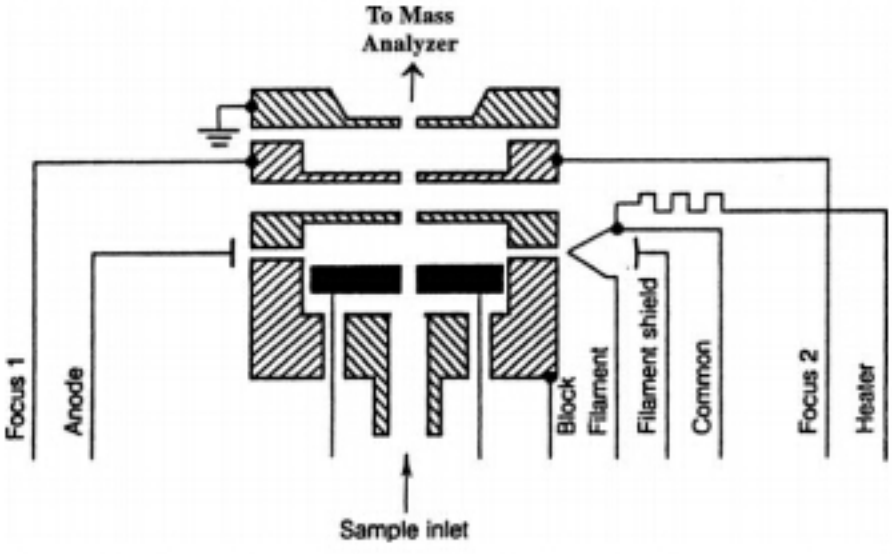

Figure 9.6 Cross-section of an EI source. The filament and anode define the electron beam. The ions are formed in the space above the two repellers (the solid color blocks). A positive charge on the repellers together with a negative potential on the focus electrodes, cause positive ions to be accelerated upward in the diagram, into the mass analyzer. (Modified from Ewing, used with permission.)

Ions are accelerated out of the center of the source into the mass analyzer by an accelerating voltage of about 10^4 V.

The EI source forms both positive and negative ions, so it can be used as a source for negative ion MS. Negative ions form from molecules containing acid groups or electronegative atoms. The high energy imparted to the ions by the EI source causes significant fragmentation of organic molecules. This type of high-energy ionization source is referred to as a *hard* ionization source. The fragmentation of the molecular ion into smaller ions is very useful in deducing the structure of a molecule. However, fragmentation can be so significant for some types of molecules that no molecular ions remain. This means that the molecular weight of the compound cannot be determined although deduction of the structure of an unknown compound is greatly facilitated by knowing the molecular weight.

Collisions between ions and molecules in the source can result in the formation of ions with higher m/z values than the molecular ion. A common ion–molecule reaction is that between a proton, H^+, and an analyte molecule, M, to give a protonated molecule, MH^+ or $(M+H)^+$. Such a species has a +1 charge and a mass that is 1 u greater than that of the molecule and is called an $(M+1)$ peak. One reason for keeping the sample pressure low in the EI ionization source is to prevent reactions between ions and molecules that would complicate interpretation of the mass spectrum.

(The electron ionization source used to be called the electron impact source and the term EI meant electron impact ionization. The use of the term *impact* is now considered archaic. Ionization and fragmentation are more often induced by the close passage of the energetic electron and the consequent large fluctuation in the electric field as opposed to a physical "impact", and an energetic electron may well perform this function multiple times on different molecules. As the student will note throughout the chapter, MS terminology has changed in recent years as a result of agreements by professional scientific organizations to standardize definitions and use of terms to avoid confusion but not all organizations have agreed to the same terms and definitions. The recommendations

from Sparkman (see the bibliography) have been followed but even current literature will be found that uses "archaic" terminology. The old or alternate terms will be provided when necessary.)

9.2.2.2. *Chemical Ionization (CI)*

A chemical ionization (CI) source is considered a *soft* ionization source; it results in less fragmentation of analyte molecules and a simpler mass spectrum than that resulting from EI. Most importantly, the molecular ion is much more abundant using CI, allowing the determination of the molecular weight. If the CI process is "soft" enough the spectrum may consist almost entirely of only the molecular ion. Such a lack of fragmentation provides less structural information than an EI spectrum. Concentration of the charge on mostly this one ion in CI-MS improves the sensitivity of detection when the method of selected ion monitoring (SIM) is employed for quantitative measurements in GC-MS (Chapter 12, Section 12.8.1). If a fragmented EI spectrum is absent a molecular ion, then combining data from a CI spectrum containing a strong molecular ion will greatly assist interpretation of an unknown compound's spectra. The two modes complement one another for identification and quantitation of unknowns.

