chapter**TWENTY**

Molecular Mass Spectrometry

Mass spectrometry is perhaps the most widely applicable of all the analytical tools available because the technique can provide information about (1) the elemental composition of samples of matter; (2) the structures of inorganic, organic, and biological molecules; (3) the qualitative and quantitative composition of complex mixtures; (4) the structure and composition of solid surfaces; and (5) isotopic ratios of atoms in samples.

We have already discussed in Chapter 11 how mass spectrometry is used by chemists for the identification and quantitative determination of one or more elements in a sample of matter. This chapter describes how mass spectrometry is used to collect the type of information listed in items (2) and (3) in the previous paragraph. Chapter 21 describes how mass spectrometry is used for elucidating the structure and composition of surfaces. Finally, in Section 20D, we discuss the use of isotopic ratios determined by mass spectrometry.

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The first general application of molecular mass spectrometry for routine chemical analysis occurred in the early 1940s, when the techniques began to be adopted by the petroleum industry for the quantitative analysis of hydrocarbon mixtures produced in catalytic cracking units. Before this time, analyses of mixtures of this type, which often contained as many as nine hydrocarbon components, were carried out by fractional distillation followed by refractive-index measurements of the separated components. Typically, 200 hours or more of operator time was required to complete an analysis. It was found that similar information could be obtained in a few hours or less with a mass spectrometer. This improved efficiency led to the appearance and rapid improvement of commercial mass spectrometers. Beginning in the 1950s, these commercial instruments began to be adapted by chemists for the identification and structural elucidation of a wide variety of organic compounds. This use of the mass spectrometer combined with the invention of nuclear magnetic resonance and the development of infrared spectrometry revolutionized the way organic chemists identify and determine the structure of molecules. This application of mass spectrometry is still extremely important.

Applications of molecular mass spectrometry dramatically changed in the decade of the 1980s, as a result of the development of new methods for producing ions from nonvolatile or thermally labile molecules, such as those frequently encountered by biochemists and biologists. Since about 1990, explosive growth in the area of biological mass spectrometry has occurred because of these new ionization methods. Now, mass spectrometry is being applied to the determination of the structure of polypeptides, proteins, and other high-molecular-mass biopolymers.

In this chapter, we first describe the nature of molecular mass spectra and define some terms used in molecular mass spectrometry. We next consider the various techniques used to form ions from analyte molecules in mass spectrometers and the types of spectra produced by these techniques. We then describe in some detail the various types of mass spectrometers used in molecular mass spectrometry (other than the quadrupole and time-of-flight instruments, which received detailed treatment

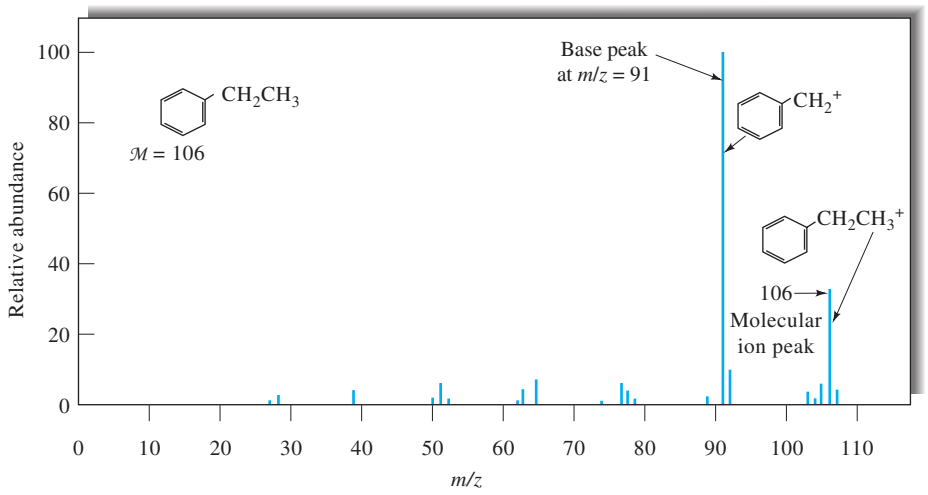

FIGURE 20-1 Mass spectrum of ethyl benzene.

in Section 11B). Finally, we describe several of the current applications of molecular mass spectrometry.[1]

20A MOLECULAR MASS SPECTRA

Figure 20-1 illustrates how mass spectral data are usually presented. The analyte was ethyl benzene, which has a nominal molecular mass of 106 daltons (Da). To obtain this spectrum, ethyl benzene vapor was bombarded with a stream of electrons that led to the loss of an electron by the analyte and formation of the ion $C_6H_5CH_2CH_3^{\cdot+}$ as shown by the reaction

$$C_6H_5CH_2CH_3 + e^- \rightarrow C_6H_5CH_2CH_3^{\cdot+} + 2e^- \quad (20\text{-}1)$$

The charged species $C_6H_5CH_2CH_3^{\cdot+}$ is the *molecular ion*. As indicated by the dot, the molecular ion is a radical ion that has the same molecular mass as the molecule.

The collision between energetic electrons and analyte molecules usually imparts enough energy to the molecules to leave them in an excited state. Relaxation then often occurs by fragmentation of part of the molecular ions to produce ions of lower masses. For example, a major product in the case of ethyl benzene is $C_6H_5CH_2^+$, which results from the loss of a CH_3 group. Other smaller positively charged fragments are also formed in lesser amounts.

The positive ions produced in electron ionization (EI) are attracted through the slit of a mass spectrometer where they are sorted according to their mass-to-charge ratios and displayed in the form of a mass spectrum. Note that the plot shown in Figure 20-1 is in the form of a bar graph that relates the relative intensity of mass peaks to their mass-to-charge ratio. The largest peak in a spectrum, termed the *base peak*, is arbitrarily assigned a value of 100. The heights of the remaining peaks are then computed as a percentage of the base-peak height. Modern mass spectrometers are programmed to automatically recognize the base peak. They then normalize the remaining peaks in the spectrum relative to the base peak.

20B ION SOURCES

The starting point for a mass spectrometric analysis is the formation of gaseous analyte ions, and the scope and the utility of a mass spectrometric method is dictated by the ionization process. The appearance of mass spectra for a given molecular species strongly depends on the method used for ion formation. Table 20-1 lists many of the ion sources that have been used in molecular mass spectrometry.[2] Note that these methods fall into three major categories: *gas-phase sources, desorption sources*, and *ambient desorption sources.* With a gas-phase source, which includes the first three sources in the table, the sample is first vaporized and then ionized. With a desorption source, however, the solid- or liquid-state sample is converted directly into gaseous ions. An advantage of desorption sources is that they

[1]For detailed discussions of mass spectrometry, see J. H. Gross, *Mass Spectrometry: A Textbook*, Heidelberg, Germany: Springer, 2011; J. T. Watson and O. D. Sparkman, *Introduction to Mass Spectrometry*, 4th ed., Chichester, UK: Wiley, 2007; R. M. Smith, *Understanding Mass Spectra: A Basic Approach*, 2nd ed., New York: Wiley, 2004; E. de Hoffman and V. Stroobant, *Mass Spectrometry: Principles and Applications*, 2nd ed., Chichester, UK: Wiley, 2002.

[2]For more information about ion sources, see J. Greaves and J. Roboz, *Mass Spectrometry for the Novice*, Boca Raton, FL: CRC Press, 2014; J. T. Watson and O. D. Sparkman, *Introduction to Mass Spectrometry*, 4th ed., Chichester, UK: Wiley, 2007; E. de Hoffman and V. Stroobant, *Mass Spectrometry: Principles and Applications*, 2nd ed., Chichester, UK: Wiley, 2002; for an historical perspective, see F. W. McLafferty, *Ann. Rev. Anal. Chem.*, **2011**, *4*, 1, **DOI**: 10.1146/annurev-anchem-061010-114018.

TABLE 20-1 Ion Sources for Molecular Mass Spectrometry

Basic Type	Name and Acronym	Ionizing Agent
Gas phase	Electron ionization (EI)	Energetic electrons
	Chemical ionization (CI)	Reagent gaseous ions
	Field ionization (FI)	High-potential electrode
Desorption	Field desorption (FD)	High-potential electrode
	Electrospray ionization (ESI)	High electrical field
	Matrix-assisted laser desorption/ionization (MALDI)	Laser beam
	Plasma desorption (PD)	Fission fragments from ^{252}Cf
	Fast atom bombardment (FAB)	Energetic atomic beam
	Secondary-ion mass spectrometry (SIMS)	Energetic beam of ions
	Thermospray ionization (TS)	High temperature
Ambient desorption	Desorption electrospray ionization (DESI)	Charged droplet spray
	Direct analysis in real time (DART)	Excited atoms or molecules

are applicable to nonvolatile and thermally unstable samples. Ambient sources allow desorption ionization with minimal sample pretreatment and without the enclosures of typical ionization sources.[3] Currently, commercial mass spectrometers are equipped with accessories that permit interchangeable use of several ionization sources.

Gas-phase sources are generally restricted to the ionization of thermally stable compounds that have boiling points less than about 500°C. In most cases, this requirement limits gaseous sources to compounds with molecular masses less than roughly 10^3 Da. Desorption and ambient sources, which do not require volatilization of analyte molecules, are applicable to analytes having molecular masses as large as 10^5 Da.

Ion sources are also classified as being *hard sources* or *soft sources*. Hard ionization sources impart enough energy to analyte molecules to leave them in a highly excited energy state. Relaxation then involves rupture of bonds, producing fragment ions that have mass-to-charge ratios less than that of the molecular ion. Soft ionization sources cause little fragmentation. Thus, the mass spectrum from a soft ionization source often consists of the molecular ion peak and only a few, if any, other peaks. Figure 20-2 illustrates the difference in spectra obtained from a hard ionization source and a soft ionization source.

Both hard- and soft-source spectra are useful for analysis. The many peaks in a hard-source spectrum provide useful information about the kinds of functional groups and thus structural information about analytes. Soft-source spectra are useful because they supply accurate information about the molecular mass of the analyte molecule or molecules.

20B-1 Electron Ionization

Historically, ions for mass analysis were produced by *electron ionization,* formerly called electron-impact ionization.[4] In this process, the sample is brought to a temperature high enough to produce a molecular vapor, which is then ionized by bombarding the resulting molecules with a beam of energetic electrons. Despite certain disadvantages, this technique is still of major importance and is the one on which many libraries of mass spectral data are based.

Figure 20-3 is a schematic of a basic EI source. Electrons are emitted from a heated tungsten or rhenium filament and accelerated by applying approximately 70 V between the filament and the anode. As shown in the figure, the paths of the electrons and molecules are at right angles and intersect near the center of the source, where collision and ionization occur. The primary product is singly charged positive ions formed when the energetic electrons approach molecules closely enough to cause them to lose electrons by electrostatic repulsion. Electron ionization is not very efficient, and only about one molecule in a million undergoes the primary reaction

$$M + e^- \rightarrow M^{\cdot +} + 2e^- \qquad (20\text{-}2)$$

Here, M represents the analyte molecule, and $M^{\cdot +}$ is its molecular ion. The positive ions produced by electron ionization are attracted through the slit in the first accelerating plate by a small potential difference (typically 5 V) that is applied between this plate and the repellers shown in Figure 20-3. With magnetic sector instruments, high voltages (10^3 to 10^4 V) are applied to the accelerator plates, which give the ions their final velocities before

[3]See G. A. Harris, A. S. Galhena, and F. M. Fernandez, *Anal. Chem.*, **2011**, *83*, 4508, **DOI**: 10.1021/ac200918u.

[4]The term *electron impact* is no longer in favor because it gives an erroneous idea of the processes that occur in this method of ionization.

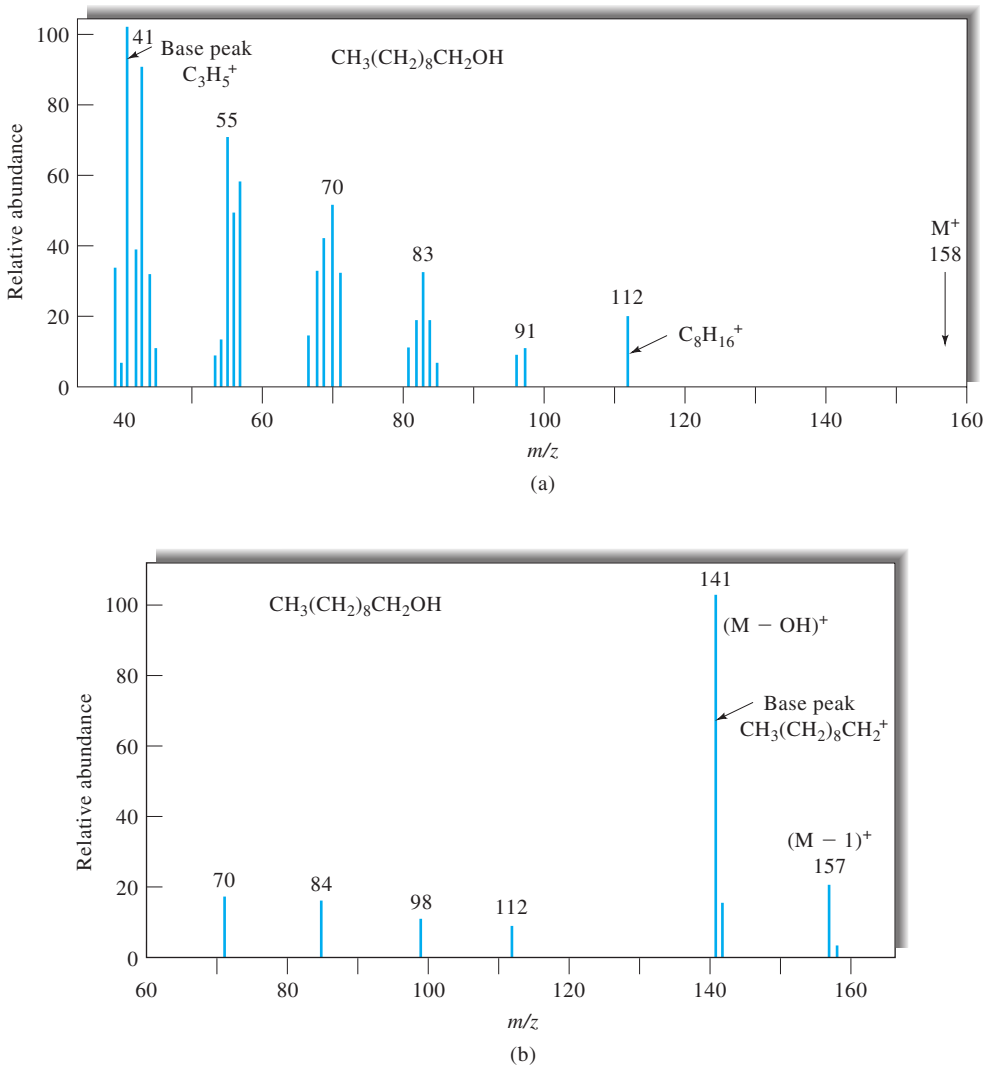

FIGURE 20-2 Mass spectrum of 1-decanol from (a) a hard ionization source (EI) and (b) a soft ionization source (chemical ionization [CI]).

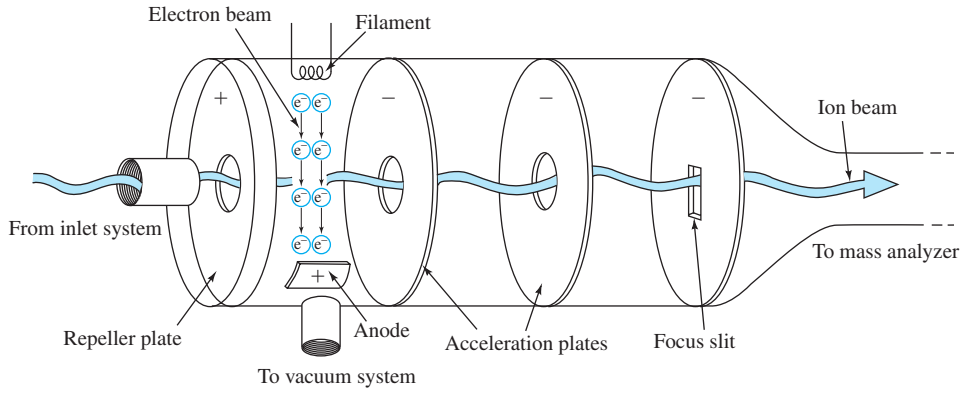

FIGURE 20-3 Diagram of an EI source. (Adapted from D. L. Pavia, G. M. Lampman, G. S. Kriz, and J. R. Vyvyanh, *Introduction to Spectroscopy*, 5th ed., Stamford, CT: Cengage Learning, 2015, p. 110.)

they enter the mass analyzer. Commercial EI sources are more complex than that shown in Figure 20-3 and may use additional electrostatic or magnetic fields to manipulate the electron or ion beam. A typical kinetic energy produced in an EI source is calculated in Example 20-1.

EXAMPLE 20-1

(a) Calculate the kinetic energy that a singly charged ion ($z = 1$) will acquire if it is accelerated through a potential of 10^3 V in an EI source. (b) Does the kinetic energy of the ion depend on its mass? (c) Does the velocity of the ion depend on its mass?

Solution

(a) The kinetic energy (KE) added to the ion is due to the accelerating potential V and is given by the equation

$$\text{KE} = qV = zeV$$

where e is the electronic charge (1.6×10^{-19} coulombs). Thus, for $z = 1$

$$\text{KE} = 1 \times 1.6 \times 10^{-19}\,\text{C} \times 10^3\,\text{V} = 1.6 \times 10^{-16}\,\text{J}$$

(b) The kinetic energy that an ion acquires in the source is independent of its mass and depends only on its charge and the accelerating potential.

(c) The translational component of the kinetic energy of an ion is a function of the ion mass m and its velocity v as given by the equation

$$\text{KE} = (1/2)mv^2 \quad \text{or} \quad v = (2\,\text{KE}/m)^{1/2}$$

Thus, if all ions acquire the same amount of kinetic energy, those ions with largest mass must have the smallest velocity.

EI Spectra

To form a significant number of gaseous ions at a reproducible rate, electrons from the filament in the source must be accelerated by a voltage of greater than about 50 V. The low mass and high kinetic energy of the resulting electrons cause little increase in the translational energy of impacted molecules. Instead, the molecules are left in highly excited vibrational and rotational states. Subsequent relaxation then usually takes place by extensive fragmentation, giving a large number of positive ions of various masses that are less than (and occasionally, because of collisions, greater than) that of the molecular ion. These lower-mass ions are called *product* or *fragment ions*. Table 20-2 shows some typical fragmentation reactions that follow formation of a *molecular ion* from a hypothetical molecule ABCD. In Example 20-2, the kinetic energy of electrons accelerated through a potential difference of 70 V is calculated.

EXAMPLE 20-2

(a) Calculate the energy (in J/mol) that electrons acquire as a result of being accelerated through a potential of 70 V. (b) How does this energy compare to that of a typical chemical bond?

Solution

(a) The kinetic energy KE of an individual electron is equal to the product of the charge on the electron e times the potential V through which it has been accelerated. Multiplying the kinetic energy of a single electron by Avogadro's number, N, gives the energy per mole:

$$\begin{aligned}\text{KE} &= eVN \\ &= (1.60 \times 10^{-19}\,\text{C/e}^-)(70\,\text{V})N \\ &= (1.12 \times 10^{-17}\,\text{CV/e}^-)(6.02 \times 10^{23}\,\text{e}^-/\text{mol}) \\ &= 6.7 \times 10^6\,\text{J/mol} \quad \text{or} \quad 6.7 \times 10^3\,\text{kJ/mol}\end{aligned}$$

(b) Typical bond energies fall in the 200- to 600-kJ/mol range. Therefore, an electron that has been accelerated through 70 V generally has considerably more energy than that required to break a chemical bond.

