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

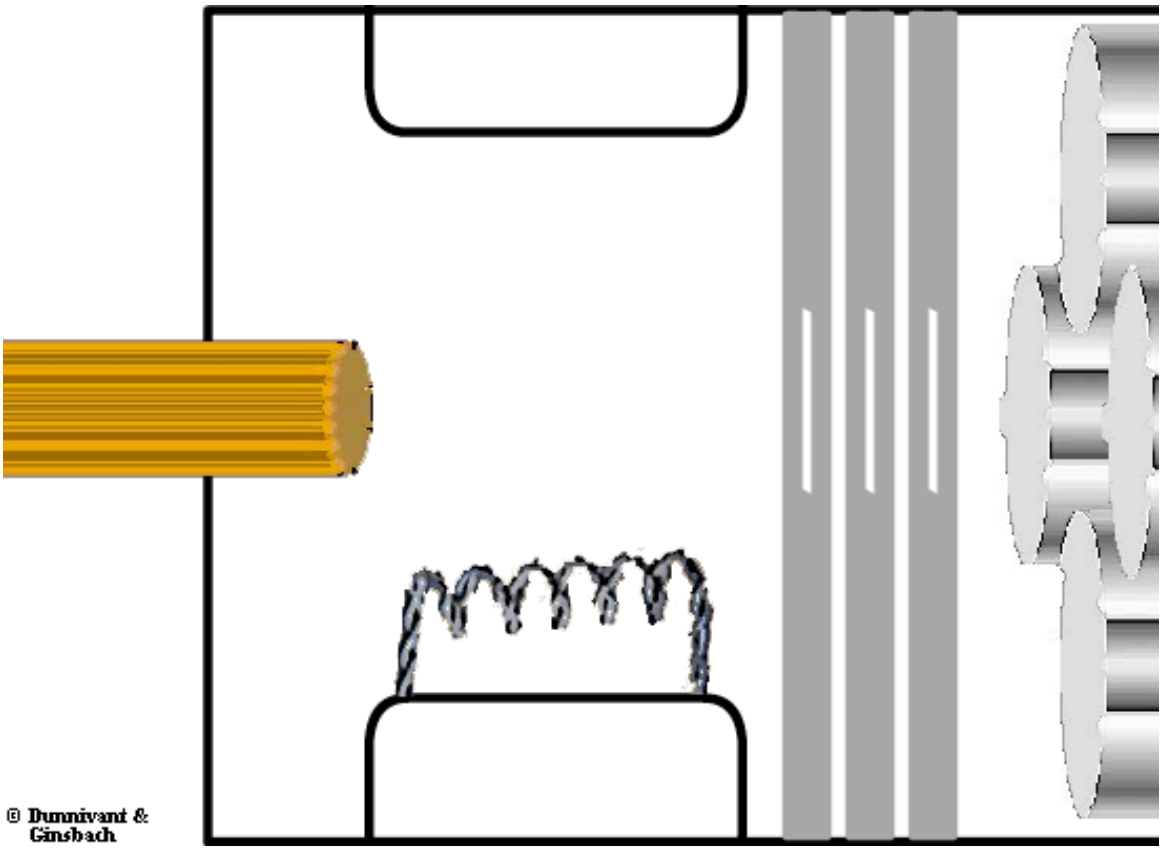
5.2.1.2a *Electron Ionization or Electron Impact (EI):* Electron ionization of analytes is referred to as a hard ionization technique since it causes bonds to be broken within a sample molecule (fragmentation). Neutral, radical, and positively charged species are produced from fragmentation. Neutral 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.

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.

