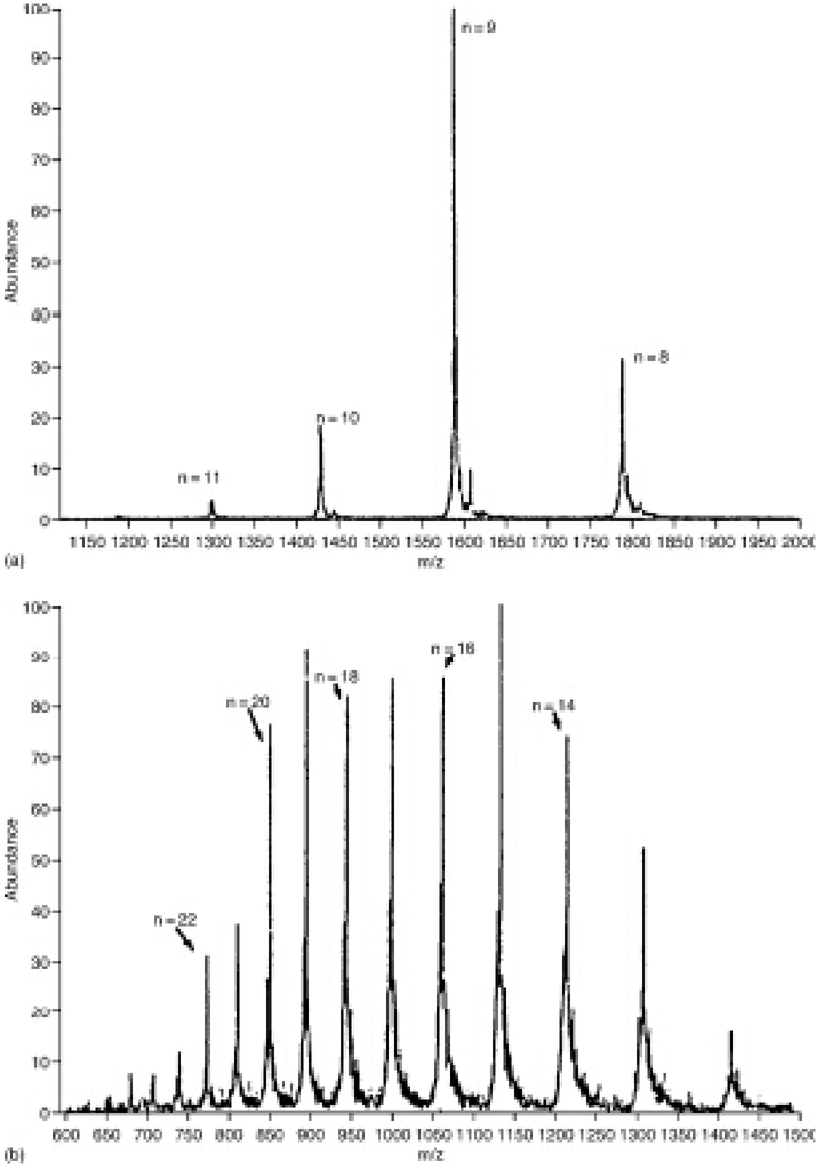

Figure 9.9 ESI mass spectra of two proteins: (a) hen egg-white lysozyme and (b) equine myoglobin.

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

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 Fig. 9.10.

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, must not react chemically and must have a low enough mass to sublime. 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. This heating causes desorption of entire analyte molecules along with the matrix molecules. Desolvation and ionization of the analyte occurs; 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.

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 may occur (Fig. 9.11).

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 a vacuum interlock.

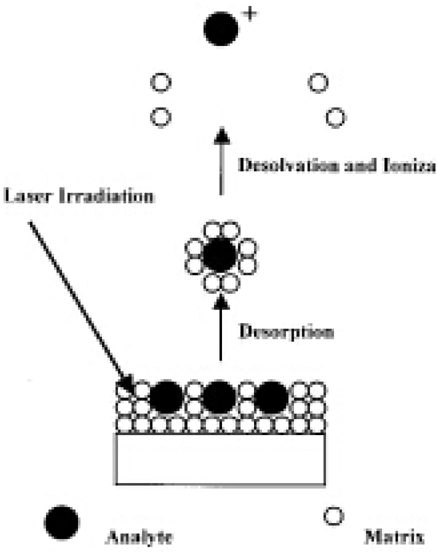

Figure 9.10 The MALDI process. Isolated analyte molecules are desorbed from a bed of matrix molecules by laser irradiation of the matrix. Subsequent desolvation and ionization of the analyte molecule occur by processes that are not completely understood.

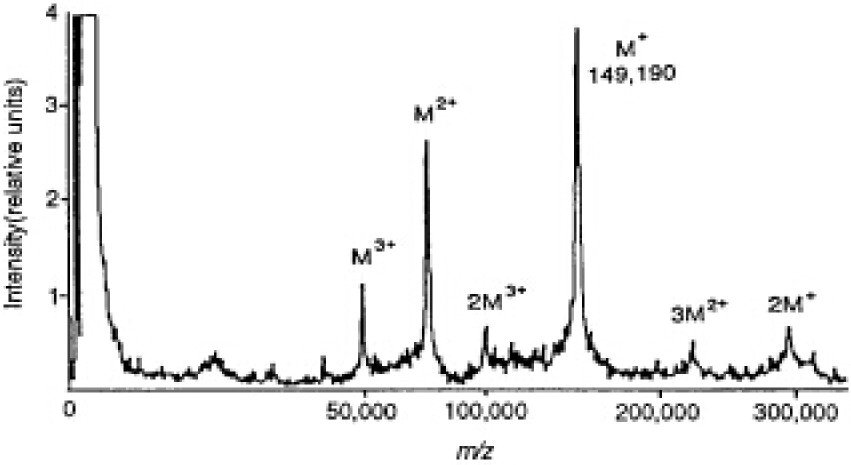

Figure 9.11 The MALDI mass spectrum of a mouse monoclonal antibody. The matrix used was nicotinic acid; the laser radiation used was 266 nm. (Reprinted from Karas and Bahr, with permission from Elsevier.)

