g., B\(_{12}\))
- Transport of oxygen
- Structure of macromolecules

When any are present in less than optimal concentrations, symptoms of morbidity will be evident — indeed, the severity of deficiency may be such that death is the eventual outcome (Figure 23.1). At the same time, all elements, whether essential or nonessential, are toxic if they accumulate in tissues to sufficiently large concentrations.\(^3\)

Therefore, it is important that accurate measurements in biological and clinical specimens may be obtained for fundamental research involving mechanistic aspects of trace element biology and so that deficiencies or excess may be detected in various situations.\(^1\,^4\) In addition to these concepts of essentiality
and nonessentiality and deficiency and toxicity, elements are also used therapeutically and concentrations in body fluids may be required to monitor the effectiveness and safety of the treatment. The analytical techniques used must afford the sensitivity necessary to measure concentrations below 1 ppm, often low ppb levels, in specimens of just a few microliters or milligrams with almost total specificity and relatively few interferences. These demands are met by the atomic spectrometry techniques described in this chapter.

Put very simply, spectroscopy is concerned with the study of interactions between electromagnetic radiation and matter, while spectrometry is the exploitation of these interactions to gain analytical (quantitative or qualitative) information. As indicated by the terminology, the interactions studied in atomic spectrometry involve atoms (rather than molecules) and the purpose is to determine the concentration or, more rarely, simply the presence of an element within a sample. The appropriate energy required to interact with atoms is that derived from the UV-visible section, and by high-energy particles, of the electromagnetic spectrum. In practice, atomic spectrometry involves the emission, absorption, or fluorescence of such energy. Thus this chapter describes atomic emission spectrometry (AES), atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), and x-ray fluorescence spectrometry (XRF). Although these procedures are used extensively, elements may also be determined by a number of other techniques; of particular relevance to biological and clinical specimens are those involving inorganic mass spectrometry, activation analysis, and anodic stripping voltammetry. Recognizing the importance of these techniques, brief reference will be included, particularly where features overlap with the more conventional atomic spectrometry.

### 23.2 Atomic Spectrometry: Principles

Analytical AES, AAS, and AFS are quantitative techniques that exploit interactions between UV-visible light and the outer shell electrons of free, gaseous, uncharged atoms. In XRF and related techniques,
high-energy particles interact with inner shell electrons to initiate further electron transitions within the atom. Each element has a characteristic atomic structure with a positively charged nucleus surrounded by electrons in orbital shells to provide neutrality. These electrons occupy discrete energy levels, but it is possible for an electron to be moved from one level to another within the atom by the introduction of energy (Figure 23.2). This energy may be supplied by collisions with other atoms, i.e., heating (for AES), as photons of light (for AAS and AFS), or as high-energy particles (for XRF). Such transitions occur only if the available energy is equal to the difference between two levels ($\Delta E$). Uncharged atoms may exist at the lowest energy level or ground state ($E_0$), or at any one of a series of excited states ($E_n$) depending on how certain electrons have been moved to higher energy levels, although it is usual to consider just the first transition. Energy levels and the $\Delta E$s associated with electron transitions are unique for each element.

The $\Delta E$ for movements of outer shell electrons in most elements correspond to the energy equivalent to UV-visible radiation and these transitions are used for AES, AAS, and AFS. The energy of a photon ($E$) is characterized by

$$E = h\nu$$  \hspace{1cm} (23.1)

where $h = $ Planck's constant and $\nu = $ the frequency of the waveform corresponding to that photon. Furthermore, frequency and wavelength are related as

$$\nu = c/\lambda$$  \hspace{1cm} (23.2)

where $c = $ the velocity of light and $\lambda = $ the wavelength. Therefore,

$$E = hc/\lambda$$  \hspace{1cm} (23.3)

and it follows that a specific transition, $\Delta E$, is associated with a unique wavelength.6

Under appropriate conditions, outer shell electrons of vaporized atoms may be excited by thermal energy (i.e., collisions with other atoms). As these electrons return to the more stable ground state, energy is lost. As Figure 23.2 shows, some of this energy will be in the form of emitted light, which can be measured with a detector; this is AES. When light (radiant energy) of a characteristic wavelength enters an analytical system, outer shell electrons of the corresponding atoms will be excited as energy is absorbed. Consequently, the amount of light transmitted from the system to the detector will be attenuated; this is understood as AAS. Finally, some of the radiant energy absorbed by ground state atoms can be emitted as light as the atom returns to the ground state, i.e., AFS.