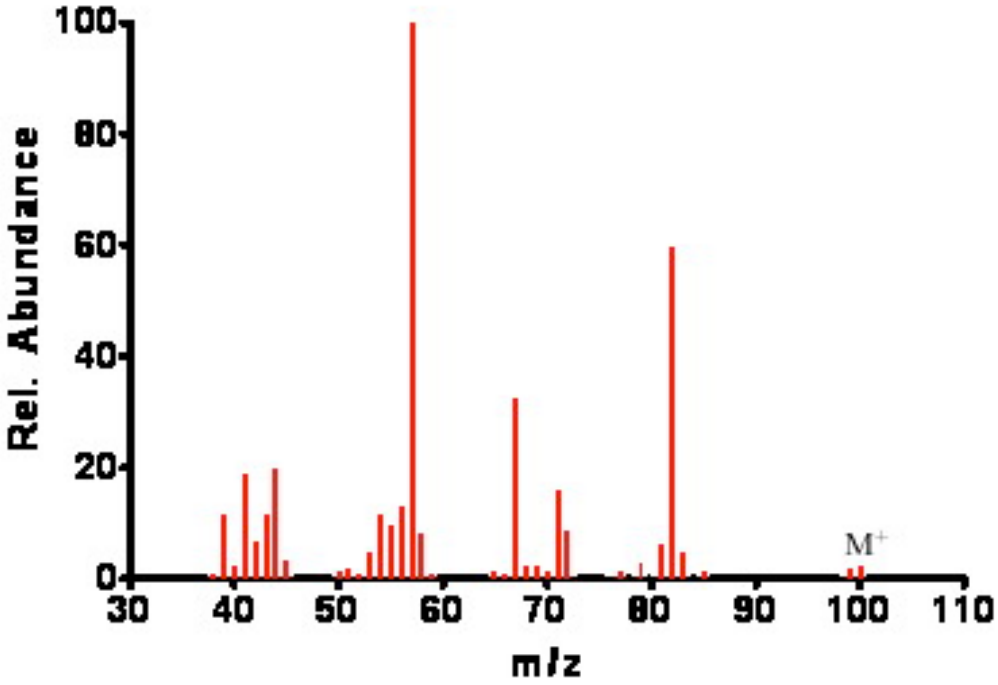


Figure 5.1. Fragmentation of Cyclohexanol by EI.

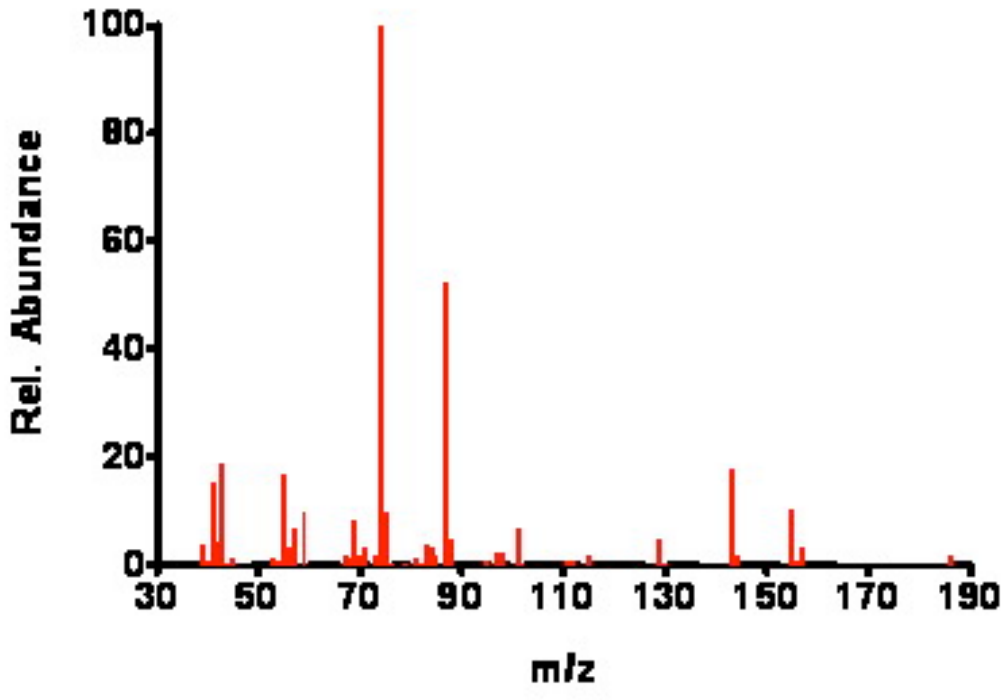


Figure 5.2. Fragmentation of Decanoic Acid Methyl Ester by EI.

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:

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

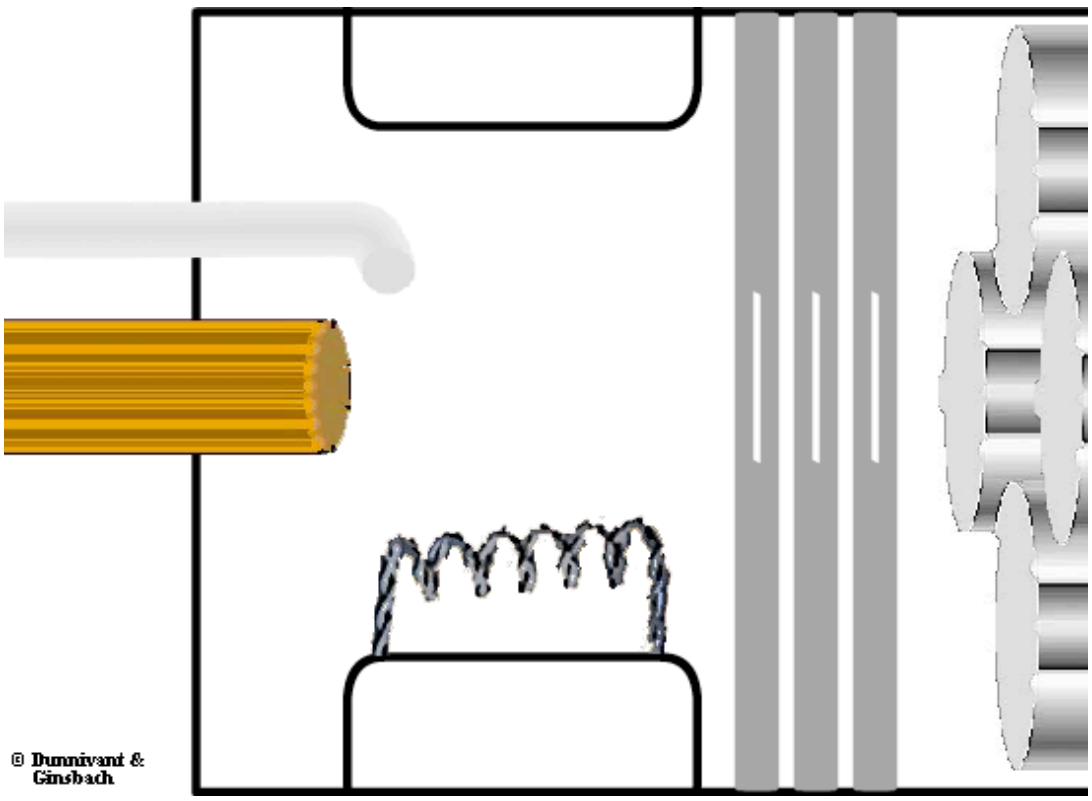
The CH_5^+ and $C_2H_5^+$ ions collide with the analytes (represented by M) and form MH^+ and $(M-1)^+$ by proton and hydride transfer

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

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.

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.

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

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

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

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.

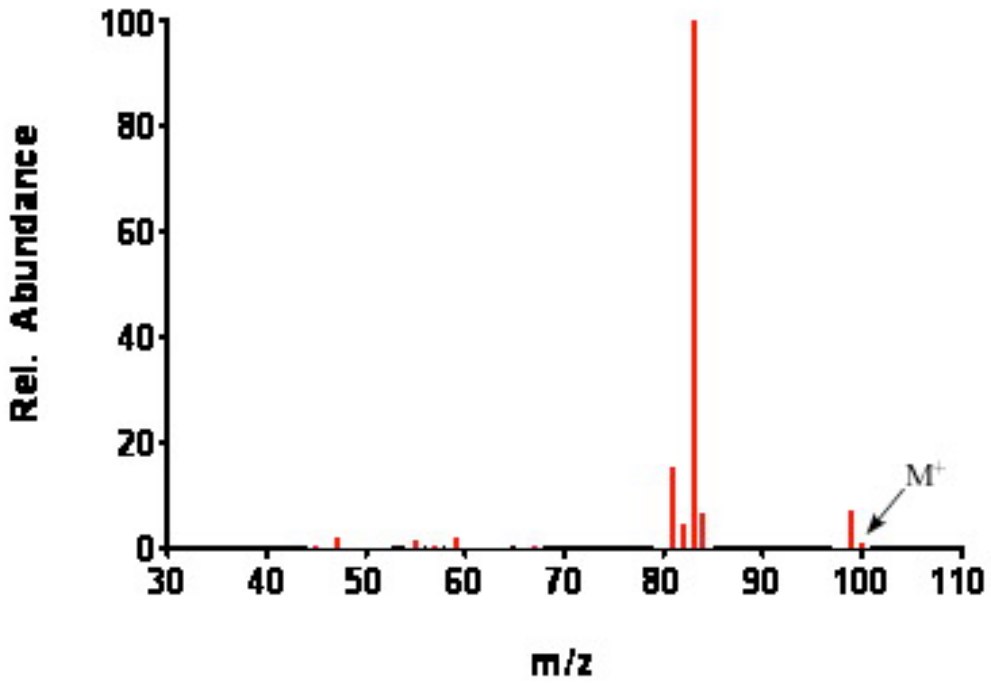


Figure 5.3. Fragmentation of Cyclohexanol by CI.

