Chapter 5
Basic Mass Spectrometry

5.1 Introduction and History

The earliest forms of mass spectrometry go back to the observation of canal rays by Goldstein in 1886 and again by Wien in 1899. Thompson's later discovery of the electron also used one of the simplest mass spectrometers to bend the path of the cathode rays (electrons) and determine their charge to mass ratio. Later, in 1928, the first isotopic measurements were made by Aston. These basic experiments and instruments were presented to most readers in first-year general chemistry. More modern aspects of mass spectrometry are attributed to Arthur Jeffrey Dempster and F.W. Aston in 1918 and 1919. Since this time there has been a flurry of activity [not only concerning minor advances in components of mass spectrometers such as different types of instrument interfaces (direct injection, GC, and HPLC)] to different ionization sources (electron and chemical ionization) but also new types of ion separators. For example, double focusing magnetic sector mass filters were developed by Mattauch and Herzog in 1934 (and recently revised into a new type of mass filter), time of flight MS by Stephens in 1946, ion cyclotron resonance MS by Hipple and Thomas in 1949, quadrupole MS by Steinwedel in 1953, and ion trap MS by Paul and Dehmelt in the 1960s.

Mass spectrometry was first coupled with GC as a means of sample introduction in 1956 by Golhke et al. and with HPLC via electro-spray ionization in the mid 1980s (Blakely and Vestal, 1983; Yamashita and Fenn, 1984). New methods of mass spectrometry are constantly under development and even as recent as 1985, Hillenkamp and Michael Karas developed the MALDI technique (a laser-based sample introduction device) that radically advanced the analysis of protein structures and more types of mass analyzers will certainly be developed. This chapter will deal only with basic mass spectrometer instruments

used in the analysis of organic chemicals exiting GC and HPLC systems, and is also applicable to effluents from ion chromatographic systems. One of the most comprehensive Internet summaries of the history of mass spectrometry can be found at http://masspec.scripps.edu/mshistory/timeline/timeline.php.

5.2 Sample Introduction from GC and Analyte Ionization

The purpose of coupling GC with MS is to provide confirmatory identification with minimal effort. Prior to the common availability of mass spectrometers, confirmatory identification was possible but required twice the effort. GC analysis alone can provide confirmatory analysis, but it is usually necessary to analyze a sample using two different columns. With capillary systems, it is possible to perform two independent analyses by installing two different capillary columns into one injector system and monitoring each column effluent with a separate detector. If the same retention time and concentration are obtained, the identity of a compound is determined and the results are considered confirmatory.

Capillary column systems are more easily interfaced with a mass spectrometer than packed columns. The high flow rate of packed columns (30 to 60 mL/min) created problems in maintaining the necessary low pressure of a mass spectrometer. On the other hand, capillary columns typically have a flow rate between 1 and 5 mL/min which has a minimal effect on the low pressure MS requirements. The GC and MS are interfaced by inserting the effluent end of the capillary column into the MS with a standard nut and ferrule system near the ionization source (Section 5.1.2a). Since GC analytes are volatile, the interface and MS must be maintained at temperatures and pressures that keep the analyte (or ionized form) in a volatile form.

As implied in the previous paragraph, mass spectrometer systems require a low operating pressure, typically 10^{-5} to 10^{-6} Torr through out the system

(ionization source, mass analyzer, and detector). This is necessary to avoid collisions between ionized molecules. If collisions are prominent, the mass resolving capabilities will be effected which decreases the detection limit and the resolution. Collisions also affect the interpretative value of the mass spectrum preventing identification.

The MS works by (1) ionizing each analyte as it exits the GC column, (2) accelerating and focusing the ionized compound and its fragments into the mass analyzer, (3) separating the fragments in the mass analyzer based on mass to charge (m/z) ratios, and (4) detecting the fragments as they exit the mass analyzer. There are a variety of ionization systems and mass analyzers that achieve these results. The following sections are dedicated to a simple description of most common ones.

5.2.1 Analyte ionization

Analytes can be introduced into the ionization zone of a MS in two states, a solid or a vapor. Solids can be introduced by depositing milligram quantities of pure analyte onto a metal probe or in a matrix that is inserted into the ionization chamber. These more direct forms of ionization do not require the interfacing of a separatory instrument such as GC or LC since relatively pure analytes are directly placed into the MS. More commonly, analytes enter the MS system in a pure form (a peak) after separation by a capillary column GC. The MALDI technique, an increasingly popular tool described below, does not neatly fit into either of these categories but is included below due to its powerful applications for biological systems. Irrespective of the samples state, analytes must be ionizated into positively charged ions, and are in some cases broken into fragments before they can be detected. Almost every compound has a unique fragmentation pattern that can subsequently be used for conclusive identification purposes. This pattern is dependent on the type of ionization source used and the stability of the energized analyte molecule. Below we will divide the

ionization techniques into those for solid, non-volatile analytes and volatile analytes entering the MS from a GC.

5.2.1.1 Ionization Techniques for Solid Non-Volatile Analytes

Field Desorption: Field Desorption (FD) techniques are relatively simple and do not require analyte separation in a GC since only one compound is introduced into the MS at a time. As noted in the heading above, compounds analyzed by this technique tend to be non-volatile, have high molecular weights, and degrade at higher temperatures. Analytes are introduced to the system on a probe made of carbon fibers that has been lightly coated with pure analyte. A high current is applied between the probe and an adjacent electrode. The current moves the ionized analyte towards the end of the carbon fibers by charge attraction, where the molecules are ionized into the vapor (plasma) phase. Then they enter the mass analyzer and then the detector. The breaking of bonds within the analyte (fragmentation) is rare in FD techniques, thus the spectrum only contains the molecular ion. Many older inexpensive bench-top systems used to come with a direct probe build into EI systems. However, this feature has been removed due to the high number of service calls to clean out the MS units when too much analyte was placed on the probe. Service technicians refer to these analyte-rich probes as having “peanut butter” placed on them.

