MASS SPECTROMETRY

Part One: Basic Theory, Instrumentation and Sampling Techniques

The principles that underlie mass spectrometry pre-date all of the other instrumental techniques described in this book. The fundamental principles date to the late 1890s when J. J. Thomson determined the mass-to-charge ratio of the electron, and Wien studied magnetic deflection of anode rays and determined the rays were positively charged. Each man was honored with the Nobel Prize (Thomson in 1906 and Wien in 1911) for their efforts.

In 1912–1913, J. J. Thomson studied the mass spectra of atmospheric gases and used a mass spectrum to demonstrate the existence of neon-22 in a sample of neon-20, thereby establishing that elements could have isotopes. The earliest mass spectrometer, as we know it today, was built by A. J. Dempster in 1918. However, the method of mass spectrometry did not come into common use until about 50 years ago, when inexpensive and reliable instruments became available.

Continued development of sample introduction and ionization techniques for high molecular weight (MW) compounds and biological samples in the 1980s and 1990s introduced mass spectrometry to a new community of researchers. The introduction of easily maintained lower-cost commercial instruments has made mass spectrometry an indispensable technique in numerous fields far removed from the laboratories of Thomson and Wien.

Today, the biotechnology industry uses mass spectrometry to assay and sequence proteins, oligonucleotides, and polysaccharides. The pharmaceutical industry uses mass spectrometry in all phases of the drug development process, from lead compound discovery and structural analysis, to synthetic development and combinatorial chemistry, and to pharmacokinetics and drug metabolism.

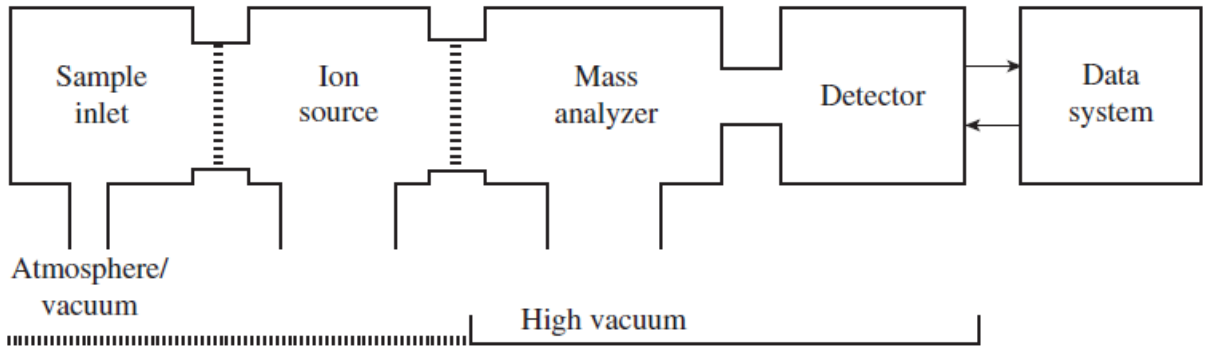
In health clinics around the world, mass spectrometry is used in testing blood and urine for everything from the presence and levels of certain compounds that are "markers" for disease states, including many cancers, to detecting the presence and quantitative analysis of illicit or performance-enhancing drugs.

Environmental scientists rely on mass spectrometry to monitor water and air quality, and geologists use mass spectrometry to test the quality of petroleum reserves. Mass spectrometry is also used routinely in airport security screening and forensic investigations to detect traces of explosives.

To date, no fewer than five Nobel Prizes have been awarded for work directly related to mass spectrometry: J. J. Thomson (Physics, 1906) for "theoretical and experimental investigations on the conduction of electricity by gases"; F. W. Aston (Chemistry, 1922) for "discovery, by means of a mass spectrograph, of isotopes, in a large number of nonradioactive elements"; W. Paul (Physics, 1989) "for the development of the ion trap technique"; and most recently J. B. Fenn and K. Tanaka (Chemistry, 2002) "for the development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules."