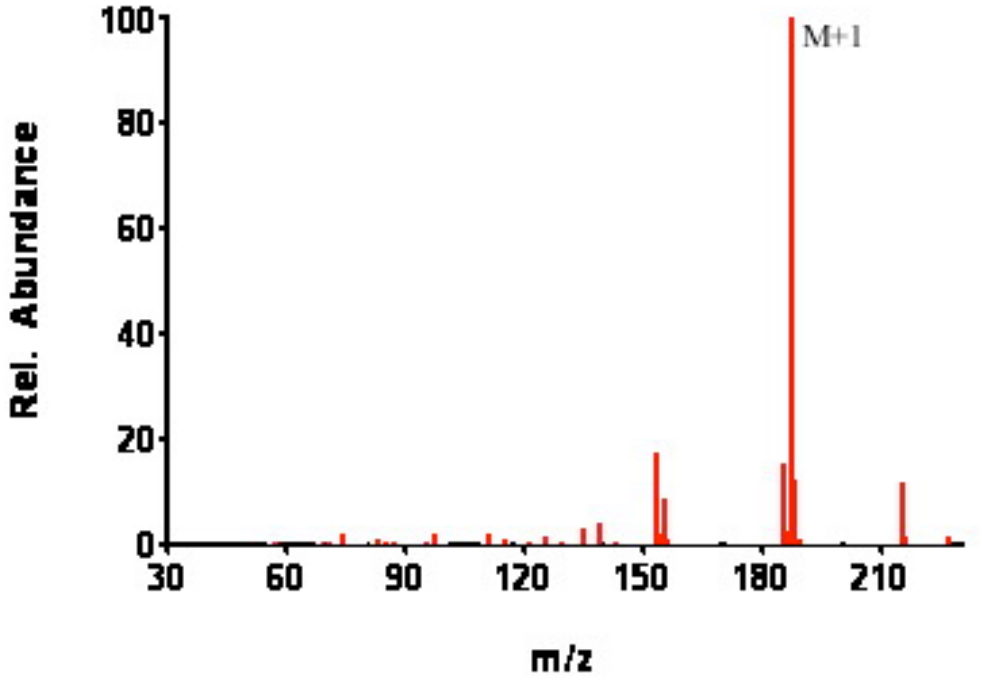


Figure 5.4. Fragmentation of Decanoic Acid Methyl Ester by CI.

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.

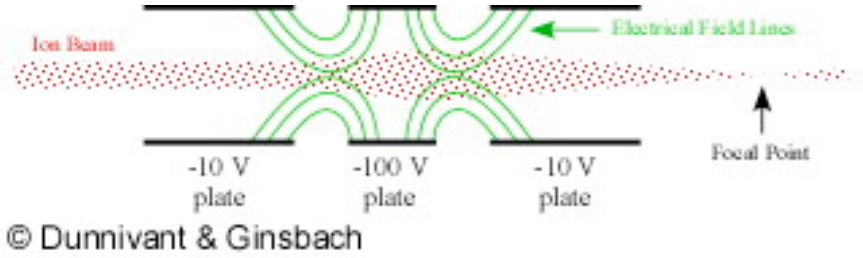


Figure 5.5. An Einzel Lens (Electronically Focusing Lens).

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.

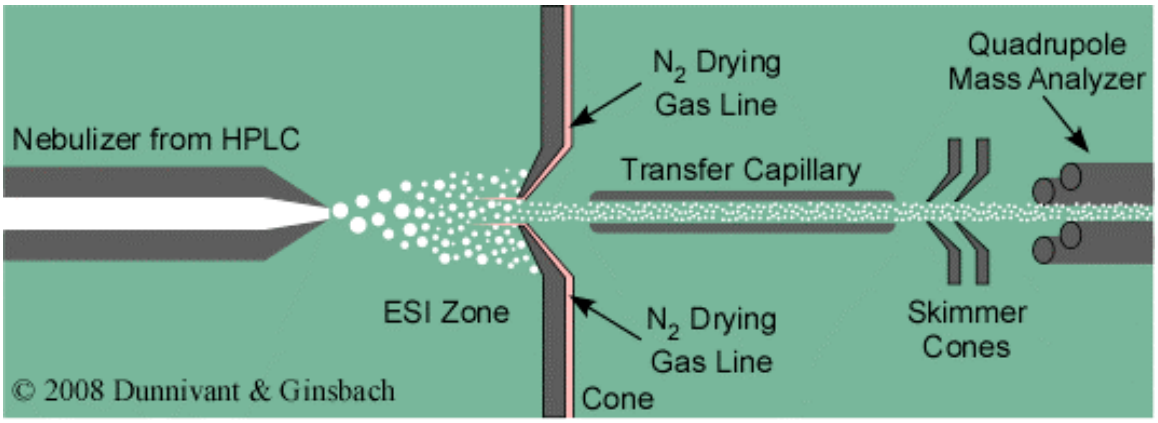


Figure 5.6 Overview of an Electro Spray Ionization (LC-MS) Interface.

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.