In CI, a large excess of **reagent gas** such as methane, isobutane, or ammonia is introduced into the ionization region. The pressure in the ion source is typically several orders of magnitude higher than in EI ion sources. A CI source design will be more enclosed with smaller orifices for the source vacuum pump to remove the reagent gas, allowing the higher source pressure to be maintained. The mixture of reagent gas and sample is subjected to electron bombardment. The reagent gas is generally present at a level 1000–10,000× higher than the sample; therefore the reagent gas is most likely to be ionized by interaction with the electrons. Ionization of the sample molecules occurs indirectly by collision with ionized reagent gas molecules and proton or hydride transfer. A series of reactions occurs. Methane, for example, forms $CH_4^{\bullet+}$ and CH_3^+ on interaction with the electron beam. These ions then react with additional methane molecules to form ions as shown:

$$CH_4^{\bullet+} + CH_4 \longrightarrow CH_5^+ + CH_3^{\bullet}$$
$$CH_3^+ + CH_4 \longrightarrow C_2H_5^+ + H_2$$

Collisions between the ionic species CH_5^+ or $C_2H_5^+$ and a sample molecule M causes ionization of the sample molecule by proton transfer from the ionized reagent gas species to form MH^+ or by hydride transfer from the sample molecule to form $(M - H)^+$, also written as $(M - 1)^+$:

$$M + CH_5^+ \longrightarrow MH^+ + CH_4$$
$$M + C_2H_5^+ \longrightarrow MH^+ + C_2H_4$$
$$M + C_2H_5^+ \longrightarrow (M - H)^+ + C_2H_6$$
$$M + CH_5^+ \longrightarrow (M - H)^+ + CH_4 + H_2$$

Hydride transfer from M occurs mainly when the analyte molecule is a saturated hydrocarbon. In addition, the ionized reagent gas can react with M to form, for example, an $(M + C_2H_5)^+$ ion with $m/z = (M + 29)$. The presence of such an *adduct* ion of mass 29 Da above a candidate molecular ion in a methane CI mass spectrum is a good confirmation of the identity of the molecular ion.

Many commercial sources are designed to switch from EI to CI rapidly to take advantage of the complementary information obtained from each technique. The main

advantage of CI is that fragmentation of the sample molecule is greatly reduced and significant peaks at $m/z = (M + 1)$ or $(M - 1)$ are seen, permitting the identification of the molecular weight of the analyte.

It is possible to introduce a sample directly into the chemical ionization source on a tungsten or rhenium wire. A drop of sample in solution is applied to the wire, the solvent is allowed to evaporate, and the sample inserted into the CI source. The sample molecules are desorbed by passing a current through the wire, causing it to heat. The analyte molecules then ionize by interaction with the reagent gas ions as has been described. This technique is called **desorption chemical ionization** and is used for nonvolatile compounds.

9.2.2.3. *Atmospheric Pressure Ionization (API) Sources*

There are two major types of ionization sources that operate at atmospheric pressure, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). A modified version of the ESI source is the ion spray source. These sources are described in detail in Chapter 13, Section 13.1.6.1, because they are used to interface LC with MS for the separation and mass spectrometric analysis of mixtures of nonvolatile high molecular weight compounds, especially in the fields of pharmaceutical chemistry, biochemistry, and clinical biomonitoring. ESI will be described briefly so that its use may be demonstrated but more detail will be found in Chapter 13.

When a strong electric field is applied to a liquid passing through a metal capillary, the liquid becomes dispersed into a fine spray of positively or negatively charged droplets, an electrospray. The electric field is created by applying a potential difference of 3–8 kV between the metal capillary and a counter electrode. The highly charged droplets shrink as the solvent evaporates until the droplets undergo a series of "explosions" due to coulombic interactions. Each "explosion" forms smaller and smaller droplets. When the droplets become small enough, the analyte ions desorb from the droplets and enter the mass analyzer. A schematic *ESI source* is shown in Fig. 9.7. The ESI source is at atmospheric pressure. The droplets and finally the analyte ions pass through a series of orifices and skimmers. These serve to divert and exclude unevaporated droplets and excess vaporized solvent from the higher vacuum regions where analyte ions are accelerated and analyzed

