9
chapter

Atomic Absorption Spectroscopy, Atomic Emission Spectroscopy, and Inductively Coupled Plasma-Mass Spectrometry

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9.1 INTRODUCTION

The development of accurate methods for measuring concentrations of mineral elements in foods and other biological samples has a long history. The major challenge is to accurately measure these elements in a food matrix that contains much higher concentrations of other components (i.e., carbohydrates, proteins, and fats) as well as other mineral elements that may interfere. Table 9.1 lists mineral elements of interest in foods [1, 2]. The USDA nutrient database [1] for calcium, iron, sodium, and potassium in foods is quite complete, but the database for the trace elements and toxic heavy metals is lacking for some food groups.

As the names imply, atomic absorption spectroscopy (AAS) quantifies the **absorption** of electromagnetic radiation by well-separated neutral atoms, while atomic emission spectroscopy (AES) measures **emission** of radiation from atoms in excited states. AAS and AES allow accurate measurements of mineral elements even in the presence of other components because the atomic absorption and emission spectra are unique for each individual element. The use of inductively coupled plasma (ICP), originally developed in the 1960s [3, 4], as an excitation source for emission spectroscopy has further expanded our ability to rapidly measure multiple elements in a single sample. In theory, virtually all of the elements in the periodic chart can be determined by AAS or AES. In practice, **atomic spectroscopy** is used primarily to determine mineral elements. Table 8.2 in Chap. 8 shows a comparison of different spectroscopy methods commonly available for food analysis, including AAS and AES.

More recently, ICP has been mated with mass spectrometry (MS) to form ICP-MS instruments that are capable of measuring mineral elements with extremely low detection limits. Moreover, mass spectrometers have the added advantage of being able to separate and quantify multiple isotopes of the same element. Taken together, these instrumental methods have largely replaced traditional wet chemistry methods for food mineral analysis, although traditional methods for calcium, chloride, fluoride, and phosphorus remain in use today (see Chap. 21).

This chapter deals with the basic principles that underlie analytical atomic spectroscopy and provides an overview of the instrumentation available for measuring atomic absorption and emission. A discussion of ICP-MS is also included. Readers interested in a more thorough treatment of the topic are referred to references 5–8.

9.1 table Elements in foods classified according to nutritional essentiality, potential toxic risk, and inclusion in USDA Nutrient Database for Standard Reference

Essential nutrient[a]	*Toxicity concern*	*USDA Nutrient Database*[b]
Sodium	Lead	Calcium
Potassium	Mercury	Iron
Chloride	Cadmium	Magnesium
Calcium	Nickel	Phosphorous
Chromium	Arsenic	Potassium
Copper	Thallium	Sodium
Fluoride		Zinc
Iodine		Copper
Iron		Manganese
Magnesium		Selenium
Manganese		Fluoride
Molybdenum		
Phosphorous		
Selenium		
Zinc		
Arsenic		
Boron		
Nickel		
Silicon		
Vanadium		

Compiled based on U.S. Department of Agriculture, Agricultural Research Service [1] and Institute of Medicine (IOM) [2]

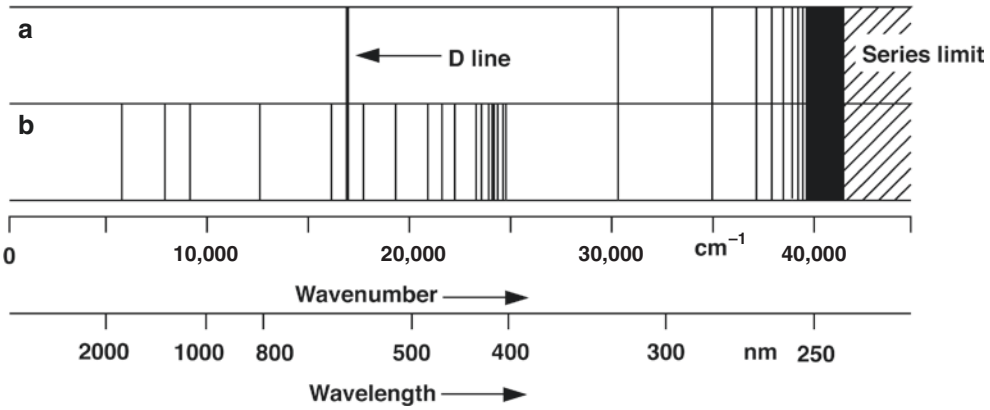
[a]Nutrients are considered essential if their removal from the diet causes some adverse change in physiological function. For arsenic, boron, nickel, silicon, and vanadium, there is evidence that trace amounts may have a beneficial role in some physiological processes in some species, but the available data is limited and often conflicting. Dietary Reference Intakes (DRIs) have been established by Institute of Medicine (IOM) for these minerals [2]

[b]Values for copper, manganese, selenium, and fluoride are not included for many foods in the USDA Nutrient Database for Standard Reference due to limited data

9.2 GENERAL PRINCIPLES

9.2.1 Energy Transitions in Atoms

Atomic absorption spectra are produced when ground-state atoms absorb energy from a radiation source. Atomic emission spectra are produced when neutral atoms in an excited state emit energy on returning to the ground state or a lower-energy state. As discussed in Chap. 6, atoms absorb or emit radiation of discrete wavelengths because the allowed energy levels of electrons in atoms are fixed and distinct. In other words, each element has a unique set of allowed electronic transitions and therefore a unique spectrum, enabling accurate identification and quantification even in the presence of other elements. The absorption and emission spectra of sodium are shown in Fig. 9.1. For absorption, transitions involve primarily the excitation of electrons in the ground state, so the number of transitions is relatively small. Emission, on the other hand, occurs

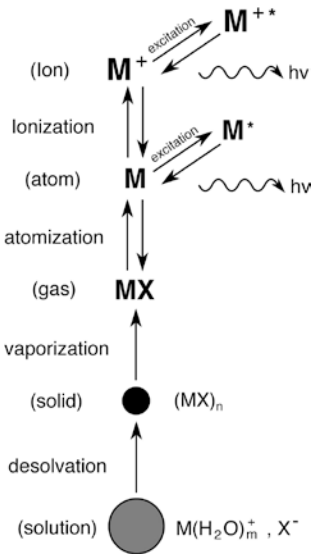


9.1 figure Spectra for sodium. The upper spectrum (**a**) is the absorption spectrum, and the lower (**b**) is the emission spectrum (From Welz [26], reprinted with permission of VCH Publishers (1985)

when electrons in various excited states fall to lower-energy levels including, but not limited to, the ground state. Therefore, the emission spectrum has more lines than the absorption spectrum of the same element as illustrated in Fig. 9.1. When a transition is from or to the ground state, it is termed a **resonance transition**, and the resulting spectral line is called a **resonance line**.

9.2.2 Atomization

Atomic spectroscopy requires that atoms of the element of interest to be in the atomic state (not combined with other elements in a compound) and to be well separated in space. In foods, virtually all elements are present as compounds or complexes and therefore must be converted to neutral atoms (i.e., atomization) before atomic absorption or emission measurements can be made. Atomization is usually accomplished by exposing a solution containing the analyte (the substance being measured) as a fine mist to high temperatures, typically in a flame or plasma. The solvent quickly evaporates, leaving solid particles of the analyte that vaporize and decompose to atoms that may absorb radiation (atomic absorption) or become excited and subsequently emit radiation (atomic emission). This process is shown schematically in Fig. 9.2. Three common methods for atomizing samples, including their atomization temperature ranges, are summarized in Table 9.2.



9.2 figure A schematic representation of the atomization of an element in a flame or plasma. The *large circle* at the bottom represents a tiny droplet of a solution containing the element (*M*) as part of a compound (From Boss and Fredeen [6], used with permission. Courtesy of the PerkinElmer Corporation, Shelton, CT)

9.3 ATOMIC ABSORPTION SPECTROSCOPY

AAS is an analytical method based on the absorption of ultraviolet-visible (UV-Vis) radiation by free atoms in the gaseous state. It is a relatively simple method and was the most widely used form of atomic spectroscopy in food analysis for many years. It has been largely replaced by the more powerful ICP-based

9.2 table Methods and temperature ranges for atomization of analytes

Source of energy for atomization	*Approximate atomization temperature range (K)*	*Analytical method*
Flame	2,000–3,400	AAS, AES
Electrothermal	1,500–3,300	AAS (graphite furnace)
Inductively coupled argon plasma	6,000–7,000	ICP-OES, ICP-MS

spectroscopy. Two types of atomization are commonly used in AAS: **flame atomization** and **electrothermal (graphite furnace) atomization**.

9.3.1 Principles of Flame Atomic Absorption Spectroscopy

A schematic diagram of a flame atomic absorption spectrometer is shown in Fig. 9.3. In flame AAS, a nebulizer-burner system is used to convert a sample solution into an atomic vapor. It is important to note that the sample must be in solution (usually an aqueous solution) before it can be analyzed by flame AAS. The sample solution is **nebulized** (dispersed into tiny droplets), mixed with fuel and an oxidant, and burned in a flame produced by oxidation of the fuel. Atoms and ions are formed within the hottest portion of the flame as the sample solution goes through the process of desolvation, vaporization, atomization, and ionization (Fig. 9.2). Atoms and ions of the same element absorb radiation of different wavelengths and produce different spectra. Therefore, it is desirable to choose a flame temperature that will maximize atomization and minimize ionization because atomic absorption spectrometers are tuned to measure *atomic* absorption, not *ionic* absorption.

Once the sample is atomized in the flame, the quantity of the analyte element is measured by determining the attenuation (decrease in intensity) of a beam of radiation passing through the flame, due to atomic absorption of incident radiation by the analyte element. For the measurement to be specific for the analyte element, the radiation source ideally should emit radiation of the exact discreet wavelengths that only the analyte element is capable of absorbing. This can be accomplished by using lamps with cathodes fabricated with the element to be determined. Thus, the beam of radiation emitted from the lamp is the emission spectrum of the element. The spectral line of interest is isolated by passing the beam through a monochromator so that only radiation of a very narrow band width reaches the detector. Usually, one of the strongest spectral lines is chosen; for example, for sodium the monochromator is set to pass radiation with a wavelength of 589.0 nm. The principle of this process is illustrated in Fig. 9.4. Note that the intensity of the radiation leaving the flame is less than the intensity of radiation coming from the source. This is because sample atoms in the flame absorb some of the radiation. Note also that the line width of the radiation from the source is narrower than the corresponding line width in the absorption spectrum. This is because the higher temperature of the flame causes a broadening of the line width.

