Mass Spectrometry

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Mass Spectrometry

This is an instrumental technique which conver sample to positive ion by different ionization technique and different particles are separated on the basis of their mass.

No radiation used and therefore called spectrometry

Mass spectrum provides the mass/charge ratio and intensity tells about the stability of specific fragments.

In General mass instrument have following sections

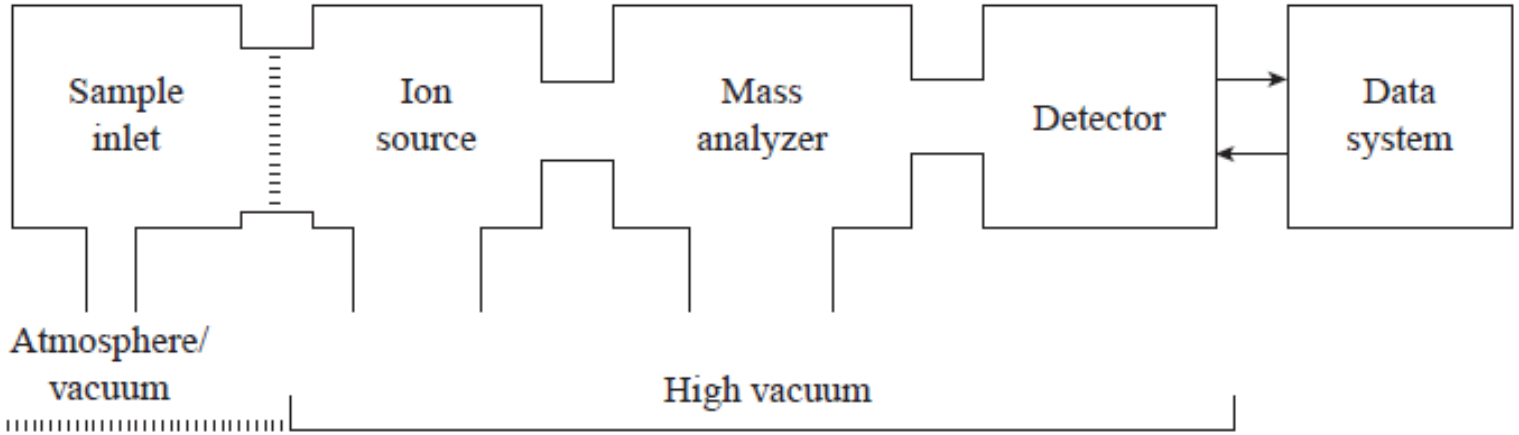

FIGURE 8.1 The components of a mass spectrometer. (From Gross, J. H., *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004. Reprinted by permission.)

1 The first component of the mass spectrometer is the **sample inlet** (Section 8.2), which brings the sample from the laboratory environment (1 atm) to the lower pressure of the mass spectrometer.

2. The sample inlet leads to the **ion source (Section 8.3), where** the sample molecules are transformed into gas phase ions. The ions are then accelerated by an electromagnetic field.

3. Next, the **mass analyzer (Section 8.4) separates the sample ions based on their**
mass-to-charge (*m/z) ratio.*

4. The ions then are counted by the detector (Section 8.5).

5. The signalis recorded and processed by the **data system, typically a personal computer (PC). The output from** the data system is the **mass spectrum—a graph of the number of ions detected as a function of their** *m/z ratio.*

Sample inlet used for incorporation of sample to the ionization chamber and detail can be read from pavia section 8.2.
In the ionzation chamber various mode of ionization can be used as per requirement some of them are.

A. Electron Ionization (EI)

1. **The simplest and most common method for converting** the sample to ions is **electron ionization (EI).**
2. **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.
3. The electron–molecule collision strips an electron from the molecule, creating a cation.
4. 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. 8.2).**

5. Non ionized samples are continuously drawn off by vacuum pumps that are connected to the ionization chamber and negative ions absorbed by repeller plate

6. 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.

7. 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.

8. 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.

9. To acquire reproducible spectral features, including fragmentation patterns, that can be readily compared with electronic databases, a standard 70-eV electron beam is used.

