9.2 INSTRUMENTATION

All mass spectrometers require a sample input system, an ionization source, a mass analyzer, and a detector. All of the components with the exception of some sample input systems or ion source volumes are under vacuum (10^{-6}–10^{-8} torr for that portion where ions are separated by mass, i.e., the **analyzer**, or 10^{-4}–10^{-5} torr in some **ion sources**, where the ions are initially formed), so vacuum pumps of various types are required. Other ion sources, such as the direct analysis in real time (DART) (discussed in Section 9.2.2.3), electrospray ionization (ESI) (Sections 9.2.2.3 and 13.1.6.1), or chemical ionization (CI) (Section 9.2.2.2), operate at atmospheric pressure and use extraction lenses set to a polarity opposite that of the ions to draw them into subsequent stages of the MS instrument. Modern mass spectrometers have all of the components under computer control, with a computer-based data acquisition and processing system. A block diagram of a typical mass spectrometer is shown in Figure 9.4.

9.2.1 Sample Input Systems

9.2.1.1 Gas Expansion

This method of sample introduction is useful for gases and for liquids with sufficiently high vapor pressures. The gas or vapor is allowed to expand into an evacuated, heated vessel. The sample is then "leaked" into the ionization source through pin holes in a gold foil seal. This is sometimes termed a **molecular leak inlet**. Vacuum pumps control the system so that the pressure in the ionization source is at the required 10^{-6}–10^{-8} torr.

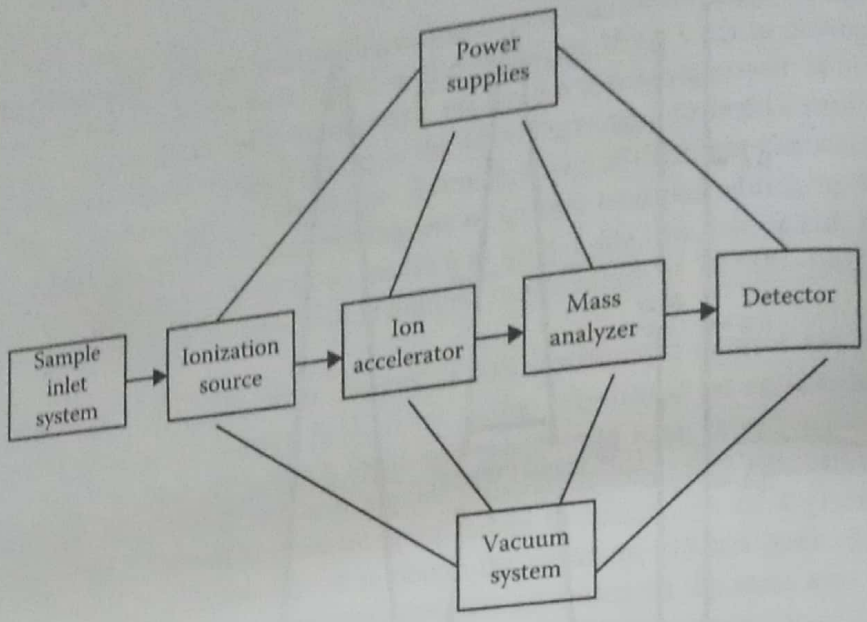

Figure 9.4 Block diagram of a mass spectrometer.

9.2.1.2 *Direct Insertion and Direct Exposure Probes*

A **direct insertion probe** is used for introduction of liquids with high boiling points and solids with sufficiently high vapor pressure. The sample is put into a glass capillary that fits into the tip of the probe shown in Figure 9.5. The probe is inserted into the ionization source of the mass spectrometer and is heated electrically, vaporizing sample into the electron beam where ionization occurs. A problem with this type of sample introduction is that the mass spectrometer can be contaminated because of the volume of sample ionized.

A **direct exposure probe** usually has a rounded glass tip. The sample is dissolved in solvent, a drop of the solution is placed on the end of the probe, and the solvent is allowed to evaporate. A thin film of sample is left on the glass tip. The tip is inserted into the ion source and heated in the same

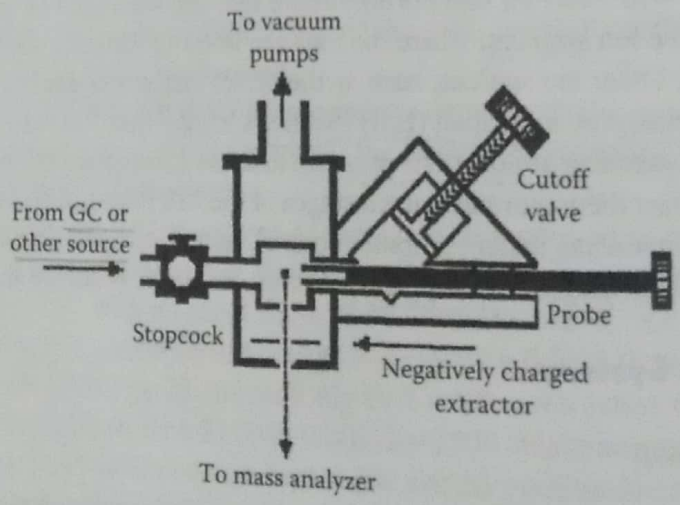

Figure 9.5 Schematic diagram of a direct insertion probe for solids and high boiling liquids. The sample is placed into the cavity in the tip of the probe. When not in use, the cutoff valve is closed, and gas samples can be introduced by opening the stopcock shown. The black dot at the center of the square shows the point at which the electron beam impacts the sample. (From Ewing, G.W., Mass spectrometry, in Ewing, G.W. (ed.), *Analytical Instrumentation Handbook*, 2nd edn., Marcel Dekker, Inc., New York, 1997. Used with permission.)

manner as the direct insertion probe. Much less sample is introduced into the ion source and the spectrometer is less likely to be contaminated as a result.