When high-energy photons, electrons or protons strike a solid sample, an electron from the inner shells (K, L, or M) of a constituent atom may be displaced. The resulting orbital vacancy is filled by an outer shell electron, with an accompanying emission of an x-ray photon; its energy is equal to the difference between the energy levels involved. This emission is known as x-ray fluorescence (XRF). The energy of the emission, i.e., the wavelength, is characteristic of the atom (element) from which it originated, while the intensity of the emission is related to the concentration of the atoms in the sample.
Depending on the principle of the spectrometer employed to measure the emission, XRF is divided into wavelength dispersive XRF (WDXRF) or energy dispersive XRF (EDXRF). Total reflection XRF (TXRF) is usually described as a separate technique although it may be seen as a modification of EDXRF. High-energy hydrogen or helium ions may also be used as incident radiation to displace an electron from a K or L shell with an emission of characteristic x-rays. This is known as particle-induced x-ray emission (PIXE). In addition to being an independent analytical technique, PIXE can be used with electron microscopy to provide elemental analysis of visualized specimens.

Analogous to XRF and PIXE, when an atom is bombarded with charged particles a radioactive nuclide may be formed. Gamma radiation then emitted is characteristic of the nuclide and the intensity of the emission is proportional to the analyte concentration. Neutrons are most often used for excitation and the technique is then called neutron activation analysis. This multielement technique requires specialized facilities and is not widely available.

The high-temperature inductively coupled plasma (see below) is an effective ion source for a mass spectrometer; the technique of inductively coupled plasma-mass spectrometry (ICP-MS) is extensively used for measurements of trace elements in clinical and biological materials. It affords very sensitive multielement analysis and also provides for the determination of stable isotopes. 7,8

Anodic stripping voltammetry is an electrochemical procedure that offers exceptional sensitivity for some applications. It is ideally suited for large sample volumes such as water specimens, but it is widely applied to the measurement of lead in blood, particularly in North America.

It follows from Equations 23.1 to 23.3 that the wavelengths of the absorbed and emitted energies are unique to a given element. It is this that makes atomic spectrometric techniques specific, so that one element can be determined even in the presence of an enormous excess of a chemically similar element.6

### 23.3 Atomic Spectrometry: Instrumentation

Formation of the atomic vapor, i.e., atomization, is central to emission, absorption, and fluorescence by atoms. Atomizers and the devices for sample introduction are the heart of the instrumentation, with an associated spectrometer for wavelength separation and detection of light. Atomization involves the following steps: removal of solvent (drying), separation from anion or other components of the matrix, and reduction of ions to the ground state atom. Energy necessary to accomplish these steps is supplied as heat. The proportion of an atom population within the vapor, as the excited or the ground state atoms, is influenced by the temperature and the atomic structure of the element. At the temperatures of flame and electrothermal atomizers, around 2 to 3000 K, the ratio is at least 10^-6:1 for most elements and AAS affords superior sensitivity to AES. With the much higher temperatures provided by an inductively coupled plasma atomizer, the proportion changes and AES may be favored.

#### 23.3.1 Flame Atomizers

The flame provides for simple, rapid measurements with few interferences and is preferred wherever the analyte concentration is suitable. The typical pneumatic nebulizer for sample introduction is inefficient and although elements such as Na and K may be measured in biological specimens by flame AES, flame atomization is more usually suited to AAS and AFS. With AAS, measurements are possible with specimens where concentrations are around 1 µg/ml or more. Devices have been developed that overcome the limitations of the pneumatic nebulizer by by-passing the nebulizer so that 100% of the sample is atomized; they also introduce the sample as a single, rapid pulse rather than by continuous flow. These approaches are also features of electrothermal atomization and vapor generation procedures (see below). Lower detection limits are obtained with AFS but various constraints restrict this technique, with a few exceptions, to the hydride-forming elements (see below).9
23.3.2 Electrothermal Atomizers