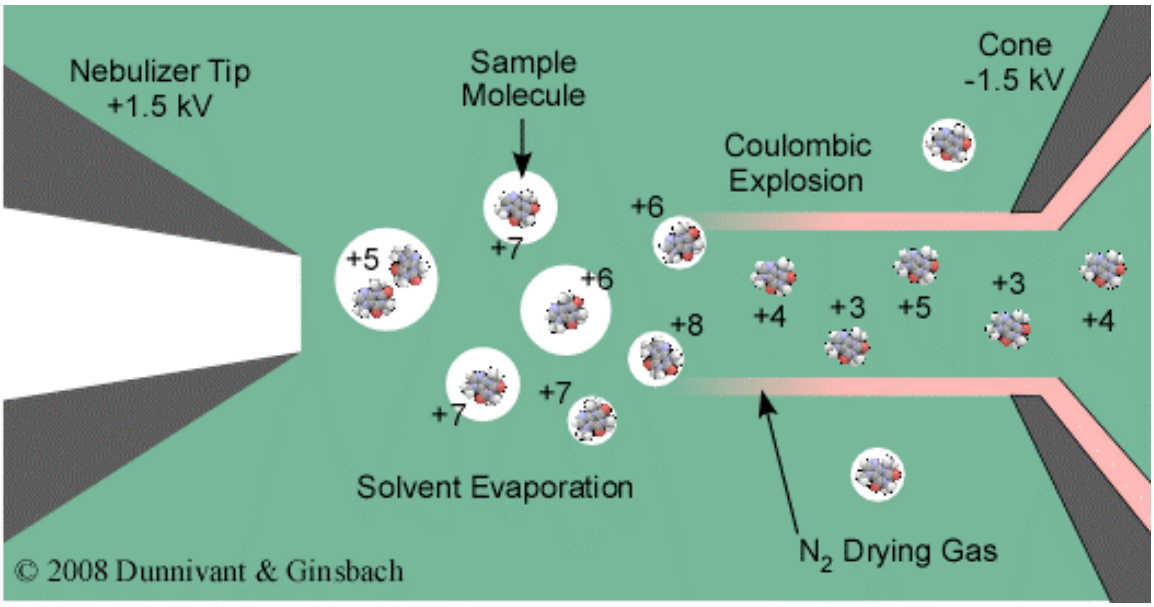


Figure 5.7 Charge Formation in ESI.

5.3.2 (NEW) Atmospheric Pressure Chemical Ionization

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

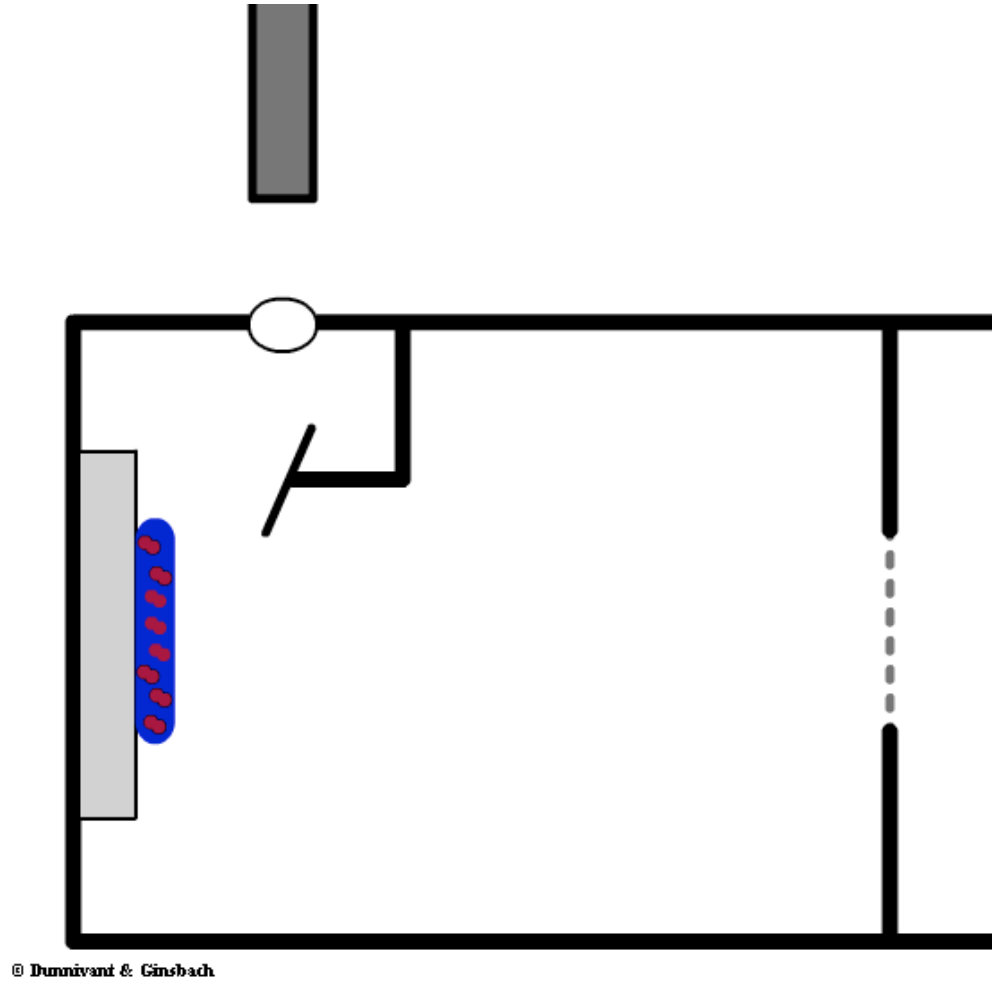
(sublimated/abulated) and carry the individual analyte molecules into a low pressure plasma. The wavelength of the laser is selected to heat and volatize the matrix and to avoid significant heat or degrade the analyte molecules. Analyte molecules are mostly ionized in the vapor phase by photoionization, 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.