9.2.1.3 Chromatography and Electrophoresis Systems

The appropriate chromatographic instrument can separate mixtures of gases and liquids, and the separated components are then introduced sequentially into a mass spectrometer for detection. Mass spectrometrists consider the chromatograph to be a "sample inlet" for their spectrometers while chromatographers consider the mass spectrometer to be "a detector" for their chromatographs. The truth is that these hyphenated (or coupled) chromatography-MS systems are extremely powerful analytical techniques, much more powerful than either instrument alone. The major problem to be overcome in coupling these two types of instruments is that the chromatography systems run at atmospheric pressure with large amounts of "carrier gas" or solvent. The mass spectrometer operates at very low pressures as noted earlier. The carrier gas or solvent must be removed without losing the analyte before the analyte can be introduced into the evacuated ionization source or analyzer regions. Alternatively, one may use an ionization source that can ionize the target analytes under the conditions of higher pressure produced by the accompanying carrier fluid and then selectively extract the ions into a lower-pressure region while diverting and discarding the vast majority of that vaporized fluid. Interfaces for these systems have been developed and are described in the chapters on the individual chromatographic techniques.

Gas chromatography (GC) coupled with MS is known as GC-MS. It is a well-established method for separating gases or volatile compounds; the mass spectrum of each component in a mixture can be obtained and the components measured quantitatively. The interfacing, operation, and applications of GC-MS are discussed in Chapters 10 and 12.

Several types of liquid chromatography (LC) and one nonchromatographic separation system for liquids have been interfaced with MS. High-performance LC (HPLC) is widely used to separate nonvolatile organic compounds of all polarities and MWs. Coupled to a mass spectrometer, the technique is called LC-MS. Supercritical fluid chromatography (SFC) and the nonchromatographic separation technique of capillary electrophoresis (CE) are also used with mass spectrometric detection. The interfacing, ionization sources, operation, and applications of these hyphenated methods are covered in Chapters 10 and 13.

9.2.2 Ionization Sources

9.2.2.1 Electron Ionization

The EI source is a commonly used source for organic MS. Electrons are emitted from a heated tungsten or other metal filament. The electrons are accelerated by a potential of 50–100 V toward the anode (Figure 9.6). A standard value of 70 V is often used to produce comparable fragmentation patterns. Modern EI sources have a small permanent magnet that produces a field aligned with the electron beam. This causes the electrons to follow a spiral path, increasing the number of target atoms or molecules encountered. Still, only 1 in 10^5 or 10^6 of them are ionized. As shown in this figure, the paths of the electrons and sample molecules meet at right angles. Ionization of the sample molecules and fragmentation into smaller ions occur as a result of interaction with the high-energy electrons. 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, EI fragmentation can be so significant for some types of molecules that either no molecular ions remain or they are so few that they cannot be reliably distinguished from the ions

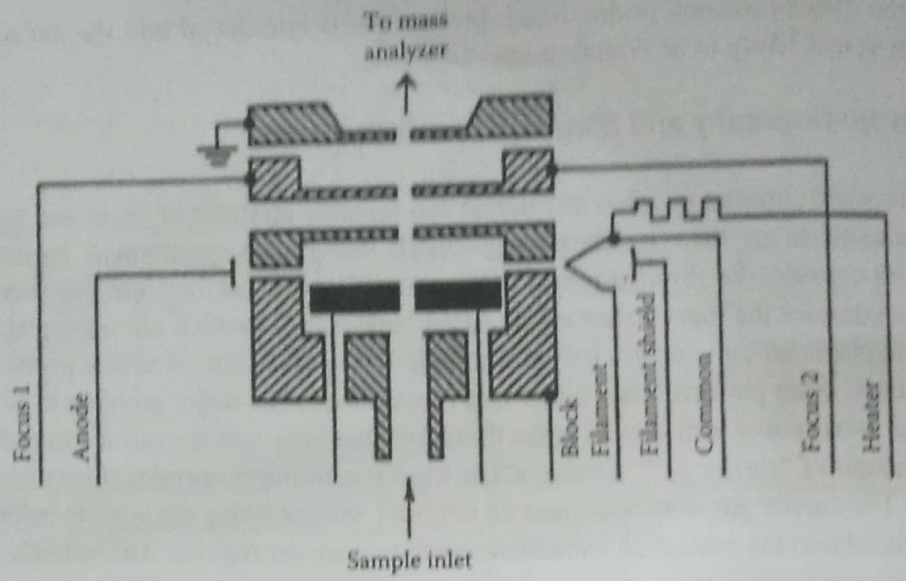

Figure 9.6 Cross section of an EI source. The filament and anode define the electron beam. The ions are formed in the space above the two repellers (the solid color blocks). A positive charge on the repellers together with a negative potential on the focus electrodes cause positive ions to be accelerated upward in the diagram, into the mass analyzer. (Modified from Ewing, G.W., Mass spectrometry, in Ewing, G.W. (ed.), *Analytical Instrumentation Handbook*, 2nd edn., Marcel Dekker, Inc., New York, 1997. Used with permission.)