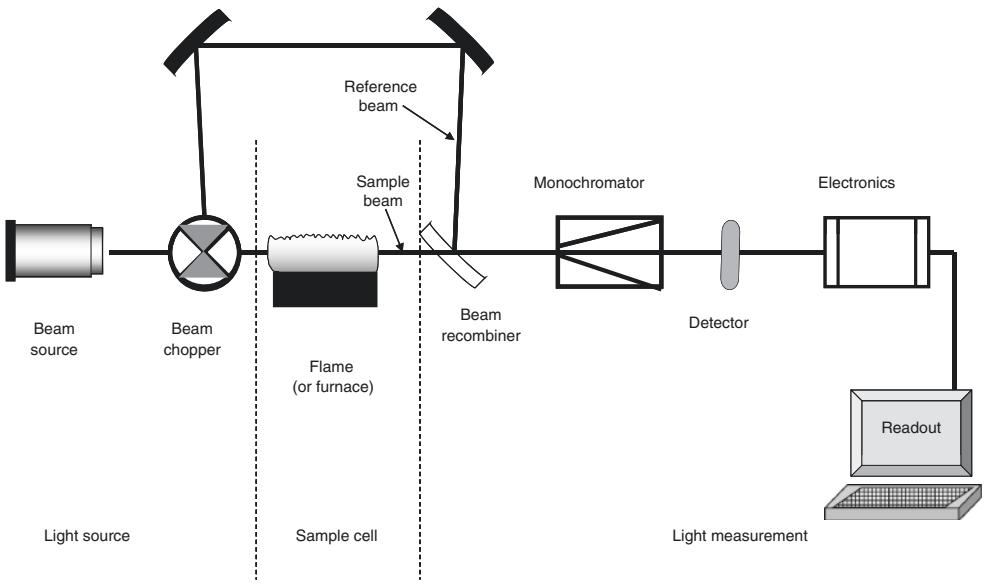
The amount of radiation absorbed by the analyte element is given by **Beer's law**:

$$A = \log(I_o / I) = abc \qquad (9.1)$$

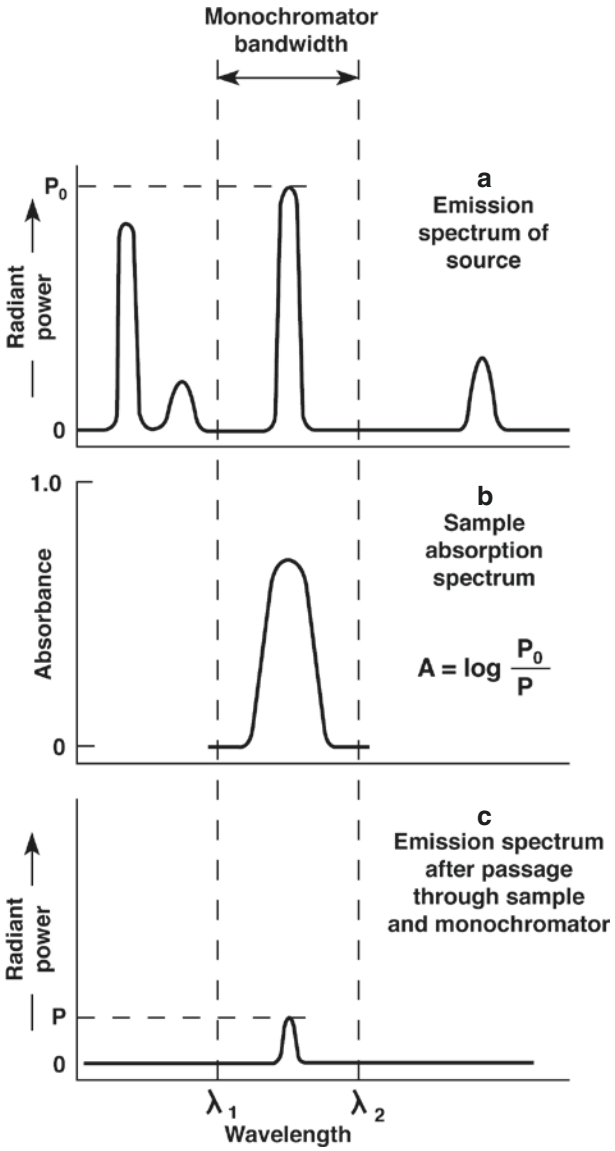
where:

A = absorbance
I_o = intensity of radiation incident on the flame
I = intensity of radiation exiting the flame
a = molar absorptivity
b = path length through the flame
c = concentration of atoms in the flame

Clearly, absorbance is directly related to the concentration of atoms in the flame.



9.3 figure Schematic representation of a double-beam atomic absorption spectrophotometer (Adapted from Beaty and Kerber [5])



9.4 figure

Schematic representation of the absorption of radiation by a sample during an atomic absorption measurement. The spectrum of the radiation source is shown in (**a**). As the radiation passes through the sample (**b**), it is partially absorbed by the element of interest. Absorbance is proportional to the concentration of the element in the flame. The radiant power of the radiation leaving the sample is reduced because of absorption by the sample (**c**) (From Skoog et al. [8], used with permission. Illustration from Principles of Instrumental Analysis, 6th ed., by DA Skoog, F. J. Holler, S.R. Crouch (2007). Reprinted with permission of Brooks/Cole, a division of Cengage Learning)

9.3.2 Principles of Electrothermal (Graphite Furnace) Atomic Absorption Spectroscopy

Electrothermal AAS is identical to flame AAS except for the atomization process. In electrothermal AAS, the sample is heated electrically in stages inside a graphite tube, commonly known as **graphite furnace**, to achieve atomization. The tube is aligned to the path of the radiation beam to be absorbed by the atomized sample and absorbance is determined. Electrothermal AAS requires smaller sample sizes and offers lower detection limits. Disadvantages are the added expense of the graphite furnace, lower sample throughput, higher matrix interferences, and lower precision.

9.3.3 Instrumentation for Atomic Absorption Spectroscopy

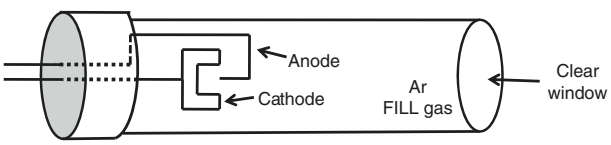
Atomic absorption spectrometers, typically with a double-beam design (see Chap. 7, Sect. 7.2.7), consist of the following components (Fig. 9.3):

1. **Radiation source**, a hollow cathode lamp (HCL) or an electrode-less discharge lamp (EDL)
2. **Atomizer**, usually a nebulizer-burner system or a graphite furnace
3. **Monochromator**, usually an UV-Vis grating monochromator
4. **Detector**, a photomultiplier tube (PMT) or a solid-state detector (SSD)
5. **Readout device**, an analog or a digital readout

(Radiation source and atomizers will be further discussed in the following paragraphs. See Chap. 7, Sects. 7.2.6.2, 7.2.6.3, and 7.2.6.4 for a more detailed discussion of monochromators, detectors, and readout devices, respectively.)

9.3.3.1 *Radiation Source*

A **hollow cathode lamp** (HCL) consists of a hollow tube filled with argon or neon gas, an anode made of tungsten, and a cathode made of the metallic form of the element being measured (Fig. 9.5). When voltage is applied across the electrodes, the lamp emits radiation



9.5 figure

Schematic representation of a hollow cathode lamp (Adapted from Beaty and Kerber [5])

characteristic of the metal in the cathode. For example, if the cathode is made of iron, an iron spectrum will be emitted. As the radiation passes through the flame containing the atomized sample, only iron atoms (not atoms of other elements) will absorb this radiation because the emitted wavelengths from the HCL are specific for iron atoms. Of course, this means that it is necessary to use a different lamp for each element analyzed (there are a limited number of multielement lamps available that contain cathodes made of more than one element). HCLs for about 60 metallic elements are commercially available, suggesting that AAS may be used for the analysis of up to 60 elements.

An **electrode-less discharge lamp** (EDL) contains no electrodes but a hollow glass vessel filled with an inert gas plus the element of interest. The discharge is produced by a high-frequency generator coil rather than an electric current [9]. EDLs are suitable for volatile elements such as arsenic, mercury, and cadmium.

Radiation reaching the monochromator comes from three sources: (1) attenuated beam from the HCL (specific emission), (2) emission from sample atoms (including both analyte and non-analyte atoms) that were excited by the flame (nonspecific emission), and (3) radiation resulting from the combustion of the fuel to create the flame (nonspecific emission). Instruments are designed to eliminate nonspecific emissions from reaching the detector. This is accomplished by positioning a monochromator between the flame and the detector. The monochromator disperses wavelengths of light that are not specific to the analyte element and isolates a line that is specific. Thus, radiation reaching the detector is the sum of radiation from the attenuated HCL beam and radiation emitted by excited analyte atoms in the flame. Since we are interested only in the amount of HCL radiation absorbed by analyte atoms in the flame, it is necessary to correct for emission from excited analyte atoms in the flame. This is accomplished by positioning a **beam chopper** perpendicular to the light path between the HCL and the flame (Fig. 9.3). A beam chopper is a disk with segments removed. The disk rotates at a constant speed so that the light emitted from the HCL reaching the detector is either unimpeded or blocked at regular intervals, i.e., it is alternating. In contrast, emission from excited analyte atoms in the flame reaching the detector is continuous. The instrument electronics subtracts the continuous emission signal from the alternating emission signal so only the signal from the attenuated HCL beam is recorded in the readout.

9.3.3.2 *Atomizers*

Flame and **graphite furnace** atomizers are the two common types of atomizers used in AAS. When applicable, a **cold vapor technique** for mercury and **a hydride generation technique** for a few elements are used to enhance sensitivity.

The **flame atomizer** consists of a **nebulizer** and a **burner**. The nebulizer is designed to convert the sample solution into a fine mist or aerosol. This is accomplished by aspirating the sample through a capillary into a chamber through which oxidant and fuel are flowing. The chamber contains baffles that remove larger droplets, leaving a very fine mist. Only about 1% of the total sample is carried into the flame by the oxidant-fuel mixture. The larger droplets fall to the bottom of the mixing chamber and are collected as waste. The burner head contains a long, narrow slot that produces a flame that may be 5–10 cm in length. This gives a long path length that increases the sensitivity of the measurement.

Flame characteristics may be manipulated by adjusting oxidant/fuel ratios and by choice of oxidant and fuel. **Air-acetylene** and **nitrous oxide-acetylene** are the most commonly used oxidant-fuel mixtures although other oxidants and fuels may be used for some elements. There are three types of flames:

1. **Stoichiometric**. This flame is produced from stoichiometric amounts (exact reaction ratios) of oxidant and fuel, so the fuel is completely burned and the oxidant is completely consumed. It is characterized by yellow fringes.
2. **Oxidizing**. This flame is produced from a fuel-lean (excess of oxygen) mixture. It is the hottest flame and has a clear blue appearance.
3. **Reducing**. This flame is produced from a fuel-rich mixture (excess of fuel compared to oxygen). It is a relatively cool flame and has a yellow color.