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 not 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

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

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

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

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

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

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.

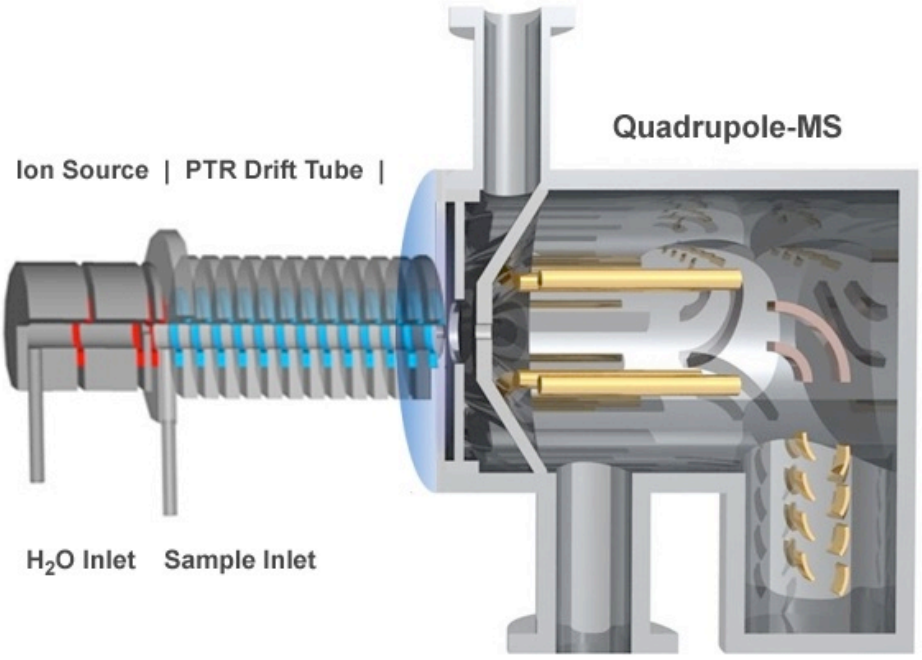


Figure 5.8 Illustration of a Proton Transfer Reaction – MS System. Reprinted with permission from Ionicon Analytik Gesellschaft, Innsbruck, Austria.

A PTR-MS is illustrated via the link in Animation 5.4.

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

Animation 5.4. Illustration of a Proton Transfer Reaction—Mass Spectrometer.

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, ss 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.

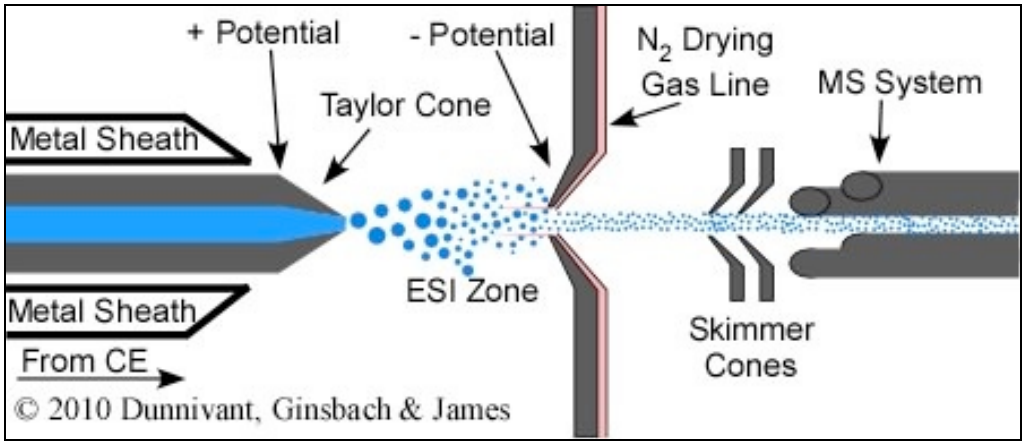


Figure 5.9. CE-MS Interface.