3.1 THE MASS SPECTROMETER: OVERVIEW

In its simplest form, the mass spectrometer has five components



The components of a mass spectrometer. (Adapted from Gross, J. H., *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004. Reprinted by permission.)

Before the ions can be formed, a stream of molecules must be introduced into the **ion source** (ionization chamber) where the ionization takes place. A **sample inlet** system provides this stream of molecules.

In the last decade, a number of open air sample introduction methods have been developed that essentially eliminate sample preparation. In various atmospheric pressure chemical ionization (APCI) techniques, the sample is placed in a stream of ionized gas (Section 3.3B) or solvent aerosol (Section 3.3D) between the ion source and the inlet to the mass analyzer.

The most versatile sample inlet systems are constructed by connecting a chromatograph to the mass spectrometer. This sample introduction technique allows a complex mixture of components to be separated by the chromatograph, and the mass spectrum of each component may then be determined individually. A drawback of this method involves the need for rapid scanning by the mass spectrometer. The instrument must determine the mass spectrum of each component in the mixture *before* the next component exits from the chromatography column so that the first substance is not contaminated by the next before its spectrum has been obtained.

In **gas chromatography–mass spectrometry (GC-MS)**, the gas stream emerging from a gas chromatograph is admitted through a valve into a tube, where it passes over a molecular leak. Some of the gas stream is thus admitted into the ionization chamber of the mass spectrometer. In this way, it is possible to obtain the mass spectrum of every component in a mixture injected into the gas chromatograph. In effect, the mass spectrometer acts in the role of detector. Similarly, **high-performance liquid chromatography–mass spectrometry (HPLC-MS,** or more simply **LC-MS)** couples an HPLC instrument to a mass spectrometer through a special interface. The substances that elute from the HPLC column are detected by the mass spectrometer, and their mass spectra can be displayed, analyzed, and compared with standard spectra found in the computer library built into the instrument.

3.3 IONIZATION METHODS

A. Electron Ionization (EI)

verting the sample to ions is **electron ionization (EI)**. In EI-MS, a beam of high-energy electrons is emitted from a **filament** that is heated to several thousand degrees Celsius. These high-energy electrons strike the stream of molecules that has been admitted from the sample inlet system. The electron–molecule collision strips an electron from the molecule, creating a cation. A **repeller plate,** which carries a positive electrical potential, directs the newly created ions toward a series of **accelerating plates.** A large potential difference, ranging from 1 to 10 kilovolts (kV), applied across these accelerating plates produces a beam of rapidly traveling positive ions. One or more **focusing slits** direct the ions into a uniform beam (Fig. 3.2).

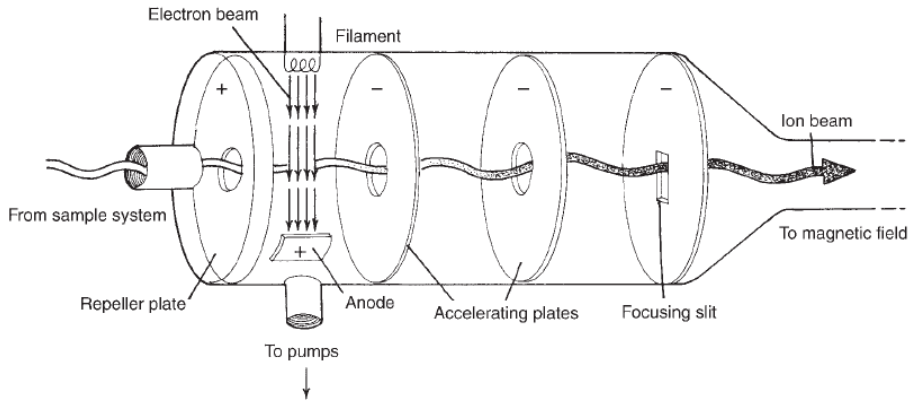


FIGURE 3.2 Electron ionization chamber.