5.2.1.2 Ionization Techniques for Volatile Analytes Entering the MS from a GC

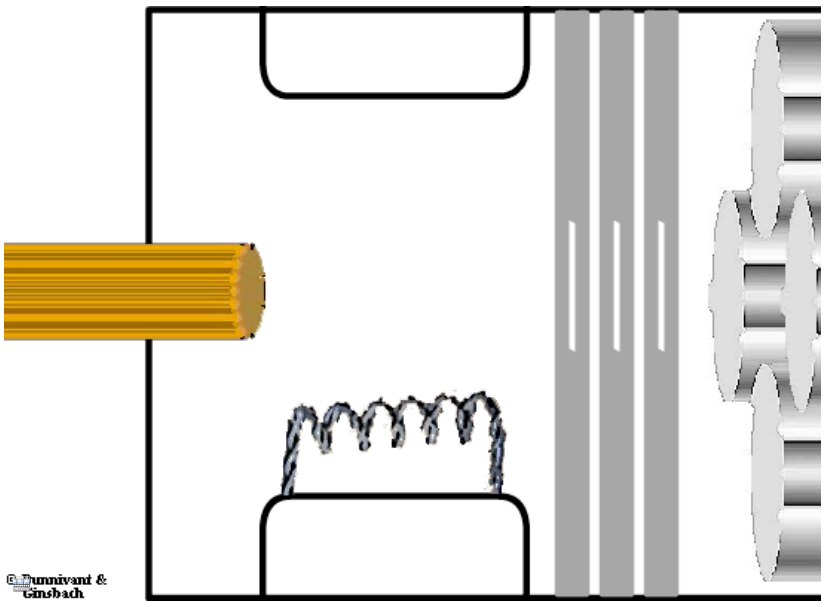
5.2.1.2a *Electron Ionization or Electron Impact (EI):* Electron ionization of analytes is referred to as a hard ionization technique since it causes bonds to be broken within a sample molecule (fragmentation). Neutral, radical, and positively charged species are produced from fragmentation. Neutral and radical species are not affected by the accelerator plates or mass analyzer and are removed by the vacuum. Positive ions are accelerated towards the mass analyzer and some either (1) collide with a surface in the source (typically the accelerator plate) or

(2) enter into the mass analyzer through the slit in the electronic lens. The ions that collide with any surface are neutralized and removed by the vacuum. The ions that enter into the mass analyzer are separated by mass to charge ratios. The high degree of fragmentation can be an advantage in compound identification. When more ion fragments are created, the more unique the fragmentation pattern, and the more confirmatory analyte identification will be. On the other hand, the detection of the molecular ion in EI can be difficult, which is often a goal of MS analysis in organic chemistry.

Electron ionization works by forcing the stream of pure analytes exiting the GC through a beam of high energy electrons in the MS. Electrons are created by heating a metal filament, usually tungsten, to a temperature high enough to expel electrons. Electrons are drawn toward an anode, passing through the stream of analyte molecules. It is important to note that electrons do not actually impact analyte molecules as implied by the name “electron impact”. The high energy of the electron (70 eV) is actually transferred to an analyte when the electronic transition of the analyte matches the frequency of the electron. The exact electron energy was selected through experimentation. It was found that a 70 eV electron energy source resulted in the most reproducible spectra and in a high degree of fragmentation. This 70 eV condition is now the standard and all computer libraries of fragmentation are based on this energy level.

The animation below shows a beam of electrons that is generated by a heated filament at the bottom of the figure that is accelerated toward the anode at the top of the figure. When different analytes (in this case butane) exit the GC column (the brown column on the left) and cross through the electron beam, an electron from the sample molecules is removed. Once the molecular ion is formed, they are forced to the right by repulsion from a positively charge accelerator plate on the left (not shown) and drawn toward the negatively charged accelerator plate to the right. Some butane molecules also fragment into smaller ions. The prevalence of this process is underestimated by the

animation due to space restraints. The molecular ion and fragments would next enter the mass analyzer (shown later).



Animation 5.1. Illustration of an Electron Impact Chamber. Go to the book's web page, download, and play An_5_1_EI_Source.mov

After the energy transfer between the electron beam and the analyte, the energy causes the molecule to become unstable and frequently cleave bonds. The fragmentation patterns are predictable because the types of bond cleavages a molecule undergoes is related to its structure (Chapter 6). The ionization rate is predicted to be between one in a thousand to one in a million of the molecules entering the ionization chamber. This level of successful ionization should be noted since MS detection limits are approximately one part per million and below (injected analyte concentration). In early systems, the instrument only ionized and detected approximately one millionth of the number of molecules that were injected; today this has been improved to about one in a thousand or more. Two examples of EI spectra are shown in Figures 5.1 and 5.2; note the extensive fragmentation of each analyte.

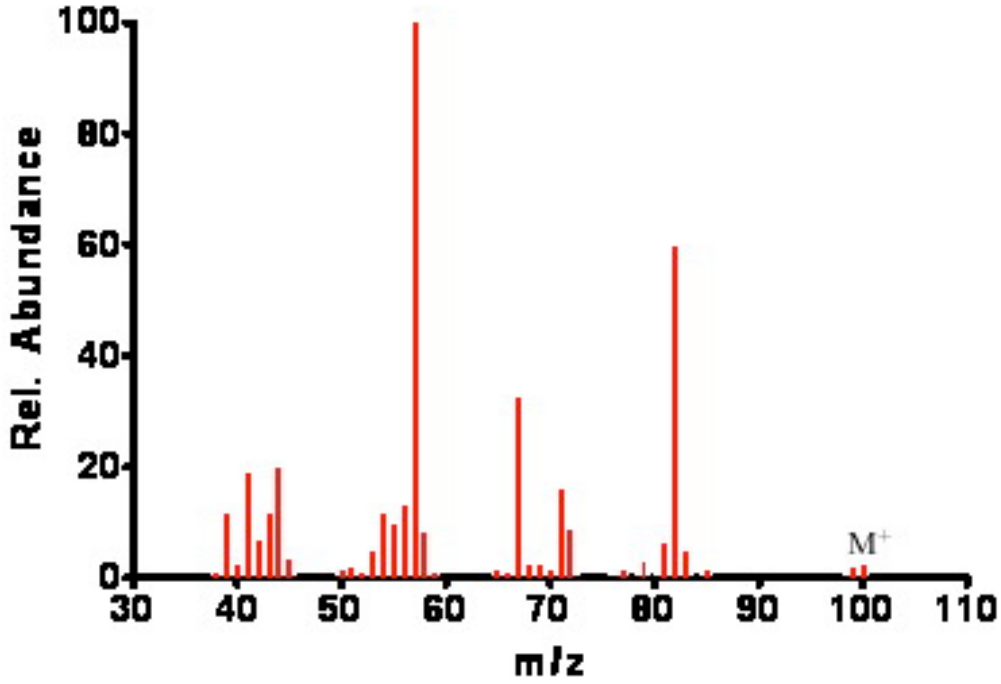


Figure 5.1. Fragmentation of Cyclohexanol by EI.