TABLE 20-2 Some Typical Reactions in an EI Source

Molecular ion formation	$\text{ABCD} + \text{e}^- \rightarrow \text{ABCD}^{\bullet+} + 2\text{e}^-$
Fragmentation	$\text{ABCD}^{\bullet+} \rightarrow \text{A}^+ + \text{BCD}^\bullet$ $\rightarrow \text{A}^\bullet + \text{BCD}^+ \rightarrow \text{BC}^+ + \text{D}$ $\rightarrow \text{CD}^\bullet + \text{AB}^+ \rightarrow \text{B} + \text{A}^+$ or $\text{A} + \text{B}^+$ $\rightarrow \text{AB}^\bullet + \text{CD}^+ \rightarrow \text{D} + \text{C}^+$ or $\text{C} + \text{D}^+$
Rearrangement followed by fragmentation	$\text{ABCD}^{\bullet+} \rightarrow \text{ADBC}^{\bullet+} \rightarrow \text{BC}^\bullet + \text{AD}^+$ or $\text{AD}^\bullet + \text{BC}^+$
Collision followed by fragmentation	$\text{ABCD}^{\bullet+} + \text{ABCD} \rightarrow (\text{ABCD})_2^{\bullet+} \rightarrow \text{BCD}^\bullet + \text{ABCDA}^+$

The complex mass spectra that result from electron ionization are useful for compound identification. On the other hand, with certain types of molecules, fragmentation is so complete that the molecular ion is not detected. Without a molecular ion, important information for determining the molecular mass of the analyte is lost. Figure 20-4 shows typical EI spectra for two simple organic molecules: methylene chloride and 1-pentanol. Note that in each of the spectra, the base peak corresponds to a fragment of the molecule, which has a mass significantly less than the molecular mass of the original compound. For methylene chloride, the base peak occurs at a mass-to-charge ratio m/z of 49, which corresponds to the loss of one Cl atom. For 1-pentanol, the base peak is found at an m/z of 44, which is that of the product ion CH_2CHOH^+. Quite frequently, the base peaks in EI spectra correspond to fragments and not to the molecular ion.

The molecular ion peak occurs at a mass corresponding to the molecular mass of the analyte. Thus, molecular ion peaks appear at $m/z = 84$ for methylene chloride and at $m/z = 88$ for 1-pentanol. The molecular ion peak is, of course, very important in structural determinations, because its m/z value provides the molecular mass of the compound. Unfortunately, it is not always possible to identify the molecular ion peak. Indeed, electron ionization of certain molecules yields no molecular ion peak at all (see Figure 20-2a).

Isotope Peaks

It is interesting to note in the spectra shown in Figures 20-1 and 20-4 that peaks occur at mass-to-charge ratios greater than that of the molecular ion. These peaks are attributable to ions having the same chemical formula, but different isotopic compositions. For example, for methylene chloride, the more important isotopic species are $^{12}C^1H_2{}^{35}Cl_2$ ($\mathcal{M} = 84$), $^{13}C^1H_2{}^{35}Cl_2$ ($\mathcal{M} = 85$), $^{12}C^1H_2{}^{35}Cl^{37}Cl$ ($\mathcal{M} = 86$), $^{13}C^1H_2{}^{35}Cl^{37}Cl$ ($\mathcal{M} = 87$), and $^{12}C^1H_2{}^{37}Cl_2$ ($\mathcal{M} = 88$), where $\mathcal{M}$ is molecular mass. Peaks for each of these species can be seen in Figure 20-4a. The size of the various peaks depends on the relative

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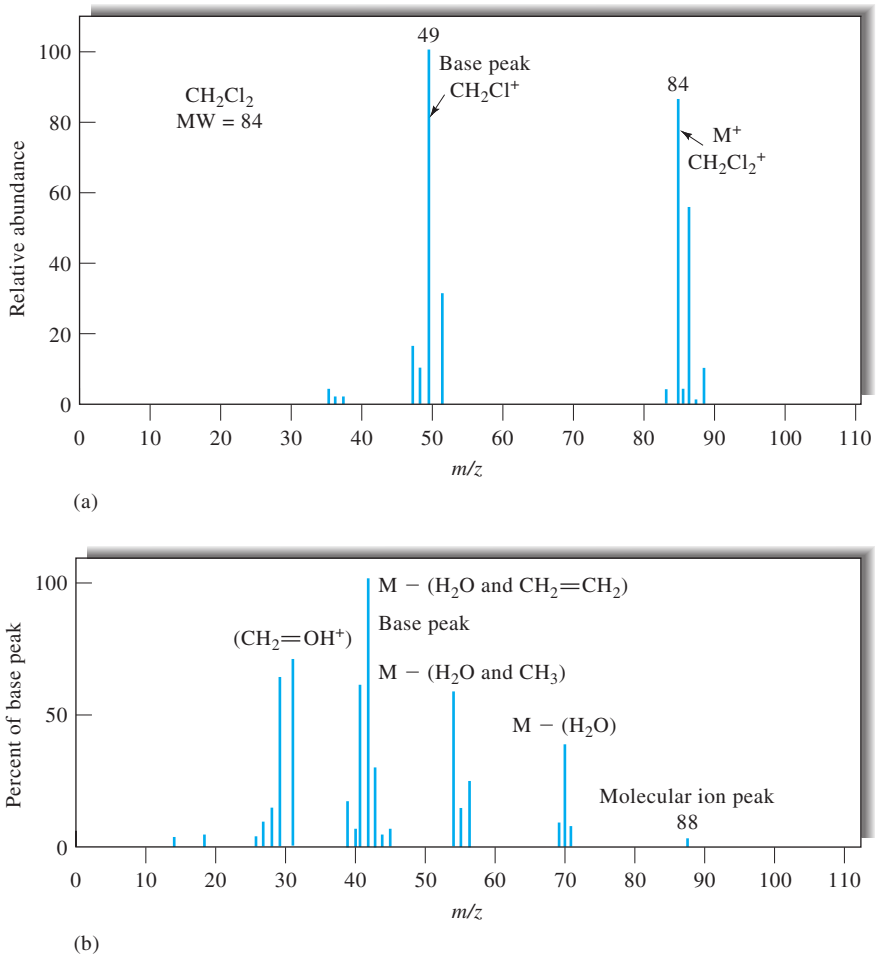

FIGURE 20-4 EI mass spectra of (a) methylene chloride and (b) 1-pentanol.

TABLE 20-3 Natural Abundance of Isotopes of Some Common Elements

Element[a]	Most Abundant Isotope	Abundance of Other Isotopes Relative to 100 Parts of the Most Abundant[b]	
Hydrogen	^{1}H	^{2}H	0.015
Carbon	^{12}C	^{13}C	1.08
Nitrogen	^{14}N	^{15}N	0.37
Oxygen	^{16}O	^{17}O	0.04
		^{18}O	0.20
Sulfur	^{32}S	^{33}S	0.80
		^{34}S	4.40
Chlorine	^{35}Cl	^{37}Cl	32.5
Bromine	^{79}Br	^{81}Br	98.0
Silicon	^{28}Si	^{29}Si	5.1
		^{30}Si	3.4

[a]Fluorine (^{19}F), phosphorus (^{31}P), sodium (^{23}Na), and iodine (^{127}I) have no additional naturally occurring isotopes.

[b]The numerical entries indicate the average number of isotopic atoms present for each 100 atoms of the most abundant isotope; thus, for every 100 ^{12}C atoms there will be an average of 1.08 ^{13}C atoms.

natural abundance of the isotopes. Table 20-3 lists the most common isotopes for atoms that occur widely in organic compounds. Note that fluorine, phosphorus, iodine, and sodium occur only as single isotopes.

The small peak for ethyl benzene at m/z 107 in Figure 20-1 is due to the presence of ^{13}C in the molecules. The intensities of peaks due to incorporation of two or more ^{13}C atoms in ethyl benzene can be predicted with good precision but are normally so small as to be undetectable because of the low probability of there being more than one ^{13}C atom in a small molecule. As will be shown in Section 20D-1, isotope peaks sometimes provide a useful means for determining the formula for a compound.

Collision Product Peaks

Ion-molecule collisions, such as that shown by the last reaction in Table 20-2, can produce peaks at higher mass numbers than that of the molecular ion. At ordinary sample pressures, however, the only important reaction of this type is one in which the collision transfers a hydrogen atom to the ion to give a protonated molecular ion. The result is an enhanced $(M + 1)^+$ peak. This transfer is a second-order reaction, and the amount of product depends strongly on the reactant concentration. Consequently, the height of an $(M + 1)^+$ peak due to this reaction increases much more rapidly with increases in sample pressure than do the heights of other peaks. This phenomenon usually makes it possible to detect this atom-transfer reaction.

Advantages and Disadvantages of EI Sources

EI sources are convenient to use. They produce high ion currents, which lead to good sensitivities. The extensive fragmentation and resulting large number of peaks is also an advantage because it often makes unambiguous identification of analytes possible. Extensive fragmentation can also be a disadvantage, however, when it results in the disappearance of the molecular ion peak so that the molecular mass of analytes cannot be easily established. Another limitation of the EI source is the need to volatilize the sample, which may result in thermal degradation of some analytes before ionization can occur. The effects of thermal decomposition can sometimes be minimized by carrying out the volatilization from a heated probe located close to the entrance slit of the spectrometer. At the lower pressure of the source area, volatilization occurs at a lower temperature. Furthermore, less time is allowed for thermal decomposition to take place. As mentioned earlier, EI sources are applicable only to analytes having molecular masses smaller than about 10^3 Da.

20B-2 Chemical Ionization Sources and Spectra

Most modern mass spectrometers are designed so that electron ionization and chemical ionization can be carried out interchangeably. Such sources are called EI-CI sources. In CI, gaseous atoms of the sample (from either a batch inlet or a heated probe) are ionized by collision with ions produced by electron bombardment of an excess of a reagent gas. Usually, positive ions are used, but negative ion CI is occasionally used with analytes that contain very electronegative atoms. Chemical ionization is probably the second-most common procedure for producing ions for mass spectrometry.[5]

[5]For a more detailed discussion of chemical ionization, see J. T. Watson and O. D. Sparkman, *Introduction to Mass Spectrometry*, 4th ed., Chap. 7, Chichester, UK: Wiley, 2007.

To carry out CI experiments, it is necessary to modify the electron beam ionization area shown in Figure 20-3 by adding vacuum pump capacity and by reducing the width of the slit to the mass analyzer. These measures allow a reagent pressure of about 100 Pa (0.75 torr) to be maintained in the ionization area while maintaining the pressure in the analyzer below 10^{-7} Pa. With these changes, a gaseous reagent is introduced into the ionization region in an amount such that the concentration ratio of reagent to sample is 10^3 to 10^4. Because of this large concentration difference, the electron beam reacts nearly exclusively with reagent molecules.

One of the most common reagents for CI is methane, which reacts with high-energy electrons to give several ions such as CH_4^+, CH_3^+, and CH_2^+. The first two predominate and represent about 90% of the reaction products. These ions react rapidly with additional methane molecules as follows:

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

Generally, collisions between the analyte molecule M and CH_5^+ or $C_2H_5^+$ are highly reactive and involve proton or hydride transfer. For example,

$$CH_5^+ + M \rightarrow MH^+ + CH_4 \qquad \text{proton transfer}$$

$$C_2H_5^+ + M \rightarrow MH^+ + C_2H_4 \qquad \text{proton transfer}$$

$$C_2H_5^+ + MH \rightarrow M^+ + C_2H_6 \qquad \text{hydride transfer}$$

Note that proton-transfer reactions give the $(M + H)^+$ ion whereas the hydride transfer produces an ion with a mass one less than the analyte, or the $(MH - H)^+$ ion. With some compounds, an $(MH + 29)^+$ peak is also produced from transfer of a $C_2H_5^+$ ion to the analyte. A variety of other reagents, including propane, isobutane, and ammonia, are used for chemical ionization. Each produces a somewhat different spectrum with a given analyte.

Figure 20-2 contrasts the CI and EI spectra for 1-decanol. The EI spectrum (Figure 20-2a) shows evidence for rapid and extensive fragmentation of the molecular ion. Thus, no detectable peaks are observed above mass 112, which corresponds to the ion $C_8H_{16}^+$.

The base peak is provided by the ion $C_3H_5^+$ at mass 41. Other peaks for C_3 species are grouped around the base peak. A similar series of peaks, found at 14, 28, and 42 mass units greater, correspond to ions with one, two, and three additional CH_2 groups.

Relative to the EI spectrum, the CI spectrum shown in Figure 20-2b is simple indeed, consisting of the $(MH - H)^+$ peak, a base peak corresponding to a molecular ion that has lost an OH group, and a series of peaks differing from one another by 14 mass units. As in the EI spectrum, these peaks arise from ions formed by cleavage of adjacent carbon-carbon bonds. As we have just noted, chemical ionization spectra generally contain well-defined $(M + H)^+$ or $(MH - H)^+$ peaks resulting from the addition or abstraction of a proton in the presence of the reagent ion.

20B-3 Field Ionization Sources and Spectra

In *field ionization* sources, ions are formed under the influence of a large electric field (10^8 V/cm). Such fields are produced by applying high voltages (10 to 20 kV) to specially formed emitters consisting of numerous fine tips having diameters of less than 1 μm. The emitter often takes the form of a fine tungsten wire (~10 μm diameter) on which microscopic carbon dendrites, or whiskers, have been grown by the pyrolysis of benzonitrile in a high electric field. The result of this treatment is a growth of many hundreds of carbon microtips projecting from the surface of the wire as can be seen in the photomicrograph of Figure 20-5.

Field ionization emitters are mounted 0.5 to 2 mm from the cathode, which often also serves as a slit. The gaseous sample from a batch inlet system is allowed to diffuse into the high-field area around the microtips of the anode. The electric field is concentrated at the emitter tips, and ionization occurs via a quantum mechanical tunneling mechanism in which electrons from the analyte are extracted by the microtips of the anode. Little vibrational or rotational energy is imparted to the analyte, and little fragmentation occurs.

FIGURE 20-5 Photomicrograph of a carbon microneedle emitter. (Courtesy of R. P. Lattimer, BF Goodrich Research and Development Center.)

Figure 20-6 shows spectra for glutamic acid obtained by (a) electron ionization and (b) field ionization. In the EI spectrum, the molecular ion peak at $m/z = 147$ is not detectable. The highest observable peak ($m/z = 129$) is due to the loss of water by the molecular ion. The base peak at $m/z = 84$ arises from a loss of water and a —COOH group. Numerous other fragments are also found at lower masses. In contrast, the field ionization spectrum is relatively simple, with an easily distinguished $(M + 1)^+$ peak at $m/z = 148$.

A limitation to field ionization is its sensitivity, which is at least an order of magnitude less than that of EI sources; maximum currents are on the order of 10^{-11} A.

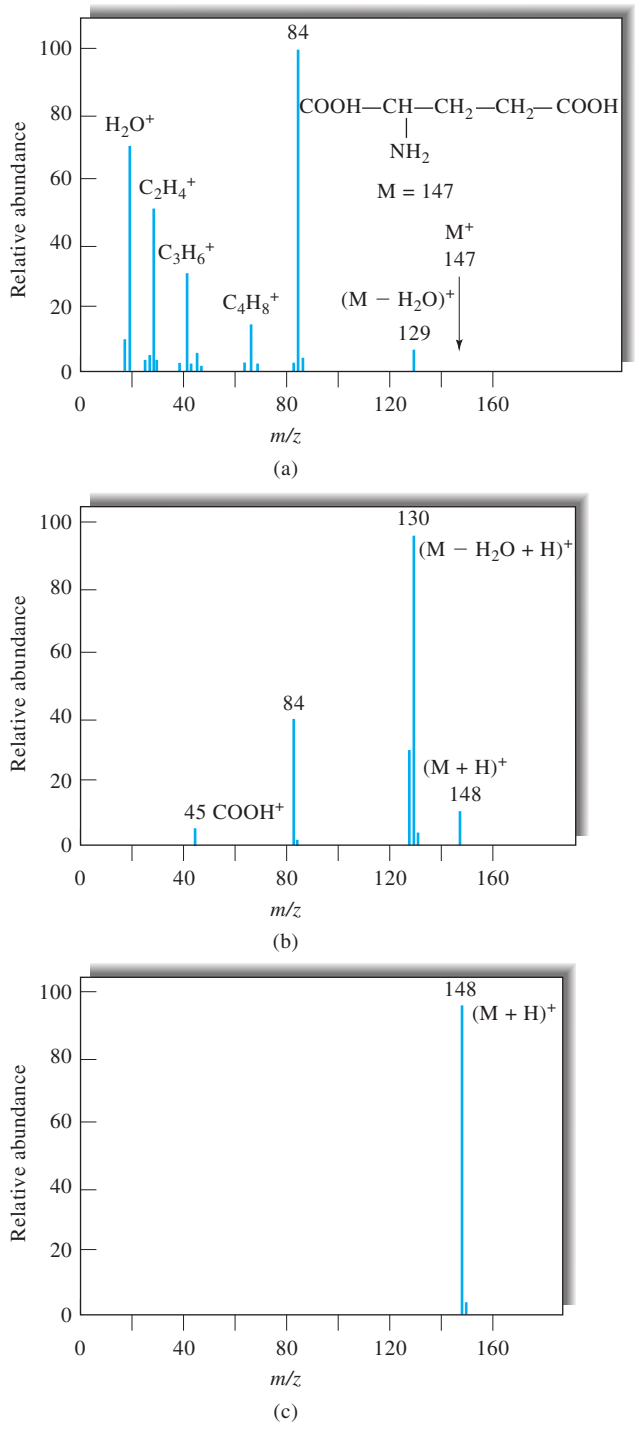

FIGURE 20-6 Mass spectra of glutamic acid with (a) EI, (b) field ionization, and (c) field desorption. (From H. D. Beckey, A. Heindrich, and H. U. Winkler, *Int. J. Mass Spec. Ion Phys.*, **1970**, *3*, A9, **DOI:** 10.1016/0020-7381(70)80009-2. With permission.)

20B-4 Desorption Sources

The ionization methods discussed so far require that the ionizing agents act on gaseous samples. Such methods are not applicable to nonvolatile or thermally unstable samples. A number of *desorption ionization* methods have been developed for dealing with this type of sample (see Table 20-1). These methods have enabled mass spectra to be obtained for thermally delicate biochemical species and species having molecular masses of greater than 100,000 Da.

Desorption methods dispense with volatilization followed by ionization of the gaseous analyte molecules. Instead, energy in various forms is introduced into the solid or liquid sample in such a way as to cause direct formation of gaseous ions. As a consequence, spectra are greatly simplified and often consist of only the molecular ion or the protonated molecular ion. In most cases the exact mechanism of how ions are formed without fragmentation is not well understood.

Field Desorption Methods

In field desorption, a multitipped emitter similar to that used in field ionization sources is used.[6] In this case, the electrode is mounted on a probe that can be removed from the sample compartment and coated with a solution of the sample. After the probe is reinserted into the sample compartment, ionization again takes place by applying a high voltage to this electrode. With some samples it is necessary to heat the emitter by passing a current through the wire. As a result, thermal degradation may occur before ionization is complete.

Figure 20-6c is a field desorption spectrum for glutamic acid. It is even simpler than the spectrum from field ionization and consists of only the protonated molecular ion peak at $m/z = 148$ and an isotope peak at $m/z = 149$.

Matrix-Assisted Laser Desorption/Ionization

Matrix-assisted laser desorption/ionization (MALDI) spectrometry is an ionization method that can be used to obtain accurate molecular mass information about polar biopolymers ranging in molecular mass from a few thousand to several hundred thousand Da.[7] The method was first described nearly simultaneously in 1988 by two research groups, one German

[6]See J. H. Gross, *Mass Spectrometry: A Textbook*, Chap. 8, Heidelberg, Germany: Springer, 2011.

[7]See reference 6, Chap. 11; J. T. Watson and O. D. Sparkman, *Introduction to Mass Spectrometry*, Chap. 9, Chichester, UK: Wiley, 2007.

and the other Japanese.[8] Commercial instrumentation is available for MALDI.

In the MALDI technique, a low concentration of the analyte is uniformly dispersed in a solid or liquid matrix deposited on the end of a stainless steel probe or placed on a metal plate. The plate is then placed in a vacuum chamber and a laser beam is focused onto the sample. In addition to the usual vacuum-chamber MALDI, atmospheric-pressure MALDI has also been described.[9] The MALDI matrix must strongly absorb the laser radiation. The matrix and analyte are then desorbed and ionized, creating an ion plume. The overall process is illustrated in Figure 20-7. The most common type of mass analyzer used with MALDI is the time-of-flight (TOF) analyzer. A mass spectrum from a MALDI-TOF instrument is shown in Figure 20-8. Here, the matrix material was nicotinic acid, and the analyte was a monoclonal antibody from a mouse having a molecular mass of approximately 150,000 Da. Note that the spectrum is characterized by very low background noise and a complete absence of fragmentation of the large analyte ion. Multiply charged ions are present as well as peaks for dimer and trimer species.