5.5 Common Mass Filters (Mass Analyzers)

Mass analyzers separate the molecular ion and its fragments by ion velocity, mass, or mass to charge ratio. A number of mass filters/analyzers are available for GC, LC and CE interfaces, but not all are commercially available. These can be used individually or coupled in a series of mass analyzers to improve mass resolution and provide more conclusive analyte identification. This text will only discuss the most common ones.

The measure of "power" of a mass analyzer is resolution, the ratio of the average mass (m) of the two adjacent peaks being separated to the mass difference (Δm) of the adjacent peaks, represented by

$$Rs = m/\Delta m$$

Resolution (R_S) is achieved when the midpoint between two adjacent peaks is within 10 percent of the baseline just before and after the peaks of interest (the valley between the two peaks). Resolution requirements can range from high resolution instruments that may require discrimination of a few ten thousands (1/10 000) of a gram molecular weight (0.0001) to low resolution instruments that only require unit resolution (28 versus 29 Daltons). Resolution values for commonly available instruments can range from 500 to 500 000.

Before introducing the various types of mass analyzers, remember our current location of the mass analyzer in the overall MS system. The analyte has been ionized, underwent fragmentation, been accelerated, and in some cases focused to a focal point with a velocity towards the mass analyzer. Now the packet of ion fragments needs to be separated based on their momentum, kinetic energy, or mass-to-charge ratio (m/z). Often the terms mass filter and mass analyzer are used interchangeable, as is done in this text. But, first a controversy in the literature needed to be addressed with respect to how a mass filter actually separates ion fragments.

Some resources state that all mass analyzers separate ions with respect to their mass to charge ratio while others are more specific and contend that only quadrupoles separate ions by mass to charge ratios. The disagreement in textbooks lies in what components of the MS are being discussed. If one is discussing the affect of the accelerator plates **and** the mass filter, then all mass filters separate based on mass to charge ratios. This occurs because the charge of an ion will be a factor that determines the velocity a particle of a given mass has after interacting with the accelerator plate in the electronic, magnetic sector, and time of flight mass analyzers. But after the ion has been accelerated, a magnetic section mass filter actually separates different ions based momentums and kinetic energies while the time of flight instrument separates different ions based on ion velocities (arrival times at the detector after traveling a fixed length).

In the other case, no matter what the momentum or velocity of an ion, the quadrupole mass analyzer separates different ions based solely on mass to charge ratios (or the ability of the ion to establish a stable oscillation in an oscillating electrical field). These differences may seem semantic but some MS users insist on their clarification. For the discussions below, in most cases, mass to charge will be used for all mass analyzers.

5.5.1 *Magnetic sector mass filter:* It has been known for some time that the trajectory of a point charge, in our case a positively charged molecular ion or fragment, can be altered by an electrical or magnetic field. Thus, the first MS systems employed permanent magnets or electromagnets to bend the packets of ions in a semi-circular path and separated ions based on their momentum and kinetic energy. Common angles of deflection are 60, 90, and 180 degrees. The change in trajectory of the ions is caused by the external force of the magnetic field. The magnitude of the centripetal force, which is directly related to the ions velocity, resists the magnetic field's force. Since each mass to charge ratio has a distinct kinetic energy, a given magnetic field strength will separate individual mass to charge ratios through space. A slit is placed in front of the detector to aid in the selection of a single mass to charge ratio at a time.

A relatively simple mathematical description will allow for a better understanding of the magnetic field and the ions centripetal force. First, it is necessary to compute the kinetic energy (KE) of an ion with mass *m* possessing a charge *z* as it moves through the accelerator plates. This relationship can be described by

$$KE = 1/2\, mv^2 = zeV \qquad \text{Eqn 5.1}$$

where e is the charge of an electron (1.60×10^{-19} C), v is the ion velocity, and V is the voltage between the two accelerator plates (shown in the Animation 1.5 below). Fortunately in EI and CI, most ions have a charge of +1. As a result, an

ions' kinetic energy will be inversely proportional to its mass. The two forces that determine the ion's path, the magnetic force (F_M) and the centripetal force (F_C), are described by

$$F_M = B z e v \qquad \text{Eqn 5.2}$$

and

$$F_C = (mv^2)/r \qquad \text{Eqn 5.3}$$

where B is the magnetic field strength and r is the radius of curvature of the magnetic path. In order for an ion of particular mass and charge to make it to the detector, the forces F_M and F_C must be equal. This obtains