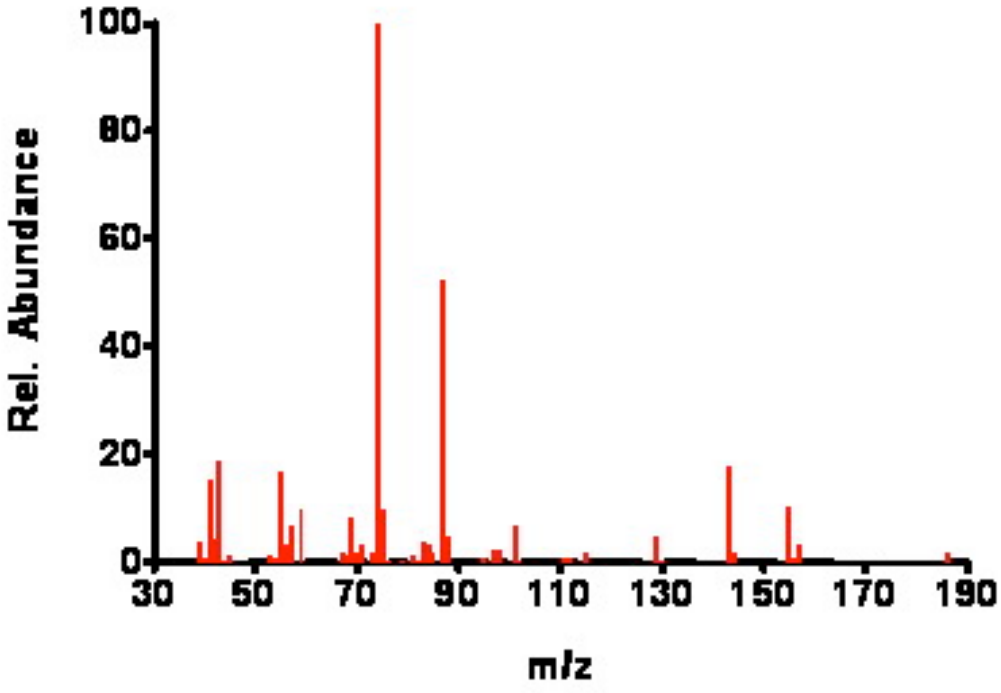


Figure 5.2. Fragmentation of Decanoic Acid Methyl Ester by EI.

5.2.1.2b *Chemical Ionization (CI):* Today, most mass spectrometers can perform both electron ionization and chemical ionization, with different interchangeable ionization units. The CI unit is less open to diffusion of the reagent gas in order to contain the reagent gas longer and promote chemical ionization. Several reagent gases are used including methane, propane, isobutane, and ammonia, with the most common being methane. CI is referred to as a soft ionization technique since less energy is transferred to the original analyte molecule, and hence, less fragmentation occurs. In fact, one of the main purposes of using CI is to observe the molecular ion, represented by $M^{\cdot+}$ or $M^{\cdot-}$, or a close adduct of it, such as MH^+, MH^{+2}, or M plus the chemical ion (i.e. $M+CH_3$ with methane as the reagent gas or $M+NH_3$ with ammonia as the reagent gas). Notice again that neutral, negative, and positive fragments are produced but only the positive fragments are of use in positive CI detection, while negative ion fragments are detected in negative CI mode.

This section will limit its discussion to CI and methane, the most common reagent gas. Methane enters the ionization chamber at about 1000 times the concentration of the analyte molecules. While the electron beam in EI is usually set at 70eV, in CI lower energy levels are used near the range of 20 to 40 eV. This energy level produces electrons that react with methane to form $CH_4^{\cdot+}$, CH_3^+, and $CH_2^{\cdot+}$. These ions rapidly react with unionized methane in the following manner:

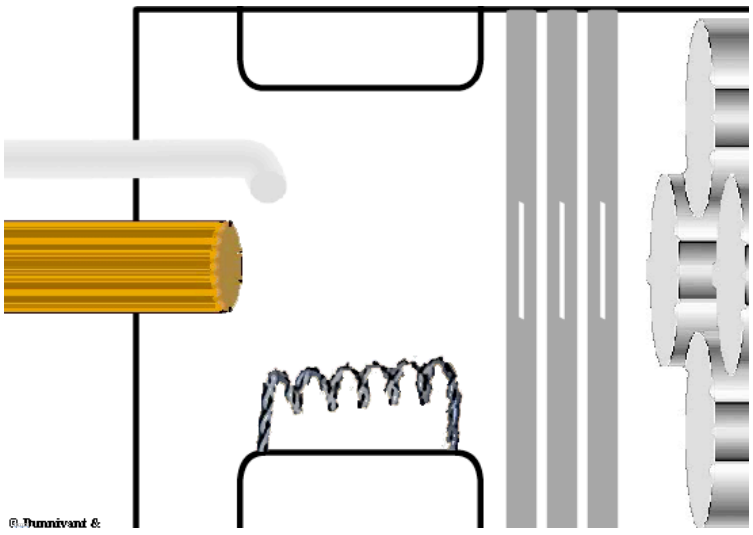
$$CH_4^+\cdot + CH_4 \rightarrow CH_5^+ + CH_3\cdot \qquad \text{Rxn 5.1}$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

The CH_5^+ and $C_2H_5^+$ ions collide with the analytes (represented by M) and form MH^+ and $(M-1)^+$ by proton and hydride transfer

$$CH_5^+ + M \rightarrow MH^+ + CH_4 \qquad \text{proton transfer}$$
$$C_2H_5^+ + M \rightarrow MH^+ + C_2H_4 \qquad \text{proton transfer} \qquad \text{Rxn 5.2}$$
$$C_2H_5^+ + M \rightarrow (M\text{-}1)^+ + C_2H_6 \qquad \text{hydride transfer}$$

Note that several types of ions can occur, $(M+1)^+$ or MH^+ from proton transfer, $(M-1)^+$ from hydride transfer, and $M+CH_3^+$ and even $M+C_2H_5^+$ from additions. By inspecting the mass spectrum for this pattern, the molecular mass of the analyte can be deduced. Similarly, if other reagent gases are used, such as propane, isobutene, and ammonia, similar proton and hydride transfer and adduct formations can occur. The usual goal of CI is to obtain a molecule weight for the molecular ion that would usually not be present in an EI spectra.