Most systems use an electrically heated graphite tube, a technique often called graphite furnace atomization, although other materials are sometimes employed.\textsuperscript{9,10} With a programmed temperature sequence, the test solution (10 to 50 µl) is dried, organic material destroyed, and the analyte ions dissociated from anions for reduction to ground state atoms. The temperatures achieved by this technique can be up to 3000 K so that refractory elements such as aluminium and chromium will form an atomic vapor. Because all of the sample is atomized and retained within the small volume of the furnace, a dense atom population is produced. The technique is, therefore, very sensitive and allows measurement of µg/l concentrations. Although the technique is widely used for AAS, electrothermal atomization is suitable for AES and for sample introduction into an inductively coupled plasma.\textsuperscript{9,11}

23.3.3 Inductively Coupled Plasmas

As stated previously, high-temperature atomizers are required to provide useful numbers of excited atoms for AES. Historical sources include arcs and sparks but modern instruments use argon, or some other inert gas, in the form of a plasma. The plasma is formed when gas atoms are ionized, \( \text{Ar} + e^{-} \rightarrow \text{Ar}^{+} + 2e^{-} \) — a process generated by seeding from a high-voltage spark — and is sustained with energy from an induction coil connected to a radio-frequency generator. This is known as an inductively coupled plasma (ICP). Plasmas exist at temperatures of up to 10,000 K and in the instrument have the appearance of a torch. Samples can be introduced via a nebulizer, by vapor generation procedures, by vaporization from a graphite atomizer or by laser ablation of solid specimens.

The main feature of AES is that it permits multielement analysis. Optical systems direct the emitted light via a monochromator to a single detector or to an array of monochromators and detectors positioned around the plasma. With the first arrangement, a sequential series of readings are made with the monochromator driven to give each of the wavelengths of interest in turn. Simultaneous readings can be made with the second arrangement. For most elements the analytical sensitivity for ICP-AES is similar to that obtained with flame AAS at the part per million level.

23.3.4 X-Ray Fluorescence

XRF requires that specimens be irradiated by high-energy photons. In most instruments the source is the polychromatic primary beam from x-ray tubes. Of interest to biological applications, however, is the use of radioactive isotopes as sources. Isotopes such as \(^{244}\text{Cm}, ^{241}\text{Am}, ^{55}\text{Fe}, \) and \(^{109}\text{Cd} \) are used.\textsuperscript{13,14} The latter is particularly important as the source in portable instruments developed for \textit{in vivo} XRF (see below). A growing number of publications refer to the use of synchrotron radiation that acts as a high-resolution highly energetic source.\textsuperscript{13}

Because sample matrix contributes considerably to signal intensity, calibration can be difficult, usually requiring the use of different reference materials and internal standardization. Fewer problems are encountered with samples prepared as very thin films and in TXRF. Together with the effect of the matrix, sensitivity is also influenced by wavelength, such that lighter elements present a difficult analytical challenge.

In WDXRF, high-intensity x-rays (e.g., from a 3-kW x-ray tube) are used to induce the fluorescence emission, which is dispersed into individual spectral lines by reflection at an analyzer crystal. The diffracted beams are collimated and directed onto a photon detector. As with ICP-AES, spectrometers may operate sequentially, with a number of interchangeable crystals, to permit the measurement of the full range of elements, or in a multichannel (simultaneous) mode usually preset for specific analytes. Detection limits for light elements are 10 to 100 times lower than with EDXRF. Resolution is good, although less so at shorter wavelengths. Sequential instruments require long analysis times to measure several elements compared with the more expensive simultaneous instruments or EDXRF technology.

For EDXRF, x-rays emitted from the sample are directed together into a crystal detector. A pulse of current is generated with a height proportional to the energy of the x-ray photon. The different energies
associated with the various atoms (elements) in the sample are sorted electronically. Lower energy sources (a low-power x-ray tube or an isotopic source) are used. The detector must be maintained at a very low temperature and in a clean vacuum. Analysis times are 10 to 30 times longer than with WDXRF but, as a truly multielement technique, the total time is not necessarily any greater.