Analysts should follow manufacturer's guidelines or consult the literature for the proper type of flame for each element.

Flame atomizers have the advantage of being stable and easy to use. However, sensitivity is relatively low because much of the sample never reaches the flame and the residence time of the sample in the flame is short.

The **graphite furnace** is typically a cylindrical graphite tube connected to an electrical power supply. The sample is injected into the tube through an inlet using a microliter syringe with sample volumes ranging from 0.5 to 100 μL. During operation, the system is flushed with an inert gas to prevent the tube from burning and to exclude air from the sample compartment. The tube is heated electrically in stages: first the sample solvent is evaporated, then the sample is ashed, and finally the temperature is rapidly increased to ~2000–3000 K to quickly vaporize and atomize the sample.

The **cold vapor technique** works only for mercury, because mercury is the only mineral element that can exist as free atoms in the gaseous state at room temperature. Mercury compounds in a sample are first reduced to elemental mercury by the action of stannous chloride, a strong reducing agent. The elemental mercury is then carried in a stream of inert gas into an absorption cell without the need for further atomization. The **hydride generation technique** is limited to elements capable of forming volatile hydrides that include arsenic, lead, tin, bismuth, antimony, tellurium, germanium, and selenium. Samples containing these elements are reacted with sodium borohydride to generate volatile hydrides, which are carried into an absorption cell and decomposed by heat. Absorbance measurements with these two techniques are conducted in the same manner as with flame atomization, but sensitivity is greatly enhanced because there is very little sample loss [5].

9.3.4 General Procedure for Atomic Absorption Analysis

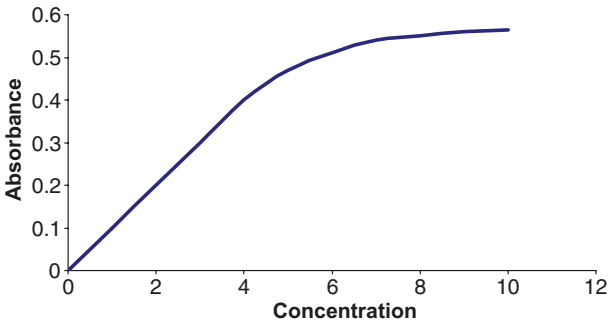
While the basic design of all atomic absorption spectrometers is similar, operation procedures do vary from one instrument to another. For any given method, it is always a good practice to carefully review standard operating procedures provided by the manufacturer before using the instrument. Most instruction manuals also provide pertinent information for the analysis of each particular element (wavelength and slit width requirements, interferences and corrections, flame characteristics, linear ranges, etc.).

9.3.4.1 *Safety Precautions*

General laboratory safety protocols and procedures as well as safety precautions recommended by the instrument manufacturers must be followed carefully to avoid personal injuries or costly accidents. The most commonly used fuel sources in flame AAS are mixtures of air-acetylene and nitrous oxide-acetylene. *ACETYLENE IS AN EXPLOSIVE GAS.* Proper ventilation must be in place before operation. The exhaust vent should be positioned directly above the burner to avoid the buildup of unburned fuel or any potentially hazardous toxic fumes. Flame atomic absorption spectrometers should never be left unattended while in operation.

9.3.4.2 *Calibration*

As illustrated in Fig. 9.6, a plot of absorbance versus concentration will deviate from linearity predicted by Beer's law when concentration exceeds a certain level. Therefore, properly constructed calibration curves using pure standards are essential for accurate quantitative measurements. If values for linear ranges are not provided by the manufacturer, the linear range of an element should be established by running a series of standards of increasing concentration and plotting absorbance versus concentration. The concentration of the unknown sample solution should be adjusted so that the measured absorbance always falls within the linear range of the calibration curve.



9.6 figure

A plot of absorbance vs. concentration showing nonlinearity above a certain concentration

Instruction manuals from the manufacturers may provide values for characteristic concentrations for each element. For example, manuals for Perkin-Elmer atomic absorption spectrophotometers state that a 5.0 mg/L aqueous solution of iron "will give a reading of *approximately* 0.2 absorbance units." If the measured absorbance reading deviates significantly from this value, appropriate adjustments (e.g., flame characteristics, lamp alignment, etc.) should be made.

9.3.5 Interferences in Atomic Absorption Spectroscopy

Two types of interferences are encountered in AAS: spectral and nonspectral. **Spectral interferences** are caused by the absorption of radiation by other elemental or molecular species at wavelengths that overlap with the spectral regions of the analyte present in the sample. **Nonspectral interferences** are caused by sample matrices and conditions that affect the atomization efficiency and/or the ionization of neutral atoms in the atomizer.

9.3.5.1 *Spectral Interferences*

An element in the sample other than the element of interest may absorb at the wavelength of the spectral band being used. Such interference is rare because emission lines from the HCL are so narrow that only the element of interest is capable of absorbing the radiation in most cases. One example of when this problem does occur is with the interference of iron in zinc determinations. Zinc has a spectral line at 213.856 nm, which overlaps the iron line at 213.859 nm. The problem may be solved by choosing an alternative spectral

line for measuring zinc or by narrowing the monochromator slit width.

The presence of alkaline earth oxide and hydroxide molecules may also lead to several specific spectral interferences. Spectra of calcium oxide and magnesium hydroxide will appear as **background absorption** for atomic absorption measurements of sodium and chromium, respectively [10]. These interferences are weak but must be taken into account when working with a complex sample matrix.

9.3.5.2 *Nonspectral Interferences*

As mentioned above, quantitative results for an unknown sample are possible only through comparison with a series of standards of known concentrations. **Transport interferences** may occur when other components present in the sample matrix influence physical properties such as viscosity, surface tension, and vapor pressure of the sample solution, leading to differences in the rate of aspiration, nebulization, or transport between the sample solution and the standards during flame atomization. Such interferences often can be overcome by using the same solvent and by matching as closely as possible the physical properties of the sample solution and the standards. The standard addition protocol (see Chap. 7, Sect. 7.2.4) may also be used. Transport interferences are rarely a problem with graphite furnace instruments but matrix interferences are a common and serious problem.

Matrix composition of the sample solution also may affect the lateral migration of an analyte, resulting in **solute vaporization interferences**. For example, it has been observed in flame absorption and emission that alkaline earth metals are depressed by elevated levels of aluminum and phosphorus [11], and aluminum also suppresses the recovery of calcium [12]. **Chemical interferences** occur when an element forms thermally stable compounds that do not decompose during atomization. **Refractory metals** such as titanium and molybdenum may combine with oxygen to form stable oxides; higher-temperature flames are usually required for their dissociation. Also, phosphate in a sample matrix reacts with calcium to form calcium pyrophosphate which is not decomposed in the flame. A **releasing agent** such as lanthanum, which binds phosphate more strongly than calcium, may be added to the sample solution and the standards to free up calcium for atomization [12].

Ionization interferences are caused by ionization of analyte atoms in the flame and thereby reduce the concentration of neutral atoms for atomic absorption measurement. (Remember that atoms and ions of the same element absorb radiation of different wavelengths and produce different spectra.) Ionization increases with increasing flame temperature and normally is not a problem in cooler air-acetylene flames. It can be a problem in hotter nitrous oxide-acetylene flames, especially with elements that have ionization potentials of 7.5 eV or less (e.g., alkali metals). The ionization of atoms results in an equilibrium situation:

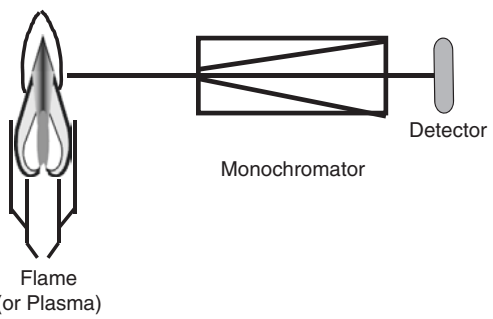
$$M \leftrightarrows M^{+} + e^{-} \tag{9.2}$$

Ionization interferences can be countered by spiking the sample solution and the standards with another **easily ionized element** (EIE) such as potassium or cesium, known as an **ionization suppressant**, to create a pool of free electrons in the flame, which shifts the above equilibrium to the left and suppresses the ionization of the analyte atoms.

9.4 ATOMIC EMISSION SPECTROSCOPY

In contrast to AAS, the source of the measured radiation in AES is the excited atoms in the sample, not radiation from a HCL. Figure 9.7 shows a simplified diagram of an atomic emission spectrometer. Sufficient energy is first applied to the sample to excite atoms to higher-energy levels; emissions of wavelengths characteristic of individual elements are then measured when electrons from excited atoms move back to the ground state or a lower-energy state. The ratio of the number of excited atoms to ground-state atoms occurring in a flame or plasma is described by the Maxwell-Boltzmann equation for resonance lines (Chap. 6, Sect. 6.4.3). This equation applies when there is thermal impact or collisions between atoms or molecules a portion of them will become excited.

Energy for excitation may be produced by **heat** (usually from a flame), **light** (from a laser), **electricity** (arcs or sparks), or **radio waves** (ICP). (*Note:* AES is also commonly called optical emission spectroscopy (OES), especially when combined with ICP. In this chapter, we will use ICP-OES rather than ICP-AES for our discussion, but the two terms are virtually interchangeable.)



9.7 figure A simplified diagram of an atomic emission spectrometer (Adapted from Boss and Fredeen [6])

The two most common forms of AES used in food analysis are **flame emission spectroscopy** and **inductively coupled plasma-optical emission spectroscopy** (ICP-OES).

9.4.1 Principles of Flame Emission Spectroscopy

Flame emission spectrometers employ a nebulizer-burner system to atomize and excite the atoms of the elements being measured. The flame with the excited atoms serves as the radiation source, so an external source (the HCL with the beam chopper) is not required. Otherwise instrumentation for flame emission spectroscopy is essentially identical to that for AAS. Many modern atomic absorption spectrometers can also be operated as flame emission spectrometers.

In some instruments, interference filters (instead of the more versatile monochromators typically found in absorption/emission spectrometers) are employed to isolate the spectral region of interest. **Flame photometers** are economical emission spectrometers equipped with interference filters and are specifically designed for the analysis of the alkali and alkaline earth metals in biological samples. Low flame temperatures are used so that only easily excited elements such as sodium, potassium, and calcium produce emissions. This results in a simpler spectrum and reduces interference from other elements present in the sample matrix.