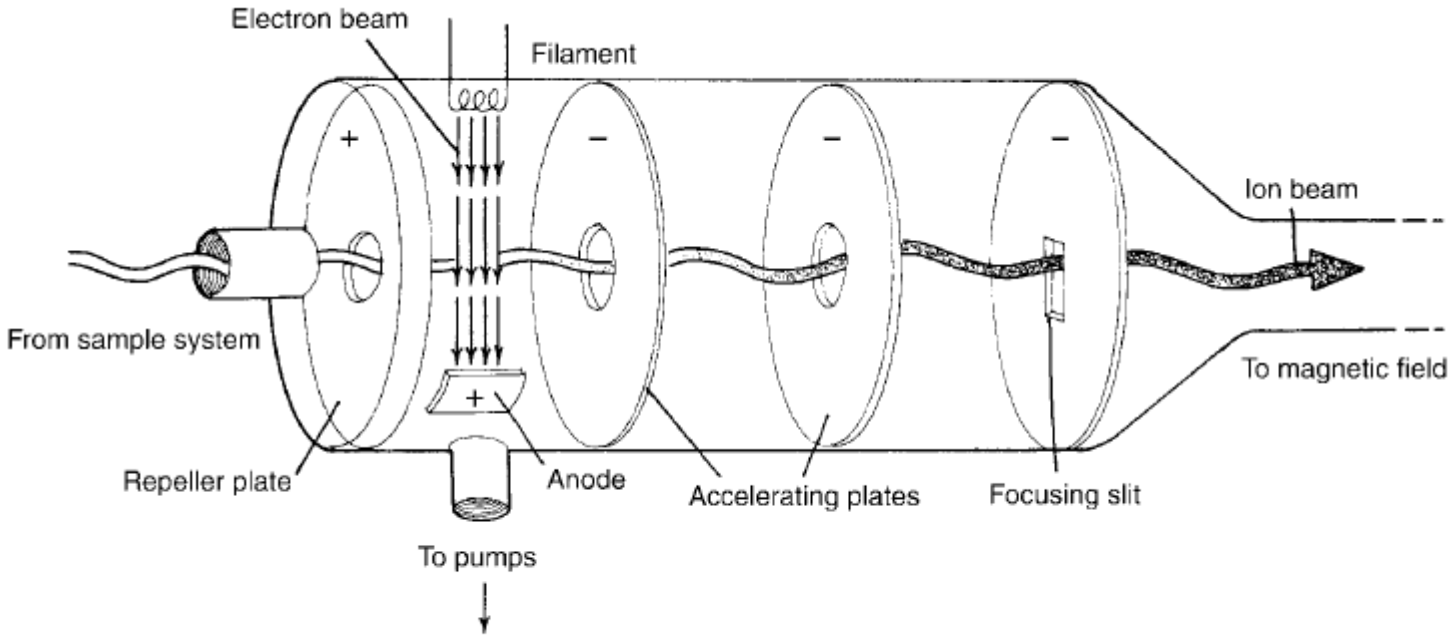

FIGURE 8.2 Electron ionization chamber.

B. Chemical ionization

1 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.

2. 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.

3. Almost any readily available gas or highly volatile liquid can be used as a reagent gas for CI-MS.

4. 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 8.l–8.4.

$$CH_4 + e^- \rightarrow CH_4^{\bullet+} + 2e^- \quad \textbf{Equation 8.1}$$

$$CH_4^{\bullet+} + CH_4 \rightarrow CH_5^+ + \cdot CH_3 \quad \textbf{Equation 8.2}$$

$$CH_4^{\bullet+} \rightarrow CH_3^+ + H\cdot \quad \textbf{Equation 8.3}$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \quad \textbf{Equation 8.4}$$