When a collimated beam of x-rays is directed against an optically flat surface at an angle of around 5°, total reflection will occur. This is the principle of TXRF in which the sample is exposed to primary and total reflected beams and is excited to fluoresce. Emitted radiation is resolved and measured as an ED spectrum. There is effectively no absorption by the matrix, so measurement and calibration are much simpler and sensitivities are greater than with other x-ray techniques.

### 23.4 Atomic Spectrometry: Sample Preparation

The objectives for preparation of biomedical specimens are to remove interfering components from the matrix and to adjust the concentration of analyte to facilitate the actual measurement. These objectives may be realized by a number of approaches (Table 23.2).

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution, protein precipitation</td>
<td>Using simple off-line arrangements or flow-injection manifold</td>
</tr>
<tr>
<td>Dry ashing</td>
<td>Using a muffle furnace or a low-temperature asher</td>
</tr>
<tr>
<td>Acid digestion</td>
<td>(1) In open vessels with convection or microwave heating</td>
</tr>
<tr>
<td></td>
<td>(2) In sealed vessels to increase the reaction pressure</td>
</tr>
<tr>
<td>Base dissolution</td>
<td>Using quaternary ammonium hydroxides</td>
</tr>
<tr>
<td>Chelation and solvent extraction</td>
<td>For analyte enhancement and removal of interferences</td>
</tr>
<tr>
<td>Trapping onto solid phase media</td>
<td>For analyte enhancement and removal of interferences</td>
</tr>
</tbody>
</table>

Methods for destruction of the organic matrix by simple heating or by acid digestion have been used extensively and are thoroughly validated. Microwave heating is now well established for this purpose, with specifically constructed apparatus to avoid dangers of excessive pressure within reaction vessels. Although the number of specimens that can be processed is not large, microwave heating affords rapid digestion and low reagent blanks. More recent developments include continuous flow systems for automated digestion linked directly to the instrument for measurement of the analytes.

Preconcentration by liquid-liquid partitioning is a widely used procedure. Analyte atoms in a large volume of aqueous specimen are complexed with an appropriate agent and then extracted into a smaller volume of organic solvent. This leads to enhancement of concentration and also removes the analyte from potential or real interferences in the original matrix. It is used to measure lead in blood, metals in urine and for other applications. Although preconcentration by trapping onto solid phase media represents the area where much of the recent interest in FAAS has been focused, it is relevant to all sample preparation work. The original work involved adsorption onto material such as charcoal or alumina but newer phases include ion-exchange resins and novel support systems to which functional groups are added to confer increased selectivity and capacity. Trapping of analyte from dilute sample and elution into a small volume of release solution may be accomplished off-line; however, developments in flow-injection analysis provide for the assembly of simple on-line manifolds so that complete measurements may be carried through automatically. Developments in these applications involving a wide range of biological and clinical sample types and elements are regularly published.

Vapor generation procedures were referred to in earlier sections. These permit the rapid introduction of 100% of the sample into the atomizer and are used for AAS, AFS, ICP-AES, and ICP-MS. Certain elements such as arsenic, selenium and bismuth readily form gaseous hydrides, e.g., arsine (AsH₃), that are transferred by a flow of inert gas to a heated silica tube positioned in the light path. The tube is heated by the air-acetylene flame or by an electric current and the temperature is sufficient to cause dissociation of the hydride and atomization of the analyte. Thus, there is no loss of specimen, all the
atoms enter the light path within a few seconds and they are trapped within the silica tube, which retards their dispersion. Hydride generation AAS allows the detection of a few nanograms of analyte from whatever sample volume is placed into the reaction flask. Mercury forms a vapor at ambient temperatures and this property is the basis for cold vapor generation. A reducing agent is added to the sample solution to convert Hg^{2+} to the elemental mercury. Agitation or bubbling of gas through the solution causes rapid vaporization of the atomic mercury, which is then transferred to a flow-through cell placed in the light path. As with hydride generation, the detection limit is a few nanograms and common instrumentation to accomplish both procedures has been developed by some manufacturers.