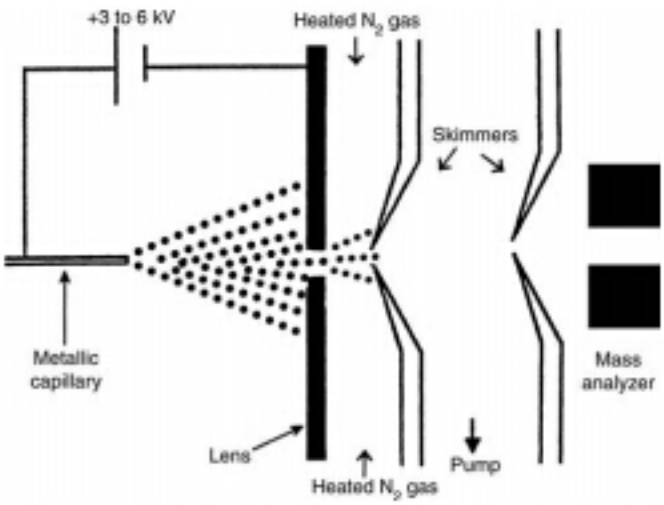

Figure 9.7 Schematic diagram of an ESI source. This source is shown with a lens system and skimmers to focus the ions and heated nitrogen gas to desolvate the ions.

by m/z. A flow of gas such as nitrogen or argon serves to desolvate the droplets and to break up ion clusters. The skimmers act as velocity filters. Heavier ions have less velocity from random thermal motions transverse to the direction of voltage acceleration through the orifices than lighter ions and continue in a straight path to the mass analyzer while the lighter ions (and solvent vapors and gases) are pumped away, permitting the pressure to be reduced without affecting the ion input to the mass analyzer. Liquid flow through the metal capillary is in the range of 1–10 μL/min for the standard ESI design. For the increasingly important HPLC-MS instrumentation used in analysis of biomolecules, orthogonal spray ESI interfaces operate at 1 mL/min and can handle flow of up to 4–8 mL/min from monolithic HPLC columns.

The advantage of ESI lies in the fact that large molecules, especially biomolecules like proteins, end up as a series of multiply charged ions, M^{n+} or $(M + nH)^{n+}$ with little or no fragmentation. For example, a given analyte M might form ions of M^{9+}, M^{10+}, M^{11+}, and so on. If the mass of the analyte M is 14,300 Da, then peaks would appear in the mass spectrum of this analyte at m/z values of (14,300/9) = 1588.9, (14,300/10) = 1430.0, and (14,300/11) = 1300.0. These ions are at much lower m/z values than would be the case if we had a singly charged M^+ ion at m/z 14300. One advantage to having multiply charged ions with low m/z values is that less-expensive mass analyzers with limited mass range can be used to separate them. Another is that high m/z ions such as high MW biomolecules with a only single charge leave a CI source with low velocities; these low velocities result in poor resolution due to dispersion and other processes in the mass spectrometer. Ions with low m/z values due to high charge are easily resolved.

Examples of mass spectra of biological molecules obtained with ESI are shown in Figs. 9.8 and 9.9. In reality, the analyst does not know the numerical charge on the peaks in the mass spectrum, but the successive peaks often vary by 1 charge unit. Computer-based algorithms have been developed for deconvoluting the sequence of m/z values of the multiply charged ions into the equivalent mass of single charged ions; such a deconvolution has been done in Fig. 9.8. This permits identification of the molecular weight of the analyte. Applications of LC-ESI-MS are described in the Chapter 13 Section 13.1.6.1. Dr. John Fenn, one of the inventors of ESI, received the Nobel Prize in Chemistry in 2002.

9.2.2.4. *Desorption Ionization*

Large molecules, such as proteins and polymers, do not have the thermal stability to vaporize without decomposing. Desorption ionization sources permit the direct ionization of solids, facilitating the analysis of large molecules. There are several types of desorption sources in which solid samples are adsorbed or placed on a support and then ionized by bombardment with ions or photons. Desorption CI, one form of desorption ionization, has already been described. The important technique of **secondary ion mass spectrometry (SIMS)** is used for surface analysis as well as characterization of large molecules; SIMS is covered in detail in Chapter 14, Section 14.4. Several other important desorption sources are described subsequently.