from background contamination. This means that the molecular mass of the compound cannot be determined from the spectrum. This is a critical loss of information, as deduction of the structure of an unknown compound is greatly facilitated by knowing its molecular mass, and this provides the starting point for assigning the mass losses to fragment ions formed.

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 a proton **adduct**, often represented as (M + 1). In LC-MS experiments, sodium salts from the LC buffer solution often produce a mass (M + 23) Na-adduct ion. 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 EI 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 direct "impact," and an energetic electron may well perform this function multiple times on different molecules. As the student will note throughout this chapter, MS terminology has changed in recent years as a result of agreements by professional scientific organizations to standardize definitions and usage of terms to avoid confusion. 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

A 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 MW. Since proton adduct

ions $(M + 1)^+$ form more easily in CI-MS, one needs to exercise care in assigning the molecular ion mass (M^+). If the CI process is "soft" enough, the spectrum may consist almost entirely of only the molecular ion or an adduct 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 (Section 12.8.1). If a fragmented EI spectrum is missing 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 1,000–10,000× 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:

$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^\bullet$$

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

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

$$M + CH_5^+ \rightarrow MH^+ + CH_4$$

$$M + C_2H_5^+ \rightarrow MH^+ + C_2H_4$$

$$M + C_2H_5^+ \rightarrow (M - H)^+ + C_2H_6$$

$$M + CH_5^+ \rightarrow (M - H)^+ + CH_4 + H_2$$

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 MW of the analyte.

It is possible to introduce a sample directly into the CI 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 CI** and is used for nonvolatile compounds.

9.2.2.3 *Atmospheric Pressure Ionization Sources*

There are two major types of ionization sources that operate at atmospheric pressure, ESI, and atmospheric pressure CI (APCI). A modified version of the ESI source is the ion spray source. These sources are described in detail in 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 MW 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. Electrospray ionization:

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 increasing coulombic repulsion of the electrons as their droplet surface density increases. 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 Figure 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 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 by addition of jets of heated gas (e.g., N_2) to increase droplet evaporation rates, they can handle flow of up to 4–8 mL/min from monolithic HPLC columns.

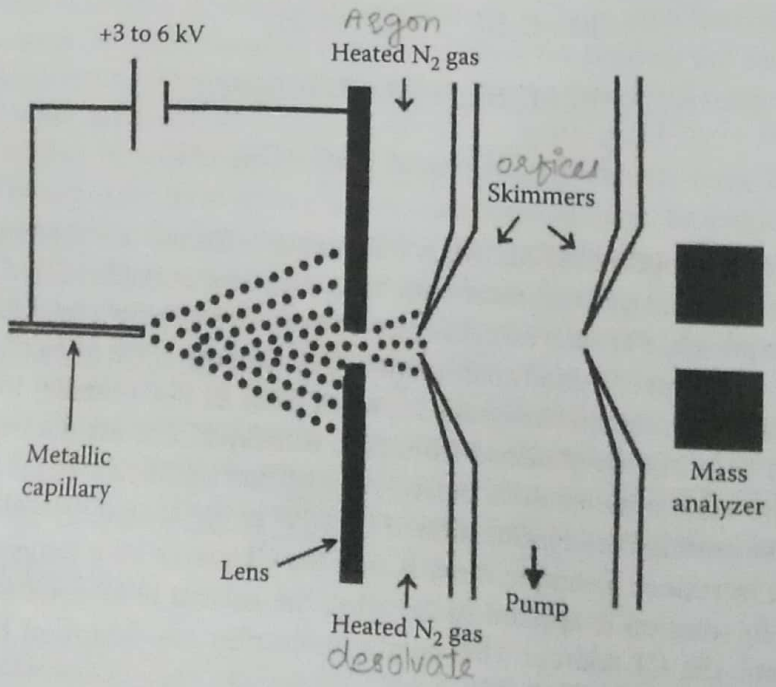

Figure 9.7 *Schematic diagram of an ESI source. This source is shown with a lens system and skimmers to focus the ions and heated nitrogen gas to desolvate the ions.*

Advantages:

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* 14,300. 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 only a 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 Figures 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