Appreciation of the importance of determining not just the total concentration of an element in a specimen, but also something of its distribution, is now well established. This concept of speciation is applied to associations with different molecules such as proteins, to different organo-metallic compounds, and to different valence states. A number of preparative procedures are available to separate or speciate the analyte, but much innovation is directed to chromatographic and electrophoretic techniques that are coupled directly to the atomic spectrometric equipment to form an integrated analytical arrangement. Examples are presented in the following section.

23.5 Atomic Spectrometry: Recent Developments and Applications

Measurements of major and trace elements in biological and clinical specimens are required in many situations:

• Work to determine mechanisms of action within biological systems at cellular and biochemical levels, of essentiality and of toxicity, involves knowing concentrations within the experimental systems.
• Determining trace element and mineral physiology, routes of absorption, tissue distribution and concentrations in normal subjects and in patients with inborn errors involves these processes, e.g., copper and Wilson's disease.
• Nutritional studies investigate possible deficiencies of essential elements, e.g., in subjects with poor diets or patients receiving long-term total parenteral feeding (where protocols for regular monitoring are generally recommended). In addition to measuring trace elements in blood and urine, such work may include analysis of foods and special investigations to assess intestinal absorption.
• Investigation of undue exposure to elements: Increased exposure to minerals and trace elements can cause morbidity and some are carcinogenic. While the function of many organs may be perturbed by accumulation of metals, the kidney, liver, nervous, intestinal, and hemopoietic systems are more likely to be involved. Accidental, (or even deliberate suicidal or homicidal) exposures to trace elements feature in the differential diagnosis when considering signs and symptoms involving these sites. Increased exposure may be consequent on sources within the environment or in the home, associated with hobbies or from unusual cosmetics and remedies. In an occupational setting, biological monitoring is important in the implementation of health and safety regulations.
• In other situations iatrogenic poisoning can occur. Profound toxicity has been observed for many elements including aluminium, bismuth, and manganese.

23.5.1 Atomic Emission Spectrometry

Sodium, potassium and lithium are usually measured by flame AES or with ion-selective electrodes although they can also be determined by flame AAS. At the temperature of the flame there is no useful emission of other biologically important elements. However, with the greater energy of the ICP, much lower detection limits, typically around 1 µg/ml, are obtained and many elements may be determined simultaneously in solutions prepared from biological tissues. Furthermore, a few elements, such as boron, phosphorus, and sulfur, cannot be measured by AAS but are determined by ICP-AES.

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Recent developments with the optical systems and array detectors have led to improvements in sensitivity and data collection. In consequence, elements such as copper, zinc, and aluminium may be measured in blood plasma, while the expanded information caught by detectors is making it possible for powerful chemometric manipulation of individual signals to be undertaken. ICP-AES now has an established role for monitoring patients with possible compromised nutritional status and those who receive hemodialysis to treat chronic renal failure and are at risk of developing aluminum toxicity. With the multielement feature of the technique, ICP-AES is widely used for analysis of foodstuffs and tissues samples. The convenience of this approach has, however, encouraged the dubious “diagnostic practice” of hair analysis among some laboratories. Vapor generation techniques for sample introduction (see below) are possible.

### 23.5.2 Atomic Absorption Spectrometry

Measurement of calcium in serum was the first analysis to which AAS was applied and is an obvious example of how the technique is useful for biomedical analysis. Elements present in biological fluids at a sufficiently high concentration to be measured by flame AAS are lithium and gold, when used to treat depression and rheumatoid arthritis, respectively, and calcium, magnesium, iron, copper, and zinc. Flame AAS is used by the large majority of laboratories needing to measure these elements. It fulfills an important role in the investigation of patients with possible nutritional problems, genetic disorders, or other relevant clinical challenges. Other elements are present in fluids at too low a concentration to be measured by conventional FAAS with pneumatic nebulization. With more exotic fluids, e.g., seminal plasma or cerebrospinal fluid, analysis may just be possible for a very few elements.