Most of the sample molecules are not ionized at all but are continuously drawn off by vacuum pumps that are connected to the ionization chamber. Some of the molecules are converted to negative ions through the absorption of electrons. The repeller plate absorbs these negative ions. It is possible to reverse the polarity of the repeller and accelerating plates in some instruments, thereby allowing for mass analysis of negative ions (anions) that are created by electron capture when the sample molecules are hit by the electron beam. A small proportion of the positive ions that are formed may have a charge greater than one (a loss of more than one electron). These are accelerated in the same way as the singly charged positive ions.

The energy required to remove an electron from an atom or molecule is its **ionization potential** or **ionization energy.** Most organic compounds have ionization potentials ranging between 8 and 15 electron volts (eV). However, a beam of electrons does not create ions with high efficiency until it strikes the stream of molecules with a potential of 50 to 70 eV. To acquire reproducible spectral features, including fragmentation patterns, that can be readily compared with electronic databases, a standard 70-eV electron beam is used.

EI-MS has distinct advantages for routine mass spectrometry of small organic molecules. Electron ionization hardware is inexpensive and robust. The excess kinetic energy imparted to the sample during the EI process leads to significant fragmentation of the molecular ion (Chapter 4).

The fragmentation pattern of a compound is reproducible, and many libraries of EI-MS data are available. This allows one to compare the mass spectrum of a sample compound against thousands of data sets in a spectral library in a few seconds using a PC, thus simplifying the process of determining or confirming a compound's identity.

The fragmentation of the molecular ion under EI conditions may also be considered a distinct disadvantage. Some compounds fragment so easily that the lifetime of the molecular ion is too short to be detected by the mass analyzer. Thus, one cannot determine a compound's molecular mass (Section 3.6) in such cases. Another drawback to EI-MS is that the sample must be relatively volatile so it can come into contact with the electron beam in the ionization chamber. This fact coupled with the fragmentation problem make it difficult to analyze high molecular weight (MW) compounds and most biomolecules using EI-MS.

B. Chemical Ionization (CI)

In **chemical ionization–mass spectrometry** (**CI-MS**), the sample molecules are combined with a stream of ionized reagent gas that is present in great excess relative to the sample. When the sample molecules collide with the preionized reagent gas, some of the sample molecules are ionized by various mechanisms, including proton transfer, electron transfer, and adduct formation. Almost any readily available gas or highly volatile liquid can be used as a reagent gas for CI-MS.

Common ionizing reagents for CI-MS include methane, ammonia, isobutane, and methanol. When methane is used as the CI reagent gas, the predominant ionization event is proton transfer from a CH_5^+ ion to the sample. Minor ions are formed by adduct formation between $C_2H_5^+$ and higher homologues with the sample. The methane is converted to ions as shown in Equations 3.l–3.4.

$$CH_4 + e^- \rightarrow CH_4^{\bullet+} + 2e^- \quad \textbf{Equation 3.1}$$

$$CH_4^{\bullet+} + CH_4 \rightarrow CH_5^+ + {\cdot}CH_3 \quad \textbf{Equation 3.2}$$

$$CH_4^{\bullet+} \rightarrow CH_3^+ + H{\cdot} \quad \textbf{Equation 3.3}$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \quad \textbf{Equation 3.4}$$

The sample molecule M is then ionized through the ion–molecule reactions in Equations 3.5 and 3.6:

$$M + CH_5^+ \rightarrow (M+H)^+ + CH_4 \quad \textbf{Equation 3.5}$$

$$M + C_2H_5^+ \rightarrow (M + C_2H_5)^+ \quad \textbf{Equation 3.6}$$

The situation is very similar for CI with ammonia as reagent gas (Equations 3.7–3.9):

$$NH_3 + e^- \rightarrow NH_3^{\bullet+} + 2e^- \quad \textbf{Equation 3.7}$$

$$NH_3^{\bullet+} + NH_3 \rightarrow NH_4^+ + {\cdot}NH_2 \quad \textbf{Equation 3.8}$$

$$M + NH_4^+ \rightarrow (M+H)^+ + NH_3 \quad \textbf{Equation 3.9}$$

Using isobutane as reagent gas produces *tert*-butyl cations (Equations 3.10 and 3.11), which readily protonate basic sites on the sample molecule (Equation 3.12). Adduct formation is also possible using isobutane in CI-MS (Equation 3.l3).