Although the mechanism of the formation of the MALDI ion plume is not completely understood, it is thought to involve absorption of the laser beam by the matrix, followed by transfer of the energy from the matrix to the analyte. Desorption of the analyte and the matrix then occurs. The analyte is thought to desorb as neutral molecules and then to be ionized by proton-transfer reactions with protonated matrix ions in a dense phase over the surface containing the matrix. A series of photochemical reactions may produce the protonated matrix ions.

Some of the matrix materials used for biomolecules are listed in Table 20-4 along with the laser wavelengths that have been used. Lasers used include nitrogen (337 nm), Nd-YAG (266 and 355 nm), excimer (308 nm), Er-YAG (2.94 μm), and CO_2 (10.6 μm).

The most common sample-preparation method is the dried-droplet technique in which a droplet of the matrix containing the analyte is deposited on the metal plate and then dried. Typically, the ratio of analyte to matrix is $1{:}10^3$ to $1{:}10^5$. Analyte concentrations are usually in the micromolar range.

Recently, the MALDI technique has been extended to imaging methods by scanning a localized laser beam over the dispersed sample.[10] Mass spectral images of a variety of biopolymers have been obtained in this manner.

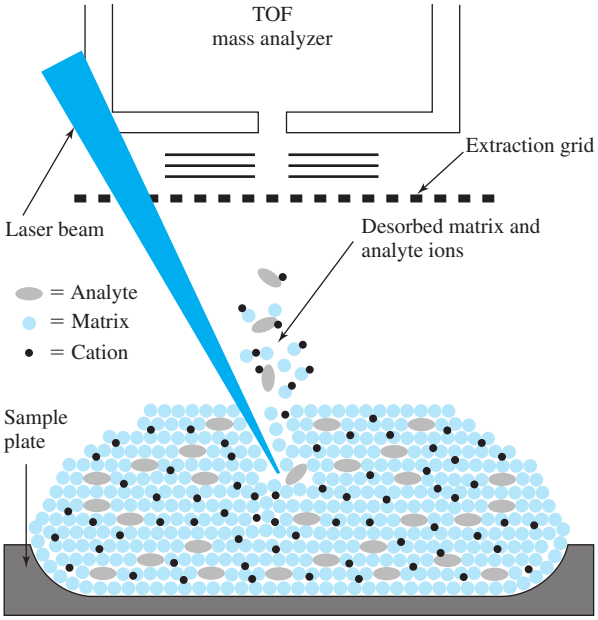

FIGURE 20-7 Diagram of the MALDI process. The analyte is uniformly dispersed in a matrix and placed on a metal sample plate. A pulsed laser beam strikes the sample causing desorption of a plume of matrix, analyte, and other ions. The analyte can be protonated, be deprotonated, or form adducts before entering the TOF analyzer.

Fast Atom Bombardment Sources

Fast atom bombardment (FAB) sources, also called *liquid secondary-ion* sources, were once very popular for producing ions for mass spectrometric studies of polar high-molecular-mass species.[11] With this type of source, samples in a condensed state, usually in a viscous solution matrix, are ionized by bombardment with energetic (several keV) xenon or argon *atoms*. Both positive and negative analyte ions are sputtered from the surface of the sample in a desorption process. This treatment provides very rapid sample heating, which reduces sample fragmentation. The liquid matrix helps to reduce the lattice energy, which must be overcome to desorb an ion from a condensed phase, and provides a means of "healing" the damage induced by bombardment. Successful matrices include glycerol, thioglycerol, *m*-nitrobenzyl alcohol, crown ethers (18-crown-6), sulfolane, 2-nitrophenyloctyl ether, diethanolamine, and triethanolamine.

A beam of fast atoms is obtained by passing accelerated argon or xenon ions from an ion source, or gun, through a chamber containing argon or xenon atoms at a pressure of about 10^{-3} Pa (10^{-5} torr). The high-velocity ions undergo a resonant electron-exchange reaction with the atoms without substantial loss of translational energy. Thus, a beam of energetic *atoms* is

[8]See M. Karas and F. Hillenkamp, *Anal. Chem.*, **1988**, *60*, 2299, **DOI**: 10.1021/ac00171a028; K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshidda, and T. Yoshidda, *Rapid Commun. Mass Spectrosc.*, **1988**, *2*, 151, **DOI**: 10.1002/rcm.1290020802; F. Hillenkamp, M. Karas, and B. T. Chait, *Anal. Chem.*, **1991**, *63*, 1193A, **DOI**: 10.1021/ac00024a716.

[9]See Section 20B-5 for other atmospheric pressure ionization techniques.

[10]See for example, S. M. Weidner and J. Falkenhagen, *Rapid Commun. Mass Spectrom.*, **2009**, *23*, 653, **DOI**: 10.1002/rcm.3919; K. A. Lunsford, G. F. Peter, and R. A. Yost, *Anal. Chem.*, **2011**, *83*, 6722, **DOI**: 10.1021/ac2013527.

[11]For more information, see J. H. Gross, *Mass Spectrometry: A Textbook*, Chap. 10, Heidelberg, Germany: Springer, 2011; J. T. Watson and O. D. Sparkman, *Introduction to Mass Spectrometry*, pp. 250–251, Chichester, UK: Wiley, 2007.

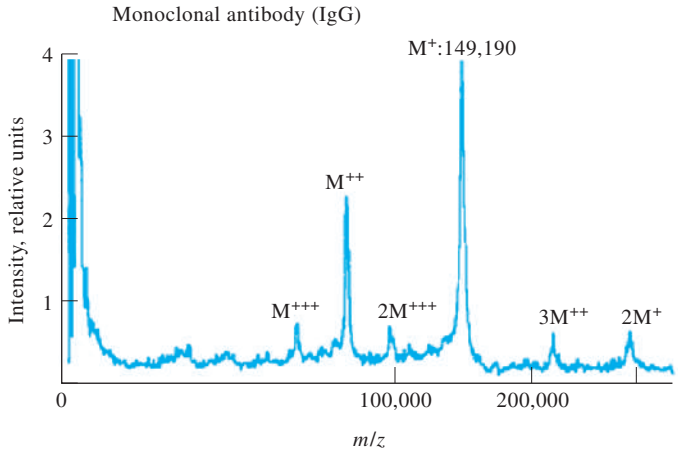

FIGURE 20-8 MALDI-TOF spectrum from nicotinic acid matrix irradiated with a 266-nm laser beam. (From M. Karas and U. Bahr, *Trends Anal. Chem.* (TRAC), **1990,** *9*, 323, **DOI:** 10.1016/0165-9936(90)85065-F.)

TABLE 20-4 Common Matrices for MALDI and Usable Wavelengths

Matrix	Analytes	Wavelength, nm
Nitropyridines:		
2-Amino-4-methyl-5-nitropyridine	Proteins, oligonucleotides	355
2-Amino-5-nitropyridine	Oligonucleotides	355
Nicotinic acid	Proteins, glycoproteins, oligonucleotides	266, 220–290
Benzoic acid derivatives:		
2,5-Dihydroxybenzoic acid	Proteins	266, 337, 355, 2940
Vanillic acid	Proteins	266
2-Aminobenzoic acid	Proteins	266, 337, 355
2-(4-Hydroxyphenylazo) benzoic acid	Proteins, gangliosides, polymers	266, 377
2-Pyrazinecarboxylic acid	Proteins	266
3-Aminopyrazine-2-carboxylic acid	Proteins	337
Cinnamic acid derivatives:		
Ferulic acid	Proteins, oligonucleotides	266, 337, 355, 488
Sinapinic acid	Proteins, industrial polymers	337, 355
Caffeic acid	Proteins, oligonucleotides	266, 337, 355, 10600
α-Cyano-4-hydroxy cinnamic acid	Proteins, oligosaccharides	337
3-Nitrobenzyl alcohol	Proteins	266
3-Nitrobenzyl alcohol with rhodamine 6G	Proteins	532
3-Nitrobenzyl alcohol with 1,4-diphenyl-1,3-butadiene	Proteins	337
3-Hydroxypicolinic acid	Oligonucleotides, glycoproteins	266, 308, 355
Succinic acid	Proteins	2940, 10600

formed. The lower-energy ions from the exchange are readily removed by an electrostatic deflector. Fast atom guns are available from several commercial sources, and modern spectrometers offer FAB sources as accessories.

The many drawbacks of FAB have led to its decrease in popularity. These include the limited molecular mass range of FAB, the need for larger sample quantities than many other ionization techniques and the necessity for finding an appropriate matrix in which the analyte is soluble. Also, FAB is best performed with double-focusing mass spectrometers (see Section 20C). For these reasons, electrospray (see next section) and MALDI methods have supplanted FAB techniques for many applications. Both of these are more sensitive and simpler to use than FAB methods.

20B-5 Atmospheric Pressure Ionization Methods

There are several ionization techniques that operate at atmospheric pressure, including electrospray, atmospheric pressure chemical ionization, and atmospheric pressure photoionization.

Electrospray Ionization

Electrospray ionization–mass spectrometry (ESI/MS), which was first described in 1984, has now become one of the most important techniques for analyzing biomolecules, such as polypeptides, proteins, and oligonucleotides, having molecular weights of 100,000 Da or more.[12] In addition, this method is finding more and more application to the characterization of inorganic species and synthetic polymers. For their development of soft desorption ionization methods, such as electrospray ionization, John B. Fenn and Koichi Tanaka shared the 2002 Nobel Prize in Chemistry.

Electrospray ionization takes place under atmospheric pressures and temperatures in an apparatus such as that shown in Figure 20-9. A solution of the sample is pumped through a stainless steel capillary needle at a rate of a few microliters per minute. The needle is maintained at several kilovolts with respect to a cylindrical electrode that surrounds the needle. The resulting charged spray of fine droplets then passes through a desolvating capillary, where evaporation of the solvent and attachment of charge to the analyte molecules take place. As the droplets become smaller as a consequence of evaporation of the solvent, their charge density becomes greater until, at a point called the *Rayleigh limit*, the surface tension can no longer support the charge. Here a so-called *Coulombic explosion* occurs and the droplet is torn apart into smaller droplets.

[12]For additional information see *Electrospray and MALDI Mass Spectrometry*, 2nd ed., R. B. Cole, ed., Hoboken, NJ: Wiley, 2010; J. H. Gross, *Mass Spectrometry: A Textbook*, Chap. 12, Heidelberg, Germany: Springer, 2011; *Applied Electrospray Ionization Mass Spectrometry*, B. N. Pramanik, A. K. Ganguly, and M. L. Gross, eds., New York: Dekker, 2002.

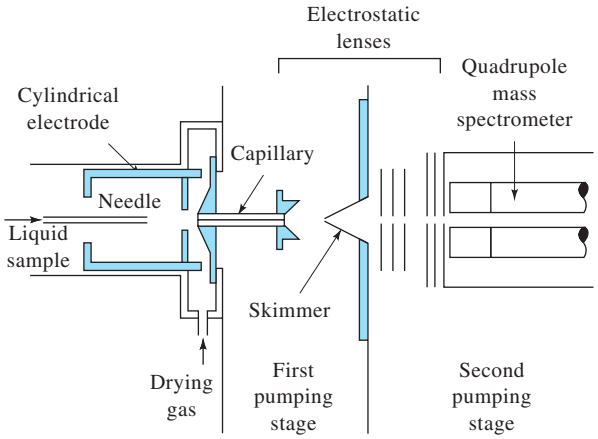

FIGURE 20-9 Apparatus for electrospray ionization. (From J. B. Fenn et al., *Science*, **1989**, *246*, 64, **DOI**: 10.1126/science.2675315. Reprinted with permission.)

These small droplets can repeat the process until all the solvent is removed from the analyte, leaving a multiply charged analyte molecule.

An interesting and useful feature of the electrospray process is that little fragmentation of large and thermally fragile biomolecules occurs because there is little extra energy retained by the analyte upon ionization. Furthermore, the ions formed are multiply charged so that their m/z values are small enough to make them detectable with a quadrupole instrument with a range of 1500 or less. This important property is demonstrated by the mass spectra of four proteins of varying molecular mass ($\mathcal{M}$) shown in Figure 20-10. In these spectra, adjacent peaks are for analyte ions that differ by one charge. A striking feature of the spectra for proteins, such as those in the figure, is that the average charge state increases in approximately linear fashion with molecular mass. The charge state corresponding to each peak can be determined from peak distribution, thus making it possible to determine the molecular mass of a protein from spectra such as those shown in Figure 20-10.

An important characteristic of electrospray ionization is that it is readily adapted to direct sample introduction from high-performance liquid chromatography and capillary electrophoresis columns. These applications are described in Chapters 28 and 30. That there is little fragmentation of the analyte makes structural elucidation a difficult task. Usually, tandem mass spectrometry (see Section 20C-5) is used for this purpose. Here, the ions from the original ionization process are separated and the ion of interest is subjected to a fragmentation step before being mass analyzed.

Atmospheric Pressure Chemical Ionization

A normal CI source, as described in Section 20B-2, operates at pressures of approximately 100 Pa. In an atmospheric pressure

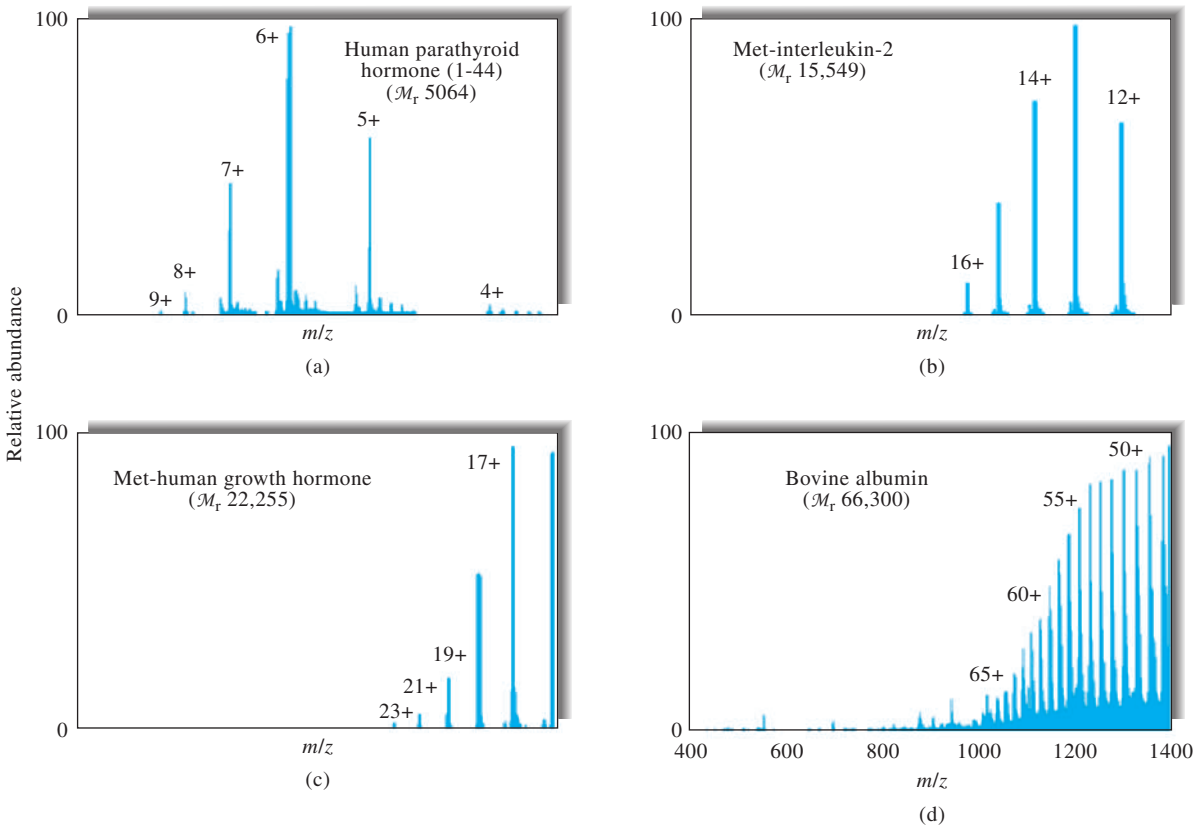

FIGURE 20-10 Typical electrospray mass spectra of proteins and peptides. The numbers above the peaks represent the molecular charge associated with each peak. (From R. D. Smith et al., *Anal. Chem.*, **1990**, *62*, 882, **DOI:** 10.1021/ac00208a002. Copyright 1990 American Chemical Society.)

chemical ionization (APCI) source, ionization is brought about by low-energy electrons emitted by a radioactive beta source or corona discharge. Usually a reagent gas (N_2, O_2, H_2O) is ionized by the low-energy electrons, which leads to subsequent ionization of the analyte by means of several complex ion-molecule reactions. Because of its operation at atmospheric pressure, molecules of the reagent gas and the analyte undergo frequent collisions, which lead to high ionization efficiencies. Just as with electrospray ionization, the mass spectrometer used with APCI must be designed to input ions at atmospheric pressure. This requires "skimmer" interfaces similar to that shown in Figure 20-9. Atmospheric pressure CI is readily adaptable to samples separated by liquid chromatography (LC).

Atmospheric Pressure Photoionization

Atmospheric pressure photoionization (APPI) uses an intense ultraviolet light source instead of low-energy electrons to ionize either the analyte directly or a dopant gas. With the latter, a series of reactions then leads to the formation of analyte ions. Many of the same compounds ionized by APCI can be ionized by APPI, but the range of APPI extends to less polar species.

20B-6 Ambient Ionization Methods

Ambient desorption ionization techniques are performed in the open environment without any sample pretreatment.[13] Almost any mass spectrometer with an atmospheric pressure sample inlet can be used. Currently, the two most popular forms of ambient mass spectrometry are desorption electrospray ionization (DESI) and direct analysis in real time (DART).[14] A generalized diagram applicable to both of these methods is shown in Figure 20-11. Mechanisms of real-time sample processing in ambient ionization methods have been reviewed.[15]

[13]For reviews of ambient mass spectrometry methods, see R. G. Cooks, Z. Ouyang, Z. Takáts, and J. M. Wiseman, *Science*, **2007**, *311*, 1566, **DOI**: 10.1126/science.1119426; J. T. Shelly and G. M. Hieftje, *J. Anal. At. Spectrom.*, **2011**, *26*, 2153, **DOI:** 10.1039/c1ja10158g.

[14]For the original report on DESI, see Z. Takáts, J. M. Wiseman, B. Gologan, and R. G. Cooks, *Science*, **2004**, *306*, 471, **DOI**: 10.1126/science.1104404. For the original description of DART, see R. B. Cody, J. A. Laramee, and H. D. Durst, *Anal. Chem.*, **2005**, *77*, 2297, **DOI**: 10.1021/ac050162j.

[15]A. R. Venter, K. A. Douglass, J. T. Shelly, G. Hasman, Jr., and E. Honarvar, *Anal. Chem.*, **2014**, *1*, 233, **DOI**: 10.1021/ac4038569.

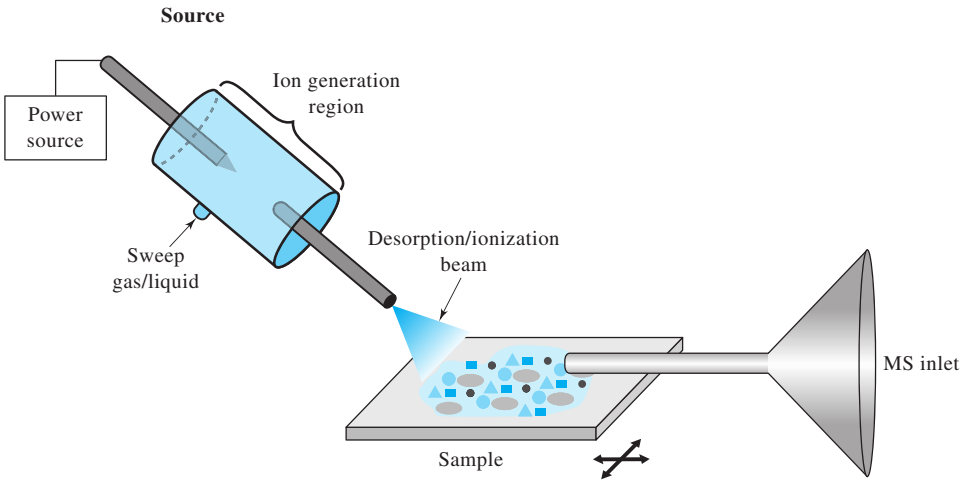

FIGURE 20-11 General diagram of an ambient mass spectrometry source. Reagent ions are generated and swept to the untreated sample by a sweep gas, liquid, or aerosol. The beam releases analytes into the gas phase and ionizes them. The ions then enter the mass spectrometer where they are separated according to their *m/z* ratio. (Adapted from J. T. Shelly and G. M. Hieftje, *J. Anal. At. Spectrom.*, **2011**, *26*, 2153, **DOI:** 10.1039/c1ja10158g.)