The concentrations of many metals in plant, animal, or human tissues are usually much higher than in biological fluids and very often the weight of an available specimen is such that a relatively large mass of analyte is recovered into a small volume of solution, thus enhancing the concentration still further. For the analysis of tissues (including specimens such as hair and the cellular fractions of blood) following sample dissolution steps, FAAS is suitable for measurement of many of the biologically important elements.

Atom traps, such as the slotted quartz tube, increase the sensitivity associated with FAAS for more volatile elements; sporadic reports appear of their use in simplified methods for analysis of biological fluids. However, if concentrations are low it is more usual to take advantage of the lower limits of detection provided by electrothermal AAS.

Virtually all the trace elements of biological interest may be determined by electrothermal AAS. Although it is relatively slow, a single-element technique, and subject to various interferences, it is extensively used throughout the world for clinical investigations and for monitoring occupational exposures, as well as in the other settings mentioned earlier. The design and construction of furnaces are subject to continuous development to improve detection limits and to reduce interferences. Devices to measure nonatomic absorption, e.g., Zeeman-effect background correction, are essential. Commercial furnaces are made from electrographite, electrographite with a pyrolytic coating or total pyrolytic graphite, although publications showing the advantages of other materials as coatings or for the furnace itself, regularly appear. Typically these refer to analysis of elements that form extremely refractory carbides in graphite furnaces, e.g., molybdenum.

Design developments are introduced with the objective of separating the appearance of the atomic vapor from components that cause an interference with the atomization signal. These innovations include graphite platforms and probes but, as with automobiles, there are continuous refinements to the overall shape and dimensions to effect improvements in performance. An authoritative review of materials suitable for use in furnace construction and of recent developments in design has been prepared by Frech. Several research groups have designed very novel atomizers with the purpose of separating the analyte from interfering species, to permit simple atomic absorption. Although some appear to be effective, none are commercially available.
It was shown some years ago that a 150-W tungsten filament from a light bulb could be used as an electrothermal atomizer. More recently, this concept has been used to develop very small portable instruments for on-site measurement of lead in blood. Excellent results have been reported but a commercial model is still awaited.

In addition, effective analysis of most biological samples requires the addition of reagents that modify the behavior of the specimen during the heating program so as to reduce interferences. The chemical modifiers most commonly employed with biological specimens are given in Table 23.3. Triton X-100 is used at a concentration of around 0.1% w/v and is included with the sample diluent. Gaseous oxygen or air is an effective ashing aid, but will cause rapid deterioration of the graphite furnace unless a desorption step is included before the temperature is increased for atomization. Other modifier solutions can be included with the sample diluent or separately added by the autosampler to the specimen inside the furnace. The choice of modifier often depends on the availability of a source material that is free from contamination.

### 23.5.3 Atomic Fluorescence Spectrometry

Recent innovations in AFS follow almost entirely from the development of commercial instruments specifically designed for use with hydride generation; the particular applications of interest will be considered in the next section. For various reasons earlier attempts to exploit the inherent sensitivity of AFS using flame systems were never fully realized. However, with improvements in light source technology and other instrumentation, this niche area has progressed rapidly in recent years.

### 23.5.4 Vapor Generation Procedures

Depending on the nature of the exposure — inorganic salts, organomercury compounds, the metal or its vapor — it may be necessary to analyze specimens of urine, blood or tissues, and foods. Mercury is used extensively in industry and occupational monitoring continues to be relevant. There is now considerable interest in two particular environmental sources of exposure, i.e., from dental amalgam and from the diet, especially fish. Although no good evidence exists for mercury leaching from amalgam fillings in amounts that can cause toxicity, many members of the public believe that their health is affected and seek to have the mercury removed, even after analysis of blood or urine has failed to show increased concentrations of the metal. Concern has been raised that undue exposure to methylmercury may occur from eating large amounts of seafoods. Those at risk are young children in utero and during early childhood. Long-term studies are in progress within communities where basic foods contain mercury and maternal hair concentrations of it are high. Mercury intakes and neurological development are being monitored within the target groups.