$$(CH_3)_3CH + e^- \rightarrow (CH_3)_3CH^{\bullet+} + 2e^- \quad \textbf{Equation 3.10}$$

$$(CH_3)_3CH^{\bullet+} \rightarrow (CH_3)_3C^+ + H{\cdot} \quad \textbf{Equation 3.11}$$

$$M + (CH_3)_3C^+ \rightarrow (M+H)^+ + (CH_3)_2C{=}CH_2 \quad \textbf{Equation 3.12}$$

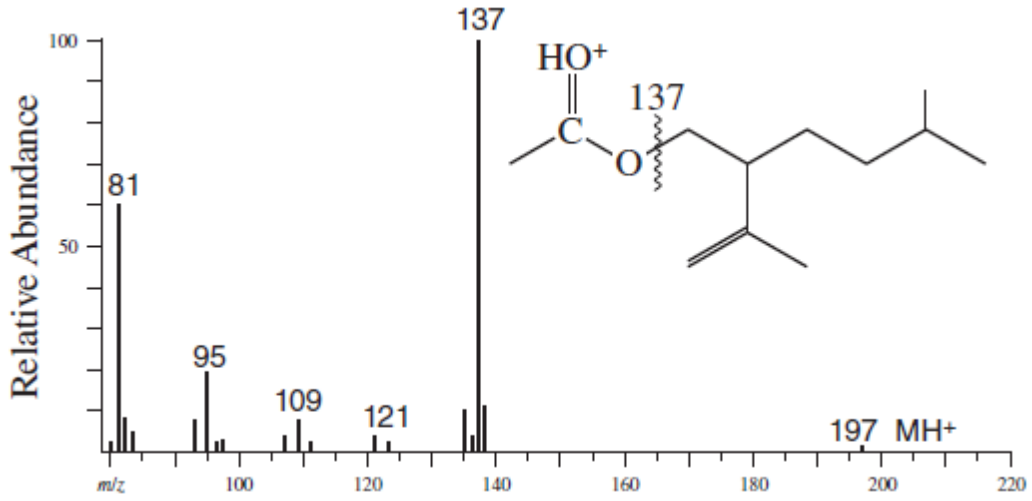
$$M + (CH_3)_3C^+ \rightarrow [M + C(CH_3)_3]^+ \quad \textbf{Equation 3.13}$$

Varying the reagent gas in CI-MS allows one to vary the selectivity of the ionization and degree of ion fragmentation. The choice of reagent gas should be made carefully to best match the **proton affinity** of the reagent gas with that of the sample to ensure efficient ionization of the sample without excessive fragmentation. The greater the difference between the proton affinity of the sample and that of the reagent gas, the more energy that is transferred to the sample during ionization. The excess energy produces an analyte ion in a highly excited vibrational state. If enough excess kinetic energy is transferred, the sample ion will fragment through the cleavage of covalent bonds.

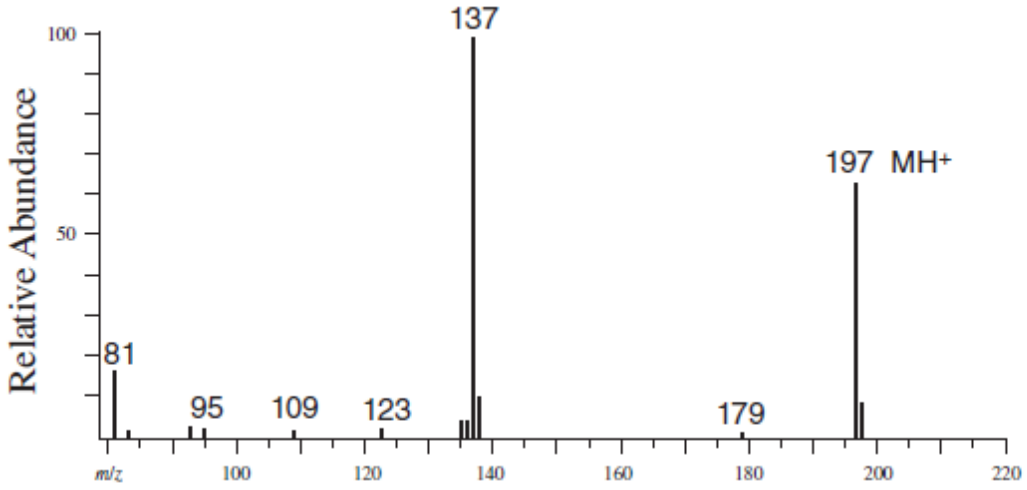
A summary of common CI reagent gases and their ions/properties is presented in Table 3.1.