Desorption Electrospray Ionization

The DESI source is similar to a normal electrospray source except that the droplets produced are not desolvated as they are in ESI/MS. The spray of charged droplets, the desorption/ionization beam shown in Figure 20-11, impinges on the sample from which the analyte is either released as ions or the ions are formed in the plume that results from the desorption/ionization process. In DESI, a goal is to keep the sample outside the mass spectrometer inlet and to allow only the desorbed ions to enter. Although the mechanism of the DESI phenomenon is still poorly understood, the accumulation of liquid on the surface is thought to play an important role in the process. Mass spectra of large biomolecules obtained with DESI resemble those obtained in normal ESI including the formation of multiply charged ions.

With open-air DESI, the distance between the spraying device and the sample is often important as is the angle at which the beam strikes the sample. Newer instrument designs have included a pressure-tight sample chamber that can minimize the angular dependence at the expense of eliminating the open-air advantage of DESI. Several variations of the DESI technique have been described.

Direct Analysis in Real Time

In DART, an excited stream of helium (sometimes nitrogen or neon) is directed at the surface where desorption/ionization occurs. To create the excited gas stream, He is introduced into a discharge chamber where a corona discharge produces a mixture of ions, electrons, and metastable atoms or molecules. Ions are removed by passing the stream through a set of grids of opposing polarities, leaving excited atoms and molecules in the stream.

The gas flow is usually reflected off the surface containing the sample into the mass spectrometer. The exact positioning and angle with respect to the surface and the mass spectrometer inlet are not critical. Limitations of DART include its incompatibility with LC and a somewhat limited mass range.

Other Desorption Methods

Examination of Table 20-1 reveals that several other desorption/ionization methods are available. Generally these produce spectra similar to the desorption methods we have described and have more limited applicability.

20C MASS SPECTROMETERS

Several types of instruments are currently used for molecular mass spectrometric measurements. Two of these, the quadrupole spectrometer and the TOF spectrometer, have been described in some detail in Sections 11B-2 and 11B-3. In this section, we consider other common types of mass spectrometers.

20C-1 General Description of Instrument Components

The block diagram in Figure 20-12 shows the major components of mass spectrometers. The purpose of the inlet system is to introduce a very small amount of sample (a micromole or less) into the mass spectrometer, where its components are converted to gaseous ions. Often the inlet system contains a means for volatilizing solid or liquid samples.

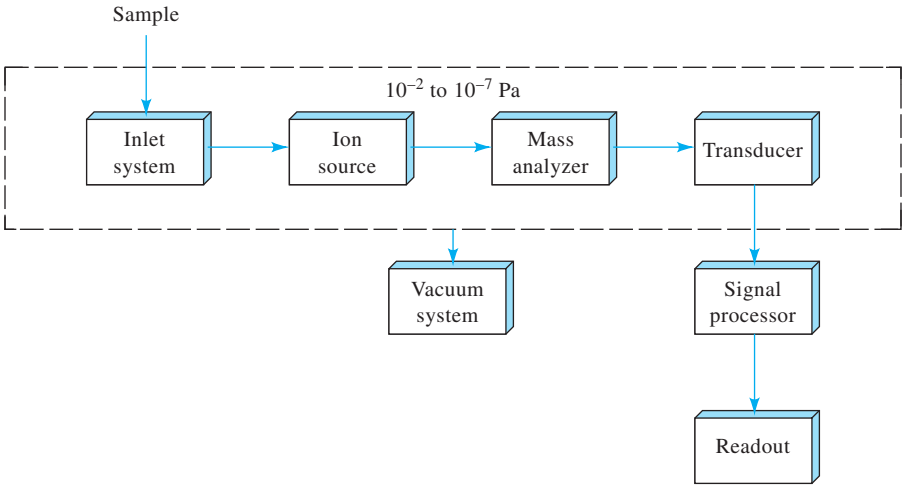

FIGURE 20-12 Components of a mass spectrometer.

Ion sources of mass spectrometers, which were discussed in some detail in the previous section and in Chapter 11, convert the components of a sample into gas-phase ions. In many cases the inlet system and the ion source are combined into a single component. In either case, the output is a stream of positive or negative ions (more commonly positive) that are then accelerated into the mass analyzer.

The function of the mass analyzer, as discussed in Chapter 11, is analogous to that of the grating in an optical spectrometer. In the mass analyzer, however, ions are dispersed according to their mass-to-charge ratios. Mass spectrometers fall into several categories, depending on the nature of the mass analyzer.

Like an optical spectrometer, a mass spectrometer contains a transducer (for ions) that converts the beam of ions into an electrical signal that can then be processed, stored in the memory of a computer, and displayed or recorded in a variety of ways. The characteristics of several types of transducers used in mass spectrometers were described in Section 11B-1.

A distinguishing feature of mass spectrometers, which is not shared by optical instruments, is the requirement of an elaborate vacuum system to create low pressures (10^{-2} to 10^{-7} Pa) in all of the instrument components except the signal processor, readout, and, in some cases, the inlet system. The need for a high vacuum arises because such conditions lead to infrequent collisions with atmospheric components and allow the production and manipulation of free electrons and ions.

In the sections that follow, we first describe inlet systems that are common to all types of mass spectrometers. We then define *resolution*, which describes the ability of a mass spectrometer to differentiate between ions with differing masses. Finally, several types of mass analyzers that lead to different categories of mass spectrometers are described.

20C-2 Sample Inlet Systems

The purpose of the inlet system is to permit introduction of a representative sample into the ion source with minimal loss of vacuum. Most modern mass spectrometers are equipped with several types of inlets to accommodate various kinds of samples; these include batch inlets, direct probe inlets, chromatographic inlets, and capillary electrophoretic inlets.

Batch Inlet Systems

The conventional (and simplest) inlet system is the batch type, in which the sample is volatilized externally and then allowed to leak into the evacuated ionization region. Figure 20-13a is a schematic of a typical system that is applicable to gaseous and liquid samples having boiling points up to about 500°C.

The first step is to obtain the mass spectrum of any residue from a previous sample. For this, the heated reservoir is evacuated, the valve to the ion source opened, and a mass spectrum scanned. The reservoir is then evacuated and isolated by closing the valves. The sample is then introduced, often through a septum, and the valve to the ion source opened. For gaseous samples a measured amount is introduced into the reservoir, sometimes with a separate introduction system. For liquid samples with boiling points greater than 150°C, the reservoir and tubing are maintained at an elevated temperature by means of an oven and/or heating tape. The sample, which is now in the gas phase, is leaked into the ionization area by means of a metal or glass diaphragm containing one or more pinholes. This simple inlet has the disadvantage of requiring rather large sample sizes. The inlet system is often lined with glass to avoid losses of polar analytes due to adsorption.

The heated reservoir inlet played an important role in the early development of mass spectrometry. This type of inlet is still used with some EI and CI sources and for the introduction of compounds for calibration of the mass scale of instruments.

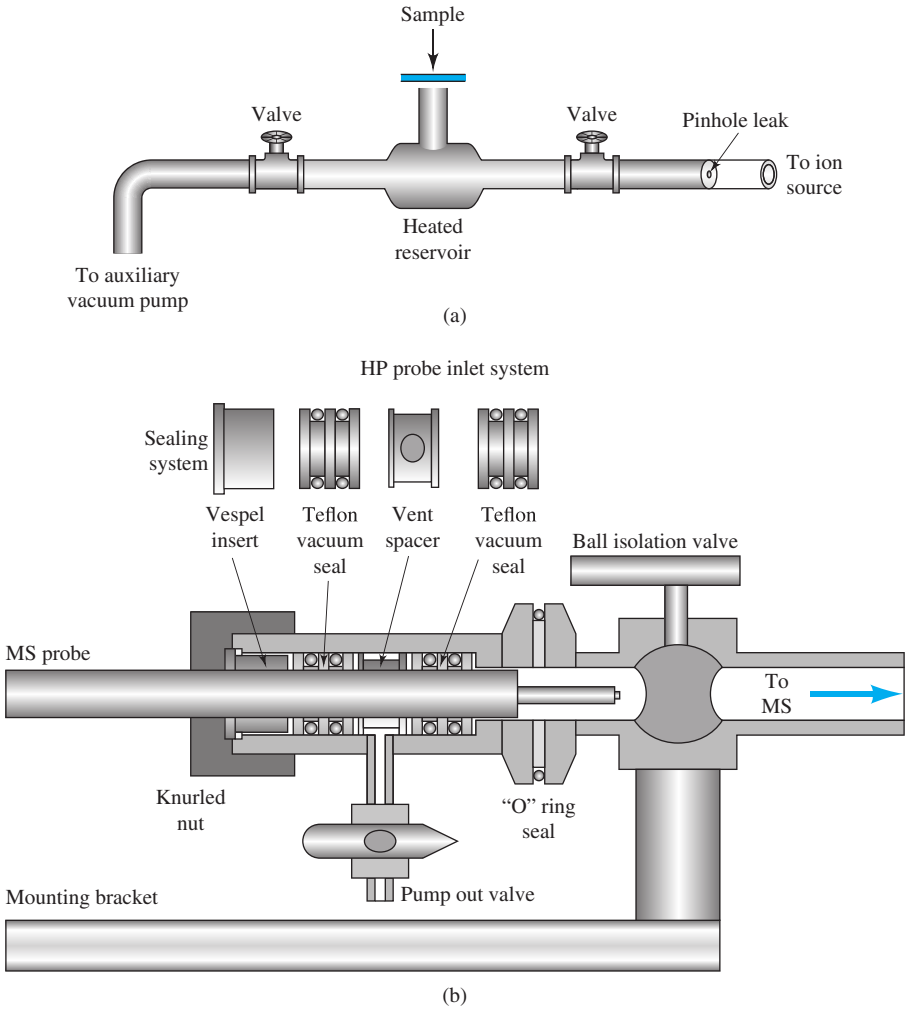

FIGURE 20-13 Schematic of a heated-reservoir sample inlet system (a) and a direct probe inlet (b). [(a) Cengage Learning; (b) Adapted from Scientific Instrument Services http://www.sisweb.com/ms/sis/probinlt.htm.]

The Direct Probe Inlet

Solids and nonvolatile liquids can be introduced into the ionization region by means of a sample holder, or probe, which is inserted through a vacuum lock (see Figure 20-13b). The lock system is designed to limit the volume of air that must be pumped from the system after insertion of the probe into the ionization region.

With a probe, the sample is generally held on the surface of a glass or aluminum capillary tube, a fine wire, or a small cup. The probe is positioned within a few millimeters of the ionization source and the slit leading to the spectrometer. Usually, provision is made for both cooling and heating the sample on the probe. Like batch inlets, direct probe inlets were used for EI and CI sources.

Direct probe inlets permitted the recording of mass spectra of relatively nonvolatile compounds. They were also used when sample amounts were limited since much less sample was wasted than with the batch inlet. Disadvantages included the possibility of venting to the atmosphere and the risk of contaminating the ion source if large quantities of nonvolatile compounds were introduced. Although direct probe inlets were once standard accessories on commercial mass spectrometers, they are no longer available for most systems.

Chromatographic and Other Inlet Systems

Mass spectrometers are often coupled with gas or high-performance liquid chromatographic systems or with capillary electrophoresis units to permit the separation and determination of the components of complex mixtures. Linking a chromatographic or electrophoretic column to a mass spectrometer requires the use of specialized inlet systems, some of which are described in Sections 27B-4, 28C-6, and 30B-4.

Many additional types of inlets have been described.[16] Among the most important are polymer membrane and atmospheric pressure inlets (see previous section).

[16]For descriptions of additional inlets, see J. T. Watson and O. D. Sparkman, *Introduction to Mass Spectrometry*, Chap. 4, Chichester, UK: Wiley, 2007.

20C-3 Mass Analyzers

Several devices are available for separating ions with different mass-to-charge ratios. Ideally, the mass analyzer should be capable of distinguishing minute mass differences. In addition, the analyzer should allow passage of a sufficient number of ions to yield readily measurable ion currents. These two properties are not entirely compatible, and design compromises must be made.

Resolution of Mass Spectrometers

The capability of a mass spectrometer to differentiate between masses is usually stated in terms of its *resolution* R, which is defined as

$$R = \frac{m}{\Delta m} \tag{20-3}$$

where Δm is the mass difference between two adjacent peaks of equal intensity that are just resolved and m is the nominal mass of the first peak (the mean mass of the two peaks is sometimes used instead). Two peaks are considered to be separated if the height of the valley between them is no more than a given fraction of their height (often 10%). Thus, a spectrometer with a resolution of 4000 would resolve peaks at m/z values of 400.0 and 400.1 (or 40.00 and 40.01).

The resolution required in a mass spectrometer depends greatly on its application. For example, discrimination among ions of the same nominal mass such as $C_2H_4^+$, CH_2N^+, N_2^+, and CO^+ (all ions of nominal mass 28 Da but exact masses of 28.0313, 28.0187, 28.0061, and 27.9949 Da, respectively) requires an instrument with a resolution of several thousand. On the other hand, low-molecular-mass ions differing by a unit of mass or more such as NH_3^+ ($m = 17$) and CH_4^+ ($m = 16$), for example, can be distinguished with an instrument having a resolution smaller than 50. Commercial spectrometers are available with resolutions ranging from about 500 to 500,000. Example 20-3 gives a calculation of the resolution needed to separate two species.

EXAMPLE 20-3

What resolution is needed to separate the ions $C_2H_4^+$ and CH_2N^+, with masses of 28.0313 and 28.0187, respectively?

Solution

Here,

$$\Delta m = 28.0313 - 28.0187 = 0.0126$$

The average of the two masses is $(28.0313 + 28.0187)/2 = 28.0250$. Substituting into Equation 20-3 gives

$$R = m/\Delta m = 28.0250/0.0126 = 2.22 \times 10^3$$

Magnetic Sector Analyzers

Magnetic sector analyzers use a permanent magnet or an electromagnet to cause the beam from the ion source to travel in a circular path, most commonly 180°, 90°, or 60°. Figure 20-14 shows a 90° sector instrument in which ions formed by EI are accelerated through slit B into the metal analyzer tube, which is maintained at an internal pressure of about 10^{-5} Pa. Ions of different mass can be scanned across the exit slit by varying the field strength of the magnet or the accelerating potential between slits A and B. The ions passing through the exit slit

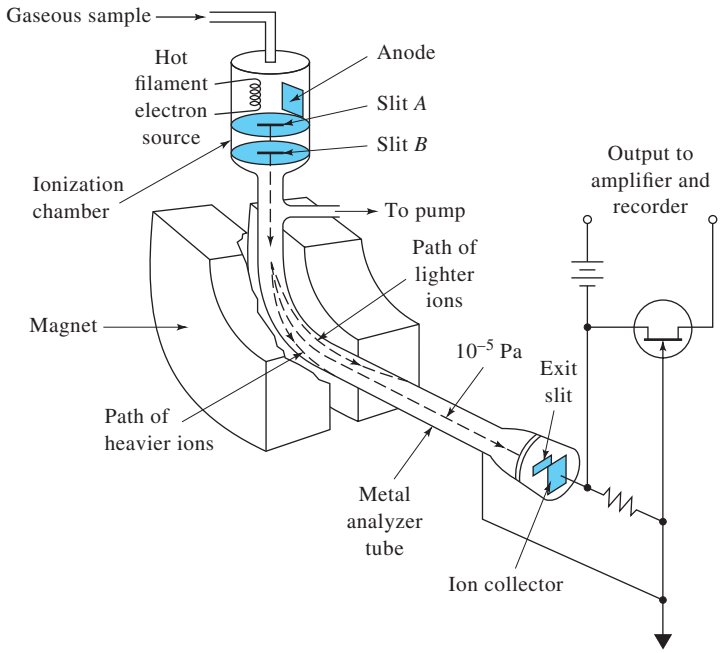

FIGURE 20-14 Schematic of a magnetic sector spectrometer.

fall on a collector electrode, resulting in an ion current that is amplified and recorded.

The translational, or kinetic, energy KE of an ion of mass m bearing a charge z on exiting slit B is given by

$$\text{KE} = zeV = \frac{1}{2}mv^2 \qquad (20\text{-}4)$$

where V is the voltage difference between A and B, v is the velocity of the ion after acceleration, and e is the electronic charge ($e = 1.60 \times 10^{-19}$ C). Note that all ions having the same number of charges z are assumed to have the same kinetic energy after acceleration regardless of their mass. This assumption is only approximately true, because before acceleration, the ions possess a statistical distribution of velocities (speeds and directions), which will be reflected in a similar distribution for the accelerated ion. The limitations of this assumption are discussed in the next section when double-focusing instruments are described. Because all ions leaving the slit have approximately the same kinetic energy, the heavier ions must travel through the magnetic sector at lower velocities.

The path in the sector described by ions of a given mass and charge represents a balance between two forces acting on them. The magnetic force F_M is given by the relationship

$$F_M = Bzev \qquad (20\text{-}5)$$

where B is the magnetic field strength. The balancing centripetal force F_c is given by

$$F_c = \frac{mv^2}{r} \qquad (20\text{-}6)$$

where r is the radius of curvature of the magnetic sector. For an ion to traverse the circular path to the collector, F_M and F_c must be equal. Thus, equating Equations 20-5 and 20-6 leads to

$$Bzev = \frac{mv^2}{r} \qquad (20\text{-}7)$$

which rearranges to

$$v = \frac{Bzer}{m} \qquad (20\text{-}8)$$

Substituting Equation 20-8 into Equation 20-4 gives, after rearranging,

$$\frac{m}{z} = \frac{B^2r^2e}{2V} \qquad (20\text{-}9)$$

Equation 20-9 reveals that mass spectra can be acquired by varying one of three variables (B, V, or r) while holding the other two constant. Most modern sector mass spectrometers contain an electromagnet in which ions are sorted by holding V and r constant while varying the current in the magnet and thus B. In older sector spectrometers that used photographic recording, B and V were constant, and r was the variable (see Figure 11-11). In Example 20-4, the use of Equation 20-9 to calculate an appropriate accelerating voltage is illustrated.

EXAMPLE 20-4

What accelerating voltage is required to direct a singly charged water molecule through the exit slit of a magnetic sector mass spectrometer if the magnet has a field strength of 0.240 T (tesla) and the radius of curvature of the ion through the magnetic field is 12.7 cm?

Solution

First, we convert all experimental variables into SI units. Thus,

charge per ion $ez = 1.60 \times 10^{-19}\ \text{C} \times 1$

radius $r = 0.127$ m

$$\text{mass } m = \frac{18.02\ \text{g}\ H_2O^+/\text{mol}}{6.02 \times 10^{23}\ \text{g/mol}} \times 10^{-3}\ \frac{\text{kg}}{\text{g}}$$

$$= 2.99 \times 10^{-26}\ \text{kg}\ H_2O^+$$

magnetic field $B = 0.240\ \text{T} = 0.240\ \text{Vs/m}^2$

We then substitute into Equation 20-9 and solve for the accelerating voltage V:

$$V = \frac{B^2r^2ez}{2m}$$

$$= \frac{[0.240\ \text{Vs/m}^2]^2[0.127\ \text{m}]^2[1.60 \times 10^{-19}\ \text{C}]}{2 \times 2.99 \times 10^{-26}\ \text{kg}}$$

$$= 2.49 \times 10^3\ \frac{(\text{Vs})^2\text{C}}{\text{m}^2\text{kg}}$$

$$= 2.49 \times 10^3\ \text{V} \qquad (1\ \text{volt} = 1\ \text{kg m}^2/\text{s}^2\ \text{C})$$

Magnetic sector mass spectrometers are limited in scan speed by the rate at which the magnetic field can be changed. This scan-rate limitation is a particular problem with capillary gas chromatography/mass spectrometry (see Section 27B-4), where narrow analyte peaks require rapid scanning.

Double-Focusing Spectrometers

The magnetic sector instruments discussed in the previous section are sometimes called *single-focusing* spectrometers. This terminology is used because a collection of ions exiting the source with the same mass-to-charge ratio but with small diverging directional distribution will be acted on by the magnetic field in such a way that a converging directional distribution is produced as the ions leave the field. The ability of a magnetic field to bring ions with different directional orientations to focus means that the distribution of translational energies of ions leaving the source is the factor most responsible for limiting the resolution of magnetic sector instruments ($R \leq 2000$).