A relatively simple illustration of a CI chamber and its reactions is shown in the animation below. This animation is similar to the EI animation, but the continuous addition of a reagent gas, methane, causes the gas to be ionized by the beam of electrons. Subsequently, the ionized methane reacts with analytes exiting the GC column. Methane is preferentially ionized by the beam of electrons due to its significantly higher concentration as compared to analytes from the GC. Positively charged fragments are drawn into the focusing lens and mass analyzer by a positively charged repeller plate (not shown) and the negatively charged accelerator plate.



Animation 5.2. Illustration of a CI Chamber and Reagent Gas-Analyte Reactions. Go to the book's web page, download, and play An_5_2_CI_Source.mov

Chemical ionization is most commonly used to create positive ions, but some analytes, such as those containing acidic groups or electronegative elements (i.e. chlorinated hydrocarbons) will also produce negative ions that can be detected by reversing the polarity on the accelerator and detector systems. Some of these analytes produce superior detection limits with CI as opposed to EI, while others only give increased sensitivity (slope of the response to concentration line). Negative ions are produced by the capture of thermal electrons (relatively slower electrons with less energy than those common in the electron beam) by the analyte molecule. Thermal electrons are present from the low energy end of the distribution of electrons produced by the lower-energy CI source (~20 eV as opposed to 70 eV in EI). These low energy electrons arise mostly from the chemical ionization process but also from analyte/electron collisions. Analyte molecules react with thermal electrons in the following manner, where R-R' is the unreacted analyte molecule and R represents an organic group.

$$R\text{-}R' + e^- \rightarrow R\text{-}R'^{-\bullet} \quad \text{(by associative resonance capture)} \qquad \text{Rxn 5.3}$$

$$R\text{-}R' + e^- \rightarrow R^\bullet + R'^- \quad \text{(by dissociative resonance capture)}$$

$$R\text{-}R' + e^- \rightarrow R^+ + R'^- + e^- \quad \text{(by ion pair production)}$$

The identification of negative ion fragmentation patterns of analytes can be used in the same manner as in EI or positive ion CI. But note that extensive fragmentation libraries exist only for 70eV electron ionization (EI). Many analysts create their own reference libraries with the analysis of reference materials that

will later be used for the identification of unknown analytes extracted from samples.

Figures 5.3 and 5.4 contain CI spectra for the same compounds analyzed by EI in Figure 5.1 and 5.2, respectively. Note the obvious lack of fragmentation with the CI source and the presence of molecular ions in the CI spectra.

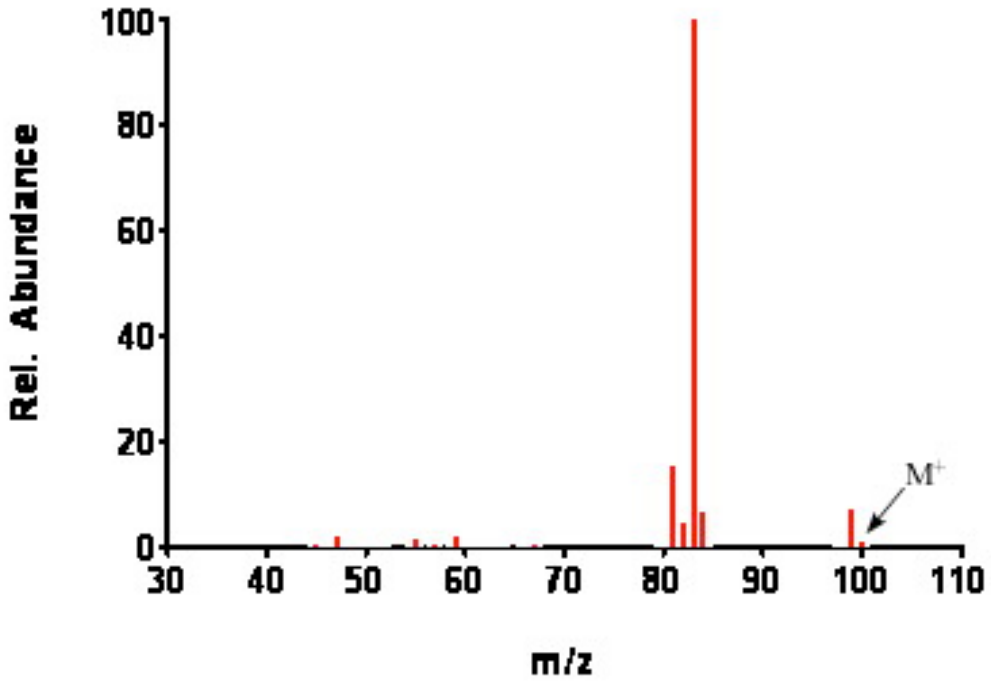


Figure 5.3. Fragmentation of Cyclohexanol by CI.

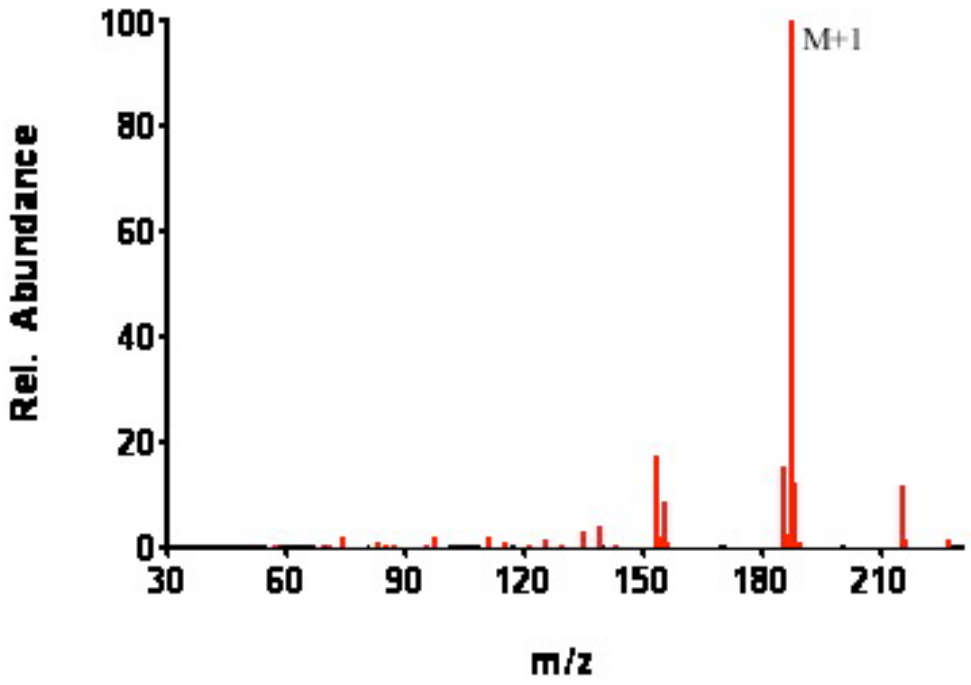


Figure 5.4. Fragmentation of Decanoic Acid Methyl Ester by CI.