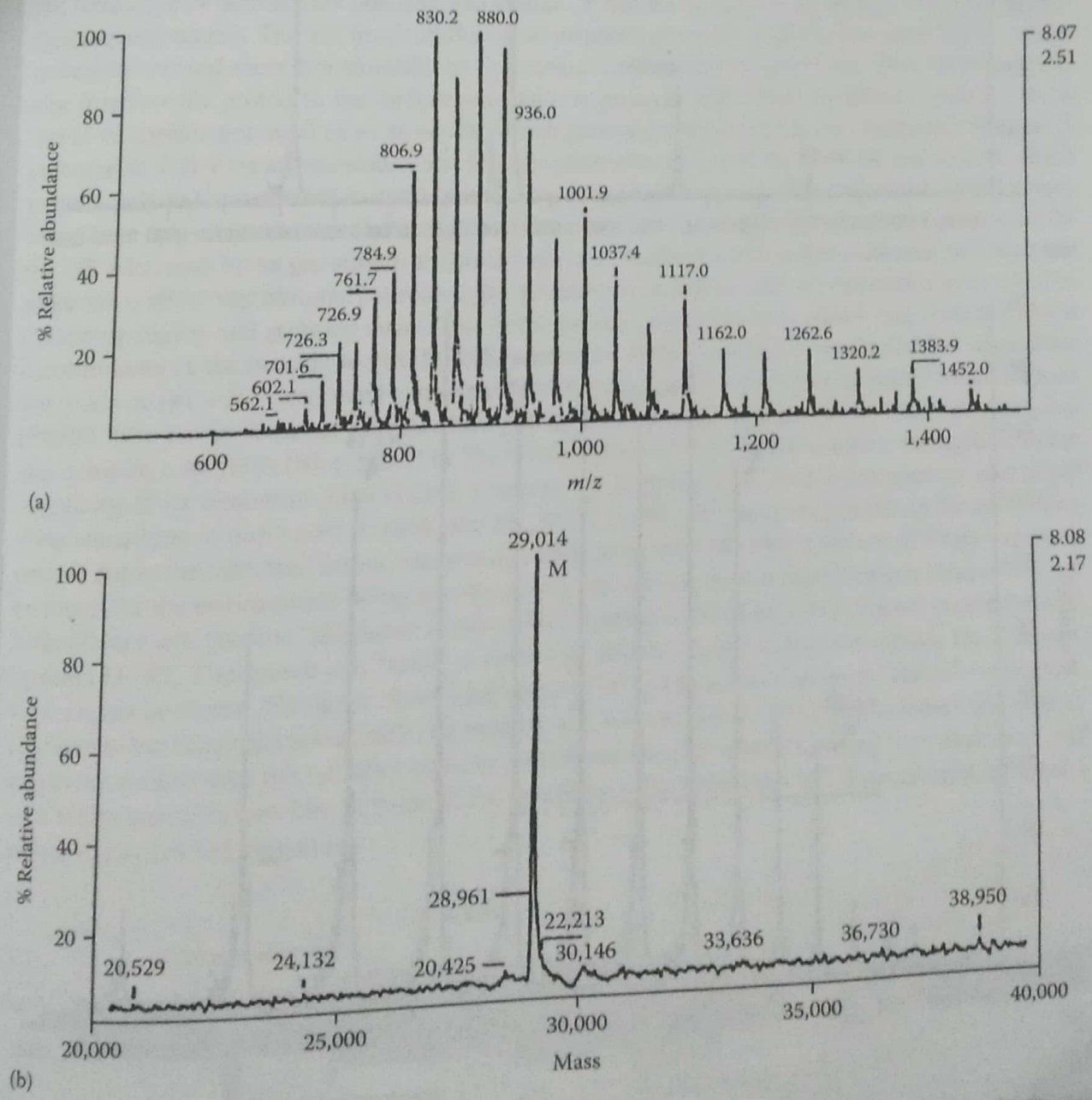

Figure 9.8 ESI mass spectrum (a) and deconvoluted mass spectrum (b) of carbonic anhydrase. (© Thermo Fisher Scientific. www.thermofisher.com. Used with permission.)

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 Figure 9.8. This permits identification of the MW of the analyte. Applications of LC-ESI-MS are described in Section 13.1.6.1. Dr. John Fenn, one of the inventors of ESI, received the Nobel Prize in Chemistry in 2002.