TABLE 3.1
SUMMARY OF CHEMICAL IONIZATION (CI) REAGENT GASES

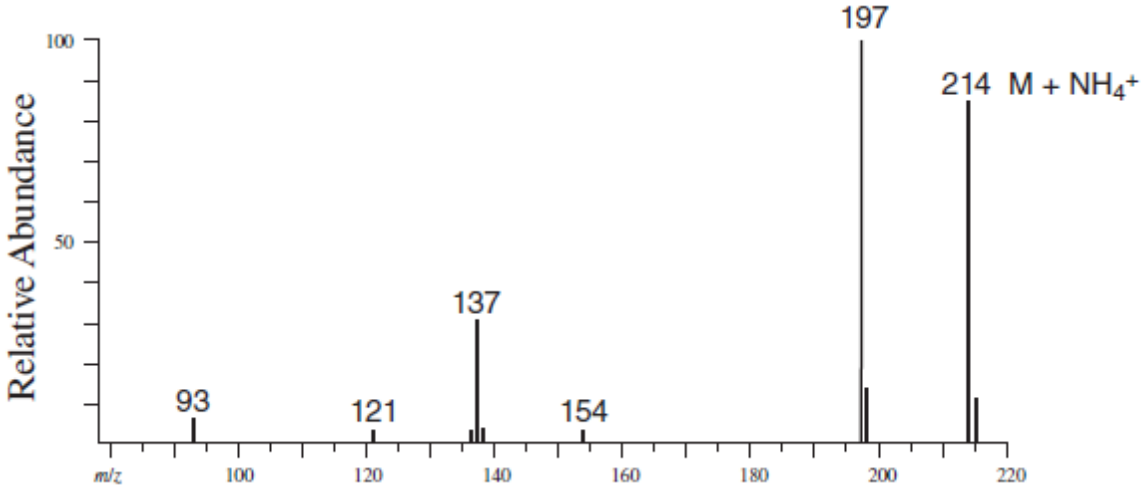
Reagent Gas	Proton Affinity (kcal/mole)	Reagent Ion(s)	Analyte Ion(s)	Comments
H_2	101	H_3^+	$(M + H)^+$	Produces significant fragmentation
CH_4	132	CH_5^+, $C_2H_5^+$	$(M + H)^+$, $(M + C_2H_5)^+$	Less fragmentation than H_2, can form adducts
NH_3	204	NH_4^+	$(M + H)^+$, $(M + NH_4)^+$	Selective ionization, little fragmentation, some adduct formation
$(CH_3)_3CH$	196	$(CH_3)_3C^+$	$(M + H)^+$, $[M + C(CH_3)_3)]^+$	Mild, selective protonation, little fragmentation
CH_3OH	182	$CH_3OH_2^+$	$(M + H)^+$	Degree of fragmentation observed between that of methane and isobutane
CH_3CN	188	CH_3CNH^+	$(M + H)^+$	Degree of fragmentation observed between that of methane and isobutane



Using CH4 as reactant gas



Using isobutene as reactant gas



Using NH3 as reactant gas.

Note the presence of an adduct ion [$(M + NH_4)^+$, $m/z = 214$] present in this spectrum.