To summarize, for GC-MS systems, individual analytes exit the GC column, are ionized, and fragmented using electron or chemical impact (ionization). Since the detector in a MS is universal (responds to any positively charged ion) it is necessary to separate the molecular ion and its fragments by their mass or mass to charge ratio. This process is completed in a mass analyzer, which is explained in the section below. But first, some mass analyzers require the beam of ion fragments to be focused and all require the ion fragments to be accelerated in a linear direction.

5.2.1.3 Repulsion and Accelerator Plates, Slits, and Electronic Focusing Lens:

Ions, regardless of the way they are generated, need to be accelerated into the mass filter/analyzer in order to separate ions of different masses. Since the majority of the ionization sources produce positively charge species, the most common way of accelerating ions is to place a positively charged plate on the

“upstream” side of the system. This plate repels the cations toward the mass filter/analyzer. Most systems require ions to have a minimum velocity, so negatively charged plates are placed on the “downstream” side of the instrument, just prior to the mass filter, to accelerate the ion in that direction (shown earlier in the EI and CI animations). The accelerator plates also act as slits since a relatively small hole is present in the middle of the plates that allow some of the ions to pass through the plate/slit and into the mass filter.

Accelerator plates/slits can also act as “gates” to the mass filter. This is accomplished by placing a positive charge on the slit that will repel the entry of an ion fragment or packet of ions to the system. Gates are used to hold up the entry of new ions to the mass filter until all of the ions have passed through to the detector. After this, the polarity on the gate is returned to negative and a new set of ion fragments is allowed to enter the mass filter. This type of gating system is important in the time-of-flight mass filters discussed in Section 5.5.4.

Some systems, especially the quadrupole mass filter require the stream of ions to be focused into a narrow point in order to allow successful mass to charge separation. One such electrical lens is the Einzel lens that is analogous to a focusing lens in an optical spectrophotometer. Figure 5.5 illustrates how an Einzel lens works. Six plates are in parallel, three on each side, and are exposed to the potentials shown below. These potentials set up a set of electrical field lines that act to bend the ions near the outside of the plates toward the center. Ions are focused to a small point for entry into the mass filter. The series of lenses stretch the length of a given beam of ions since ions on the outside (near the plates) have to travel a longer distance to reach the focal point.

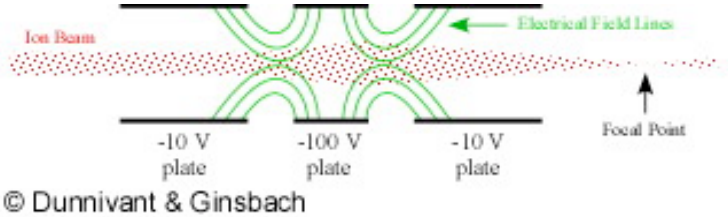


Figure 5.5. An Einzel Lens (Electronically Focusing Lens).

The Einzel lens above is shown and explained as six horizontal plates. In practice, Einzel lens are vertical plates with a hole in each plate. Thus, the applied electrical potential creates three-dimensional field lines that focuses the ion beam to a point where the entrance slit/hole to the next component is located.

Electrostatic, magnetic, and time-of-flight instruments have only repulsion and accelerator plates. In addition to these plates, quadrupole instruments have a focusing lens to help introduce the ions towards the center of the mass filter/analyzer.

5.3 The Introduction of Samples from HPLC

At this point it is noteworthy to recall the differences between GC and LC. Chapter 2 defined GC as a technique applicable to relatively volatile, thermally stable compounds. These restrictions greatly limited the types and number of compounds that could be analyzed by GC, and GC-MS. LC, discussed in Chapter 3, uses a mobile phase in the analysis of many of the compounds analyzed by GC, and also can be used to analyze the plethora of biomolecules that are non-volatile and thermally unstable at even slightly elevated temperatures. While the conditions used in LC greatly extends the applications of chromatography, it has historically suffered difficulties with mass spectrometry interfaces. Most of the various forms of LC, especially HPLC types discussed in Chapter 3, can be interfaced with MS today.

The largest difficulties in interfacing LC with MS is the removal of the mobile phase solvent prior to introduction to the MS mass analyzer and the transfer and ionization of nonvolatile analyte molecules into the gas phase. The first attempt at an LC-MS interface was to place the effluent droplets from the LC onto a supposed chemical resistant conveyer belt that transported the liquid into the MS ionization chamber. The conveyer belt was then cleaned and returned to

the HPLC effluent for more sample. However, these early attempts resulted in inefficient removal of the analytes from the conveyer belt and analyte residue being left on and released from the belt during subsequent MS runs. This problem was significantly compounded with 4.5 mm diameter HPLC columns with flow rates in the range of 1 mL/min. The later use of 300 to 75 mm long capillary columns improved flow rate problems. The invention of Electro Spray Ionization (ESI) solved all of the major problems associated with sample introduction to MS. ESI was first conceived in the 1960s by Malcolm Dole at Northwestern University, but it was not put into practice until the early 1980s by John B. Fenn of Yale University (and resulted in his Noble prize in 2002). Its common use today has been one of the most important advances in HPLC and today allows routine identification of biological macromolecules.

5.3.1 Electro-Spray Ionization (ESI) Sample Introduction

Today, the most common form of LS-MS interface is the ESI sample introduction system. An overview of this system is shown in Figure 5.6. Samples can be introduced via a syringe or an HPLC system (convention or capillary column type). A restriction in the syringe needle or HPLC column causes the solvent containing the analytes to form droplets. An electrical potential, discussed in the next paragraph, is placed between the sample inlet and the first cone. This cone separates the sample introduction from the vacuum chamber in the MS. For high flow HPLC applications N_2 gas is used to evaporate the solvent or mobile phase and de-solvate the analyte molecules. This is usually unnecessary for capillary columns or nano- applications. After desolvation and charge formation occur, as discussed below, the charged molecules enter a slightly heated transfer capillary tube and pass through two more cones that are used to control the vacuum. Finally, the positively charged ions enter a mass analyzer such as the quadrupole shown in Figure 5.6.