The sample molecule M is then ionized through the ion–molecule reactions in Equations 8.5 and 8.6:

$$M + CH_5^+ \rightarrow (M+H)^+ + CH_4 \quad \textbf{Equation 8.5}$$

$$M + C_2H_5^+ \rightarrow (M + C_2H_5)^+ \quad \textbf{Equation 8.6}$$

The situation is very similar for CI with ammonia as reagent gas (Equations 8.7–8.9):

$$NH_3 + e^- \rightarrow NH_3^{\bullet+} + 2e^- \quad \textbf{Equation 8.7}$$

$$NH_3^{\bullet+} + NH_3 \rightarrow NH_4^+ + \cdot NH_2 \quad \textbf{Equation 8.8}$$

$$M + NH_4^+ \rightarrow (M+H)^+ + NH_3 \quad \textbf{Equation 8.9}$$

Using isobutane as reagent gas produces *tert*-butyl cations (Equations 8.10 and 8.11), which readily protonate basic sites on the sample molecule (Equation 8.12). Adduct formation is also possible using isobutane in CI-MS (Equation 8.13).

$$(CH_3)_3CH + e^- \rightarrow (CH_3)_3CH^{\bullet+} + 2e^- \quad \textbf{Equation 8.10}$$

$$(CH_3)_3CH^{\bullet+} \rightarrow (CH_3)_3C^+ + H\cdot \quad \textbf{Equation 8.11}$$

$$M + (CH_3)_3C^+ \rightarrow (M+H)^+ + (CH_3)_2C{=}CH_2 \quad \textbf{Equation 8.12}$$

$$M + (CH_3)_3C^+ \rightarrow [M + C(CH_3)_3]^+ \quad \textbf{Equation 8.13}$$

TABLE 8.1
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

1. Both EI and CI methods require a relatively volatile (low molecular weight) sample.
2. 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.**
3. **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).
4. Beams of Ar+ or Cs+ are often used in SIMS, and beams of neutral Ar or Xe atoms are common in FAB.
5. 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.
6. The collision of these ions/atoms/photons with the sample ionizes some of the sample molecules and ejects them from the surface (Fig. 8.5).
7. The ejected ions are then accelerated toward the mass analyzer as with other ionization methods.

8. Since FAB uses neutral atoms to ionize the sample, both positive-ion and negative-ion detection are possible.
9. 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.
10. SIMS and FAB ionization methods may be used on sample compounds with molecular weights up to about 20,000, such as polypeptides and oligonucleotides.