The translational energy distribution of ions leaving a source arises from the Boltzmann distribution of energies of the molecules from which the ions are formed and from field inhomogeneities in the source. The spread of kinetic energies causes a broadening of the beam reaching the transducer and thus a

loss of resolution. To measure atomic and molecular masses with a precision of a few parts per million, it is necessary to design instruments that correct for both the directional distribution and energy distribution of ions leaving the source. The term *double focusing* is applied to mass spectrometers in which the directional aberrations and the energy aberrations of a population of ions are simultaneously minimized. Double focusing is usually achieved by the use of carefully selected combinations of electrostatic and magnetic fields. In the double-focusing instrument, shown schematically in Figure 20-14, the ion beam is first passed through an electrostatic analyzer (ESA) consisting of two smooth curved metallic plates across which a dc voltage is applied. This voltage has the effect of limiting the kinetic energy of the ions reaching the magnetic sector to a closely defined range. Ions with energies greater than average strike the upper side of the ESA slit and are lost to ground. Ions with energies less than average strike the lower side of the ESA slit and are thus removed.

Directional focusing in the magnetic sector occurs along the focal plane labeled *d* in Figure 20-14; energy focusing takes place along the plane labeled *e*. Thus, only ions of one m/z are double focused at the intersection of *d* and *e* for any given accelerating voltage and magnetic field strength. Therefore, the collector slit is located at this locus of the double focus.

Many different types of double-focusing mass spectrometers are available commercially. The most sophisticated of these are capable of resolution in the 10^5 range. More compact double-focusing instruments can also be purchased (for considerably less money). A typical instrument of this type will have a 6-in. electrostatic sector and a 4-in. 90° magnetic deflector. Resolutions of about 2500 are common with such instruments. Often they are used as detection systems for gas or liquid chromatography.

The spectrometer shown in Figure 20-15 is based on the so-called *Nier-Johnson* design. Another double-focusing design, of the *Mattauch-Herzog* geometry, is shown in Figure 11-11. The geometry of this type of instrument is unique in that the energy and direction focal planes coincide; for this reason, the Mattauch-Herzog design can use an array detector or a photographic plate for recording the spectrum. The transducer is located along the focal plane, where all of the ions are in focus, regardless of mass-to-charge ratio.

Quadrupole Mass Spectrometers

Quadrupole mass spectrometers are usually less expensive and more rugged than their magnetic sector counterparts. They are generally considerably more compact than magnetic sector instruments and are commonly found in commercial benchtop mass spectrometers. They also offer the advantage of low scan times (that is, <100 ms), which is particularly useful for real-time scanning of chromatographic eluents. Quadrupole analyzers are the most common mass analyzers in use today. A detailed discussion of quadrupole mass spectrometers is found in Section 11B-2.

TOF Mass Analyzers

As shown in Section 11B-3, in TOF instruments, positive ions are produced periodically by bombardment of the sample with brief pulses of electrons, secondary ions, or laser-generated photons. The ions produced in this way are then accelerated into

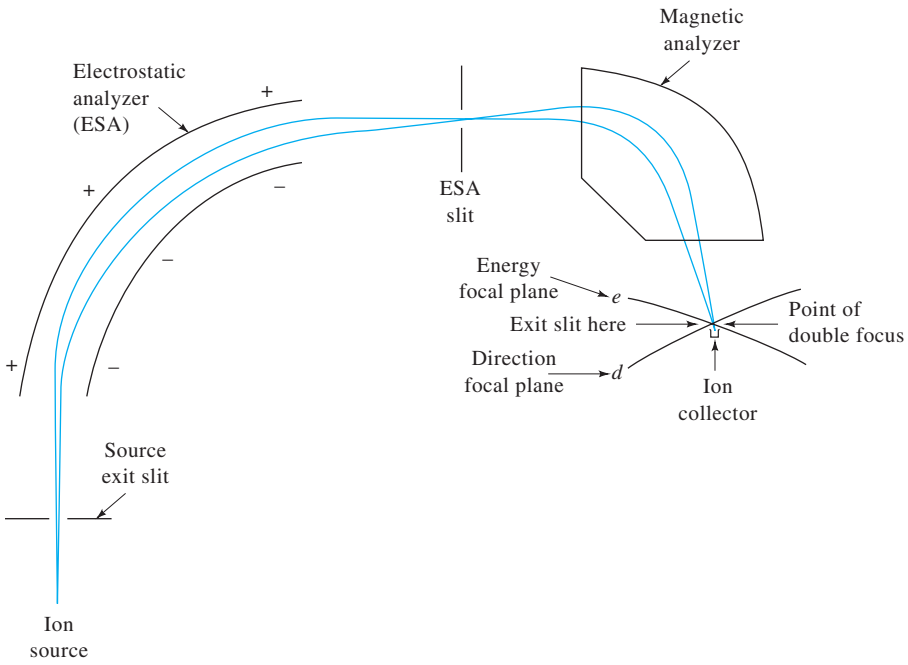

FIGURE 20-15 Nier-Johnson design of a double-focusing mass spectrometer.

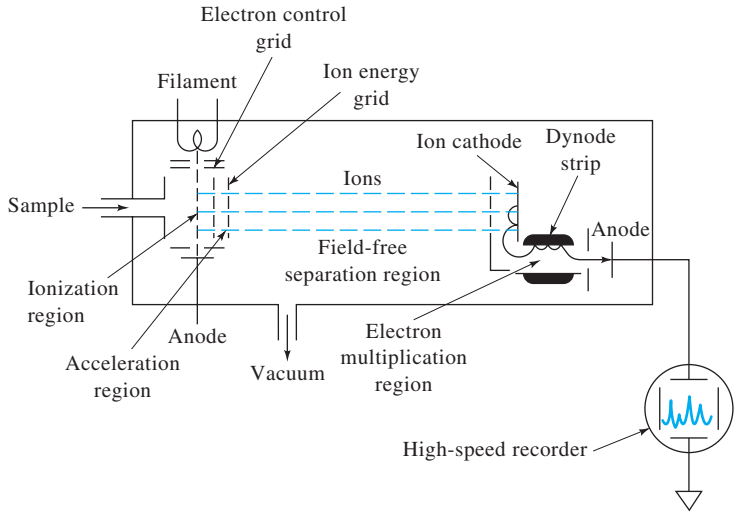

FIGURE 20-16 Schematic of a TOF mass spectrometer.

a field-free drift tube by an electric field pulse of 10^3 to 10^4 V (see Figure 20-16). Separation of ions by mass occurs during the transit of the ions to the detector located at the end of the tube. Because all ions entering the tube have the same kinetic energy, their velocities in the tube vary inversely with their masses (Equation 20-4), with the lighter particles arriving at the detector earlier than the heavier ones. The flight time t_F is given by

$$t_F = \frac{L}{v} = L\sqrt{\frac{m}{2zeV}} \qquad (20\text{-}10)$$

where L is the distance from the source to the detector. Typical flight times are 1 to 50 μs.

TOF instruments offer several advantages over other types of mass spectrometers, including simplicity and ruggedness, ease of accessibility of the ion source, and virtually unlimited mass range. They suffer, however, from limited resolution and sensitivity. TOF instruments also require fast electronics because ions often arrive at the transducer only fractions of microseconds apart. Several instrument manufacturers offer TOF instruments, but they are less widely used than are magnetic sector and quadrupole mass spectrometers.

Ion-Trap Analyzers

An ion trap is a device in which gaseous anions or cations can be formed and confined for extended periods by electric and magnetic fields. The quadrupole ion trap was first introduced by Paul in 1953.[17] Since that time several types of ion traps have been developed.[18] Ion-trap mass spectrometers are now used as chromatography detectors and to obtain mass spectra of a variety of analytes. For their work in developing the ion-trap technique, Wolfgang Paul and Hans Dehmelt were awarded the Nobel Prize in Physics in 1989.

Figure 20-17 is a cross-sectional view of a simple, commercially available ion trap. It consists of a central doughnut-shape ring electrode and a pair of end-cap electrodes. A variable radio-frequency voltage is applied to the ring electrode while the two end-cap electrodes are grounded. Ions with an appropriate m/z value circulate in a stable orbit within the cavity surrounded

Tutorial: Learn more about **mass analyzers** at www.tinyurl.com/skoogpia7

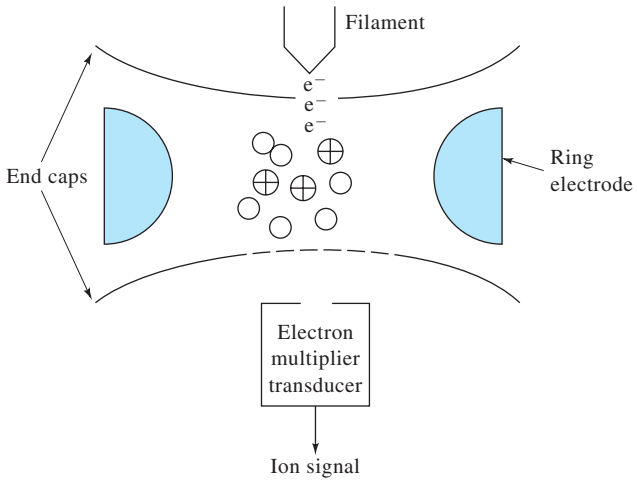

FIGURE 20-17 Ion-trap mass spectrometer. (Adapted from Watson, *Introduction to Mass Spectrometry*, p. 89, Philadelphia: Lippincott-Raven Press, 1997.)

[17]W. Paul and H. Steinwedel, *Z. Naturforsch*, **1953**, *8A*, 448.

[18]R. E. March and J. F. J. Todd, eds., *Quadrupole Ion Trap Mass Spectrometry*, 2nd ed., Hoboken, NJ: Wiley, 2005; R. E. March, *Mass Spectrom. Rev.*, **2009**, *28*, 961; **DOI**: 10.1002/mas.20250; R. E. March, *Int. J. Mass Spectrom.*, **2000**, *200*, 285, **DOI**: 10.1016/S1387-3806(00)00345-6; C. Hao and R. E. March, *Int. J. Mass Spectrom.*, **2001**, *212*, 337, **DOI:** 10.1016/S1387-3806(01)00465-1.

by the ring. As the radio-frequency voltage is increased, the orbits of heavier ions become stabilized, and those for lighter ions become destabilized, causing them to collide with the wall of the ring electrode.

When the device is operated as a mass spectrometer, ions produced by an EI or a CI source are admitted through a grid in the upper end cap. The ionization source is pulsed so as to create a burst of ions. The ions over a large mass range of interest are trapped simultaneously. The ion trap can store ions for relatively long times, up to 15 minutes for some stable ions. A technique called *mass-selective ejection* is then used to sequentially eject the trapped ions in order of mass by increasing the radio-frequency voltage applied to the ring electrode in a linear ramp. As trapped ions become destabilized, they leave the ring electrode cavity via openings in the lower end cap. The emitted ions then pass into a transducer such as the electron multiplier shown in Figure 20-17.

Ion-trap mass analyzers have the advantage of being rugged, compact, and less costly than other mass analyzers. They have the potential for achieving low detection limits. In addition to EI sources, such analyzers have been interfaced to electrospray ionization sources as well as MALDI sources.

The latest development in ion-trap analyzers is the orbitrap analyzer.[19] Although the principles of orbital trapping have been known since the 1920s, recent developments in ion optics and other areas led to the use of the orbitrap in mass spectrometry. The orbitrap analyzer is an electrostatic trap consisting of an inner electrode that is wide in the middle and tapered at both ends (spindle-shaped) and an outer coaxial split electrode as shown in Figure 20-18. A constant potential is applied between these two electrodes. Since the opposing surfaces of the outer electrode are not parallel to each other, the electric field varies with position reaching a minimum in the center of the trap. Prior to injection, the ions are collected in a C-trap that tightly focuses them in time and space. The ions injected into the trap follow a circular orbit around the inner electrode with an axial oscillation that is proportional to the m/z ratio of the injected ions. An image current is generated, recorded, and decoded using Fourier Transform analysis (see next section).

Orbitraps can have high resolution (>200,000) and a high dynamic range. Mass measurements can be made with high accuracy. The orbitrap analyzer is small and less expensive than many other high resolution analyzers. The number of ions that can be held in the analyzer is, however, limited, which can influence detection of minor components in the sample. Another disadvantage is that the orbitrap needs the lowest pressure of any mass analyzer to ensure a large mean-free path for the orbiting ions. Orbitrap analyzers have been commercially available since 2005.

[19]For a review of orbitrap analyzers, see R. A. Zubarev and A. Makarov, *Anal. Chem.*, **2013**, *85*, 5288, **DOI**: 10.1021/ac4001223.

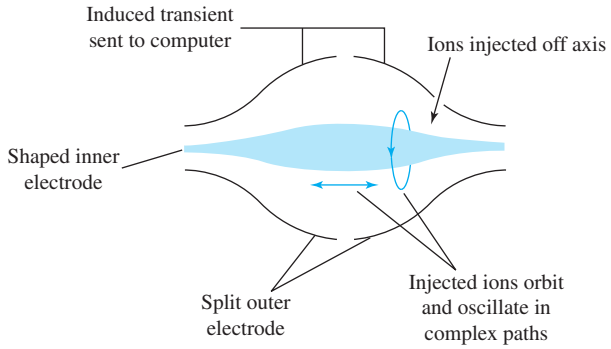

FIGURE 20-18 Orbitrap analyzer. When ions are injected into the analyzer, they follow a complex path composed of rotary motion around the inner electrode and an axial oscillation at right angles to the rotary component. Prior to injection, the ions are collected in a C-trap that tightly focuses them in time and space. (Adapted from J. Greaves and J. Roboz, *Mass Spectrometry for the Novice*, Boca Raton, FL: CRC Press, 2014, p. 84.)

20C-4 Fourier Transform Spectrometers

As was true with infrared and nuclear magnetic resonance instruments, Fourier transform mass spectrometers provide improved signal-to-noise ratios, greater speed, and higher sensitivity and resolution.[20] Commercial Fourier transform mass spectrometers appeared on the market in the early 1980s and are offered by a few manufacturers.

The heart of a Fourier transform instrument is an ion trap within which ions can circulate in well-defined orbits for extended periods. Such cavities are constructed to take advantage of a phenomenon known as *ion cyclotron resonance*.

Ion Cyclotron Resonance

When a gaseous ion drifts into or is formed in a strong magnetic field, its motion becomes circular in a plane perpendicular to the direction of the field. The angular frequency of this motion is called the cyclotron frequency, ω_c. Equation 20-8 can be rearranged and solved for v/r, which is the cyclotron frequency in radians per second.

$$\omega_c = \frac{v}{r} = \frac{zeB}{m} \qquad (20\text{-}11)$$

Note that in a fixed field, the cyclotron frequency depends only on the inverse of the m/z value. Increases in the velocity of an ion will be accompanied by a corresponding increase in the radius of rotation of the ion. A measurement of ω_c can provide an accurate indication of z/m and thus the mass-to-charge ratio of the ion.

[20]For reviews of Fourier transform mass spectrometry, see R. M. A. Heeren, A. J. Kleinnijenhuis, L. A. McDonnell, and T. H. Mize, *Anal. Bioanal. Chem.*, **2004**, *378*, 1048, **DOI**: 10.1007/s00216-003-2446-4; A. G. Marshall, *Int. J. Mass Spectrom.*, **2000**, *200*, 331, **DOI**: 10.1016/S1387-3806(00)00324-9; A. G. Marshall, C. L. Hendrickson, and G. S. Jackson, *Mass Spectrom. Rev.*, **1998**, *17*, 1, **DOI**: 10.1002/(sici)1098-2787(1998)17:1<1::aid-mas1>3.0.co;2-k.

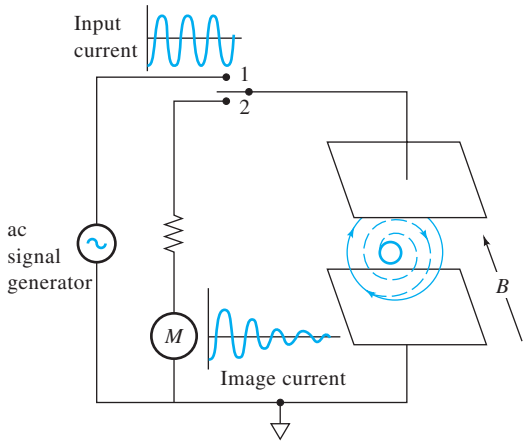

FIGURE 20-19 Path of an ion in a strong magnetic field. Inner solid line represents the original circular path of the ion. Dashed line shows spiral path when switch is moved briefly to position 1. Outer solid line is new circular path when switch is again opened.

An ion trapped in a circular path in a magnetic field is capable of absorbing energy from an ac electric field, provided the frequency of the field matches the cyclotron frequency. The absorbed energy then increases the velocity of the ion (and thus the radius of its path) without disturbing ω_c. This effect is illustrated in Figure 20-19. Here, the original path of an ion trapped in a magnetic field is depicted by the inner solid circle. Brief application of an ac voltage creates a fluctuating field between the plates that interacts with the ion, provided the frequency of the source is resonant with the cyclotron frequency of the ion. Under this circumstance, the velocity of the ion increases continuously as does the radius of its path (see dashed line). When the ac electrical signal is terminated, the radius of the path of the ion again becomes constant, as is suggested by the outer solid circle in the figure.

When the region between the plates in Figure 20-19 contains an ensemble of ions of the same mass-to-charge ratio, application of the ac signal having the cyclotron resonance frequency sets all of the particles into coherent motion in phase with the field. Ions of different cyclotron frequency, that is, those with different mass-to-charge ratios, are unaffected by the ac field.

Measurement of the ICR Signal

The coherent circular motion of resonant ions creates a so-called *image current* that can be conveniently observed after termination of the frequency sweep signal. Thus, if the switch in Figure 20-19 is moved from position 1 to position 2, a current is observed that decreases exponentially with time. This image current is a capacitor current induced by the circular movement of a packet of ions with the same mass-to-charge ratios. For example, as a packet of positive ions approaches the upper plate in Figure 20-19, electrons are attracted from circuit common to this plate, causing a momentary current. As the packet continues around toward the other plate, the direction of external electron flow is reversed. The magnitude of the resulting alternating current depends on the number of ions in the packet. The frequency of the current (the cyclotron resonance frequency) is characteristic of the mass-to-charge value of the ions in the packet. This current is used in ion cyclotron spectrometers to measure the concentration of ions brought into resonance at various applied signal frequencies.

The induced image current just described decays over a period of a few tenths of a second to several seconds as the coherent character of the circulating packet of ions is lost. Collisions between ions provide the mechanism by which the coherently circulating ions lose energy and the ions return to a condition of thermal equilibrium. This decay of the image current provides a time-domain signal that is similar to the free-induction decay signal encountered in Fourier transform–nuclear magnetic resonance experiments (see Section 19A-3).

Fourier Transform Spectrometers

Fourier transform mass spectrometers are generally equipped with a trapped-ion analyzer cell such as that shown in Figure 20-20. Gaseous sample molecules are ionized in the center of the cell by electrons that are accelerated from the filament through the cell to a collector plate. A pulsed voltage applied at the grid serves as a gate to periodically switch the electron beam on and off. The ions are held in the cell by a 1 to 5 V potential applied to the trap plate. The ions are accelerated by a radio-frequency signal applied to the transmitter plate as shown. The receiver plate is connected to a preamplifier that amplifies the image current. This approach for confining ions is highly efficient, and storage times of up to several minutes have been observed. The dimensions of the cell are not critical but are usually a few centimeters on a side.

The basis of the Fourier transform measurement is illustrated in Figure 20-21. Ions are first generated by a brief electron beam pulse (not shown) and stored in the trapped ion cell. After a brief delay, the trapped ions are subjected to a short radio-frequency pulse that increases linearly in frequency during its lifetime. Figure 20-21a shows a pulse of 5 ms, during which time the frequency increases linearly from 0.070 to 3.6 MHz. After the frequency sweep is discontinued, the image current, induced by the various ion packets, is amplified, digitized, and stored in memory. The time-domain decay signal, shown in Figure 20-21b, is then transformed to yield a frequency-domain signal that can be converted to the mass domain via Equation 20-11. Figure 20-22 illustrates the relationship between a time-domain spectrum, its frequency-domain counterpart, and the resulting mass spectrum.