As a practical note, spectra acquired under CI conditions are usually acquired over a mass range above the m/z of the reagent gas ions. The ionized reagent gas is also detected by the spectrometer, and because the reagent gas is present in great excess relative to the sample, its ions would dominate the spectrum. Thus, CI (methane) spectra are typically acquired above $m/z = 50$ (CH_5^+ is $m/z = 17$, of course, but $C_2H_5^+$ [$m/z = 29$] and $C_3H_5^+$ [$m/z = 41$] are also present), and CI (isobutane) spectra are typically acquired above $m/z = 60$ or 70.

The main advantage of CI-MS is the selective production of intact quasi-molecular ions [$(M + H)^+$]. Figure 3.4 shows the mass spectrum of butyl methacrylate acquired under different ionization conditions. The molecular ion (m/z = 142) is barely visible in the EI-MS, but the $(M + H)^+$ ion (m/z = 143) is prominent in the CI-MS spectra.

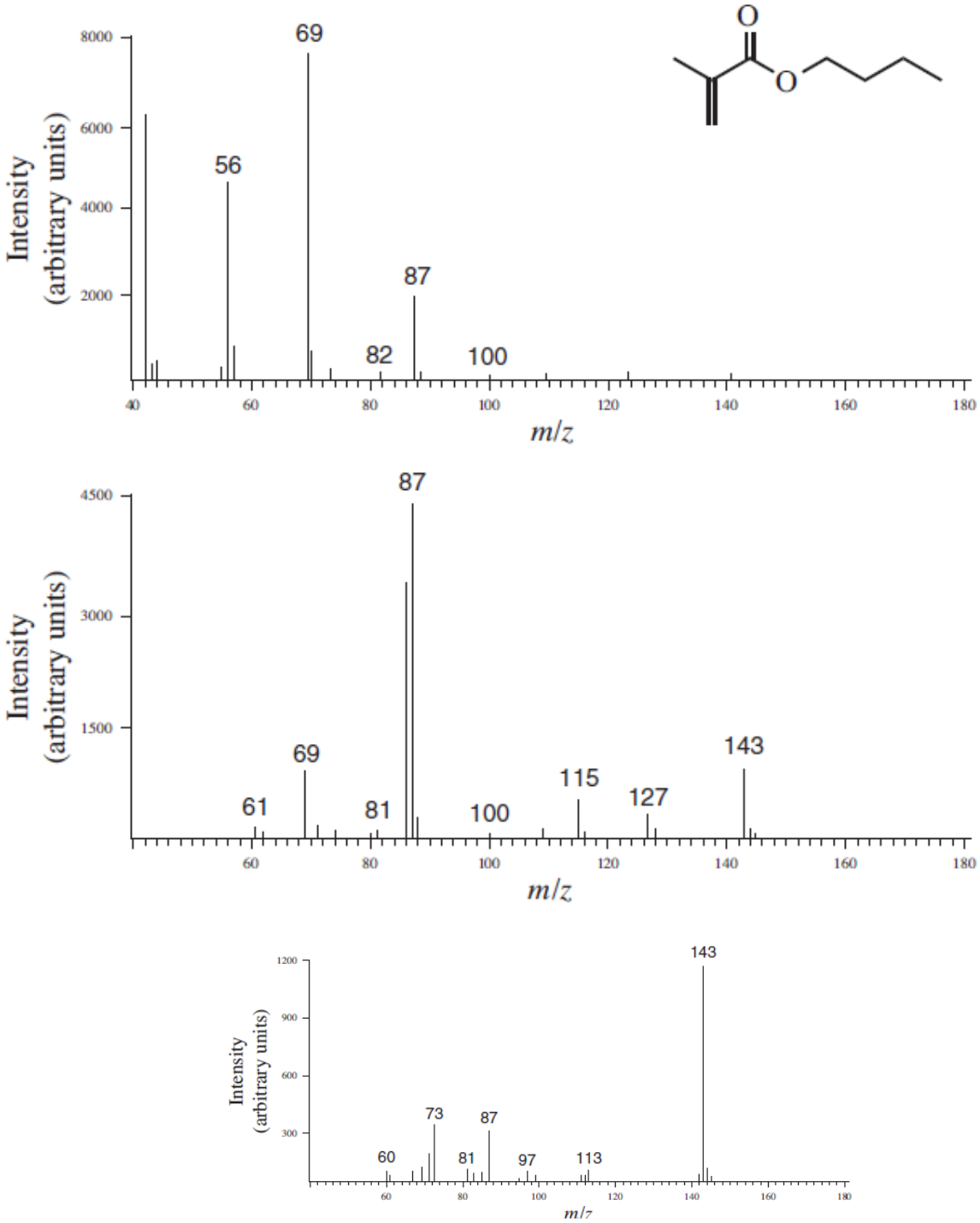


FIGURE 3.4 MS of butyl methacrylate acquired under EI (top) and CI (methane, middle; isobutane, bottom) conditions. (From DeHoffmann, E., and V. Stroobant, *Mass Spectrometry: Principles and*

The CI-MS acquired using isobutane has much less fragmentation than the CI-MS acquired using methane as the reagent gas. Other advantages to CI-MS include inexpensive and robust hardware. Like in EI-MS, however, the sample must be readily vaporized to be subjected to chemical ionization, which precludes the analysis of high molecular weight compounds and many biomolecules. CI ion sources are very similar in design to EI sources, and most modern mass spectrometers can switch from EI to CI mode in a matter of minutes.