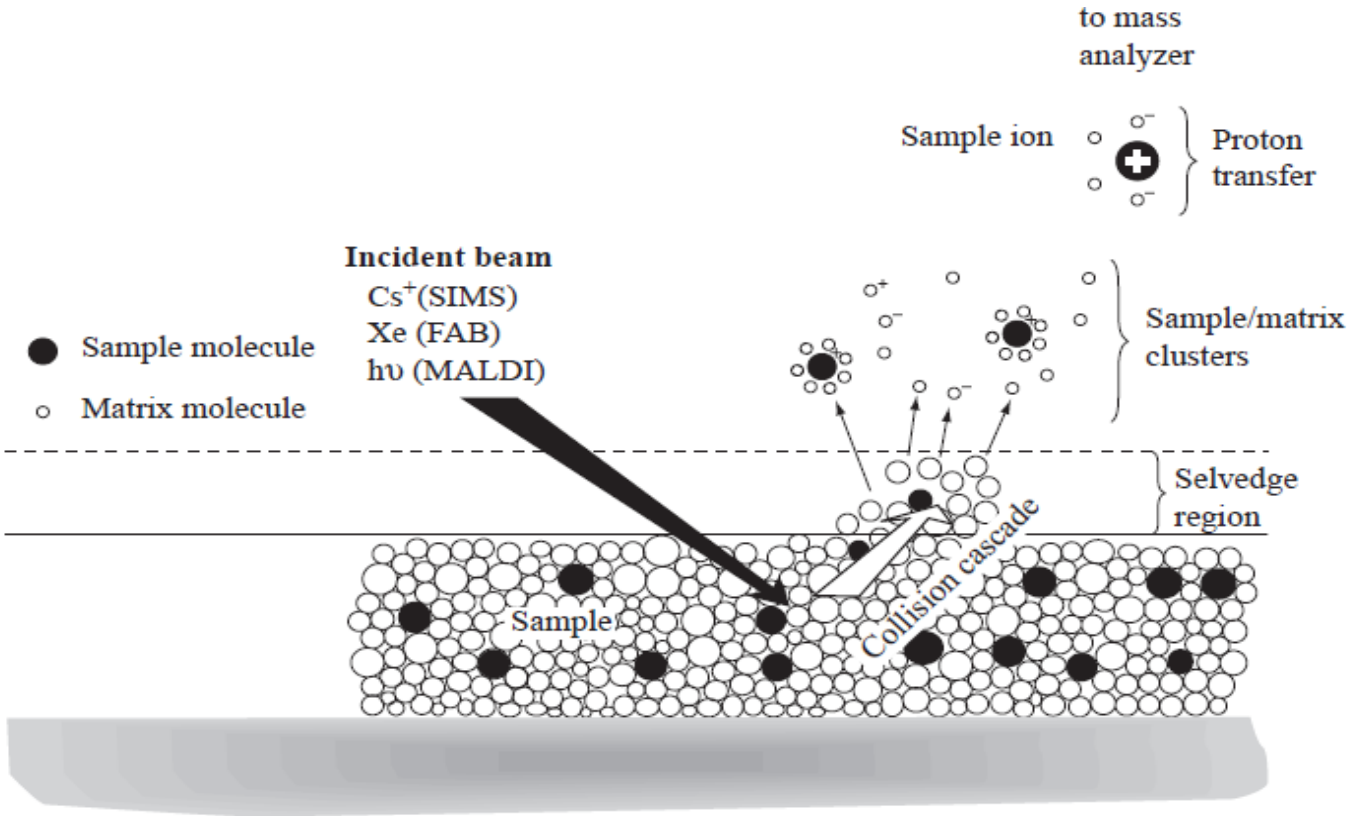

FIGURE 8.5 Schematic representations of desorption ionization techniques.

11. The matrix should be nonvolatile, relatively inert, and a reasonable electrolyte to allow ion formation.
12. If the matrix compound is more acidic than the analyte, then predominantly (M + H)+ ions will be formed, while mostly (M – H)– ions will result when the matrix is less acidic than the analyte.
13. The matrix absorbs much of the excess energy imparted by the beam of ions/atoms and produces ions that contribute a large amount of background ions to the mass spectrum
14. Common matrix compounds for SIMS and FAB include glycerol, thioglycerol, 3-nitrobenzyl alcohol, di- and triethanolamine, and mixtures of dithiothreitol (DTT) and dithioerythritol (Fig. 8.6)
15. The matrix compounds used in MALDI are chosen for their ability to absorb the ultraviolet (UV) light from a laser pulse (337 nm for N2 laser). Substituted nicotinic, picolinic, and cinnamic acid derivatives are often used in MALDI techniques (Fig. 8.7). The