Considerable interest in methods to measure arsenic and other hydride-forming elements has been evident in recent years. The basic procedure involves careful digestion of the specimens to convert all the

<table>
<thead>
<tr>
<th>Modifier</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton X-100</td>
<td>To promote drying of protein-rich specimens, avoid a dried crust around a liquid core</td>
</tr>
<tr>
<td>Gaseous oxygen</td>
<td>To promote destruction of organic matrix, reduce smoke formation and particulates which give nonatomic absorption</td>
</tr>
<tr>
<td>Ni, Cu, Pd</td>
<td>To stabilize volatile elements, e.g., Se, As, during the dry and ash phases</td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td>Stabilizes Hg up to a temperature of 200°C</td>
</tr>
<tr>
<td>HNO₃ or NH₄NO₃</td>
<td>To stabilize analyte atoms by removal of halides as HCl or NH₄Cl during the ash phase</td>
</tr>
<tr>
<td>Mg(NO₃)₂</td>
<td>Becomes reduced to MgO, which traps the metals to reduce volatilization losses, delays atomization and separates the analyte signal from the background absorption</td>
</tr>
<tr>
<td>NH₄H₂PO₄ or (NH₄)₂HPO₄</td>
<td>Usually used with Mg(NO₃)₂, reduces volatilization losses and delays atomization to separate the analyte signal from the background absorption</td>
</tr>
</tbody>
</table>

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different species to a single valency form, reduction with BH$_4^-$ and vaporization to the hydride, which is then transferred by a stream of inert gas to a quartz tube heated in an air-acetylene flame or with electrical thermal wire. Atomization is then achieved by the high temperature. Some work has been reported in which the hydride is transferred into a cold graphite furnace where it is trapped onto the surface. Atomization takes place as the furnace is rapidly heated and, as with conventional electrothermal AAS, improved sensitivity is observed due to the high atom density within the small volume of the furnace.

Trapping is more efficient when the graphite is coated with a metal salt, e.g., Ag, Pd, Ir.$^{35,36}$ The chemical hydride reaction is impaired by other hydride-forming elements and by transition metals so that careful calibration is essential. An emerging development involves an electrolytic process in which nascent hydrogen is produced as an alternative to chemical hydride generation. With this arrangement the interferences are much less and the reagents employed introduce less contamination.$^{37-39}$

The biological and clinical importance of selenium receives much current interest; more attention is focused on measurement of this element than on any other. The stimuli to this flurry of activity are (1) the association between selenium status and cardiovascular disease coupled to the demonstration that dietary intakes are low in some regions and are declining in others,$^{40}$ and (2) epidemiological data suggesting a relationship between selenium status and the incidence of certain carcinomas.$^{41}$ Measurements of selenium in foods, biological fluids and tissues, using vapor generation techniques, electrothermal AAS, or ICP-MS, are integral to many large-scale studies now in progress or recently completed.

There is also much interest in the determination of arsenic. This element is important within the microelectronics industry and in other occupations, but extensive environmental exposure is also associated with naturally high concentrations in drinking water. Arsenic in drinking water is a problem in several areas of the world; however, the situation that now exists in Bangladesh and West Bengal, India, is extraordinary, with millions of people consuming highly toxic and carcinogenic water.$^{42}$ Measurement of total arsenic is not always entirely helpful. Fish contain large amounts of organoarsenic species that are absorbed and excreted without further metabolism and with no adverse health effects. These species will be included in a total arsenic determination and can mask attempts to measure toxic As$^{3+}$ and metabolites. Thus, methods to measure the individual species or related groups of compounds in urine or other samples provide more meaningful results. These methods include separation by chromatography or solvent extraction and pretreatment steps that transform only the species of interest into the reducible form.