While protonation is the most commonly encountered ionization method in CI-MS, other ionization processes may be exploited. For example, use of methyl nitrite/methane mixtures as reagent gas produces CH_3O^- that abstracts a proton from the sample, leading to a $(M - H)^-$ parent ion. Similarly, use of NF_3 as reagent gas produces F^- ion as a proton abstraction agent, also leading to $(M - H)^-$ ions. It is also possible to form negatively charged adducts under CI conditions.

Chemical ionization is also used in the open air technique known as direct analysis in real time (DART). In this ionization method (Figure 3.5) a reagent gas, usually He or Ar, is passed through a needle electrode with a 1–5 kV potential, creating excited gas atoms. The excited-state gas collides with atmospheric water to generate protonated water clusters (Equation 3.14). The sample is placed in the ion beam in any number of ways including a wire screen, glass capillary, or other solid surface. Collisions between the sample and these protonated water clusters transfer a proton to the sample (Equation 3.15). Analyte molecular ions then enter the mass analyzer through a small orifice.

$$He^* + n\,H_2O \rightarrow [(H_2O_{n-1})H]^+ + OH^- + He \qquad \textbf{Equation 3.14}$$

$$[(H_2O_{n-1})H]^+ + M \rightarrow (M + H)^+ + n\,H_2O \qquad \textbf{Equation 3.15}$$

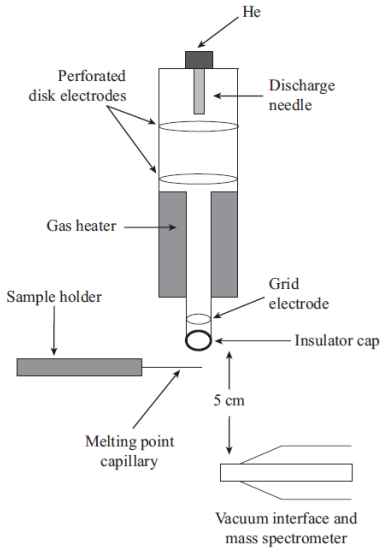


FIGURE 3.5 Schematic representation of a direct analysis in real time (DART) source. (From Petucci, C. et al., *Analytical Chemistry 79*, (2007): 5064.)

C. Desorption Ionization Techniques (SIMS, FAB, and MALDI)

Both EI and CI methods require a relatively volatile (low molecular weight) sample. More recently developed ionization techniques allow the analysis of large, nonvolatile molecules by mass spectrometry. Three of these methods, **secondary ion mass spectrometry** (**SIMS**), **fast atom bombardment** (**FAB**), and **matrix-assisted laser desorption ionization** (**MALDI**) are all **desorption ionization** (**DI**) techniques.