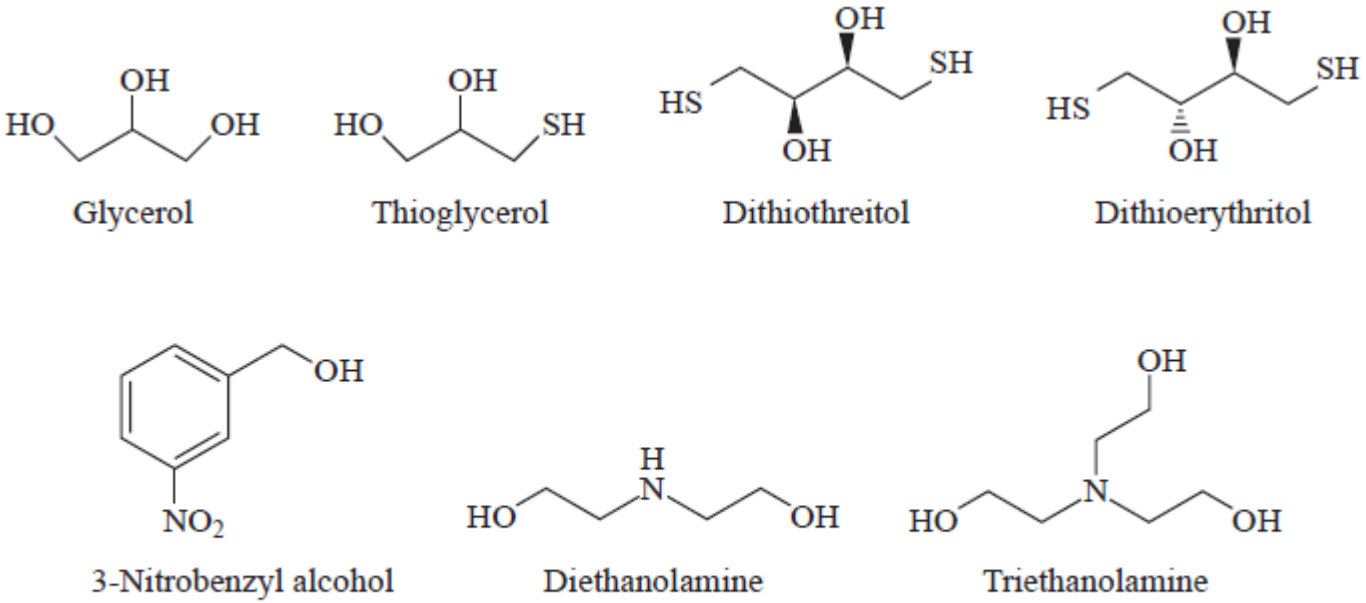

FIGURE 8.6 Common matrices for SIMS and FAB mass spectrometry.

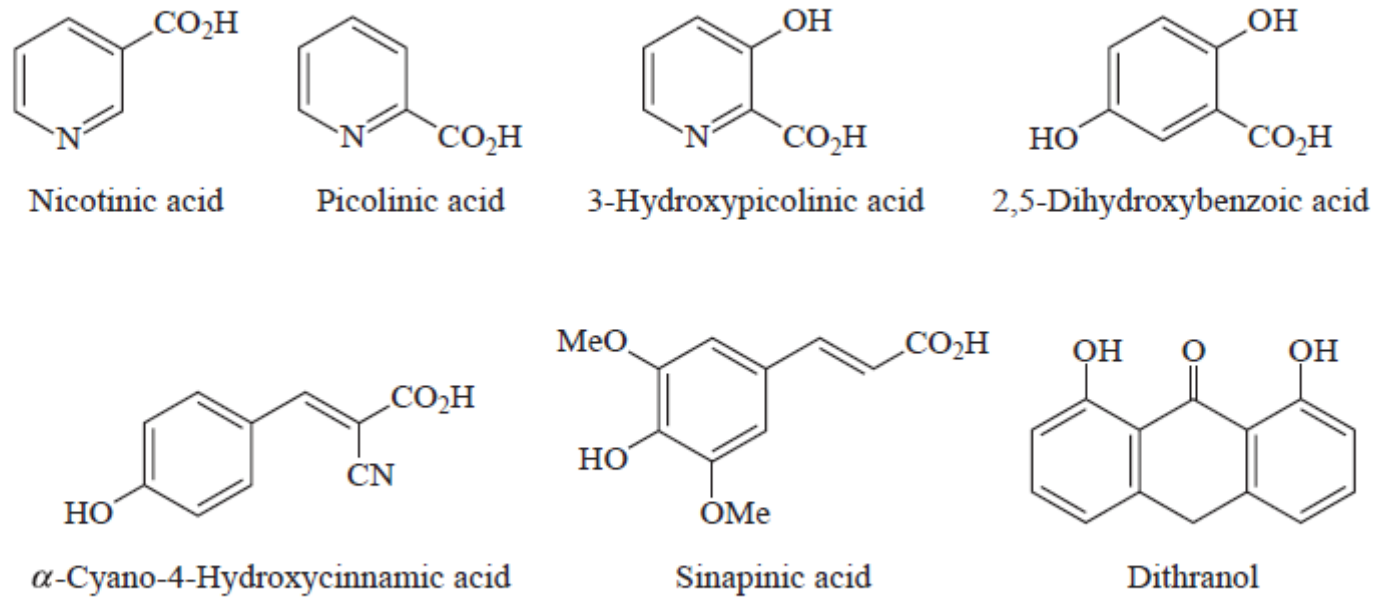

FIGURE 8.7 Common matrices for MALDI applications.