Fourier transform spectrometers can be interfaced to a variety of ionization sources, including MALDI, electrospray, FAB, and EI-CI. Resolution in Fourier transform mass spectrometry is limited by the precision of the frequency measurement rather than slits or field measurements. The resolution and mass range also depend on the magnitude and stability of the magnetic

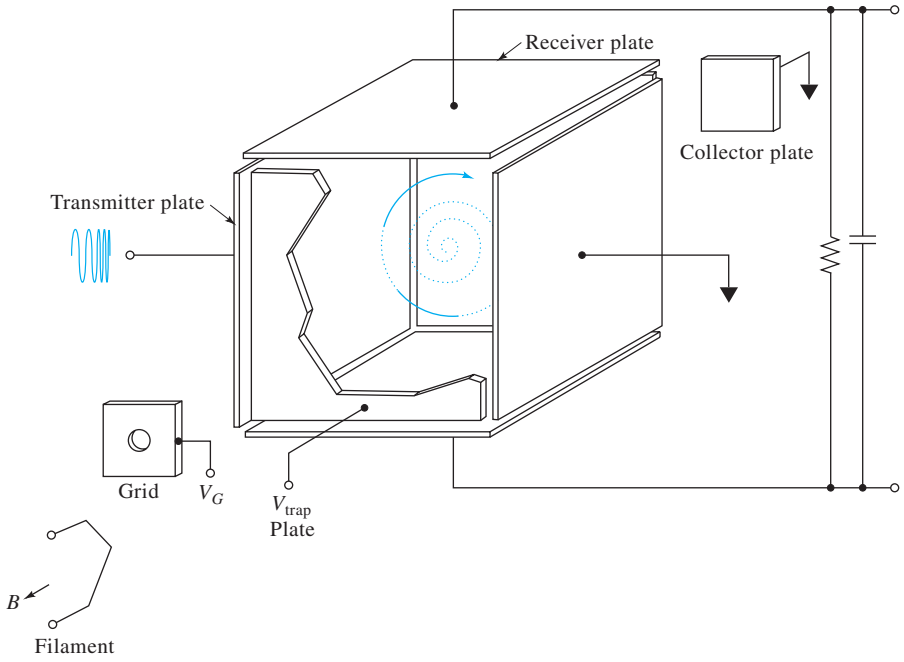

FIGURE 20-20 A trapped-ion analyzer cell. (Reprinted from E. B. Ledford Jr., R. L. White, S. Ghaderi, and C. L. Wilkins, *Anal. Chem.*, **1980**, *52*, 1090, **DOI**: 10.1021/ac50057a022. Copyright 1980 American Chemical Society.)

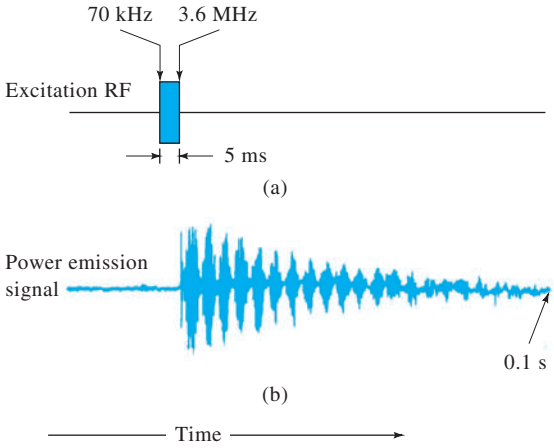

FIGURE 20-21 Schematic showing the timing of (a) the radio-frequency signal and (b) the transient image signal (lower). (Reprinted with permission from R. T. McIver Jr., *Amer. Lab.*, **1980**, *12* (11), 26. Copyright 1980 by International Scientific Communications, Inc.)

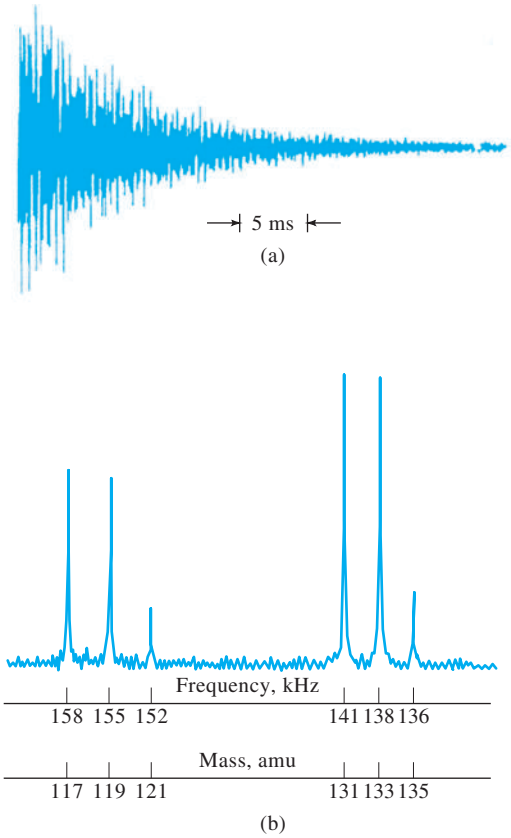

FIGURE 20-22 Time-domain (a) and (b) frequency- or mass-domain spectrum for 1,1,1,2-tetrachloroethane. (Reprinted with permission from E. B. Ledford Jr. et al., *Anal. Chem.*, **1980**, *52*, 463, **DOI**: 10.1021/ac50053a021. Copyright 1980 American Chemical Society.)

field. Because frequency measurements can be made with high precision, extremely high resolution is possible (in excess of 10^6). The accuracy with which mass measurements can be made with Fourier transform instruments is also superb. Fourier transform spectrometers can be very expensive with prices exceeding $500,000. Commercial models are available with superconducting magnets with fields varying from 1.2 to 12 T. The high-field models are quite useful in biological applications, including proteomics.

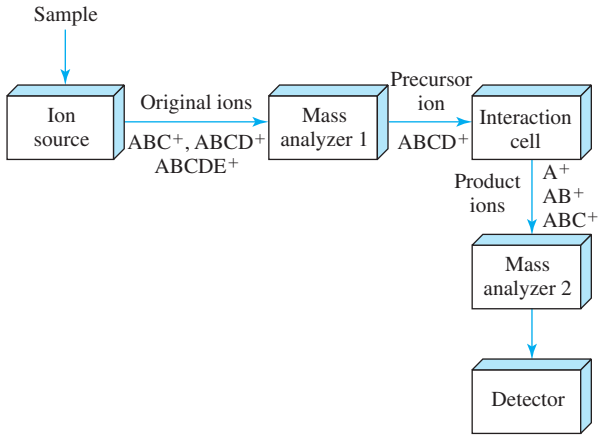

FIGURE 20-23 Block diagram of a tandem mass spectrometer.

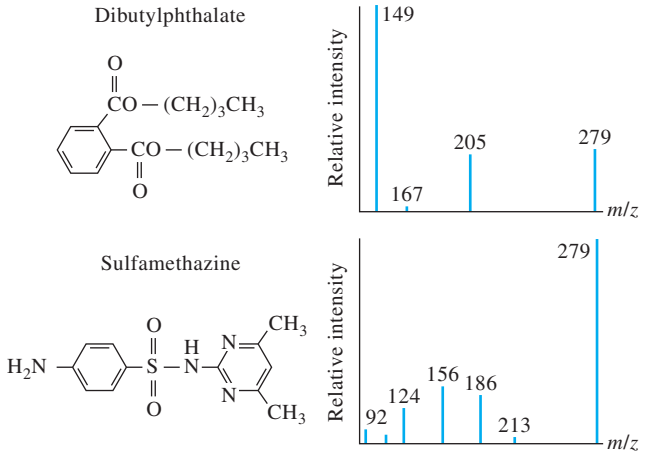

FIGURE 20-24 Product-ion spectra for dibutylphthalate and sulfamethazine obtained after the protonated precursor ion peaks at 279 Da were isolated by the first mass analyzer of an MS/MS instrument. (Reprinted from K. L. Busch and G. C. DiDonato, *Amer. Lab.*, **1986**, *18* (8), 17. Copyright 1986 by International Scientific Communications, Inc.)

20C-5 Tandem Mass Spectrometry

Tandem mass spectrometry, sometimes called *mass spectrometry–mass spectrometry* (MS/MS), is a method that allows the mass spectrum of preselected and fragmented ions to be obtained. The basic idea is illustrated in Figure 20-23. Here, an ionization source, often a soft ionization source, produces ions and some fragments. These are then the input to the first mass analyzer, which selects a particular ion called the *precursor ion* and sends it to the interaction cell. In the interaction cell, the precursor ion can decompose spontaneously, react with a collision gas, or interact with an intense laser beam to produce fragments, called *product ions*. These ions are then mass analyzed by the second mass analyzer and detected by the ion detector.

Types of Tandem Mass Spectra

Several different types of spectra can be obtained from the MS/MS experiment. First, the *product-ion spectrum* can be obtained by scanning mass analyzer 2, while mass analyzer 1 is held constant, acting as a mass selector to select one precursor ion. Figure 20-24 shows product-ion spectra for dibutylphthalate and sulfamethazine. Both compounds produce molecular ions with m/z values of 279. However, the product-ion spectra of the two compounds are very different.

A *precursor ion spectrum* can be obtained, in addition to product-ion spectra, by scanning the first mass analyzer while holding the second mass analyzer constant to detect a given product ion. In a mixture of compounds, those that give the same products are readily identified by precursor ion spectra. Closely related compounds often give several of the same product ions, so that this method of operation provides a measure of identity and concentration of the *members of a class* of closely related compounds. Consider, for example, a mixture of ABCD, BCDA, IJKL, and IJMN in the sample. To identify species containing the IJ group, the second analyzer is set to the mass corresponding to the IJ^+ ion and the molecular ions $ABCD^+$, $BCDA^+$, $IJKL^+$, and $IJMN^+$ are sequentially selected by the first analyzer. Ion signals in the detector would be observed only when $IJKL^+$ and $IJMN^+$ are selected by the first analyzer, indicating the presence of the IJ group.

By scanning both analyzers simultaneously with an offset in mass between them, a *neutral loss spectrum* can be obtained. This gives the identity of those precursor ions that undergo the same loss such as the loss of a H_2O or CO neutral. Finally, by scanning mass analyzer 1 and obtaining the product-ion spectrum for each selected precursor ion, a complete *three-dimensional MS/MS spectrum* can be obtained.

Dissociative Interactions in the Interaction Cell

Several types of interactions can be used to produce fragmentation in the interaction cell. In some cases, the ions selected by mass analyzer 1 in Figure 20-23 are themselves *metastable* and decompose into fragments after a certain time. In general, however, the kinetics of the decomposition process can greatly limit the applicability and sensitivity of the process. In such cases, fragmentation can be induced by adding a collision gas to the interaction cell so that interaction with precursor ions occurs, leading to decomposition into product ions. In this case, the cell is called a *collision cell*, and the interactions are termed *collisionally activated dissociation* (CAD) or alternatively *collision-induced dissociation* (CID).

Another type of interaction is *surface-induced dissociation* (SID), in which precursor ions interact with a surface to induce dissociation. Ions have been reflected off cell walls or trapping plates to increase their internal energy and promote dissociation. Chemically modified surfaces such as thin films have also been used.

Another dissociation technique that has been applied to large multiply charged ions is *electron-capture dissociation* (ECD), in which precursor ions capture a low-energy electron to

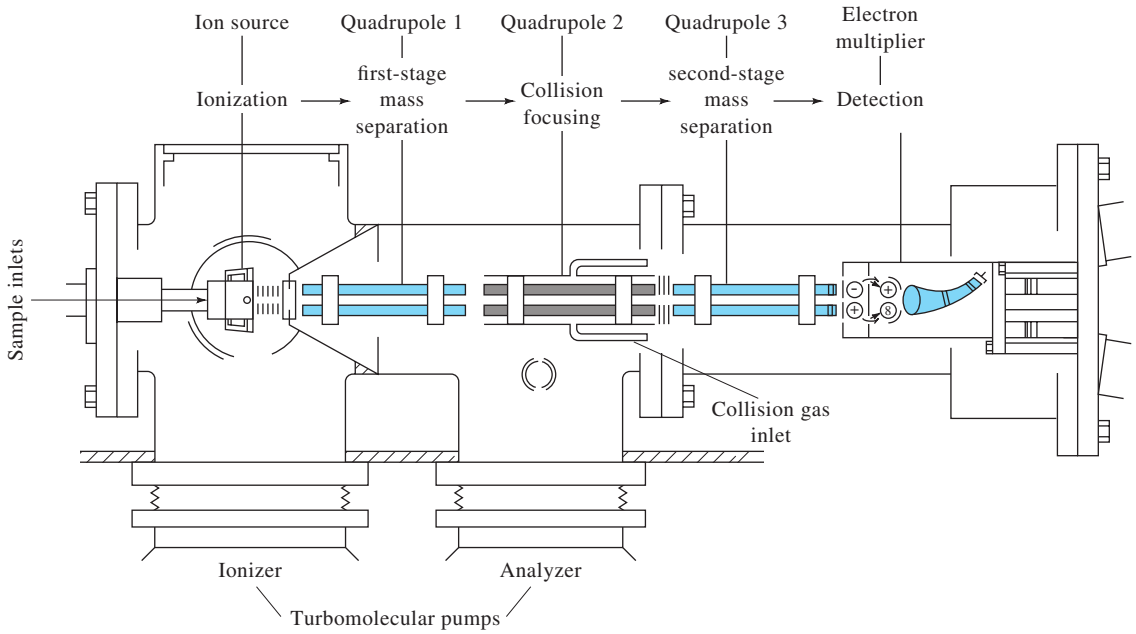

FIGURE 20-25 Schematic of a triple quadrupole mass spectrometer. (Courtesy of Thermo-Finnigan Corp.)

produce an intermediate that rapidly dissociates. In some cases, a background gas is added to aid in the dissociation process.

Photo-induced dissociation (PID) is another process to stimulate decomposition of precursor ions. In most PID methods, an intense laser beam is used in the interaction cell to promote the dissociation. A difficulty with PID is that the ion beam and photon beam must overlap in the interaction region for a time long enough for absorption and bond rupture to occur. In some cases ion-trap cells have been used to allow for long periods of overlap.

Instrumentation for Tandem Mass Spectrometry

Tandem mass spectrometry has been implemented in a number of ways.[21] These can be classified as *tandem in space* and *tandem in time*.[22]

Tandem-in-Space Spectrometers. In tandem-in-space instruments, two independent mass analyzers are used in two different regions in space. The triple quadrupole mass spectrometer is the most common of these instruments. In commercial triple quadrupole instruments, such as the instrument illustrated in Figure 20-25, the sample is introduced into a soft ionization source, such as a CI or FAB source. The ions are then accelerated into quadrupole 1 (Q), which is an ordinary quadrupole mass filter. The selected fast-moving ions pass into quadrupole 2 (q), which is a collision chamber where dissociation of the ions selected by quadrupole 1 occurs. This quadrupole is operated in a radio-frequency-only mode in which no dc voltage is applied across the rods. This mode basically traps the precursor and product ions in a relatively high concentration of collision gas so that CAD can occur. Quadrupole 3 (Q) then allows mass analysis of the product ions formed in the collision cell. The configuration is known as the QqQ configuration. Recently, the triple-quadrupole mass spectrometer has been miniaturized to an overall size of about 9.5 inches in length.[23] Such a small analyzer could be widely used in field applications.

Sector instruments and hybrid quadrupole-sector instruments have also been used in a tandem manner. The first tandem mass spectrometers were sector instruments that combined an electric sector spectrometer with a magnetic sector spectrometer, either in forward geometry (electric sector followed by magnetic sector, or EB) or in reverse geometry (magnetic sector followed by electric sector, or BE). The reverse geometry is sometimes called a *mass-analyzed ion kinetic energy spectrometer* (MIKES). Such instruments were not very efficient, but they allowed the principles of tandem mass spectrometry to be demonstrated.

Hybrid instruments include the BEqQ spectrometer (magnetic sector, B; electric sector, E; RF-only quadrupole, q; quadrupole mass analyzer, Q) and the BTOF (magnetic sector, B; TOF analyzer, TOF) spectrometer. The QqTOF spectrometer is similar to the triple quadrupole (QqQ) instrument except that the final quadrupole mass analyzer is replaced with a TOF analyzer. In another variant, the Qq section can be replaced by a quadrupole ion trap to yield an ion-trap-TOF instrument.

[21]For a description of these implementations, see J. T. Watson and O. D. Sparkman, *Introduction to Mass Spectrometry*, 4th ed., Chichester, UK: Wiley, 2007, pp. 184–196.

[22]See J. W. Hager, *Anal. Bioanal. Chem.*, **2004**, *378*, 845, **DOI**: 10.1007/s00216-003-2287-1; S. A. McLuckey and J. M. Wells, *Chem. Rev.*, **2001**, *101*, 571, **DOI**: 10.1021/cr990087a.

[23]C. Wright et al., *Anal. Chem.*, **2015**, *87*, 3115, **DOI**: 10.1021/acs.analchem.5b00311.

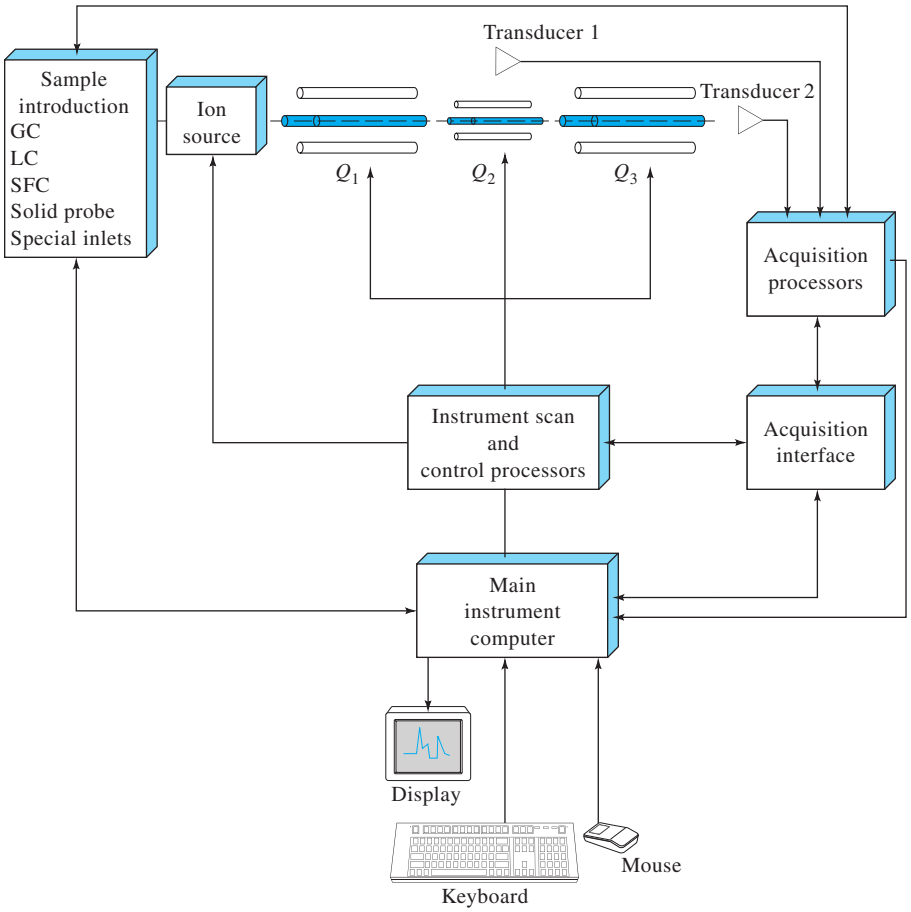

FIGURE 20-26 Instrument control and data processing for a triple quadrupole mass spectrometer. SFC = supercritical fluid chromatography (see Chap. 29).

A final type of tandem-in-space spectrometer is the TOF-TOF spectrometer, in which a TOF instrument followed by a timed ion selector separates the precursor ions. A collision cell then induces fragmentation, and the product ions are mass analyzed in the final TOF stage.[24] Mass resolution of several thousand was reported.

Tandem-in-Time Spectrometers. Tandem-in-time instruments form the ions in a certain spatial region and then at a later time expel the unwanted ions and leave the selected ions to be dissociated and mass analyzed in the same spatial region. This process can be repeated many times over to perform not only MS/MS experiments, but also MS/MS/MS and MS^n experiments. Fourier transform ICR and quadrupole ion-trap instruments are well suited for performing MS^n experiments. In principle, tandem-in-time spectrometers can perform MS/MS experiments much more simply than tandem-in-space instruments because of the difficulty in providing different ion focal positions in the latter. Although tandem-in-time spectrometers can readily provide product-ion scans, other scans, such as precursor ion scans and neutral loss scans, are much more difficult to perform than they are with tandem in space instruments.