(**DI**) techniques. In desorption ionization, the sample to be analyzed is dissolved or dispersed in a matrix and placed in the path of a high-energy (1- to 10-keV) beam of ions (SIMS), neutral atoms (FAB), or high-intensity photons (MALDI). Beams of Ar^+ or Cs^+ are often used in SIMS, and beams of neutral Ar or Xe atoms are common in FAB. Most MALDI spectrometers use a nitrogen laser that emits at 337 nm, but some applications use an infrared (IR) laser for direct analysis of samples contained in gels or thin-layer chromatography (TLC) plates. The collision of these ions/atoms/photons with the sample ionizes some of the sample molecules and ejects them from the surface (Fig. 3.6). The

ejected ions are then accelerated toward the mass analyzer as with other ionization methods. Since FAB uses neutral atoms to ionize the sample, both positive-ion and negative-ion detection are possible. Molecular ions in SIMS and FAB are typically $(M + H)^+$ or $(M - H)^-$, but adventitious alkali metals can create $(M + Na)^+$ and $(M + K)^+$ ions also. SIMS and FAB ionization methods may be used on sample compounds with molecular weights up to about 20,000, such as polypeptides and oligonucleotides.

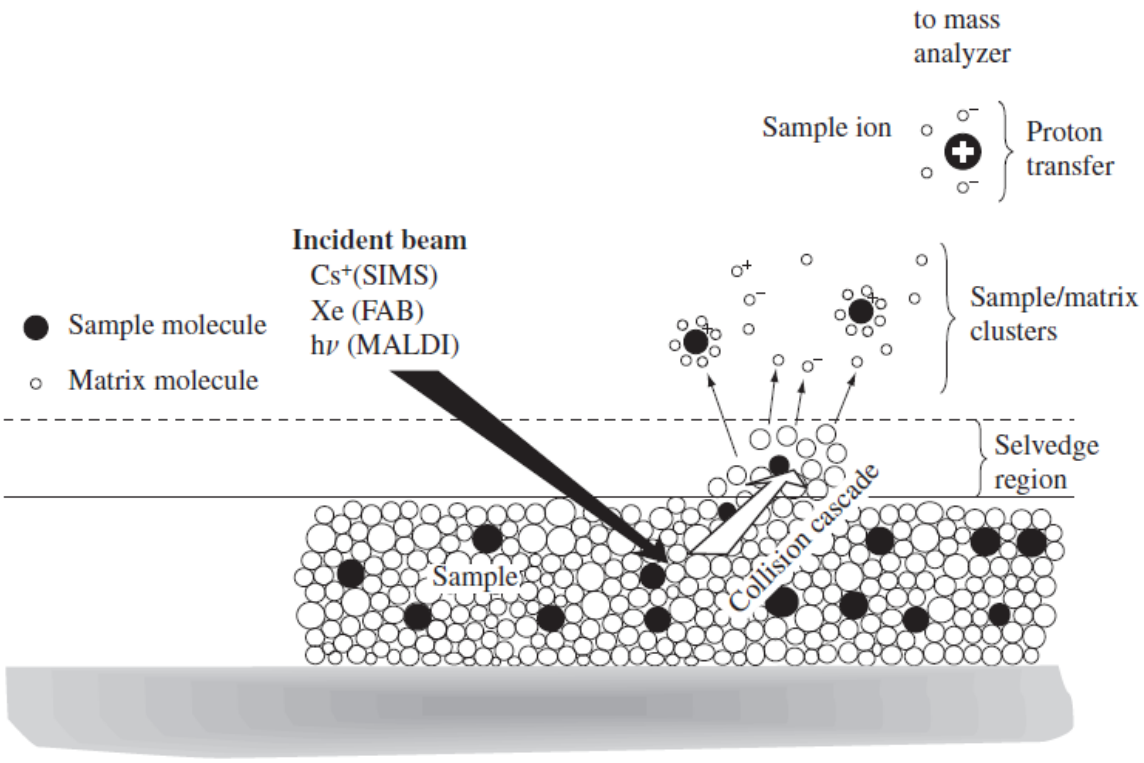


FIGURE 3.6 Schematic representations of desorption ionization techniques.