ESI (Electrospray ionization)

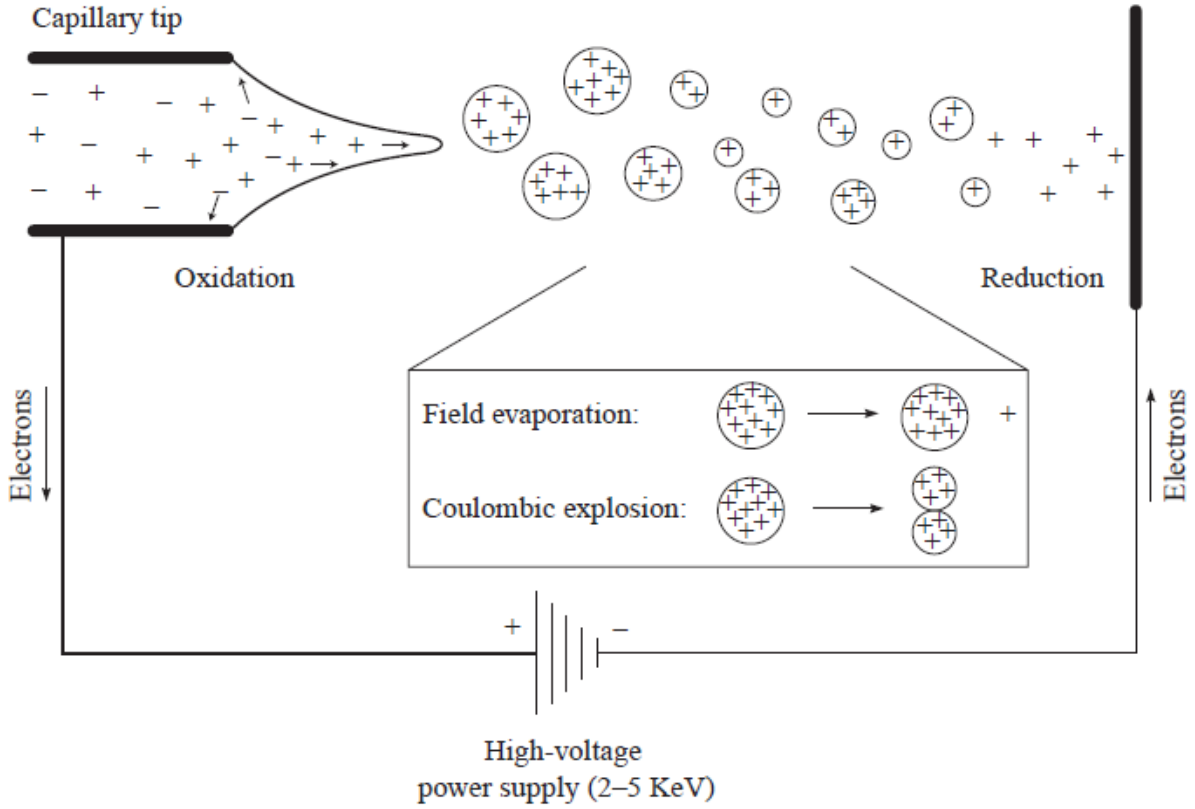

FIGURE 8.8 Schematic representation of electrospray ionization (ESI) showing both field evaporation and coulombic explosion. (From Gross, J. H., *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004. Reprinted by permission.)

1. More useful technique for studying high molecular weight biomolecules and other labile or nonvolatile compounds is **electrospray ionization (ESI) and its cousin thermospray ionization** (**TSI).**
2. **In ESI, a solution containing the sample molecules is sprayed out the end of a fine capillary into** a heated chamber that is at nearly atmospheric pressure.
3. The capillary through which the sample solution passes has a high voltage potential across its surface, and small, charged droplets are expelled into the ionization chamber.
4. The charged droplets are subjected to a counterflow of a drying gas (usually nitrogen) that evaporates solvent molecules from the droplets. Thus, the charge density of each droplet increases until the electrostatic repulsive forces exceed the surface tension of the droplet (the Rayleigh limit), at which point the droplets break apart into smaller droplets. This process continues until solvent-free sample ions are left in the gas phase (Fig. 8.8).
5. TSI occurs by a similar mechanism but relies on a heated capillary rather than one with an electrostatic potential to initially form the charged droplets.