9.2.2.3.1 *Direct Analysis in Real Time (the DART Source)*

This source was originally developed by Japan Electron Optics Laboratory (JEOL)—a major MS vendor—and is now owned by Ion Sense. This ion production process reacts electronically excited atoms or vibrationally excited molecules to form energetic metastable species M^*. When M* collides with a sample, surface energy is transferred to the analyte molecule A, causing it to lose an electron and become a radical cation $A^{+\cdot}$. This process is called Penning ionization. In Figure 9.10 for the DART source, we see a gas stream of N_2 or Ne, which carries the ions thus formed to the inlet cone of an ambient pressure MS source. When He is used as a carrier, a different ionization process occurs. The He first collides at atmospheric pressure with H_2O to form $H_2O^{+\cdot}$, which undergoes several more transformations to become a protonated water cluster. This cluster eventually transfers the proton to the analyte molecule to produce MH^+. The excitation in either case is due to an applied potential of +1 to +5 kV, which generates the ionized gas or metastable species. A potential of 100 V on an electrode lens removes other charged particles from the gas stream, which permits only excited species to continue on. The excited species can react with solid, liquid, or gas samples to desorb and ionize the analytes. After entry into the ambient pressure sampling cone of the MS inlet, ions in the gas stream are pulled out at an angle by a charged lens into the MS analyzer stage inlet, while the obliquely directed gas stream containing neutral contaminants is directed to a trapping region and pumped away. This helps greatly to keep the ion optics free from the major contaminants of the outside world. DART spectra are simple, mainly protonated MH^+ in positive ion mode or $[M - H]^-$, and even sometimes just M^+ for some polynuclear aromatic hydrocarbons (PAHs). One never observes multiply charged or alkali metal cation adducts, although ammonia and chloride may form $[M + NH_4]^+$ or $[M + Cl]^-$ adducts. The DART source's strength is in the simplicity of its operation: One simply presents the sample to the source entrance as one might bring something to one's nose to sniff, and M^+, MH^+, or $[M - H]^-$ ions are picked up for everything present above the detection limits. The use of a high-resolution MS with a library of likely vapors to be found for the environment being tested can provide almost instant identification. Major hits not in the library will become "elephants in the room" begging for further consideration, hence the designation DART. The source can "sniff" a variety of simple sample collection media, for example, wipe papers or cloths, thin-layer chromatography (TLC) plate spots, and even "stains"—just present them to the input and stand back (figuratively). A tale told by the DART developers was that an employee walked into the lab after passing a construction site where blasting was underway and on a whim waved his necktie in front of the sampling orifice, and the MS immediately reported a nitrate-explosive MS signature.

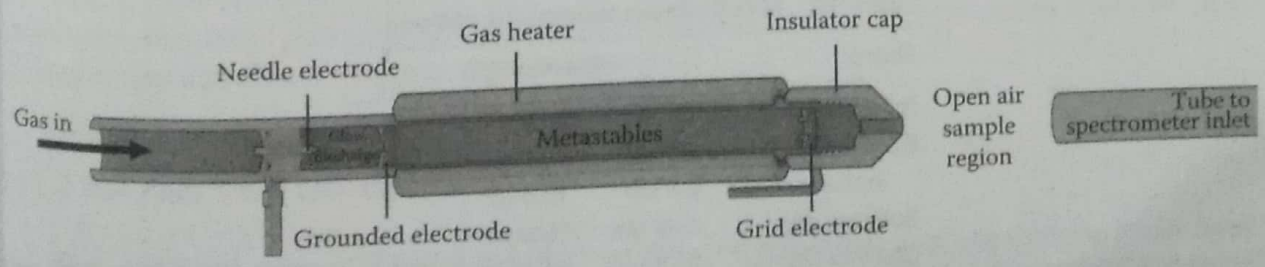

Figure 9.10 Diagram of the DART source. (Used with permission from Ion Sense. www.ionsense.com.)

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 (DI) 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 DI, has already been described. The important technique of **secondary ion MS (SIMS)** is used for surface analysis as well as characterization of large molecules; SIMS is covered in detail in Section 14.4. Several other important desorption sources are described subsequently.

9.2.2.4.1 Laser Desorption and Matrix-Assisted Laser Desorption Ionization

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 infrared (IR) lasers such as the CO_2 laser ($\lambda = 10.6$ μm) and ultraviolet (UV) lasers such as Nd: YAG ($\lambda = 266, 355$ 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 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 (TOF) mass analyzer or a Fourier transform (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 MWs 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 DI 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.

A typical sample is prepared for MALDI by mixing 1–2 μL of sample solution with 5–10 μL of matrix solution. A drop (<2 μL) of the mixture is placed on the MALDI probe and allowed to dry at room temperature. The solvent evaporates and the now-crystallized solid is placed into the mass spectrometer. The analyte molecules are completely separated from each other by the matrix, as shown in Figure 9.11.

Table 9.3 MALDI Experimental Conditions

Matrix	Wavelength		
Nicotinic acid	266 nm	2.94 μm	10.6 μm
2,5-Dihydroxy	266 nm	337 nm	355 nm
benzoic acid	2.8 μm	10.6 μm	
Succinic acid	2.94 μm	10.6 μm	
Glycerol (liquid)	2.79 μm	2.94 μm	10.6 μm
Urea (solid)	2.79 μm	2.94 μm	10.6 μm