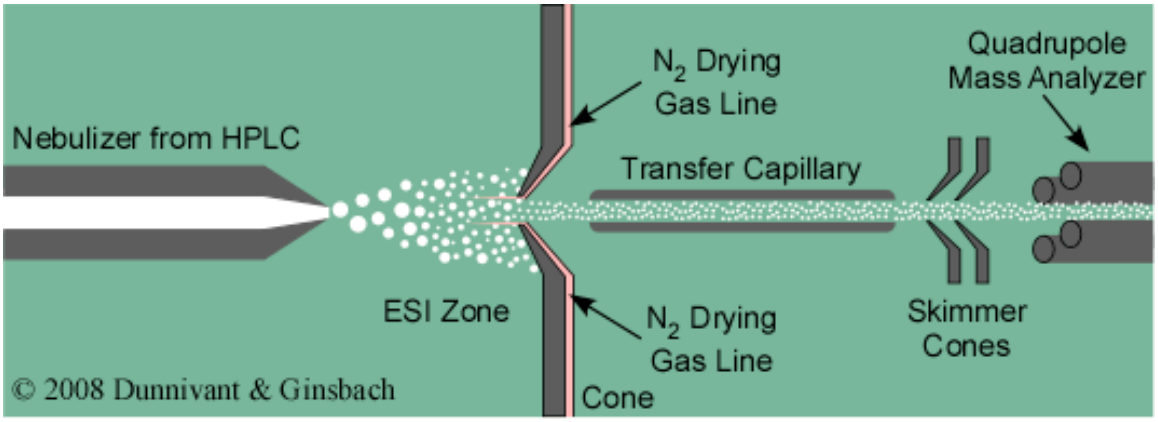


Figure 5.6 a) Overview of an Electro Spray Ionization (LC-MS) Interface. One of the most important advancements in LC-MS interfaces in the last 10 years has been the replacement of the transfer capillary with an ion funnel. The ion funnel allows more carrier gas to be removed and ions to be focused to enter the mass spectrometer. The new result is a ten fold improvement in detection limits.

The heart of ESI is the desolvation and charge formation shown in Figure 5.7. "Ionization" in ESI is referred to as a soft ionization and is really not ionization but charge formation since no real ionization source is present. Charge formation occurs by evaporating the solvent by passing a dry gas counter current to the movement of droplets. While at the same time the droplets are passed along a charged field (from 2.5 to 4 kV) between the tip of the sample introduction point and the first cone. Charge formation occurs by one of two proposed mechanisms, (1) Ion Evaporation Model where the droplet reaches a certain radius such that the field strength at the surface of the droplet becomes large enough to assist the field desorption of solvated ions and (2) Charged Residue Model where electrospray droplets undergo evaporation and fission cycles, resulting in gas-phase ions that form after the remaining solvent molecules evaporate.

The Charged Residue Model is the most accepted theory and is explained in the following. As the droplets pass from left to right, desolvation occurs in the

present of the dry N_2 gas. At the same time, the charged field results in the collection of a positive charge on the droplet. As this process continues, from left to right, the droplet shrinks until it reaches a point where the surface tension can no longer sustain the charge accumulation, this point is referred to as the Rayleigh limit. Above the Rayleigh limit, Rayleigh fission (also known as Coulombic explosion) occurs and the droplet is ripped apart forming smaller charged droplets containing the analyte molecules. This process continues until desolvation is complete and the charge is transferred to the ionized and now gaseous analyte molecule. The resulting charged molecules can be singly or multiply charged (refer to Figure 5.7). The positively charged ions enter the mass analyzer. Simple molecules result in a single mass to charge ion while complex molecules result in a Gaussian distribution of mass to charge ions yielding a single molecule molecular mass for identification purposes. As noted above, the ionization process is considered to be a soft ionization, thus, if structural identification is required the parent ion is usually analyzed by tandem MS where it is fragmented into smaller fragments for identification. Nano-spray versions of this process have recently become available.

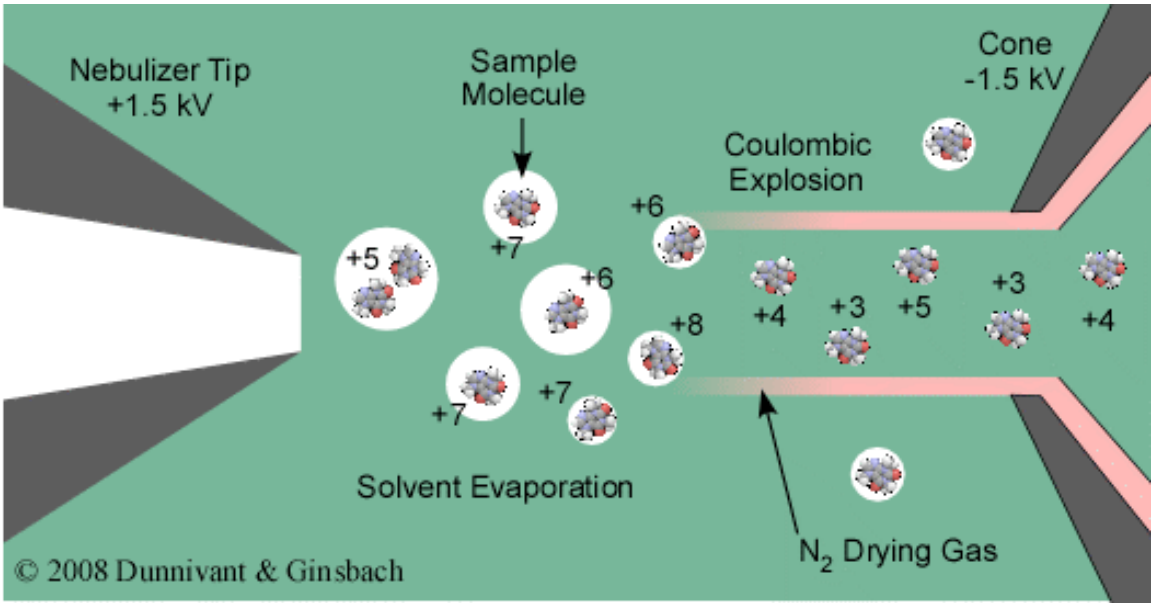


Figure 5.7 Charge Formation in ESI.