9.4.2 Principles of Inductively Coupled Plasma-Optical Emission Spectroscopy

ICP-OES differs from flame emission spectroscopy in that it uses an argon plasma as the excitation source. A **plasma** is defined as a gaseous mixture containing significant concentrations of cations and electrons. Temperatures in argon plasmas could be as high as 10,000 K, with analyte excitation temperature typically ranging from 6,000 to 7,000 K.

The extremely high temperatures and the inert atmosphere of argon plasmas are ideal for the atomization, ionization, and excitation of the analyte atoms in the sample. The low oxygen content reduces the formation of oxides, which is sometimes a problem with flame methods. The nearly complete atomization of the sample minimizes chemical interferences. The relatively uniform temperatures in plasmas (compared to nonuniform temperatures in flames) and the relatively long residence time give good linear responses over a wide concentration range (up to 6 orders of magnitude).

9.4.3 Instrumentation for Inductively Coupled Plasma-Optical Emission Spectroscopy

Inductively coupled plasma-optical emission spectrometers typically consist of the following components (Fig. 9.8):

1. **Argon plasma torch**
2. **Monochromator**, **polychromator**, or **echelle optical system**
3. **Detector(s)**, a single or multiple PMT(s) or solid-state array detector(s)
4. **Computer** for data collection and treatment

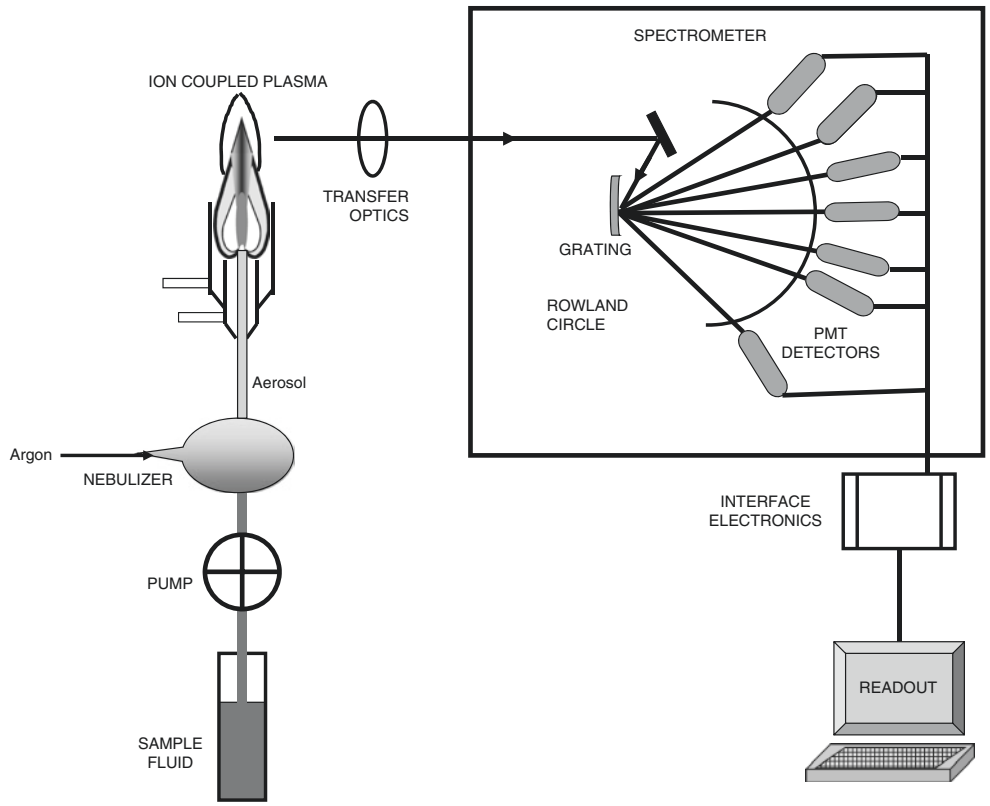
9.4.3.1 *Argon Plasma Torch*

9.4.3.1.1 Characteristics of an Argon Plasma Torch

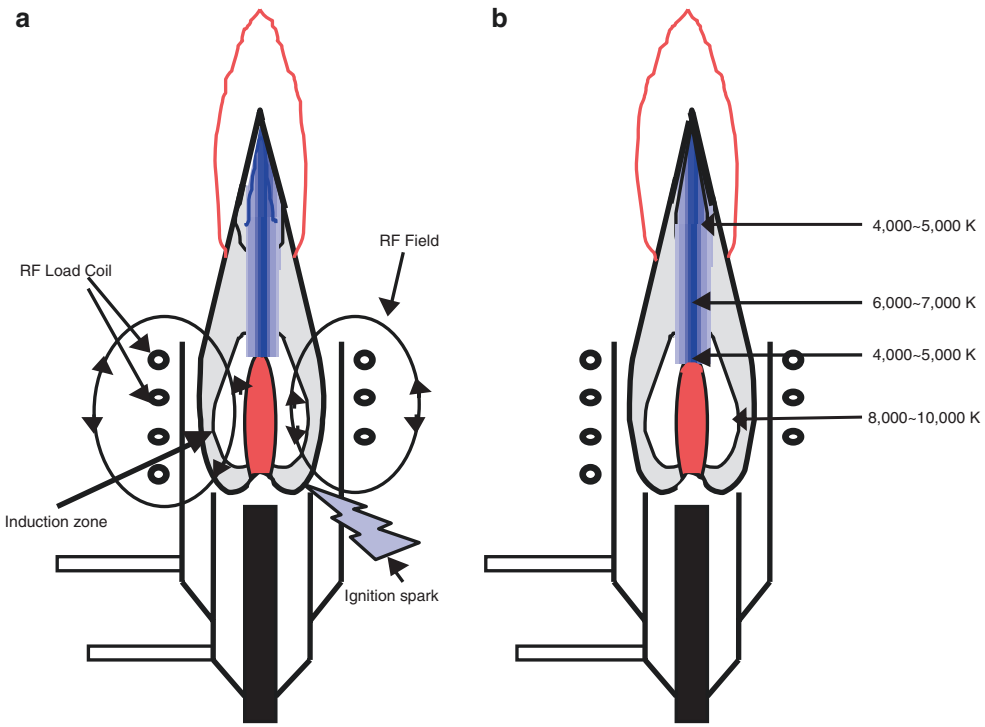
The plasma torch (Fig. 9.9) consists of three concentric quartz tubes centered in a copper coil, called the **load coil**. During operation of the torch, a stream of argon gas flows through the outer tube, and **radio frequency** (RF) power is applied to the load coil, creating a magnetic field oscillating at the frequency of the RF generator (usually 27 MHz or 40 MHz). The plasma is started by ionizing argon atoms with an electric spark to form argon ions and electrons. The oscillating magnetic field couples with the argon ions and electrons, forcing them to flow in an annular (ring-shaped) path. Heating does not involve burning fuel to directly heat and atomize the sample, as is the case with flame AAS (argon is a noble gas and will not combust). Rather, heating is accomplished by transferring RF energy to free electrons and argon ions in a manner similar to the transfer of microwave energy to water in a microwave oven. These high-energy electrons in turn collide with argon atoms, generating even more electrons and argon ions and causing a rapid increase in temperature to approximately 10,000 K. The process continues until about 1 % of the argon atoms are ionized. At this point the plasma is very stable and self-sustaining for as long as the RF field is applied at constant power. The transfer of energy to a system through the use of electromotive forces generated by magnetic fields is known as **inductive coupling** [13], hence the name ICP.

9.4.3.1.2 Sample Introduction and Analyte Excitation

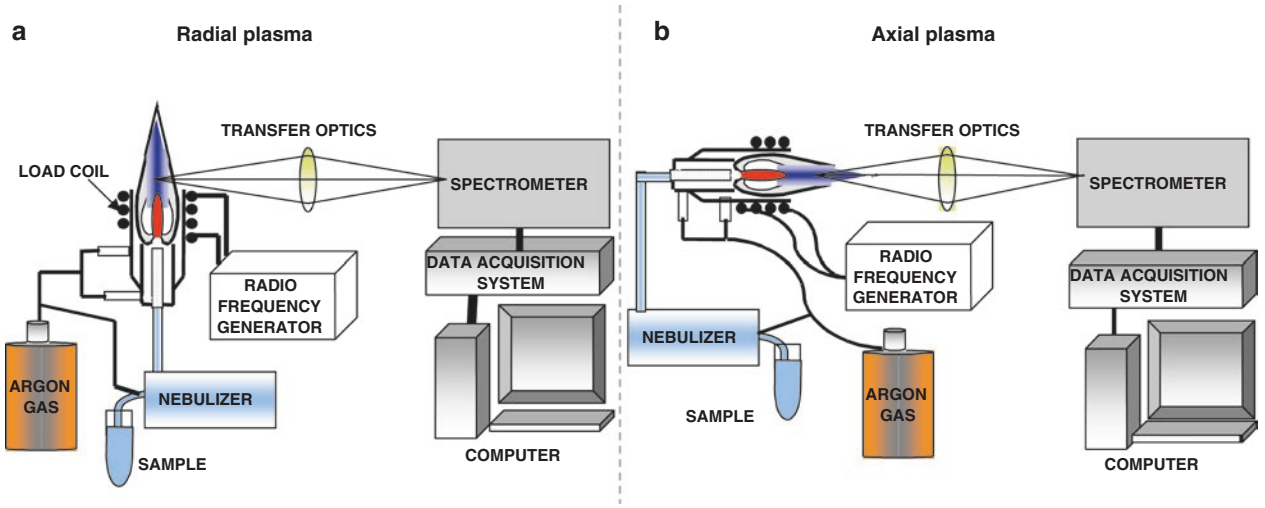
Samples are nebulized and introduced as aerosols carried by another stream of argon gas in the inner tube inside the annulus of the plasma at the base of the RF load coil. The sample goes through the process



9.8 figure Schematic of an inductively coupled plasma-optical emission simultaneous spectrometer



9.9 figure The ICP plasma: (**a**) the process by which the plasma is formed and sustained and (**b**) the temperature distribution of the plasma



9.10 figure Major components and typical layout of (**a**) a radially viewed ICP-OES instrument and (**b**) an axially viewed ICP-OES instrument

of desolvation, vaporization, atomization, ionization, and excitation as shown in Fig. 9.2. The exact mechanisms by which the analyte atoms and ions are excited in a plasma are not understood fully. Nevertheless, there is general agreement that excitation is dependent primarily on the number and temperature of the electrons in the plasma [14]. Presumably, when electrons are accelerated in a magnetic field, they acquire enough kinetic energy to excite analyte atoms and ions upon collision [14]. Exceptions to this mechanism include the excitation of neutral atoms of magnesium, copper, and a few other elements that are believed to be excited when an argon ion (Ar^+) extracts an electron from the analyte atom (M) to yield M^{+*} and Ar^0, where M is a generic abbreviation for a ground-state metal atom and M^{+*} is an excited metal ion. This mechanism is termed "charge transfer" [15, 16].