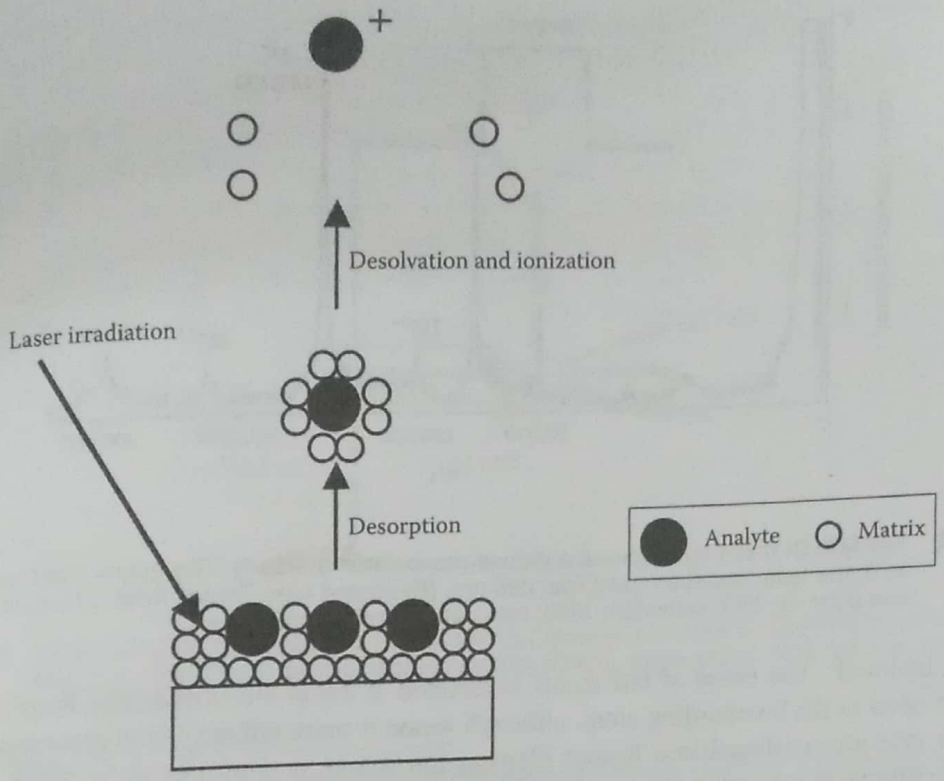

Figure 9.11 The MALDI process. Isolated analyte molecules are desorbed from a bed of matrix molecules by laser irradiation of the matrix. Subsequent desolvation and ionization of the analyte molecules occur by processes that are not completely understood.

Matrix Compounds

The matrix material must absorb strongly at the wavelength of the laser used for irradiation. In addition, the matrix must be stable in a vacuum and must not react chemically. Matrix compounds used for MALDI include 2,5-dihydroxybenzoic acid, 3-hydroxypicolinic acid, and 5-chlorosalicylic acid for the UV region of the spectrum and carboxylic acids, alcohols, and urea for the IR region of the spectrum. Intense pulses of laser radiation are aimed at the solid on the probe. The laser radiation is absorbed by the matrix molecules and causes rapid heating of the matrix. The heating causes desorption of entire analyte molecules along with the matrix molecules. Desolvation and ionization of the analyte occur; several processes have been suggested for the ionization, such as ion–molecule reactions, but the MALDI ionization process is not completely understood. A useful, if simplistic, analogy is to think of the matrix as a mattress and the analyte molecules as china plates sitting on the mattress. The laser pulses are like an energetic person jumping up and down on the mattress. Eventually, the oscillations of the mattress will cause the china to bounce up into the air without breaking. The plates (i.e., molecules) are then whisked into the mass analyzer intact.

Advantages:

MALDI acts as a soft ionization source and generally produces singly charged molecular ions from even very large polymers and biomolecules, although a few multiple-charge ions and some fragment ions and cluster ions may occur (Figure 9.12).

9.2.2.4.2 *Fast Atom Bombardment*

Fast atom bombardment (FAB) uses a beam of fast-moving neutral inert gas atoms to ionize large molecules. In this technique, the sample is dissolved in an inert, nonvolatile solvent such as glycerol and spread in a thin layer on a metal probe. The probe is inserted into the mass spectrometer through

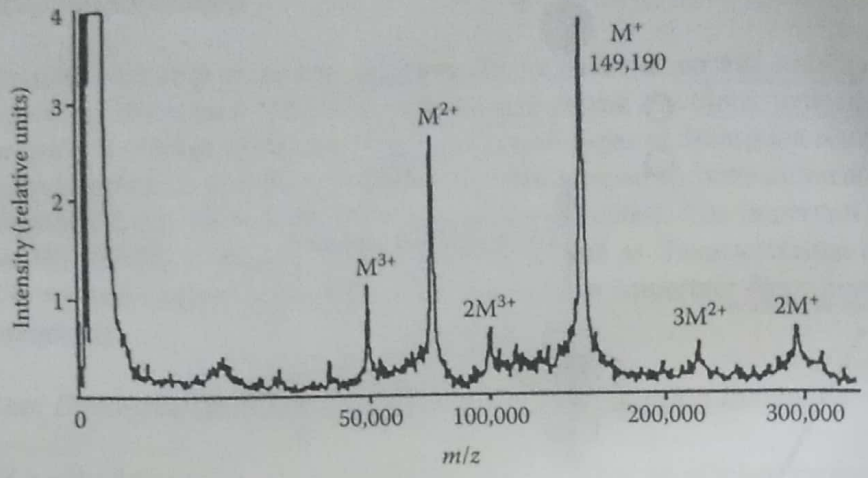

Figure 9.12 The MALDI mass spectrum of a mouse monoclonal antibody. The matrix used was nicotinic acid; the laser radiation used was 266 nm. (Reprinted from *Trends Anal. Chem.*, 9, Karas, M. and Bahr, U., 323, Copyright 1990, with permission from Elsevier.)

a vacuum interlock. The beam of fast atoms is directed at the probe surface (the target). Argon is commonly used as the bombarding atom, although xenon is more effective (and more expensive).