5.3.2 *Matrix Assisted Laser Desorption/Ionization, MALDI:* The MALDI technique has revolutionized the analysis of large molecular weight non-volatile compounds, especially synthetic polymers and biopolymers with molecular weights up to 300 000 Daltons. Unlike the Field Desorption technique that desorb and ionize pure analyte from a probe, MALDI volatilizes a mixture of a matrix and analyte in order to "transport" the non-volatile analyte into a vapor phase.

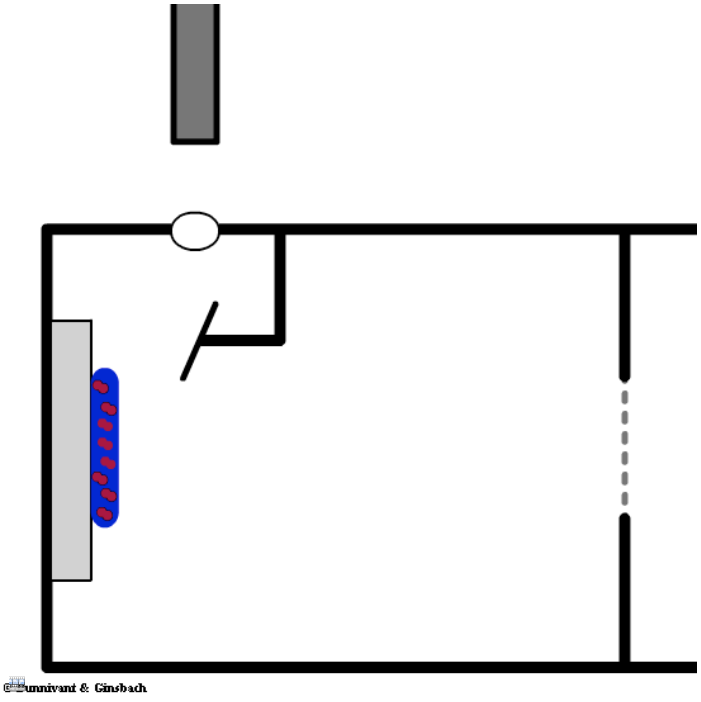
The MALDI technique is completed in two steps. First, a solution of solvent, analyte, and matrix compound are thoroughly mixed and placed on a disk to dry. As the solvent evaporates crystals of matrix containing evenly dispersed analyte molecules are formed. For the second step, the coated disk is placed in the vacuum chamber of the MS. Then the disk is repeatedly pulsed with a laser in the UV or visible spectrum depending on the matrix (Table 1.2). During each laser pulse, the matrix molecules are rapidly volatilized (sublimated/abulated) and carry the individual analyte molecules into a low pressure plasma. The wavelength of the laser is selected to heat and volatize the matrix and to avoid significant heat or degrade the analyte molecules. Analyte molecules are mostly ionized in the vapor phase by photoionization, excited-state proton transfer, ion-molecule reactions, desorption of preformed ions and most commonly by gas-phase proton transfer in the expanding plume by photoionized matrix molecules.

After the analyte molecules are ionized (to cations) they are drawn toward the negative accelerator plate and into the mass filter. A time-of-flight mass filter is always used because of its rapid scanning abilities and large mass range. The introduction of ions into the flight tube is controlled so that all ions reach the detector before the next group enters into the TOF tube. This requires carefully spacing the laser pulses and electric gates (discussed in Section 5.5.4). The spectrum of the analysis is considerably "clean" since only pure analyte is introduced into the MS and essentially no fragmentation occurs (matrix

molecules/ions can be ignored by the mass filter due to their relatively low mass). Ionized analytes can acquire +1, +2, and +3 charges and multiple molecules can form dimer and trimer peaks (combined fragments of two or three molecular ions), so the confirmational molecular weights can easily be determined. A very simple illustration of a MALDI-Time-of-Flight MS (the most common combination) is shown in Animation 5.3.

Table 5.1. Frequently Used Matrix Compounds

Matrix Compound	**Active Wavelength (nm)**
Nicotinic acid	220-290
Benzoic acid derivatives such as Vanillic acid	266
Pyrazine-carboxylic acid	266
3-Aminopyrazine-2-carboxlic acid	337
Cinnamic acid derivatives such as Caffeic acid	266-355
3-Nitrobenzylalcohol	266



Animation 5.3. Illustration of a MALDI-TOF MS System. Go to the book's web page, download, and play An_5_3_MALDI.mov

5.3.3 Proton Transfer Reaction Ionization (PTR): PTR is a relatively recent addition to mass spectrometry (1995) that was originally developed for GC and LC, there is no reason that it can not be used for CE. It was developed at the Institut für Ionenphysik at the Leopold-Franzens University in Innsbruck, Austria by Hansel et al. (1995). As shown in Figure 5.8, the PTR consists of a reaction chamber where water vapor is ionized to gas phase ions by hollow cathode discharge via the following reactions

$$e^- + H_2O \rightarrow H_2O^+ + 2\,e^- \qquad \textit{Rxn 5.4}$$
$$e^- + H_2O \rightarrow H_2^+ + O + 2\,e^-$$
$$e^- + H_2O \rightarrow H^+ + OH + 2\,e^-$$
$$e^- + H_2O \rightarrow O+ + H_2 + 2\,e^-$$

These products undergo ion-water vapor reactions in a short drift tube to form

$$H_2^+ + H_2O \rightarrow H_2O^+ + H_2 \qquad \textit{Rxn 5.5}$$
$$H+ + H_2O \rightarrow H_2O^+ + H$$
$$O+ + H_2O \rightarrow H_2O^+ + O$$
$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH$$

The hydronium ion (H_3O^+) is end product and the primary reacting ion that ionizes organic analytes in the reaction drift tube via the reaction

$$H_3O^+ + R \rightarrow RH^+ + H_2O \qquad \textit{Rxn 5.6}$$

Unlike in TOF or ion mobility MS, reaction ions are not subjected to a electrical potential in the drift tube but are moved through the system by placing a low pressure vacuum pump at the interface of the PRT drift tube and the inlet to the mass filter (refer to Figure 5.8). Analyte cations created in the drift tube enter a mass filter where they are separated by the operating parameters of each mass filter and are detected with an electron mulitiplier.