9.4.3.1.3 Radial and Axial Viewing

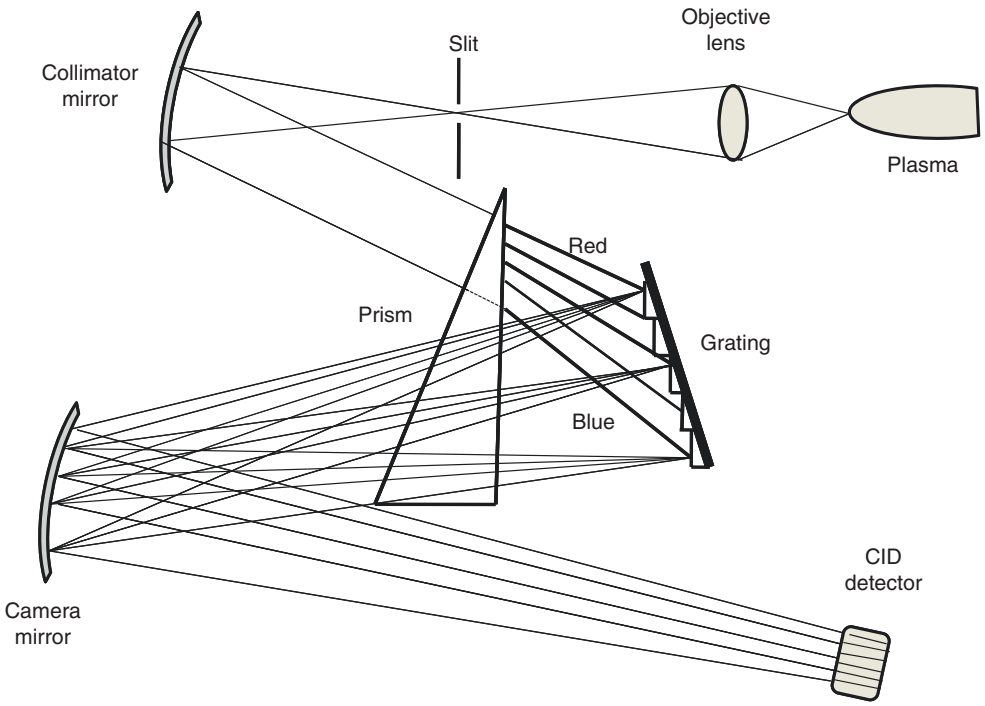
Emissions from ICP torches can be viewed either radially or axially. In the **radial view**, the optics are aligned perpendicular to the torch (Fig. 9.10a). In the **axial view**, the light is viewed by looking down the center of the torch (Fig. 9.10b). Axial viewing offers lower detection limits but is more prone to matrix interferences. Manufacturers of modern ICP-OES instruments have mostly combined the radial and axial configurations into single "dual-view" units, offering greater flexibility to the end user.

9.4.3.2 *Detectors and Optical Systems*

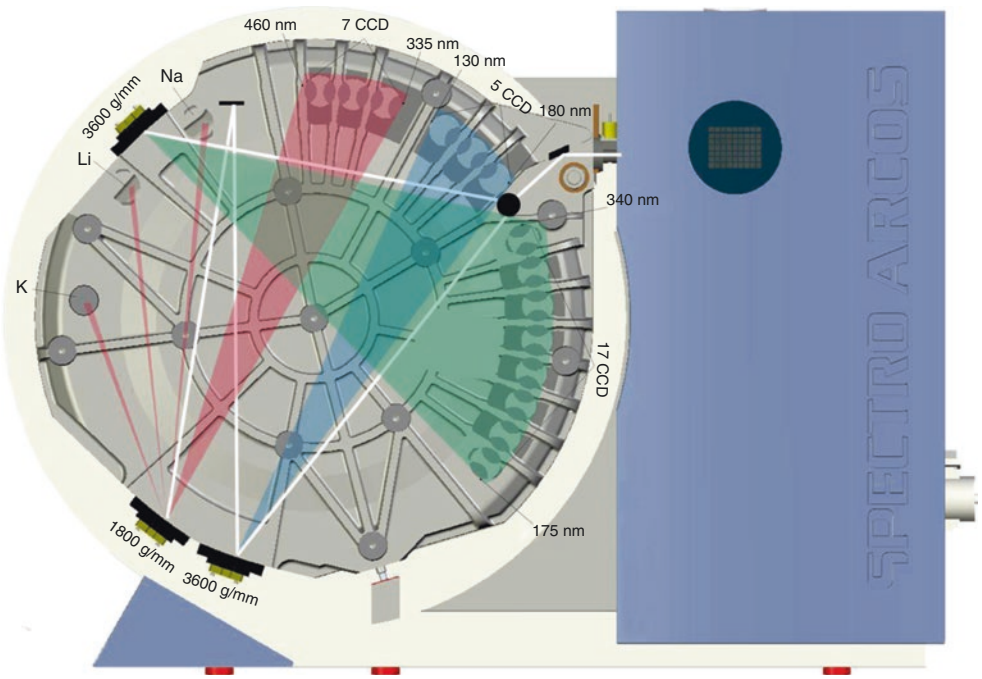
Older ICP-OES instruments were configured to focus emission lines from each of the analyte elements on separate PMTs arranged in a semicircle (the Rowland circle) as shown in Fig. 9.8. PMT-based instruments are relatively large and bulky, and while some are still in use today, they have been mostly replaced by modern instruments equipped with an **echelle optical system** and a solid-state array detector (Fig. 9.11), capable of measuring continuous emission spectra over a wide wavelength range.

The echelle optical system employs two dispersing components in series, a prism and a diffraction grating. The prism first disperses the radiation from the plasma torch without any wavelength overlap (in the x-direction). The radiation then strikes a low-density ruled grating (about 53 groves per mm). This further separates the radiation in a direction perpendicular to the direction of radiation dispersed by the prism (in the y-direction), producing a two-dimensional spectrum with a wavelength range of 166–840 nm. When the radiation passing through the echelle optical system is focused onto the solid-state array detector, electrons are liberated proportionally to the intensity of the incident radiation and trapped in the silicon-based, light-sensitive elements called pixels for signal processing. ICP-OES instruments typically use one of three types of solid-state array detectors: **charge coupled device** (CCD), **complementary metal oxide semiconductor** (CMOS), or **charge injection device** (CID). A description of these detectors is beyond the scope of this chapter. Interested readers are referred to the comparisons between different types of solid-state array detectors provided in references [17, 18].

More recent development in ICP-OES instrumentation involves the use of the Optimized Rowland Circle Alignment (ORCA) design to further extend measurement over a wavelength range of 130–800 nm. This allows for the measurement of chlorine which emits a spectral line at 134 nm, below the range of an echelle system. In an ORCA system, detectors are positioned in a semicircular arrangement (Fig. 9.12). A holographic grating is used to separate wavelengths of



9.11 figure A simplified example of an echelle spectrometer. The echelle spectrometer combines two light dispersing elements in series. A prism first disperses the light in the *x*-direction without overlapping orders, followed by a grating which disperses the light in the *y*-direction, producing a two-dimensional spectrum on the detector



9.12 figure The Optimized Rowland Circle Alignment (ORCA) design using three concave gratings. Each concave grating has the same curvature of the Rowland circle and will disperse the light according to its wavelength. The dispersed light will then be focused back onto one of the detectors placed along the diameter of the Rowland circle. The system uses CCD detectors in place of photomultiplier tubes (PMTs) and allows for the determination of nearly all the elements listed in the periodic chart (Used with permission. Courtesy of SPECTRO Analytical Instruments GmbH Boschstr., Kleve, Germany)

the light coming from the plasma torch. The ORCA system has fewer light dispersing elements (gratings and prisms) than an echelle system. This reduces light loss in the system and therefore increases sensitivity. It also allows for a more uniform resolution and greater stability.

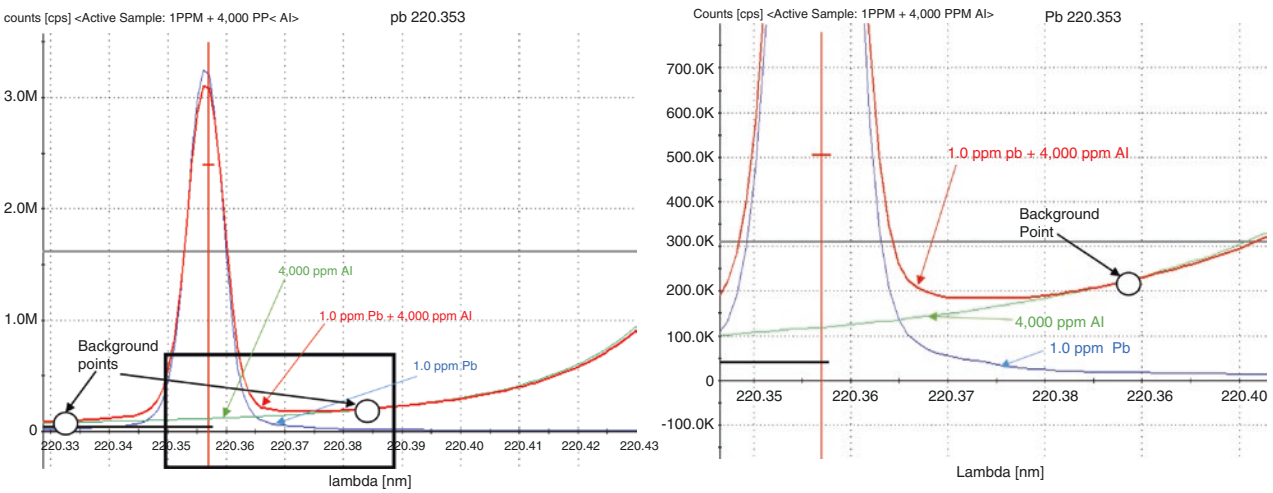
9.4.4 General Procedure for Inductively Coupled Plasma-Optical Emission Spectroscopy Analysis

As is the case with atomic absorption spectrometers, operating procedures for atomic emission spectrometers vary somewhat from instrument to instrument. ICP-OES instruments are almost always interfaced with computers. The software contains methods that specify instrument operating conditions. The computer may be programmed by the operator, or in some cases, default conditions may be used. Once the method is established, operation is highly automated.