Argon ions are produced in a heated filament ion source or gun, just as in SIMS, a surface analysis technique discussed in Chapter 14. The ions are accelerated through a cloud of argon atoms under an electrostatic field of 3–8 kV toward the target. The fast-moving ions exchange their charge with slow-moving argon atoms but lose no kinetic energy in the process. This results in a beam of fast-moving argon atoms and slow-moving argon ions. The latter are repelled and excluded from the system using a negatively charged deflection plate. The fast-moving atoms now strike the target, liberating molecular ions of the sample from the solvent matrix. The process may again be visualized using the analogy of china dishes atop a mattress. Instead of responding to repetitive laser pulses, the matrix (mattress) absorbs, moderates, and transfers impact energy from the heavy fast atoms to the analyte molecules (dishes). If the analytes have surfactant character, they will preferentially concentrate at the liquid matrix surface, in a location optimal for being lofted into the vapor state. Positively charged $(M + H)^+$ or negatively charged $(M - H)^-$ ions may be produced, so positive ion or negative ion mass spectra may be collected. The process is shown schematically in Figure 9.13.

Advantages There are several advantages to the FAB technique. The instrumentation is simple and the sensitivity is high. Analytes such as surfactants have been measured quantitatively at concentrations as low as 0.1 ppb. It is difficult to get very large molecules into the gas phase because of their low volatility, and it is difficult to ionize large molecules and retain the molecular ion in many ionization sources. The FAB process works at room temperature; volatilization is not required, so large molecules and thermally unstable molecules can be studied. The duration of the signal from the sample is continuous and very stable over a long period.

Sample fragmentation is greatly reduced in the FAB process, resulting in a large molecular ion, even with somewhat unstable molecules. This provides information on the MW of the molecule, which is particularly important in biological samples such as proteins. Spectra from molecules with MWs greater than 10,000 have been obtained. Although a strong molecular ion is obtained with FAB, fragmentation patterns are also obtained, providing structural information on biologically important molecules such as proteins.

Sample quantity:

An important advantage of FAB is that only those analyte molecules sputtered from the glycerol are lost; the remainder can be recovered for other analyses. Consequently, samples as small as 1 μg can be placed on the probe, and after the mass spectrum is obtained, a significant amount of the sample can be recovered.

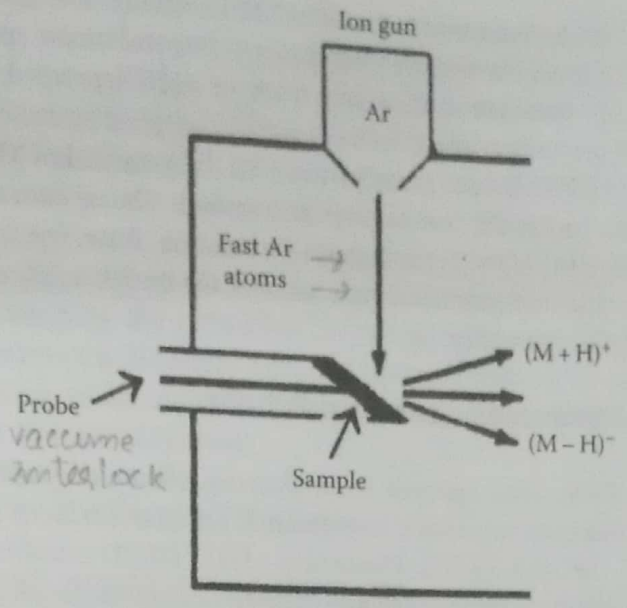

Figure 9.13 Schematic FAB ionization source. The sample, dissolved in solvent, is spread in a thin film on the end of a metal probe and bombarded by fast-moving argon atoms. Both positive and negative ions are produced.

modified FAB:

A modification of the FAB technique is **continuous flow FAB (CFFAB)**. In this approach, the sample in solution is introduced into the mass spectrometer through a fused silica capillary. The tip of the capillary is the target. The solution is bombarded by fast atoms produced as described earlier. Solvent is flowing continuously, and the liquid sample is introduced by continuous flow injection (Figure 9.14). The mass spectrum produced has the same characteristics as that from conventional FAB, but with low background. Typically, the solvent used is 95% water and 5% glycerol. The ability to inject aqueous samples is an enormous advantage in biological and environmental studies. Very frequently, these types of samples are aqueous in nature, such as blood, urine, and other body fluids; water; and wastewater. Automated background correction:

The sensitivity at the lower MW range (1500 Da) is increased by two orders of magnitude over conventional FAB. Further, the background is reduced because of the reduced amount of glycerol present. In addition, when the solvent alone is injected, a background signal can be recorded. This can be subtracted from the signal due to sample plus solvent, and the net signal of the sample is obtained. This is especially valuable for trace analysis; concentrations as low as 10^{-12} g have been detected using CFFAB.