20C-6 Computerized Mass Spectrometers

Computers are an integral part of modern mass spectrometers. A characteristic of a mass spectrum is the wealth of structural data that it provides. For example, a molecule with a molecular mass of 500 may be fragmented by an EI source into 100 or more different ions, each of which leads to a discrete spectral peak. For a structural determination, the heights and mass-to-charge ratios of each peak must be determined, stored, and ultimately displayed. Because the amount of information is so large, it is essential that acquisition and processing be rapid; computers are ideally suited for these tasks. Moreover, for mass spectral data to be useful, several instrumental variables must be closely controlled or monitored during data collection. Computers are much more efficient than a human operator in exercising such controls.

Figure 20-26 is a block diagram of the computerized control and data-acquisition system of a triple quadrupole mass spectrometer. This figure shows two features encountered in any modern instrument. The first is a computer that serves as

[24]K. F. Medzihradszky, J. M. Campbell, M. A. Baldwin, A. M. Falick, P. Juhasz, M. L. Vestal, and A. L. Burlingame, *Anal. Chem.*, **2000**, *72*, 552, **DOI**: 10.1021/ac990809y.

the main instrument controller. The operator communicates via a keyboard with the spectrometer by selecting operating parameters and conditions via easy-to-use interactive software. The computer also controls the programs responsible for data manipulations and output. The second feature common to almost all instruments is a set of microprocessors (often as many as six) that are responsible for specific aspects of instrument control and the transmission of information between the computer and spectrometer.

The interface between a mass spectrometer and a computer usually has provisions for digitizing the amplified ion-current signal plus several other signals that are used for control of instrumental variables. Examples of the latter are source temperature, accelerating voltage, scan rate, and magnetic field strength or quadrupole voltages.

The digitized ion-current signal ordinarily requires considerable processing before it is ready for display. First, the peaks must be normalized, a process by which the height of each peak relative to some reference peak is calculated. Most often the *base peak*, which is the largest peak in a spectrum, serves as the reference and is arbitrarily assigned a peak height of 100 (sometimes 1000). The m/z value for each peak must also be determined. This assignment is frequently based on the time of the peak's appearance and the scan rate. Data are acquired as intensity versus time during a carefully controlled scan of the magnetic or electric fields. Conversion from time to m/z requires careful periodic calibration; for this purpose, perfluorotri-*n*-butylamine (PFTBA) or perfluorokerosene is often used as a standard. For high-resolution work, the standard may be admitted with the sample. The computer is programmed to then recognize and use the peaks of the standard as references for mass assignments. For low-resolution instruments, the calibration must generally be obtained separately from the sample, because of the likelihood of peak overlaps.

With most systems the computer stores all spectra and related information on a disk. In routine applications, bar graphs of the normalized spectra can be sent directly to a printer or display. However, in many cases, the user will use data-reduction software to extract specific information prior to producing a printed copy of a spectrum. Figure 20-27 is an example of the printout from a computerized mass spectrometer. The first and next-to-last columns in the table list m/z values in increasing order. The second and last columns contain the corresponding ion currents normalized to the largest peak found at mass 156. The current for this ion is assigned the number of 100, and all other peaks are expressed relative to this one. Thus, the height of the peak at mass 141 is 53% of the base peak.

As is true with infrared and nuclear magnetic resonance spectroscopy, large libraries of mass spectra (>150,000 entries) are available in computer-compatible formats.[25] Most commercial mass spectrometer computer systems have the ability to rapidly search all or part of such files for spectra that match or closely match the spectrum of an analyte.

Sample: Unknown compound
Date: 11/15/2004

m/z	Rel. Int.		*m/z*	Rel. Int.
41	9		141	53
43	14		142	4
55	5		155	6
69	4		156	100
71	5		157	21
98	5		197	5

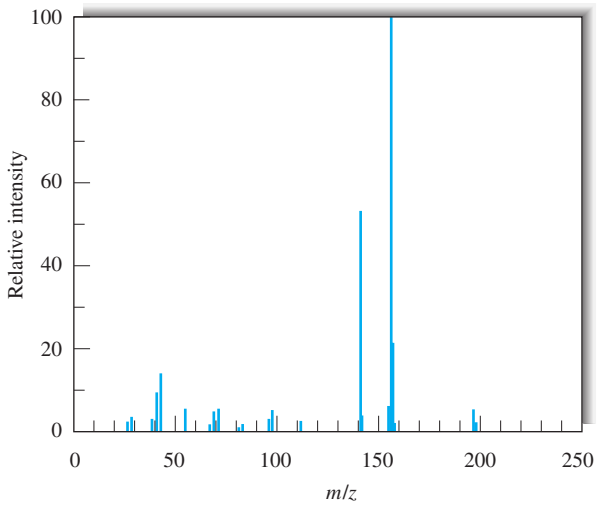

FIGURE 20-27 A computer display of mass-spectral data. The compound was isolated from a blood serum extract by chromatography. The spectrum showed it to be the barbiturate pentobarbital.

20D APPLICATIONS OF MOLECULAR MASS SPECTROMETRY

The applications of molecular mass spectrometry are so numerous and widespread that describing them adequately in a brief space is not possible. Table 20-5 lists several of these applications to provide some idea of the capabilities of mass spectrometry. In this section we describe a few of the most widely used and important of these applications.

20D-1 Identification of Pure Compounds

The mass spectrum of a pure compound provides several kinds of data that are useful for its identification.[26] The first is the molecular mass of the compound, and the second is its molecular formula. In addition, study of fragmentation patterns revealed by the mass spectrum often provides information about

[25]For example, see F. W. McLafferty, *Wiley Registry of Mass Spectral Data*, 10th ed., New York: Wiley, 2012. The registry is available online and combined with the *NIST/EPA/NIH Mass Spectral Library*.

[26]R. M. Silverstein, F. X. Webster, D. Kiemle, and D. L. Bryce, *The Spectrometric Identification of Organic Compounds*, 8th ed., New York: Wiley, 2015; F. W. McLafferty and F. Turecek, *Interpretation of Mass Spectra*, 4th ed., Mill Valley, CA: University Science Books, 1993.

TABLE 20-5 Applications of Molecular Mass Spectrometry

1. Elucidation of the structure of organic and biological molecules
2. Determination of the molecular mass of peptides, proteins, and oligonucleotides
3. Identification of components in thin-layer and paper chromatograms
4. Determination of amino acid sequences in sample of polypeptides and proteins
5. Detection and identification of species separated by chromatography and capillary electrophoresis
6. Identification of drugs of abuse and metabolites of drugs of abuse in blood, urine, and saliva
7. Monitoring gases in patient's breath during surgery
8. Testing for the presence of drugs in blood in racehorses and in Olympic athletes
9. Dating archaeological specimens
10. Analyses of aerosol particles
11. Determination of pesticide residues in food
12. Monitoring volatile organic species in water supplies

the presence or absence of various functional groups. Finally, the actual identity of a compound can often be established by comparing its mass spectrum with those of known compounds until a close match is realized.

Molecular Masses from Mass Spectra

For compounds that can be ionized to give a molecular ion or a protonated or a deprotonated molecular ion by one of the methods described earlier, the mass spectrometer is an unsurpassed tool for the determination of molecular mass. This determination, of course, requires the identification of the molecular ion peak, or in some cases, the $(M + 1)^+$ or the $(M - 1)^+$ peak. The location of the peak on the abscissa then gives the molecular mass with an accuracy that cannot be realized easily by any other method.

To determine a molecular mass by mass spectrometry, the identity of the molecular ion peak must be known. Caution is therefore always advisable, particularly with EI sources, when the molecular ion peak is absent or small relative to impurity peaks. When there is doubt, additional spectra by chemical, field, and desorption ionization are particularly useful.

Molecular Formulas from Exact Molecular Masses

Molecular formulas can be determined from the mass spectrum of a compound, provided the molecular ion peak can be identified and its *exact* mass determined. This application, however, requires a high-resolution instrument capable of detecting mass differences of a few thousandths of a dalton. Consider, for example, the mass-to-charge ratios of the molecular ions of the following compounds: purine, $C_5H_4N_4$ ($\mathcal{M} = 120.044$); benzamidine, $C_7H_8N_2$ ($\mathcal{M} = 120.069$); ethyltoluene, C_9H_{12} ($\mathcal{M} = 120.096$); and acetophenone, C_8H_8O ($\mathcal{M} = 120.058$). If the measured mass of the molecular ion peak is 120.070 (± 0.005), then all but $C_7H_8N_2$ are excluded as possible formulas. Note that the precision in this example is about 40 ppm. Uncertainties on the order of a few parts per million are routinely achievable with high-resolution, double-focusing instruments. Tables that list all reasonable combinations of C, H, N, and O by molecular mass to the third or fourth decimal place have been compiled.[27] A small portion of such a compilation is shown in the fifth column of Table 20-6.

Molecular Formulas from Isotopic Ratios

The data from a low-resolution instrument that can discriminate only between ions differing in mass by whole mass numbers can also yield useful information about the formula of a compound, provided that the molecular ion peak is sufficiently intense that its height and the heights of the $(M + 1)^+$ and $(M + 2)^+$ isotope peaks can be determined accurately. Example 20-5 illustrates this type of analysis.

The use of relative isotope peak heights for the determination of molecular formulas is greatly expedited by the tables referred to in note 21 and by compilations available for computer analysis. In Table 20-6, a listing of all reasonable combinations of C, H, O, and N is given for mass numbers 83 and 84 (the original tables extend to mass number 500). The heights of the $(M + 1)^+$ and $(M + 2)^+$ peaks reported as percentages of the height of the M^+ peak are tabulated. If a reasonably accurate experimental determination of these percentages can be made, a likely formula can be deduced. For example, a molecular ion peak at mass 84 with $(M + 1)^+$ and $(M + 2)^+$ values of 5.6 and 0.3% of M^+ suggests a compound having the formula C_5H_8O.

EXAMPLE 20-5

Calculate the ratios of the $(M + 1)^+$ to M^+ peak heights for the following two compounds: dinitrobenzene, $C_6H_4N_2O_4$ ($\mathcal{M} = 168$), and an olefin, $C_{12}H_{24}$ ($\mathcal{M} = 168$).

Solution

From Table 20-3, we see that for every 100 ^{12}C atoms there are 1.08 ^{13}C atoms. Because nitrobenzene contains six carbon

[27] J. H. Beynon and A. E. Williams, *Mass and Abundance Tables for Use in Mass Spectrometry*, New York: Elsevier, 1963.

TABLE 20-6 Isotopic Abundance Percentages and Molecular Masses for Various Combinations of C, H, O, and N

		Abundance, % M Peak Height		
	Formula	$\mathcal{M}+1$	$\mathcal{M}+2$	Molecular Mass
$\mathcal{M}=83$	C_2HN_3O	3.36	0.24	83.0120
	$C_2H_3N_4$	3.74	0.06	83.0359
	C_3HNO_2	3.72	0.45	83.0007
	$C_3H_3N_2O$	4.09	0.27	83.0246
	$C_3H_5N_3$	4.47	0.08	83.0484
	$C_4H_3O_2$	4.45	0.48	83.0133
	C_4H_5NO	4.82	0.29	83.0371
	$C_4H_7N_2$	5.20	0.11	83.0610
	C_5H_7O	5.55	0.33	83.0497
	C_5H_9N	5.93	0.15	83.0736
	C_6H_{11}	6.66	0.19	83.0861
$\mathcal{M}=84$	CN_4O	2.65	0.23	84.0073
	$C_2N_2O_2$	3.00	0.43	83.9960
	$C_2H_2N_3O$	3.38	0.24	84.0198
	$C_2H_4N_4$	3.75	0.06	84.0437
	C_3O_3	3.36	0.64	83.9847
	$C_3H_2NO_2$	3.73	0.45	84.0085
	$C_3H_4N_2O$	4.11	0.27	84.0324
	$C_3H_6N_3$	4.48	0.08	84.0563
	$C_4H_4O_2$	4.46	0.48	84.0211
	C_4H_6NO	4.84	0.29	84.0449
	$C_4H_8N_2$	5.21	0.11	84.0688
	C_5H_8O	5.57	0.33	84.0575
	$C_5H_{10}N$	5.94	0.15	84.0814
	C_6H_{12}	6.68	0.19	84.0939
	C_7	7.56	0.25	84.0000

Taken from R. M. Silverstein, G. C. Bassler, and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, 4th ed., p. 49, New York: Wiley, 1981.

atoms, we would expect each of $6.48 = 6 \times 1.08$ molecules of nitrobenzene to have one ^{13}C atom for every 100 molecules having none. Thus, from this effect alone the $(M + 1)^+$ peak will be 6.48% of the M^+ peak. The isotopes of the other elements also contribute to this peak; we may tabulate their effects as follows:

$C_6H_4N_2O_4$	
^{13}C	$6 \times 1.08 = 6.48\%$
^{2}H	$4 \times 0.015 = 0.060\%$
^{15}N	$2 \times 0.37 = 0.74\%$
^{17}O	$4 \times 0.04 = 0.16\%$
	$(M + 1)^+/M^+ = 7.44\%$

$C_{12}H_{24}$	
^{13}C	$12 \times 1.08 = 12.96\%$
^{2}H	$24 \times 0.015 = 0.36\%$
	$(M + 1)^+/M^+ = 13.32\%$

Thus, if the heights of the M^+ and $(M + 1)^+$ peaks can be measured, it is possible to discriminate between these two compounds that have identical whole-number molecular masses.

The isotopic ratio is particularly useful for the detection and estimation of the number of sulfur, chlorine, and bromine atoms in a molecule because of the large contribution they make to the $(M + 2)^+$ (see Table 20-3). For example, an $(M + 2)^+$ that is about 65% of the M^+ peak is strong evidence for a molecule

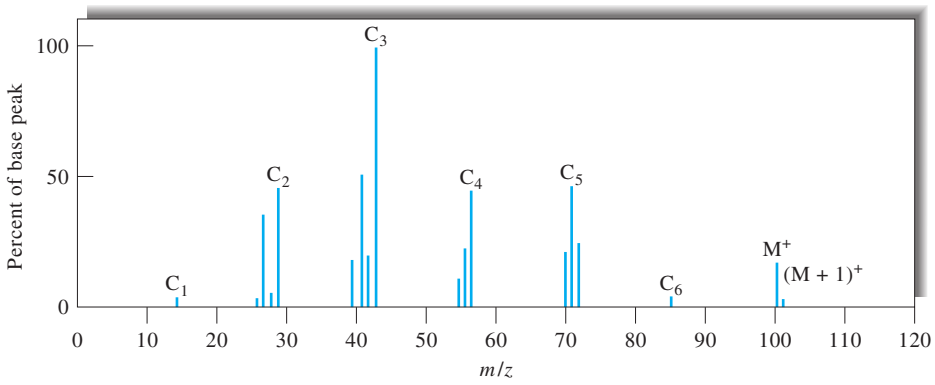

FIGURE 20-28 EI spectrum of *n*-heptanal. The peaks labeled C_6, C_5, . . . , C_1 correspond to the successive losses of a CH_2 group.

containing two chlorine atoms; an $(M + 2)^+$ peak of 4%, on the other hand, suggests the presence of one atom of sulfur.

Structural Information from Fragmentation Patterns

Systematic studies of fragmentation patterns for pure substances have led to rational guidelines to predict fragmentation mechanisms and a series of general rules helpful in interpreting spectra.[28] It is seldom possible (or desirable) to account for all of the peaks in the spectrum. Instead, characteristic patterns of fragmentation are sought. For example, the spectrum in Figure 20-28 is characterized by clusters of peaks differing in mass by 14. Such a pattern is typical of straight-chain paraffins, in which cleavage of adjacent carbon-carbon bonds results in the loss of successive CH_2 groups having this mass. Quite generally, the most stable hydrocarbon fragments contain three or four carbon atoms, and the corresponding peaks are thus the largest.

Alcohols usually have a very weak or nonexistent molecular ion peak but often lose water to give a strong peak at $(M - 18)^+$. Cleavage of the C—C bond next to an oxygen is also common, and primary alcohols always have a strong peak at mass 31 due to the CH_2OH^+. Extensive compilations of generalizations concerning the use of mass spectral data for the identification of organic compounds are available, and the interested reader should consult the references in note 22.

Compound Identification from Comparison Spectra

Generally, after determining the molecular mass of the analyte and studying its isotopic distribution and fragmentation patterns, the experienced mass spectroscopist is able to narrow the possible structures down to a handful. When reference compounds are available, final identification is then based on a comparison of the mass spectrum of the unknown with spectra for authentic samples of the suspected compounds. The procedure is based on the assumptions that (1) mass fragmentation patterns are unique and (2) experimental conditions can be sufficiently controlled to produce reproducible spectra. The first assumption often is not valid for spectra of stereo- and geometric isomers and occasionally is not valid for certain types of closely related compounds. The probability that different compounds will yield the same spectrum becomes markedly smaller as the number of spectral peaks increases. Assumption 2 can also be problematic. EI spectra are fairly reproducible from laboratory to laboratory. However, spectra from other sources can vary significantly. For this reason, electron-ionization is the method of choice for spectral comparison and for building spectral libraries.

Unfortunately, heights of mass spectral peaks strongly depend on such variables as the energy of the electron beam, the location of the sample with respect to the beam, the sample pressure and temperature, and the general geometry of the mass spectrometer. As a result, significant variations in relative abundances are observed for spectra acquired in different laboratories and from different instruments. Nevertheless, it has proven possible in a remarkably large number of cases to identify unknowns from library spectra obtained with a variety of instruments and operating conditions. Generally, however, it is desirable to confirm the identity of a compound by comparing its spectrum to the spectrum of an authentic compound obtained with the same instrument under identical conditions.

Computerized Library Search Systems. Although libraries of mass spectral data are available in text form,[29] most modern mass spectrometers are equipped with highly efficient computerized library search systems. There are two basic types of

Simulation: Learn more about **MS spectral interpretation** at www.tinyurl.com/skoogpia7

[28]For example, see R. M. Silverstein, F. X. Webster, D. Kiemle, and D. L. Bryce, *The Spectrometric Identification of Organic Compounds*, 8th ed., New York: Wiley, 2015.

[29]F. W. McLafferty and D. A. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data*, 7 vols., New York: Wiley, 1989.

mass spectral libraries: large comprehensive ones and small specific ones. The largest commercially available mass spectral library (>300,000 spectra) is marketed by John Wiley and Sons.[30] A unique feature of this compilation is that it is available on CD-ROM and can be searched on a personal computer. Small libraries usually contain a few hundred to a few thousand spectra for application to a limited area, such as pesticide residues, drugs, or forensics. Small libraries are often part of the equipment packages offered by instrument manufacturers, and it is almost always possible for the instrument user to generate a library or to add to an existing library. Mass spectra for some 33,000 compounds are available from the National Institute of Standards and Technology (NIST) on the Internet.[31]

For large numbers of spectra, such as are obtained when a mass spectrometer is coupled with a chromatograph for identifying components of a mixture, the instrument's computer system can be used to perform a library search on all, or any subset, of the mass spectra associated with a particular sample. The results are reported to the user, and if desired, the reference spectra can be displayed on a monitor or printed for visual comparison.

20D-2 Analysis of Mixtures by Hyphenated Mass Spectral Methods

Although ordinary mass spectrometry is a powerful tool for the identification of pure compounds, its usefulness for analysis of all but the simplest mixtures is limited because of the immense number of fragments of differing m/z values produced. It is often impossible to interpret the resulting complex spectrum. For this reason, chemists have developed methods in which mass spectrometers are coupled with various efficient separation devices in so-called *hyphenated methods*.

Chromatography–Mass Spectrometry

Gas chromatography–mass spectrometry (GC/MS) has become one of the most powerful tools available for the analysis of complex organic and biochemical mixtures. In this application, spectra are collected for compounds as they exit from a chromatographic column. These spectra are then stored in a computer for subsequent processing. Mass spectrometry has also been coupled with liquid chromatography (LC/MS) for the analysis of samples that contain nonvolatile constituents. A major problem that had to be overcome in the development of both of these hyphenated methods is that the sample in the chromatographic column is highly diluted by the gas or liquid carrying it through the column. Thus, methods had to be developed for removing the diluent before introducing the sample into the mass spectrometer. Instruments and applications of GC/MS and LC/MS are described in Sections 27B-4 and 28C-6, respectively.

[30]See reference 21.

[31]http://webbook.nist.gov/.