9.4.5 Interferences in Inductively Coupled Plasma-Optical Emission Spectroscopy

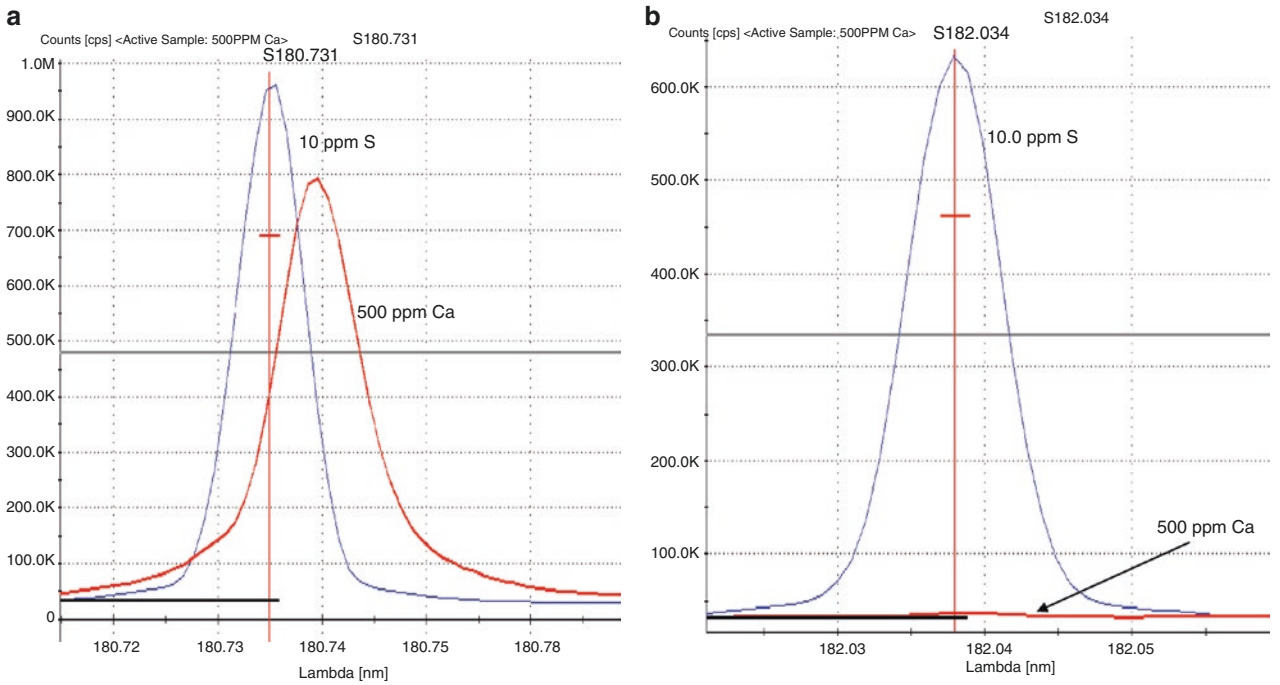
Generally, interferences in ICP-OES analyses are less of a problem than with AAS, but they do exist and must be taken into account. **Spectral interferences** are the most common. Samples containing high concentrations of certain ions may cause an increase (shift) in background emissions at some wavelengths. This will cause a positive error in the measurement, referred to as **background shift interference** (Fig. 9.13). Correction is relatively simple. Two additional emission measurements are made at a wavelength above and below the emission line of the analyte. The average of these two emissions is then subtracted from the emission of the analyte. (*Note*: In the example in Fig. 9.13, the intensity of the background shift from the aluminum is *increasing*, and this is why it is necessary to make measurements above and below the emission line for lead. If the intensity of the background shift is constant, then only one measurement near the wavelength of the analyte's emission line is required for correction.) Alternatively, another emission line could be chosen in a region where there is no background shift.

A more problematic type of spectral interference, called **spectral overlap interference** (Fig. 9.14), occurs when the resolution of the instrument is insufficient to prevent overlap of the emission line (i.e., a very narrow bandwidth) of one element with that of another. For example, when determining sulfur in a sample containing high concentrations of calcium, some of the emission from one of the calcium lines overlaps with the sulfur emission line at 180.731 nm. This will cause an apparent increase in the measured concentration of sulfur. This problem may be overcome by either choosing a different sulfur emission line or by calculating the **inter-element correction** (IEC) factor. In the above example, this would require first measuring the emission of calcium at a different wavelength to determine its concentration in the sample. A standard solution of pure calcium is then prepared at that same concentration, and the apparent sulfur concentration in the pure calcium standard solution, presumably containing no sulfur, is determined. Finally, the sample is analyzed for sulfur, and the contribution of calcium to the sulfur signal is subtracted, thereby giving an accurate estimate of the true sulfur concentration.



9.13 figure

Background shift interference of aluminum on lead. The two graphs show that aluminum will increase the background signal near the lead line at 220.35 nm. This is corrected by placing background subtraction points on both sides of the lead peak. The graph on the right is a blowup of the area that shows the background shift interference



9.14 figure

Spectral overlap interference of calcium on sulfur. (**a**) The overlapping spectra of sulfur and calcium indicate that the resolution of the instrument is insufficient to resolve the emission from sulfur at 180.731 nm and calcium at 180.734 nm. (**b**) If the sample is being analyzed for sulfur, it is best to use the sulfur line at 182.034 nm where there is no spectral overlap

9.5 APPLICATIONS OF ATOMIC ABSORPTION AND EMISSION SPECTROSCOPY

9.5.1 Uses

Atomic absorption and emission spectroscopy are widely used for the quantitative measurement of mineral elements in foods. In principle, any food may be analyzed with any of the atomic spectroscopy methods discussed. In most cases, it is necessary to ash the food to destroy organic matter and to dissolve the ash in a suitable solvent (usually water or dilute acid) prior to analysis (see Chap. 16 for details on ashing methodology). Proper ashing is critical to accuracy. Some elements may be volatile at temperatures used in **dry ashing** procedures. Volatilization is less of a problem in **wet ashing**, except for the determination of boron, which is recovered better using a dry ashing method. However, ashing reagents may be contaminated with the analyte. It is therefore wise to carry blanks through the ashing procedure.

Some liquid products may be analyzed without ashing, provided appropriate precautions are taken to avoid interferences. For example, vegetable oils may be analyzed by dissolving the oil in an organic solvent such as acetone or ethanol and aspirating the solution directly into a flame atomic absorption spectrometer. Milk samples may be treated with trichloroacetic acid to precipitate the protein; the resulting supernatant is analyzed directly. A disadvantage of this approach is that the sample is diluted in the process and the analyte can become entrapped or complexed to the precipitated proteins. This may be a problem when analytes are present in low concentrations. An alternative approach is to use a graphite furnace for atomization. For example, an aliquot of an oil may be introduced directly into a graphite furnace for atomization. The choice of method will depend on several factors, including instrument availability, cost, precision/sensitivity, and operator skill.

9.5.2 Practical Considerations

9.5.2.1 *Reagents*

Since concentrations of many mineral elements in foods are at the trace level, it is essential to use highly pure chemical reagents and water for preparation of samples and standard solutions. Only reagent grade chemicals should be used. Water may be purified by distillation, deionization, or a combination of the two. **Reagent blanks** should always be carried through the analysis.

9.5.2.2 *Standards*

Quantitative atomic spectroscopy depends on comparison of the sample measurement with appropriate standards. Ideally, standards should contain the ana-

lyte metal in known concentrations in a solution that closely approximates the sample solution in composition and physical properties. Because many factors can affect the measurement, such as flame temperature, aspiration rate, and the like, it is essential to run standards frequently, preferably right before and/or right after running the sample. Standard solutions may be purchased from commercial sources, or they may be prepared by the analyst. Obviously, standards must be prepared with extreme care since the accuracy of the analyte determination depends on the accuracy of the standard. Perhaps the best way to check the accuracy of a given assay procedure is to analyze a reference material of known composition and similar matrix. Standard reference materials may be purchased from the United States National Institute of Standards and Technology (NIST) [19].

9.5.2.3 *Labware*

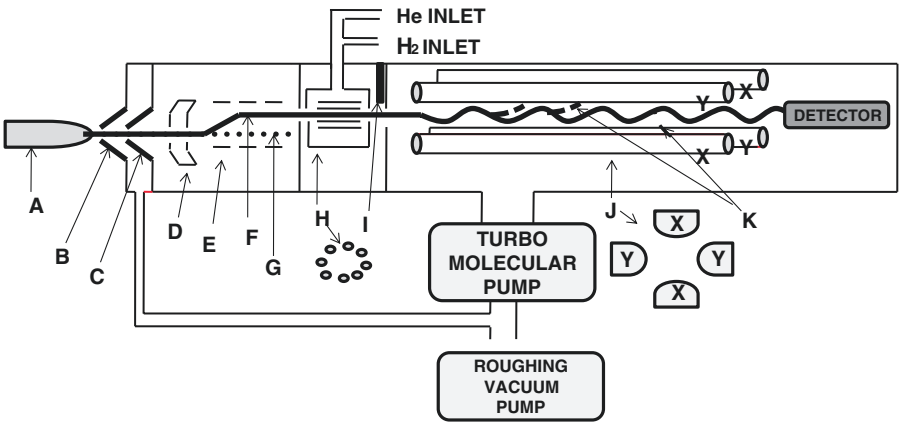
Vessels used for sample preparation and storage must be clean and free of the elements of interest. Plastic containers are preferable because glass has a greater tendency to adsorb and later leach metal ions. All labware should be thoroughly washed with a detergent, carefully rinsed with distilled or deionized water, soaked in an acid solution (1*N* HCl is sufficient for most applications), and rinsed again with distilled or deionized water.

9.6 INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

As described above, atomic absorption and emission spectrometers are designed to quantify mineral elements in a sample by measuring either the absorption or emission of radiation by the element of interest at a wavelength unique to that element. Another approach is to directly measure the atoms (as ions in this case) of the element in the sample. This is possible with an **inductively coupled plasma-mass spectrometry** (ICP-MS) instrument that combines ICP with a mass spectrometer (Fig. 9.15). This allows extremely low detection limits at the single part per trillion (ppt) levels, enhanced multielement capabilities, and the ability to quantify individual isotopes present in multi-isotope elements. Note that isotopic analysis is not possible with atomic absorption or emission spectroscopy since absorption and emission lines are the same for all isotopes of a given element.