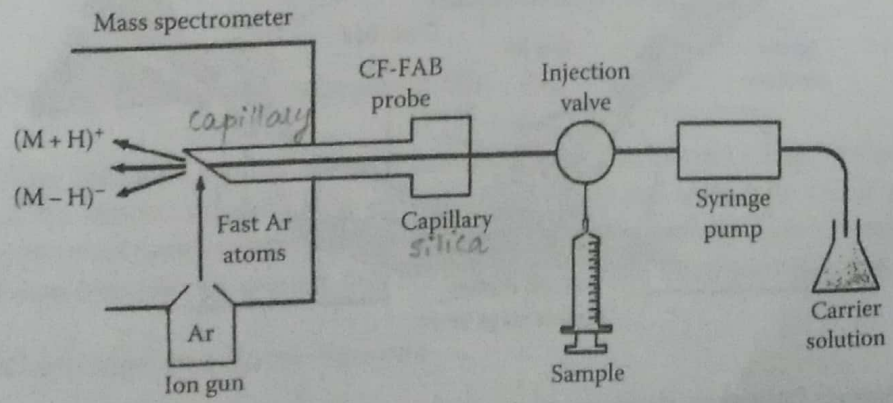

Figure 9.14 Schematic of CFFAB MS operated in the flow-injection mode.

Role in LC-MS:

The CFFAB system can be incorporated into LC-MS systems. The mobile phase is the solvent used. The effluent from the LC is transported directly into the mass spectrometer and the MS obtained by CFFAB. This provides a mass spectrum of each separated peak in mixtures. (See Chapter 13 for a detailed discussion of LC-MS.) Applications:

In summary, FAB and CFFAB have greatly increased the potential of MS by increasing the MW range of molecules whose molecular ion can be determined. The system can be directly attached to LC, permitting identification of the components of a solution. Also, trace analysis is possible. The method can be applied to the important research areas of the health sciences, biology, and environmental science, as well as to chemistry.

9.2.2.4.3 *Desorption Electrospray Ionization Source*

While ESI, APCI, FAB, etc., operate from flowing chromatographic effluents or dipped point sources, other techniques introduce ions from 2D planar surfaces such as tissue thin sections or TLC plates (cf. Section 13.7.1). Desorption ESI (DESI) combines ESI and DI. Under ambient atmospheric conditions, an electrically charged cloud of droplets is directed at a small spot on a sample surface only a few mm distant. This charged mist is pulled to the surface by a voltage of opposite polarity applied to it. The droplet charge is transferred to analytes on the surface, and the resultant ions travel into an atmospheric pressure interface similar to that in APCI or ESI. For high MW targets such as large protein molecules, multiple charges can be transferred with the subsequent behavior of the droplets essentially the same as the series of coulomb explosions and desolvation steps seen in ESI. By contrast, for low MW analytes, the ionization occurs by charge transfer of either an electron or a proton (thus yielding MH^+ ions, but not adducts such as $M + Na^+$), similar to the APCI or DART ionization mechanisms. The geometry of the DESI source is illustrated in Figure 9.15. The key features are the two angles: α and β, d (*distance tip to surface*), and the unmarked distance (*surface to center of MS inlet*). Optimal conditions for high MW analytes are high $\alpha = 70°–90°$ and short $d = 1–3$ mm. For low MW analytes, conditions are reversed: $\alpha = 35°–50°$ and $d = 7–10$ mm. This is consistent with and predicted for the two different ionization mechanisms described earlier. The spray emitter and the sample surface holder are each attached to a 3D stage that moves with three orthogonal degrees of freedom, which allows variation of all four of those geometric parameters. One can take full-scan mass spectra from a raster of points covering the whole 2D surface to discover

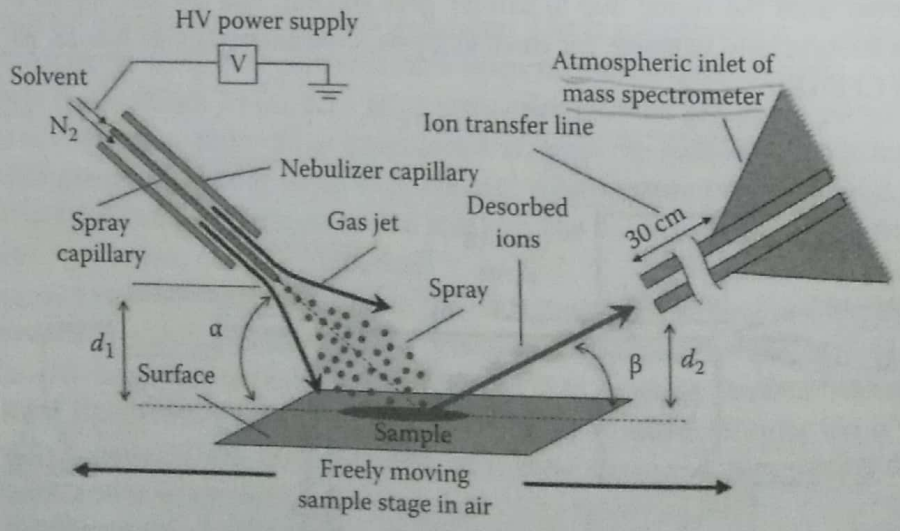

Figure 9.15 Prosolia DESI Omni Spray ion source geometry and operation diagram. The family of different (Manual, 1D and 2D) ion sources can be used on MS equipment from Agilent, Thermo, Waters, ABSciex, Bruker, and LECO. (Used with permission from Prosolia, Inc. www.prosolia.com.)