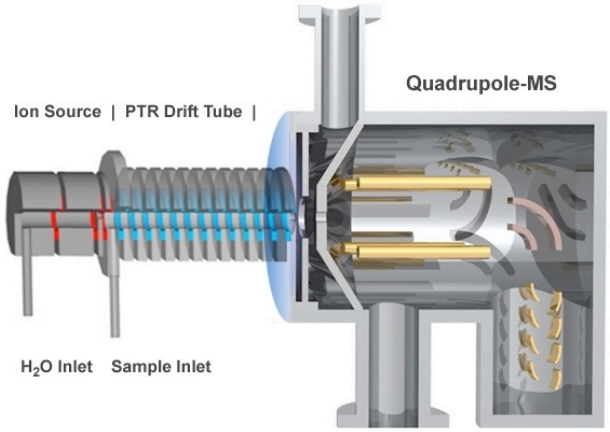


Figure 5.8 Illustration of a Proton Transfer Reaction – MS System. Reprinted with permission from Ionicon Analytik Gesellschaft, Innsbruck, Austria.

A PTR-MS is illustrated via the link in Animation 5.4. Illustration of a Proton Transfer Reaction—Mass Spectrometer

http://www.uibk.ac.at/ionen-angewandte-physik/umwelt/research/pics/animation.gif

Advantages of the PTR-MS include (1) low fragmentation with allows improved detection limits due to the formation of more molecular ions, (2) direct sampling of atmospheric gases (no sample preparation), (3) real time measurements, (4) high mobility due to the lack of gas cylinders, relative ease of

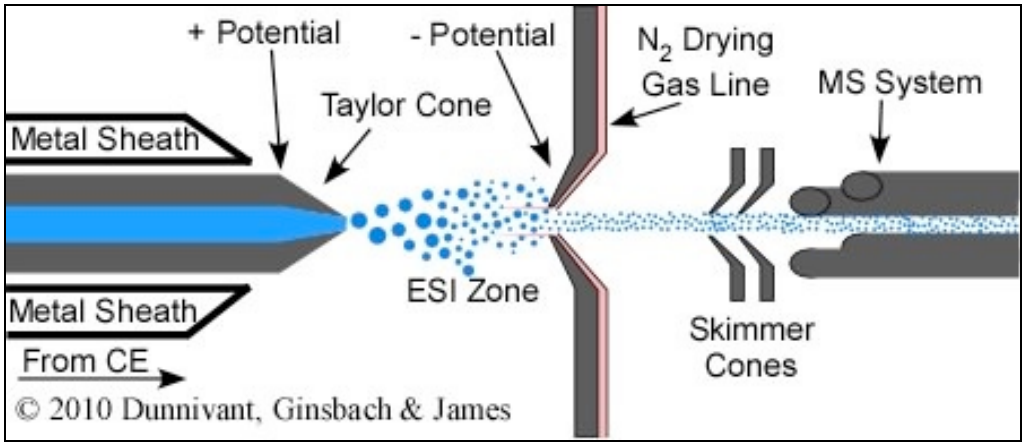
operation only requiring electrical power and distilled water, and part per billion detection limits.

5.3.4 Fast Ion Bombardment (FAB): Another technique for ionizing large bio-molecules (up to and greater then 10,000 Daltons) is to bombard them with ions of argon or xenon; this is also referred to as a liquid secondary ion source. First, analytes are embedded in a matrix such as glycerol, thioglycerol, m-nitrobenzyl alcohol, crown ethers, sulfolane, 20-nitrophenyloctyl ether, diethanolamine or triethanolamine. An electronic impact (EI) source similar to that described in the GC ionization section is used to ionize Ar or Xe gas at a pressure of 10^{-5} torr. Ar and Xe ions are accelerated towards the matrix containing the analytes and their impact sputters off positive and negative analytes ions (mostly molecular ions) that enter a mass spectrometer for mass determination.

5.4 The Introduction of Samples from a Capillary Electrophoresis System

Years ago, if you wanted to own a CE-MS system you had to purchase the CE and MS separately and hire the MS manufacturer or vender to interface the two instruments. Recently (~2008) you are now able to purchase off-the-shelf interfaced instruments from chromatography vendors. CE-MS interfaces are designed and operate in much the same way as the HPLC-MS interface, with two exceptions. While HPLC columns can be composed of metal that readily conduct the electrical potential to ionize the analytes, the CE columns are only composed of fused silica. As a result the effluent of the CE column must be coated with a conducting metal sheath. Also, as you will recall from Chapter 4 on CE, minimal solvent flow results in CE, only from the dragging of solvent by the electrophoretic mobility of the buffer ions. Thus, CE is almost ideal for MS interfaces and is far superior to HPLC interfacing since very little solvent must be removed prior to entry into the MS vacuum system. Other than these two

differences, CE-MS operates like HPLC-MS. Solvent droplets, containing analytes, are created at the end of the fused silica column, and are charged by the electrical potential placed between the metal sheath and the metal cone at the entry to the MS system (Figure 5.9). Solvent is evaporated with a drying gas that flows counter current to the movement of the solvent droplets. Charge transfer occurs through Coulombic explosion and the de-solvated and ionized anionic or cations (depending on the potential) are accelerated through the MS interface cone. CE-MS has finally reached a level of maturity and dependability that promises significant advances in many areas of analytical separation and quantification, especially protein studies.



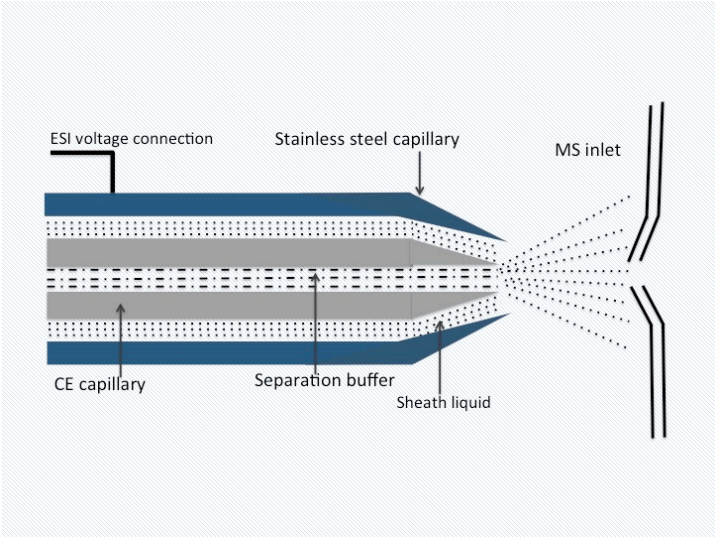


Figure 5.9. a) an older CE-MS Interface, b) a newer CE-MS Interface (credit WikiCommons, image from https://en.wikipedia.org/wiki/Capillary_electrophoresis%E2%80%93mass_spectrometry#/media/File:Sheath_Flow_Interface.jpg