Capillary Electrophoresis–Mass Spectrometry

The first report on coupling capillary electrophoresis with mass spectrometry was published in 1987.[32] Since then, this hyphenated method has become a powerful and important tool in the analysis of large biopolymers, such as proteins, polypeptides, and DNA species. In most of the applications reported to date, the capillary effluent is passed directly into an electrospray ionization device, and the products then enter a quadrupole mass filter for analysis. Continuous flow FAB has also been used for ionization in some applications. Capillary electrophoresis–mass spectrometry is discussed in more detail in Section 30B-4.

Applications of Tandem Mass Spectrometry

Dramatic progress in the analysis of complex organic and biological mixtures began when the mass spectrometer was first combined with gas chromatography and subsequently with liquid chromatography. Tandem mass spectrometry offers some of the same advantages as GC/MS and LC/MS but is significantly faster. Separations on a chromatographic column are achieved in a time scale of a few minutes to hours, but equally satisfactory separations in tandem mass spectrometers are complete in milliseconds. In addition, the chromatographic techniques require dilution of the sample with large excesses of a mobile phase and subsequent removal of the mobile phase, which greatly enhances the probability of introduction of interferences. Consequently, tandem mass spectrometry is potentially more sensitive than either of the hyphenated chromatographic techniques because the chemical noise associated with its use is generally smaller. A current disadvantage of tandem mass spectrometry with respect to the two chromatographic procedures is the greater cost of the required equipment; this gap appears to be narrowing as tandem mass spectrometers gain wider use.

For some complex mixtures the combination of GC or LC and MS does not provide enough resolution. In recent years, it has become feasible to couple chromatographic methods with tandem mass spectrometers to form GC/MS/MS and LC/MS/MS systems. There have also been reports of LC/MS^n instruments.[33]

To date, tandem mass spectrometry has been applied to the qualitative and quantitative determination of the components of a wide variety of complex materials encountered in nature and industry. Some examples include the identification and determination of drug metabolites, insect pheromones, alkaloids in plants, trace contaminants in air, polymer sequences, petrochemicals, polychlorinated biphenyls, prostaglandins, diesel exhausts, and odors in air. One of the most promising areas of

[32]J. A. Olivares, N. T. Nguyen, N. T. Yonker, and R. D. Smith, *Anal. Chem.*, **1987**, *59*, 1230, **DOI**: 10.1021/ac00135a034. See also, D. C. Simpson and R. D. Smith, *Electrophoresis*, **2005**, *26*, 1291, **DOI**: 10.1002/elps.200410132.

[33]See, for example, J. C. A. Wuilloud, S. R. Gratz, B. M. Gamble, and K. A. Wolnik, *Analyst*, **2004**, *129*, 150, **DOI**: 10.1039/b311030c; E. W. Taylor, W. Jia, M. Bush, and G. D. Dollinger, *Anal. Chem.*, **2002**, *74*, 3232, **DOI**: 10.1021/ac020035c; L. Howells and M. J. Sauer, *Analyst*, **2001**, *126*, 155, **DOI**: 10.1039/b008305o.

applications is that of *proteomics*, the study of proteins produced by a cell or by a species.[34]

20E QUANTITATIVE APPLICATIONS OF MASS SPECTROMETRY

Applications of mass spectrometry for quantitative analyses fall into two categories. The first involves the quantitative determination of molecular species or types of molecular species in organic, biological, and occasionally inorganic samples. The second involves the determination of the concentration of elements in inorganic and, less commonly, organic and biological samples. In the first type of analysis, all of the ionization sources listed in Table 20-1 are used. Mass spectroscopic elemental analyses, which are discussed in detail in Chapter 11, are currently based largely on inductively coupled plasma sources, although glow discharge, radio-frequency spark, laser, thermal, and secondary ion sources have also found use.

20E-1 Quantitative Determination of Molecular Species

Mass spectrometry has been widely applied to the quantitative determination of one or more components of complex organic (and sometimes inorganic) systems such as those encountered in the petroleum and pharmaceutical industries and in studies of environmental problems. Currently, such analyses are usually performed by passing the sample through a chromatographic or capillary electrophoretic column and into the spectrometer. With the spectrometer set at a suitable m/z value, the ion current is then recorded as a function of time. This technique is termed *selected ion monitoring*. In some instances, currents at three or four m/z values are monitored in a cyclic manner by rapid switching from one peak to another. The plot of the data consists of a series of peaks, with each appearing at a time that is characteristic of one of the several components of the sample that yields ions of the chosen value or values for m/z. Generally, the areas under the peaks are directly proportional to the component concentrations and are used for determinations. In this type of procedure, the mass spectrometer simply serves as a sophisticated selective detector for quantitative *chromatographic* or *electrophoretic analyses*. Further details on quantitative gas and liquid chromatography are given in Sections 27B-4 and 28C-6. The use of a mass spectrometer as a detector in capillary electrophoresis is described in Section 30B-4.

In the second type of quantitative mass spectrometry for molecular species, analyte concentrations are obtained directly from the heights of the mass spectral peaks. For simple mixtures, it is sometimes possible to find peaks at unique m/z values for each component. Under these circumstances, calibration curves of peak heights versus concentration can be prepared and used for analysis of unknowns. More accurate results can ordinarily be realized, however, by incorporating a fixed amount of an internal standard substance in both samples and calibration standards. The ratio of the peak intensity of the analyte species to that of the internal standard is then plotted as a function of analyte concentration. The internal standard tends to reduce uncertainties arising in sample preparation and introduction. These uncertainties are often a major source of indeterminate error with the small samples needed for mass spectrometry. Internal standards are also used in GC/MS and LC/MS. For these techniques, the ratio of peak areas serves as the analytical variable.

A convenient type of internal standard is a stable, isotopically labeled analog of the analyte. Usually, labeling involves preparation of samples of the analyte in which one or more atoms of deuterium, carbon-13, or nitrogen-15 have been incorporated. It is then assumed that during the analysis the labeled molecules behave in the same way as do the unlabeled ones. The mass spectrometer easily distinguishes between the two. Another type of internal standard is a homolog of the analyte that yields a reasonably intense ion peak for a fragment that is chemically similar to the analyte fragment being measured.

With low-resolution instruments, it is seldom possible to locate peaks that are unique to each component of a mixture. In this situation, it is still possible to complete an analysis by collecting intensity data at a number of m/z values that equal or exceed the number of sample components. Simultaneous equations are then developed that relate the intensity of each m/z value to the contribution made by each component to this intensity. Solving these equations then provides the desired quantitative information. Alternatively, chemometric methods such as partial least squares or principal component analysis are used.

Precision and Accuracy

The precision of quantitative mass spectral measurements by the procedure just described usually ranges between 2% and 10% relative. The analytical accuracy varies considerably depending on the complexity of the mixture being analyzed and the nature of its components. For gaseous hydrocarbon mixtures containing five to ten components, absolute errors of 0.2 to 0.8 mole percent are typical.

Applications

The early quantitative applications of mass spectrometry tended to focus on petroleum products and on industrial materials characterization. In recent years, quantitative mass spectrometry has been applied to many diverse areas, including industrial polymers, environmental and forensic samples, and increasingly to biological materials.

Tutorial: Learn more about **quantitative applications of MS** at www.tinyurl.com/skoogpia7

[34] I. Ntai et al., *Anal. Chem.*, **2014**, *86*, 4961, **DOI**: 10.1021/ac500395k; P. Mao and D. Wang, *J. Proteom Res.*, **2014**, *13*, 1560, **DOI**: 10.1021/pr401074t; N. L. Kelleher, *Biochem.*, **2013**, *52*, 3794, **DOI**: 10.1021/bi400466p; J. P. Whitelegge, *Anal. Chem.*, **2013**, *85*, 2258, **DOI**: 10.1021/ac303064a; See also N. L. Kelleher, *Anal. Chem.*, **2004**, *76*, 196A, **DOI**: 10.1021/ac0415657.

Mass spectrometry is widely used for the characterization of high-molecular-mass polymeric materials. The method of choice for many of these applications is MALDI mass spectrometry.[35] In recent applications, MALDI-MS has been used in imaging applications to identify and determine the location and distribution of polymers on membrane substrates.[36] MALDI-TOF imaging mass spectrometry has been recently used as a detector for polymer LC. The individual retention behavior of single structural units of polyethylene and polypropylene oxide copolymers could be monitored.[37]

Mass spectrometry in conjunction with liquid chromatography has become very useful in environmental analysis.[38] The interface of choice between LC and MS is electrospray ionization. HPLC with tandem mass spectrometry has also become quite useful in the environmental area, being particularly important in the determination of pharmaceuticals in surface and wastewaters. Ultrahigh resolution FT-ICR mass spectrometry has been shown to be very useful in identifying compositional changes that occur in the weathering of crude oil components released during oil spills.[39] This technique can overcome some of the volatility limitations of gas chromatography that has been traditionally used in oil spill characterizations.

In forensic science, mass spectrometry and GC/MS are widely used in identifying and characterizing evidence including explosive materials of interest in homeland security. Mass spectrometry plays a key role in drug testing athletes and horses and in analyzing body fluids and hair in forensic investigations. In addition, paints and fibers are often subjected to mass spectral analysis.[40] Mass spectrometers have become indispensable tools in the forensic laboratory.

In the clinical laboratory, GC/MS, LC/MS, and tandem mass spectrometry are finding increasing applications. In one study, six urinary pteridines and creatinine were determined by HPLC coupled with tandem mass spectrometry in order to detect breast cancer in its early stages.[41] Tandem MS methods are now the standard for screening newborn babies for metabolic disease.[42]

Many other biological applications of mass spectrometry have appeared. Mass spectrometry has been important for many years in protein identification, particularly in the analysis of peptides derived from digestions with proteolytic enzymes such as trypsin. Recently, MS methods have been used to study intrinsically disordered proteins, that is polypeptide chains without a well-defined three-dimensional structure.[43] Electrospray ionization and MALDI are particularly useful. MALDI methods have found important applications in the imaging of lipids in biological materials.[44] Mass spectrometry is now playing a major role in the expanding field of proteomics as discussed in section 20D-2. It has also become of major importance in plant science to investigate the many functions and mechanism of plant biochemistry.[45]

[35]See *MALDI-MS: A Practical Guide to Instrumentation, Methods and Applications*, 2nd ed., F. Hillenkamp and J. Peter-Katalinic, eds., Weinheim: Wiley-Blackwell, 2013.

[36]K. Krueger et al. *Anal. Chem*., **2013**, *85*, 4998, **DOI**: 10.1021/ac4002063.

[37]S. M. Weidner and J. Falkenhagen, *Anal. Chem*., **2011**, *83*, 9153, **DOI**: 10.1021/ac202380n.

[38]D. Barcelo and M. Petrovic, *TRAC: Trends Anal. Chem*., **2007**, *26*, 2, **DOI**: 10.1016/j.trac.2006.11.006.

[39]A. M. McKenna et al., *Environ. Sci. Technol*., **2013**, *47*, 7530, **DOI**: 10.1021/es305284t.

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[42]K. C. Dooley, *Clin. Biochem*., **2003**, *36*, 471, **DOI**: 10.1016/S0009-9120(03)00105-X.

[43]R. Beveridge, Q. Chappuis, C. Macphee, and P. Barran, *Analyst*, **2013**, *138*, 32, **DOI**: 10.1039/c2an35665a.

[44]D. Gode and D. A. Volmer, *Analyst*, **2013**, *138*, 1289, **DOI**: 10.1039/c2an36337b.

[45]E. Gemperline, C. Keller, and L. Li, *Anal. Chem*., **2016**, *88*, 3422, **DOI**: 10.1021/acs.analchem.5b02938.

» QUESTIONS AND PROBLEMS

*Answers are provided at the end of the book for problems marked with an asterisk.

Problems with this icon are best solved using spreadsheets.

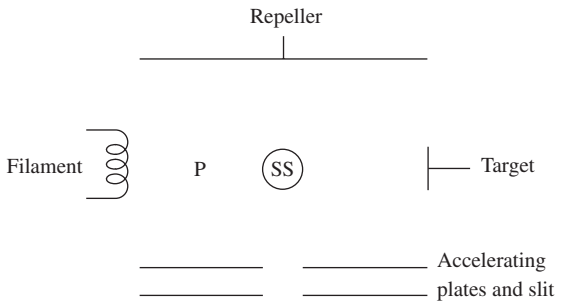
20-1 How do gaseous and desorption sources differ? What are the advantages of each?

20-2 How do the spectra for EI, field ionization, and CI sources differ from one another?

20-3 Describe the difference between gaseous field ionization sources and field desorption sources.

QUESTIONS AND PROBLEMS (continued)

20-4 The following figure is a simplified diagram of a commercially available EI source.



(a) What voltage must be applied between the filament and target so that electrons interacting with molecules at the point marked SS (sample source) will have 70 eV of kinetic energy?

(b) What will happen to a molecule that diffuses toward the filament and is ionized at point *P*?

* **20-5** When a magnetic sector instrument was operated with an accelerating voltage of 3.00×10^3 V, a field of 0.126 T was required to focus the CH_4^+ on the detector.

(a) What range of field strengths would be required to scan the mass range between 16 and 300, for singly charged ions, if the accelerating voltage is held constant?

(b) What range of accelerating voltages would be required to scan the mass range between 16 and 300, for singly charged ions, if the field strength is held constant?

* **20-6** Calculate the accelerating voltage that would be required to direct singly charged ions of mass 5000 through an instrument that is identical to the one described in Example 20-4.

* **20-7** The ion-accelerating voltage in a particular quadrupole mass spectrometer is 10.00 V. How long will it take a singly charged cyclohexane ion to travel the 15.0 cm length of the rod assembly? Assume that the initial velocity of the ion in the *z* direction is zero.

20-8 On page 259 a qualitative discussion described how a positive ion would behave in the *xz* plane (positive dc potential plane) of a quadrupole mass filter. Construct a similar argument for the behavior of positive ions in the *yz* plane (negative dc potential plane).

20-9 Why do double-focusing mass spectrometers give narrower peaks and higher resolutions than single-focusing instruments?

20-10 Discuss the differences between quadrupole ion-trap mass spectrometers and Fourier transform ICR mass spectrometers.

* **20-11** Calculate the resolution required to resolve peaks for

(a) CH_2N ($\mathcal{M} = 28.0187$) and N_2^+ ($\mathcal{M} = 28.0061$).

(b) $C_2H_4^+$ ($\mathcal{M} = 28.0313$) and CO^+ ($\mathcal{M} = 27.9949$).

(c) $C_3H_7N_3^+$ ($\mathcal{M} = 85.0641$) and $C_5H_9O^+$ ($\mathcal{M} = 85.0653$).

(d) androst-4-en-3,17,-dione (M^+) at $m/z = 286.1930$ and an impurity at 286.1240.

20-12 What mass differences can just be resolved at *m* values of 100, 1000, 2000, 3000, and 5000 if the mass spectrometer has a resolution of

(a) 500, **(b)** 1000, **(c)** 3000, **(d)** 5000?

* **20-13** Calculate the ratio of the $(M + 2)^+$ to M^+ and the $(M + 4)^+$ to M^+ peak heights for

(a) $C_{10}H_6Br_2$, **(b)** C_3H_7ClBr, and **(c)** $C_6H_4Cl_2$.

20-14 In a magnetic sector (single-focusing) mass spectrometer, it might be reasonable under some circumstances to monitor one m/z value, to then monitor a second m/z, and to repeat this pattern in a cyclic manner. Rapidly switching between two accelerating voltages while keeping all other conditions constant is called *peak matching*.

(a) Derive a general expression that relates the ratio of the accelerating voltages to the ratio of the corresponding m/z values.

(b) Use this equation to calculate m/z of an unknown peak if m/z of the ion used as a standard, CF_3^+, is 69.00 and the ratio of $V_{unknown}/V_{standard}$ is 0.965035.

(c) Based on your answer in part (b), and the assumption that the unknown is an organic compound that has a mass of 143, draw some conclusions about your answer in part (b), and about the compound.

* **20-15** Measuring the approximate mass of an ion without using a standard can be accomplished via the following variant of the peak-matching technique described in Problem 20-14. The peak-matching technique is used to alternately cause the P^+ ion and the $(P + 1)^+$ ions to reach the detector. It is assumed that the difference in mass between P^+ and $(P + 1)^+$ is due to a single ^{13}C replacing a ^{12}C atom.

(a) If the accelerating voltage for $(P + 1)^+$ is labeled V_2 and that for P^+ is V_1, derive a relationship that relates the ratio V_2/V_1 to the mass of P^+.

(b) If $V_2/V_1 = 0.987753$, calculate the mass of the P^+ ion.

20-16 Discuss the major differences between a tandem-in-space mass spectrometer and a tandem-in-time mass spectrometer. Include the advantages and disadvantages of each type.

* **20-17** Identify the ions responsible for the peaks in the mass spectrum shown in Figure 20-22b.

20-18 Identify the ions responsible for the four peaks having greater mass-to-charge ratios than the M^+ peak in Figure 20-4a.

Challenge Problem

20-19 Figure 20-29 (page 536) shows the mass spectrum of the same compound from an EI source and a CI source.

(a) Which mass spectrum would be best for determining the molecular mass of the compound? Why?

(b) Which mass spectrum would be best for determining the chemical structure? Why?

(c) The EI source was a pulsed source used with a TOF mass analyzer. If the flight tube were 1.0 m long and the accelerating voltage were 3000 V, what would the flight time be for the ion at $m/z = 58$?

(d) For two ions of m/z values m_1/z and m_2/z, derive an equation for the difference in flight times Δt_F as a function of the two masses, the charges, and the accelerating voltage.

(e) For the same TOF analyzer as in part (c), calculate the difference in flight times between ions of $m/z = 59$ and $m/z = 58$.

(f) To get more structural information, the compound of Figure 20-27 was subjected to tandem mass spectrometry. Which ionization source, EI or CI, would be most suitable for this purpose? Why?

QUESTIONS AND PROBLEMS (continued)

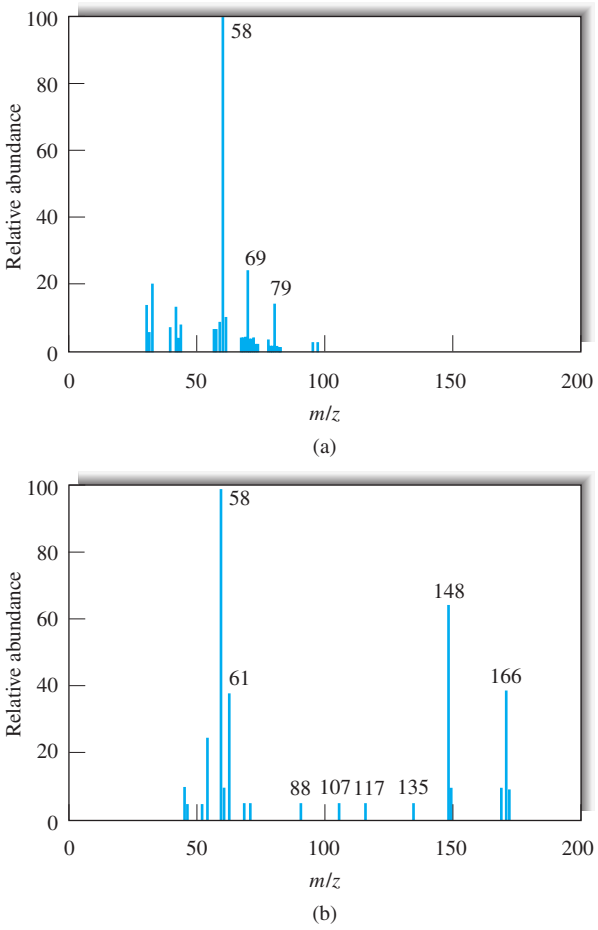


FIGURE 20-29 EI spectrum (a) and CI spectrum (b) of the same biologically important compound. (From H. M. Fales, H. A. Lloyd, and G. A. W. Milne, *J. Amer. Chem. Soc.*, **1970**, *92*, 1590–1597, **DOI:** 10.1021/ja00709a028. American Chemical Society.)

(g) Using the ionization source chosen in part (f), describe the types of mass spectra that could be obtained from an MS/MS experiment by:

(1) holding the first mass analyzer constant and scanning the second analyzer.

(2) scanning both analyzers with a small *m*/*z* offset between them.

(3) scanning the first analyzer while holding the second analyzer constant.

(4) scanning the second mass analyzer for every mass selected by the first analyzer.

In your answer, use features of the mass spectrum of Figure 20-29 to illustrate your description.