As this speciation work has become more refined additional arsenic-containing compounds have been demonstrated and, very recently, some of these have been identified as methylated As species containing As$^{3+}$. It is well known that methylated species with As$^V$ are found in blood and urine following exposure to inorganic arsenic and believed to represent steps in the detoxification pathway. Methylarsenic$^{3+}$ species are potent enzyme inhibitors and cytotoxins and their formation may be involved in the mechanism of arsenic toxicity. In one recent study,$^{43}$ biliary and urinary arsenic species were determined in rats exposed to As$^{3+}$ and As.$^V$. MonomethylAs$^{3+}$arsonate (MMA$^{3+}$) was present in bile but not in urine; the authors hypothesized that MMA$^{3+}$ was subsequently oxidized to MMA$^{5+}$ and excreted in urine. In a separate investigation, arsenic species were measured in water, urine and cultured cells and methylarsenic$^{3+}$ species were identified in the urine of individuals who had consumed water contaminated with inorganic As.$^{44}$

Other elements that form gaseous hydrides, such as antimony, bismuth, tellurium, etc., are also relevant to investigations in clinical and biological specimens.

23.5.5 X-Ray Fluorescence Spectrometry

XRF and other x-ray techniques offer no particular advantage over the many other procedures for simple quantitative measurement of minerals and trace elements in clinical and biochemical specimens. Alternative methods are widely available, well established, and relatively simple. Nevertheless, a few interesting applications have been reported with recent examples of gold and palladium in urine by TXRF, with detection limits around 2.5 ng/l, and the analysis of breast milk by PIXE.$^{45,46}$ In certain applications...
involving clinical and biochemical specimens, however, XRF does have a specific role. These include elemental mapping and in vivo analyses.

By virtue of the very narrow x-ray beam (100 µm or less) it is possible to take repeat measurements over a very small surface and develop a map of the distribution of elements within a structure. This approach is regularly applied to solid materials including hairs, teeth, and other calcareous materials. Results have also been reported in which single cells have been investigated, e.g., in a study to compare iron and other elements within neuromelanin aggregates in neuronal cells of patients with Parkinson's disease and their controls.47

Developments involving in vivo XRF have flourished in the last few years although the majority of the many publications originate from just a few centers. Most of the work is concerned with measurement of lead in bone by 109Cd-based XRF. Analytically, recent improvements refer to detection systems to reduce detection limits to low part per million levels, and to the methods for calibration. Phantoms (often plaster of Paris) containing known amounts of Pb are generally used. Recent alternative materials that are reported to behave more like bone are a synthetic apatite matrix48 and a material with polyurethane and CaCO3.49 It has been shown that the measurement location, i.e., proximal-distal sites, influences the measured XRF intensity and its uncertainty. Considerable differences in mean bone Pb concentrations between the left and right legs of the same individual (0.8 and 2.0 µg/g bone mineral) have also been demonstrated.50 Using this technique, cumulative exposures to lead at work have been assessed and results compared with other markers such as blood and urine lead concentrations.51 Blood and bone lead concentrations were determined and related to the development of hypertension. A positive association was found between the baseline bone Pb level and the incidence of hypertension but no association was found with blood Pb level.52 Bone lead has also been shown to be released into the circulation during pregnancy, with an implied risk to the fetus if the mother has a history of previous lead absorption.

There are reports of the determination of other elements by in vivo XRF. Skin iron concentrations correlated strongly with iron in the internal organs of rats injected with iron-dextran and it was concluded that this technique had great potential in the diagnosis and treatment of hereditary hemochromatosis and β-thalassemia.53 A method for measuring platinum in kidneys of patients receiving Pt-based chemotherapy drugs has been developed.54 These few examples illustrate that the potential for in vivo elemental analysis is hugely exciting.

### 23.6 Atomic Spectrometry: Quality Assurance

Apart from the actual analysis, adventitious contamination, which can occur during collection and storage of specimens, the preparative procedure, and the spectrometric measurement, has the greatest impact on the quality of results. Data from proficiency testing schemes indicate that specialist trace element centers tend to maintain the highest standards of analytical performance. This observation reflects the continuing application of practices that minimize contamination and the expertise and experience to ensure optimal functioning of equipment. Because of the stability of inorganic analytes and the purity with which standard materials can be prepared, there are reasonable numbers of reference materials available for use to validate methods and for internal and external quality control.55 Proficiency testing schemes relating to occupational and environmental laboratory medicine are organized in most countries and all laboratories involved in this work should have access to an appropriate scheme to demonstrate the reliability of their analytical data.56

### References