Laser Desorption and Matrix-Assisted Laser Desorption Ionization (MALDI). The use of a pulsed laser focused on a solid sample surface is an efficient method of ablating material from the surface and ionizing the material simultaneously. A variety of lasers have been used; examples include IR lasers such as the CO_2 laser (λ = 10.6 μm) and UV lasers such as Nd:YAG (λ = 266 nm, 355 nm). (YAG stands for yttrium aluminum garnet). Selective ionization is possible by choosing the appropriate laser wavelength. The laser can be focused to a small spot, from submicron to several

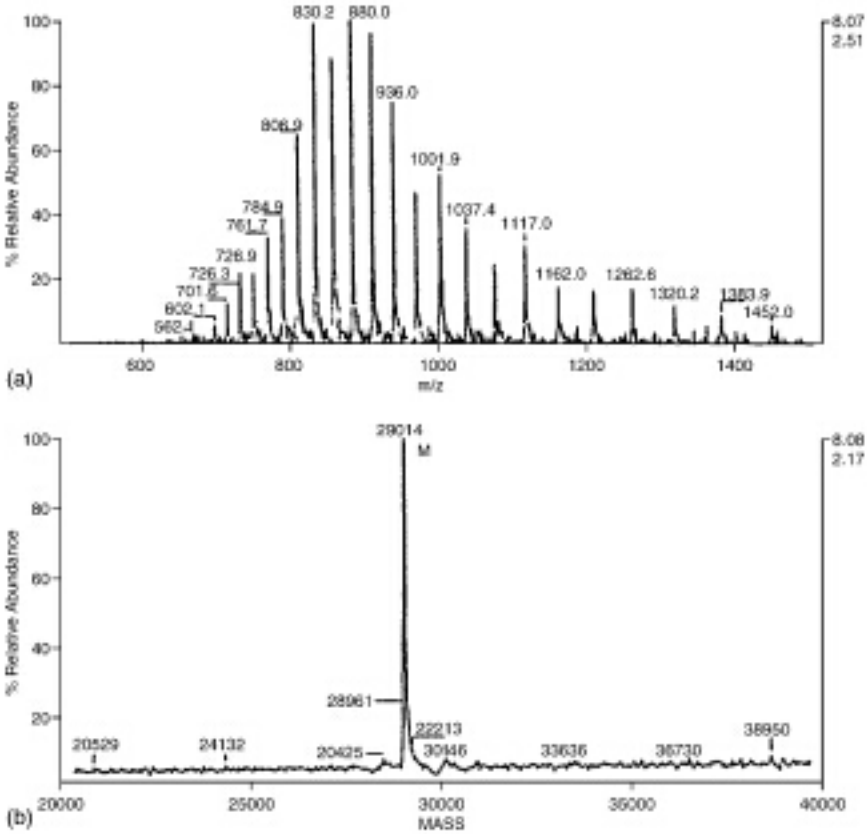

Figure 9.8 ESI mass spectrum (a) and deconvoluted mass spectrum (b) of carbonic anhydrase. (Reprinted with permission from Finnigan.)

microns in diameter. This permits the investigation of inclusions and multiple phases in solids as well as bulk analysis. The laser pulses generate transient signals, so a simultaneous detection system or a time-of-flight mass analyzer or a FT mass spectrometer is required. The laser provides large amounts of energy to the sample. This energy must be quickly dispersed within the molecule without fragmenting the molecule. Until the development of matrix-assisted laser desorption, the use of a laser resulted in fragmentation of biological molecules with molecular masses above about 1000 Da.

By mixing large analyte molecules with a "matrix" of small organic molecules, a laser can be used to desorb and ionize analyte molecules with molecular weights well over 100,000 Da with little fragmentation. The function of the matrix is to disperse the large amounts of energy absorbed from the laser, thereby minimizing fragmentation of the analyte molecule. This technique of "matrix-assisted" laser desorption ionization (MALDI) has revolutionized the mass spectrometric study of polymers and large biological molecules such as peptides, proteins, and oligosaccharides. The actual process by which ions are formed using the MALDI approach is still not completely understood.

Typical matrices and optimum laser wavelengths are shown in Table 9.3. A matrix is chosen that absorbs the laser radiation but at a wavelength at which the analyte absorbs moderately or not at all. This diminishes the likelihood of fragmenting the analyte molecule.