9.6.1 Principles of Inductively Coupled Plasma-Mass Spectrometry

The principles of mass spectrometry and descriptions of instrumentation for different types of mass spectrometers are given in detail in Chap. 11. In ICP-MS



A Plasma
B Sample Cone
C Skimmer cone
D Extraction Lenses
E Omega and Focusing Lenses
F Ion Beam
G Photons and Neutrals being separated from the ion beam
H Collision/reaction Cell (CRC) or Octopole Reaction System (ORS); also showing a cross section of the octopole
I Gate valve used to maintain vacuum in the MS for maintenance
J Quadrupole rods; also showing a cross section of the 4 molybdenum hyperbolic rods
K Rejected ions of improper mass to charge ratio

9.15 figure

A simplified diagram of an inductively coupled plasma-mass spectrometer. The ions, electrons, photons, and neutral species generated in the plasma (*A*) are guided through an interface made up of a water-cooled sampling cone (*B*) and a skimmer cone (*C*) with a partial vacuum in-between to remove argon gas and some neutral species. The remaining particles pass through the extraction lenses (*D*), which repel electrons and accelerate the positive ions further through to the off-axis ion omega lenses (*E*), bending the ion beam (*F*) as a result. The paths of the photons and neutral species (*G*) are unaffected and separated from the ion beam (see Chap. 11 for more details on the instrumentation of mass spectrometers)

mineral analyses, samples are prepared and aspirated into the ICP torch in the same manner as in ICP-OES, but instead of having an optical system and a device for separating and detecting radiation of specific wavelengths, the ICP-MS uses a mass spectrometer to separate and detect **ions** of the elements directly based on their unique **mass-to-charge (*m/z*) ratios**. As shown in Fig. 9.15, the interface between the high-temperature ICP operating at atmospheric pressure and the high vacuum mass spectrometer consists of: (1) two funnel-shaped water-cooled cones (the sampling cone and the skimmer cone) and (2) ion lenses (extraction lenses and omega lenses) to guide the analyte ions into the quadrupole while removing electrons, photons, and other neutral species from the ICP discharge.

9.6.2 Interferences in Inductively Coupled Plasma-Mass Spectrometry

Interferences in ICP-MS arise when different ionic species from the sample have the same *m/z* ratio, leading to overlapping of signals. For example, iron has four naturally occurring stable isotopes: ^{54}Fe, ^{56}Fe, ^{57}Fe, and ^{58}Fe, with natural abundances of 5.8 %, 91.75 %, 2.1 %, and 0.28 %, respectively. Nickel has five stable isotopes: ^{58}Ni, ^{60}Ni, ^{61}Ni, ^{62}Ni, and ^{64}Ni, with natural abundances of 68.04 %, 26.22 %, 1.14 %, 3.63 %, and 0.93 %, respectively. The signals for ^{58}Fe and ^{58}Ni will overlap, resulting in an **isobaric interference** because $m/z = 58$ for both isotopes. In this case, the analyst would select ^{56}Fe for the determination of iron and ^{60}Ni for the determination of nickel in the sample. (It is best to select the isotope with the highest natural abundance because the measurement precision is higher for more abundant isotopes. The concentration of the isotope in the sample is equal to the concentration of the element multiplied by the % natural abundance.) Most elements have at least one isotope with a unique mass number, thus allowing identification and quantification of elements.

The **doubly charged interference** occurs when ions of a particular element exist with double positive charges (instead of the normal single positive charge). Ions with charges greater than +1 typically have negligible impact except for ^{138}Ba, which may lose one or two electrons to produce singly and doubly charged ions with $m/z = 138$ and $m/z = 69$, respectively. The presence of doubly charged ^{138}Ba in a sample would interfere with ^{69}Ga. Another possible source of interference, referred to as **polyatomic interference**, comes from the formation of molecular species in the plasma between argon and elements from acids (e.g., H, N, O, Cl, etc.) used in dissolving the sample. Table 9.3 shows some examples of polyatomic interferences. Modern ICP-MS instruments can be equipped with devices called **collision/reaction cells** (CRC) through which gasses such as H_2, He, NH_3, or CH_4 may be introduced to remove doubly charged and polyatomic interferences based on differences in the physical size and kinetic energy between the analytes and the interfering species.

9.3 table Examples of polyatomic interference in ICP-MS

Polyatomic species	*m/z*	*Interfered element/isotope*
$^{38}Ar^{1}H^{+}$	39	$^{39}K^{+}$
$^{35}Cl^{16}O^{+}$	51	$^{51}V^{+}$
$^{40}Ar^{12}C^{+}$	52	$^{52}Cr^{+}$
$^{38}Ar^{16}O^{1}H^{+}$	55	$^{55}Mn^{+}$
$^{40}Ar^{16}O^{+}$	56	$^{56}Fe^{+}$
$^{40}Ar^{35}Cl^{+}$	75	$^{75}As^{+}$
$^{40}Ar^{40}Ar^{+}$	80	$^{80}Se^{+}$

Adapted from Thomas [7]

Another approach to reduce interferences from polyatomic species is to use a high-resolution ICP-MS, which utilizes a double focusing sector-field mass spectrometer to separate ions generated by the plasma [20]. For example, high-resolution ICP-MS has been shown to resolve the Fe peak at mass 55.935 from ArO at mass 55.957 [21, 22]. In this case the ions are measured sequentially, which is the case for most of the ICP-MS systems. However there is one ICP-MS system available that is capable of measuring all the elements from lithium to uranium simultaneously [23]. This system uses a double focusing sector-field mass spectrometer in a Mattauch-Herzog geometry, in which the ion beam energy band width is reduced using an electrostatic energy analyzer to achieve high resolution. The ion beam then passes through a magnetic field for mass separation and is focused on to one focal plane, enabling the entire spectrum to be measured with a flat surface detector simultaneously. This would be an ideal instrument for measuring isotope ratios from transient signals.

9.7 COMPARISON OF AAS, ICP-OES, AND ICP-MS

Table 9.4 provides a summary of advantages and disadvantages of AAS, ICP-OES, and ICP-MS. Flame AAS has enjoyed a long history of applications in mineral analysis. It is relatively inexpensive and easy to use, and is ideal for analyzing a single element in a given sample. The major disadvantages of flame AAS include relatively low sensitivity, narrow linear working range, the use of potentially dangerous fuel gas, and its limitations on multielement

9.4 table Advantages and disadvantages of AAS, ICP-OES and ICP-MS

	Flame AAS	*Graphite furnace AAS*	*ICP-OES*	*ICP-MS*
Detection limit[a]	Good detection limits with many elements at the part per billion (ppb) level	Better than flame AAS and better than ICP-OES for some elements	Better than flame AAS	Overall best detection limits compared to other techniques
Elemental analytical capability	Single	Single	Multiple	Multiple
Approximate analytical working range	3 orders of magnitude	2 orders of magnitude	6 orders of magnitude (could be higher with dual-view models)	9 orders of magnitude
Cost	Low	Low to medium	Medium	High
Use of explosive fuel gas	Yes (Flame AAS instruments should not be unattended while in operation.)	No	No	No
User-friendliness	Some skills required but relatively easy to use	Some skills required	Easy to use once the computer interface is set up and operation is automated	Method development requires more expertise compared to other techniques
Ideal application	Analyzing a limited number of elements in a given sample	Analyzing a limited number of elements, and requiring better detection limits than Flame AAS	Multiple elements in a large number of samples	Multiple elements at ultra-trace concentrations in a large number of samples
Isotopic analysis	N/A	N/A	N/A	Isotopic analysis possible because isotopes of the same element have different mass-to-charge ratios

[a]See Table 9.5 for more information on detection limit of each technique for different elements

analysis. Electrothermal AAS offers improved detection limits, but with the added cost of the graphite furnace, it somewhat negates the cost advantage of AAS. In fact, the cost of a high-end graphite furnace spectrometer overlaps with an entry-level ICP-OES system [24].

ICP-OES instruments are capable of determining concentrations of multiple elements in a single sample with a single aspiration. This offers a significant speed advantage and higher throughput over AAS when the objective is to quantify multiple elements (up to 70) in a given sample. The high temperature of the ICP torch also eliminates many nonspectral interferences (e.g., chemical interferences) encountered in AAS. Another advantage of ICP-OES over AAS is a much wider analytical working range. The analytical working range for ICP-OES is 4–6 orders of magnitude (i.e., 1 µg/L to 1 g/L without having to recalibrate the instrument), compared to about 3 orders of magnitude for AAS (i.e., 1 µg/L to 1 mg/L). All these advantages help explain the popularity of ICP-OES in commercial laboratories analyzing multiple elements in a large number of samples. ICP-OES is commonly used to obtain information for standard nutrition labeling.

ICP-MS retains the advantages offered by ICP-OES but, in conjunction with mass spectrometry, offers the lowest detection limits, enhanced multielement capabilities, a wider analytical working range (9 orders of magnitude, i.e., 1 ng/L to 1 g/L), and isotopic information potentially for tracking the geographical origins of food products [25]. Laboratories analyzing trace or ultra-trace concentrations of toxic heavy metals such as cadmium would be best served with an ICP-MS system. The major disadvantage for ICP-MS is perhaps the cost, which is about two to four times higher than its ICP-OES counterparts.

Table 9.5 lists the detection limits with different techniques for mineral elements of interest in foods. It should be noted that these are approximate values, and detection limits will vary depending on many factors such as the sample matrix and the stability of the instrument. A two- or threefold difference in detection limit is probably not meaningful, but an order of magnitude difference is noteworthy.

9.5 table Approximate detection limits (μg/L) for selected elements analyzed with various instruments

Element	*Flame AAS*	*Graphite furnace AAS*	*ICP-OES*	*ICP-MS*
Al	45	0.1	1	0.0004
As	150	0.05	1	0.0004
Ca	1.5	0.01	0.05	0.0003
Cd	0.8	0.002	0.1	0.00007
Cu	1.5	0.014	0.4	0.0002
Fe	5	0.06	0.1	0.0005
K	3	0.005	1	0.001
Hg	300	0.6	1	0.001
Mg	0.15	0.004	0.04	0.0001
Mn	1.5	0.005	0.1	0.0001
Na	0.3	0.005	0.5	0.0003
Ni	6	0.07	0.5	0.0002
P	75,000	130	4	0.04
Pb	15	0.05	1	0.00004
Se	100	0.05	2	0.0003
Tl	15	0.1	2	0.00001
Zn	1.5	0.02	0.2	0.0007

Adapted from Anonymous [24]
Detection limit is defined as the lowest concentration of the element in a solution that can be distinguished from the blank with 98% confidence

9.8 SUMMARY

In comparison with traditional wet chemistry methods, AAS, AES, and ICP-MS methods are capable of measuring trace concentrations of elements in complex matrices rapidly and with excellent precision. For most applications, sample preparation involves the destruction of organic matter by dry or wet ashing, followed by dissolution of the ash in an aqueous solvent, usually a dilute acid. The sample solution is introduced as a fine mist into a flame atomizer or an ICP torch (or by injection into a graphite furnace) where it encounters very high temperatures (~2000–3000 K for flame or graphite furnace, and ~6000–7000 K for plasma). The sample goes through the process of desolvation, vaporization, atomization, and ionization. Analyte atoms, now in the gaseous state, are well separated and remain mostly neutral in a flame, but a significant fraction of them lose an electron and become charged in a plasma. The final step is to measure quantitatively the concentrations of the elements either by atomic spectroscopy or mass spectrometry.