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^+ or negatively charged M^- may be produced, so positive ion or negative ion mass spectra may be collected. The process is shown schematically in Fig. 9.12.

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 molecular weight of the molecule, which is particularly important in biological samples such as proteins. Spectra from molecules with molecular weights greater than 10,000 have been obtained. Although a strong molecular ion is obtained with

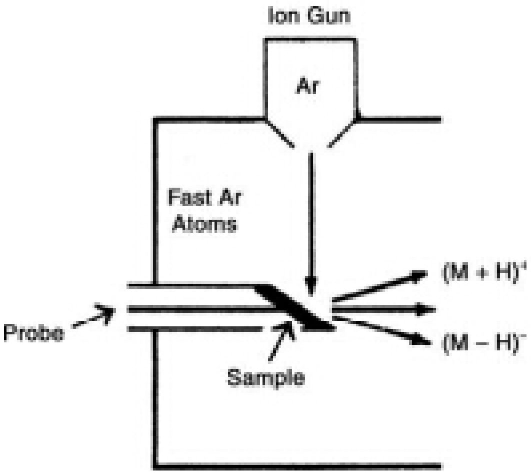

Figure 9.12 Schematic FAB ionization source. The sample, dissolved in solvent, is spread in a thin film on the end of a metal probe and bombarded by fast-moving argon atoms. Both positive and negative ions are produced.

FAB, fragmentation patterns are also obtained, providing structural information on biologically important molecules such as proteins.

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.

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 (Fig. 9.13). 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.

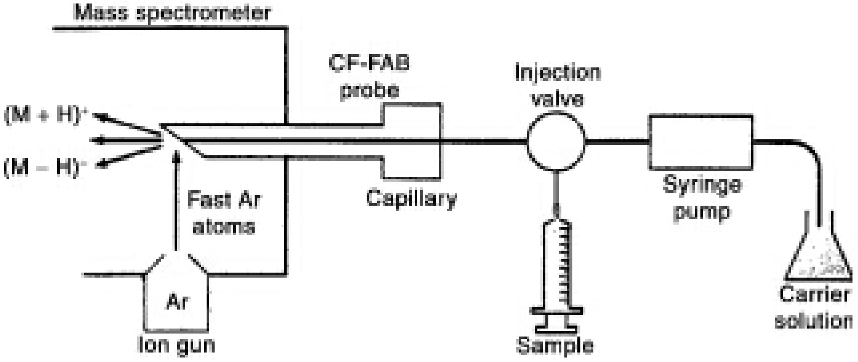

Figure 9.13 Schematic of continuous flow FAB MS operated in the flow-injection mode.

Very frequently, these types of samples are aqueous in nature, such as blood, urine, and other body fluids, water, and wastewater.

The sensitivity at the lower molecular weight 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.

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.)

In summary, FAB and CFFAB have greatly increased the potential of mass spectrometry by increasing the molecular weight 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.

9.2.2.5. *Ionization Sources for Inorganic MS*

The following ionization sources are used mainly in inorganic (atomic) MS, where the elemental composition of the sample is desired. The glow discharge (GD) and spark sources are used for solid samples, while the inductively coupled plasma (ICP) is used for solutions. All three sources are also used as atomic emission spectroscopy sources; they are described in more detail with diagrams in Chapter 7.

GD Sources and Spark Sources. The GD source and spark source are both used for sputtering and ionizing species from the surface of solid samples and have been discussed in Chapter 7 for use as atomic emission sources. As MS ionization sources, they are used primarily for atomic MS to determine the elements present in metals and other solid samples. The DC GD source has a cathode and anode in 0.1–10 torr of argon gas. The sample serves as the cathode. When a potential of several hundred volts is imposed across the electrodes, the argon gas ionizes forming a plasma. Electrons and positive argon ions are accelerated toward the oppositely charged electrodes. The argon ions impact the cathode surface, sputtering off atoms of the cathode material. The sample atoms are then ionized in the plasma by electrons or by collision with excited argon (Penning ionization). The sample ions are extracted from the plasma into the mass analyzer by a negatively charged electrode with a small aperture. The DC GD source is used for the analysis of conductive samples including metals, alloys, and semiconductors. The sample must be conductive to serve as the cathode. RF GD sources have been developed that enable the sputtering of electrically nonconductive samples such as ceramics and other insulators. Spark sources also can be used for sputtering of solids, but the GD source produces a more stable ion beam with better signal-to-noise ratio. The GD source sputters more material from a sample and gives more representative and more quantitative results of the elemental bulk composition than the spark source.

ICP Source. The argon ICP source has also been described in Chapter 7 for use with atomic emission spectrometers. The source produces ions from the elements introduced into the plasma as well as radiation; these ions can be extracted into a mass analyzer. The ICP torch is usually mounted horizontally with the tip of the plasma at the entrance to the mass analyzer as shown in Fig. 9.14. Most of the plasma gas is deflected by a metal