Atomic spectroscopy depends on the absorption or emission of electromagnetic radiation (light) by the atoms in the gaseous state. Atoms absorb or emit radiation of discrete wavelengths because the allowed energy levels of electrons in atoms are fixed and distinct. In other words, each element has a unique set of allowed electronic transitions and therefore a unique spectrum. In AAS, light of a discrete wavelength from the element-specific hollow cathode lamp will only be absorbed by the atoms of that element in the sample. Furthermore, the amount of light absorbed is directly related to the concentration of the atoms in the sample. By measuring the absorbance of light of a particular wavelength by an atomized sample, analysts can determine the concentration of an element even in the presence of other elements. In emission spectroscopy, the optical approach involves exciting the electrons in an element to a higher-energy state by a flame or plasma, and then measuring the intensity of the light emitted when the electrons fall back to the ground state or a lower-energy state. Emission spectroscopy instruments are designed to separate the light emitted from excited atoms and to quantitatively measure the intensity of the emitted light.

In contrast to atomic spectroscopy, ICP-MS instruments are designed to measure ions of the element directly. This necessitates the ionization of atoms in the plasma. The ions of the element are then guided into a mass spectrometer which separates and detects ions according to their unique mass-to-charge (m/z) ratio. Quantification of elements with high sensitivity and specificity can be achieved because most elements have at least one isotope with a unique mass number.

Atomic spectroscopy is a powerful tool for the quantitative measurement of elements in foods. The development of these technologies over the past six decades has had a major impact on food analysis. Today, accurate and precise measurements of a large number of mineral nutrients and non-nutrients in foods can be made rapidly using commercially available instrumentation. Analysts contemplating what instruments to acquire could make a decision based on an assessment of the required cost, user-friendliness, analytical working range, detection limit, multielement capability, and the need for isotopic data.

9.9 STUDY QUESTIONS

1. AAS and AES instruments rely on energy transitions in atoms of elements being measured. What is an "energy transition" in this context and why can it be used to detect and quantify a given element in a sample containing multiple elements? What is the source of energy that produces this energy transition in an AAS instrument? In an AES instrument?
2. Describe the process of "atomization" as it pertains to AAS and AES analyses.
3. Your boss wants to purchase an AAS instrument for your analytical laboratory because it is cheaper but you want an ICP-OES instrument

because it is more versatile and will greatly increase your sample throughput. To convince your boss to go with the ICP-OES, you need to educate him on the capabilities and operating principles of the two instruments. Keep in mind that your boss is a food scientist who has not worked in a lab in 20 years.

(a) Explain the underlying principles of operation for an ICP-OES instrument in language your boss can understand. Describe the instrument you want to purchase (a simple drawing of the instrument might be helpful here).
(b) Explain how AAS differs in instrumentation and principle of operation from what you described previously for ICP-OES.
(c) Can you make the case that costs for an ICP-OES would be lower over the long term?
(d) For most types of food samples other than clear liquids, what type of sample preparation and treatment is generally required before using ICP-OES or AAS for analysis?

4. You are training a new technician in your analytical laboratory on mineral analysis by AAS and ICP-OES. Briefly describe the purpose of each of the following items

(a) HCL in AAS
(b) Plasma in ICP-OES
(c) Echelle optical system in ICP-OES
(d) Nebulizer in AAS and ICP-OES

5. In the quantitation of Na by atomic absorption, KCl or LiCl was not added to the sample. Would you likely over- or underestimate the true Na content? Explain why either KCl or LiCl is necessary to obtain accurate results.
6. Give five potential sources of error in sample preparation prior to atomic absorption analysis.
7. You are performing iron analysis on a milk sample using AAS. Your results for the blank are high. What could be causing this problem and what is a possible remedy?
8. The detection limit for calcium is lower for ICP-OES than it is for flame AAS. How is the detection limit determined, and what does it mean?
9. When analyzing a sample for mineral elements using ICP-MS, the instrument is programmed to count the number of ions with a specific *m/z* ratio striking the detector. You decide to determine the concentrations of potassium and calcium in a sample of wheat flour. What *m/z* ratio would you use for potassium? For calcium? Why? (Hint, study the masses of all the naturally occurring and stable isotopes for the two elements and for argon (see table) and select isotopes with no interferences.) Why is it important to know the masses of argon isotopes as well as potassium and calcium?

Isotope	*Natural abundance (%)*
^{36}Ar	0.34
^{38}Ar	0.063
^{40}Ar	99.6
^{39}K	93.2
^{40}K	0.012
^{41}K	6.73
^{40}Ca	96.95
^{42}Ca	0.65
^{43}Ca	0.14
^{44}Ca	2.086
^{46}Ca	0.004

9.10 PRACTICE PROBLEMS

1. Your company manufactures and markets an enriched all-purpose flour product. You purchase a premix containing elemental iron powder, riboflavin, niacin, thiamin, and folate which you mix with your flour during milling. To comply with the standard of identity (see Chap. 2) for enriched flour, you specified to the supplier that the premix be formulated so that when added to flour at a specified rate, the concentration of added iron is 20 mg/lb flour. However, you have reason to believe that the iron concentration in the premix is too low so you decide to analyze your enriched flour using your new atomic absorption spectrometer. You follow the following protocol to determine the iron concentration.

(a) Weigh out 10.00 g of flour, in triplicate (each replicate should be analyzed separately).
(b) Transfer the flour to an 800-mL Kjeldahl flask.
(c) Add 20 mL of deionized water, 5 mL of concentrated H_2SO_4, and 25 mL of concentrated HNO_3.
(d) Heat on a Kjeldahl burner in a hood until white SO_3 fumes form.
(e) Cool, add 25 mL of deionized water, and filter quantitatively into a 100-mL volumetric flask. Dilute to volume.
(f) Prepare iron standards with concentrations of 2, 4, 6, 8, and 10 mg/L.
(g) Install an iron hollow cathode lamp in your atomic absorption spectrometer and turn on the instrument and adjust it according to instructions in the operating manual.
(h) Run your standards and each of your ashed samples and record the absorbances.

Calculate the iron concentration in each of your replicates, express as mg Fe/lb flour.

Absorbance data for iron standards and flour samples

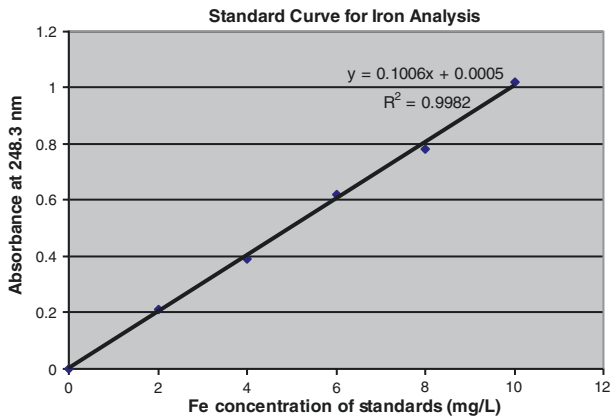
Sample	Fe Conc. (mg/L)	Absorbance	Corrected absorbance
Reagent blank	–	0.01	–
Standard 1	2.0	0.22	0.21
Standard 2	4.0	0.40	0.39
Standard 3	6.0	0.63	0.62
Standard 4	8.0	0.79	0.78
Standard 5	10.0	1.03	1.02
Flour sample 1	?	0.28	0.27
Flour sample 2	?	0.29	0.28
Flour sample 3	?	0.26	0.25

2. Describe a procedure for determining calcium, potassium, and sodium in infant formula using ICP-OES. *Note:* Concentrations of Ca, K, and Na in infant formula are around 700 mg/L, 730 mg/L, and 300 mg/L, respectively.

Answers

1. The following steps may be used to determine the iron concentration in the flour samples.

   (a) Enter the data for the standards into Excel. Using the scatter plot function, plot the standard curve and generate a trend line using linear regression. Include the equation for the line and the R^2 value. Your results should look like the standard curve shown.



   (b) Using the equation, calculate the iron concentration in the solution in the volumetric flask for each of your samples. Your answers should be 2.68 mg/L, 2.79 mg/L, and 2.48 mg/L for samples 1, 2, and 3, respectively. The mean is 2.65 mg/L; the standard deviation is 0.16.

   (c) Now determine the iron concentration in the flour. Recall that you transferred the solution from the Kjeldahl flask quantitatively into the 100-mL volumetric flask. Therefore all of the iron in the flour sample should be in the volumetric flask. The mean concentration is 2.65 mg/L. The volume is 0.1 L. Therefore, the amount of iron in the 10 g of flour is 0.265 mg. To convert this to mg/lb, multiply by 454/10: $0.265\ \text{mg}/10\ \text{g} \times 454\ \text{g/lb} = 12\ \text{mg Fe/lb flour}$

   (d) Your suspicions are confirmed; your supplier shorted you on iron in the premix. You need to correct this as soon as possible because your flour does not conform to the FDA's standard of identity for enriched flour and you may be subject to legal action by the FDA.

2. Consult AOAC Method 984.27 (see Chap. 1 for a description of AOAC International), and the following approach may be used:

   (a) Shake can vigorously.
   (b) Transfer 15.0 mL of formula to a 100-mL Kjeldahl flask. (Carry two reagent blanks through with sample.)
   (c) Add 30 mL of HNO_3-$HClO_4$ (2:1).
   (d) Leave samples overnight.
   (e) Heat until ashing is complete (follow AOAC procedure carefully – mixture is potentially explosive.)
   (f) Transfer quantitatively to a 50-mL vol flask. Dilute to volume.
   (g) Calibrate instrument. Choose wavelengths of 317.9 nm, 766.5 nm, and 589.0 nm for Ca, K, and Na, respectively. Prepare calibration standards containing 200 µg/mL, 200 µg/mL, and 100 µg/mL for Ca, K, and Na, respectively.
   (h) The ICP-OES computer will calculate concentrations in the samples as analyzed. To convert to concentrations in the formula, use the following equation:

$$\text{Concentration in formula} = \text{Concentration measured by ICP} \times (50\ \text{mL}/15\text{mL})$$

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