$$B z e v = (mv^2)/r \qquad \text{Eqn 5.4}$$

which upon rearrangement yields

$$v = (V z e r) / m \qquad \text{Eqn 5.5}$$

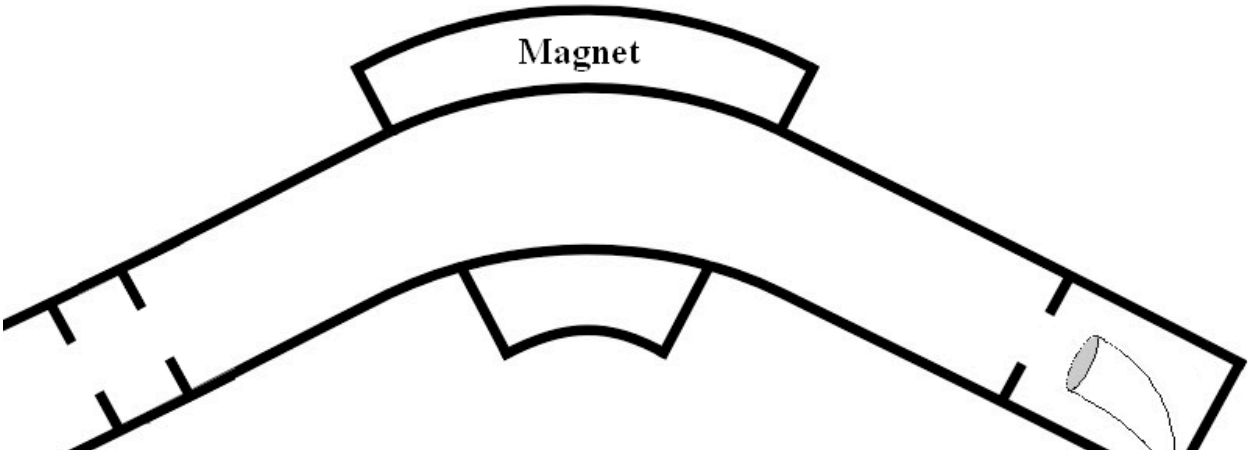
Substituting this last equation into our first KE equation yields

$$m/z = (B^2 r^2 e) / 2V \qquad \text{Eqn 5.6}$$

Since e (the charge of an electron) is constant and r (the radius of curvature) is not altered during the run, altering the magnetic field (B) or the voltage between the accelerator plates (V) will vary the mass to charge ratio that can pass through the slit and reach the detector. By holding one constant and varying the other throughout the range of m/z values, the various mass to charge ratios can be separated. One option is to vary the magnetic field strength while keeping the voltage on the accelerator plates constant.

However, it is difficult to quickly vary the magnetic field strength. The resulting slow scan rate is especially problematic with capillary column GCs since the peak width is narrow. Using a magnetic sector instrument could complicate identification of a compound if two or more peaks emerge from the GC during a single scan, especially in the relatively fast elution of peaks from a capillary column GC. Generally, several complete mass to charge scans are desired for accurate analyte identification. This can be overcome in modern magnetic sector instruments by rapidly sweeping the voltage between the accelerator plates, in order to impart different momentums on the ion fragments, as opposed to sweeping the field strength. Due to the operational advantages of this technique, most electromagnets hold the magnetic field strength (B) and vary the voltage (V) on the accelerator plates.

The magnetic sector mass filter is illustrated in Animation 5.5 below. Although B and r are normally held constant, this modern design is difficult to illustrate, so we will illustrate a magnetic sector MS where B, the magnetic field, is varied to select for different ions. As a particular peak (compound) enters the MS from a GC, it is ionized/fragmented by an EI in the animation. The ions are then uniformly accelerated by the constant voltage between the two accelerator plates/slits on the left side of the figure. As the different ions travel through the electromagnet, the magnetic field is varied to select for different m/z ratios. Ions with the same momentum or kinetic energy (and therefore mass) pass through the exit slit together and are measured by the detector, followed by the next ion, and so on.



Animation 5.5. Illustration of a Magnetic Sector MS.

While magnetic sector mass filters were once the only tool to create a mass spectrum, they are becoming less common today. This is due to the size of the instrument and its weight. As a result, many magnetic sector instruments have been replaced by quadrupole systems that are much smaller, lighter, and able to perform extremely fast scans. Magnetic sector instruments are still used in cases where extremely high resolution is required such as double-focusing instruments (Section 5.5.6).

5.5.2 *Quadrupole mass filter:* Quadrupole mass filters have become the most common type of MS used today due to their relatively small size, light weight, low cost, and rapid scan times (less than 100 ms). This type of mass filter is most commonly used in GC applications and to some extent in LC systems because they are able to operate at a relatively high pressure (5×10^{-5} torr). The quadrupole has also gained widespread use in tandem MS applications (a series of MS analyzers).

Despite the fact that quadrupoles produce the majority of mass spectra today as mentioned earlier, they are not true mass spectrometers. Actual mass spectrometers produce a distribution of ions either through time (time of flight mass spectrometer) or space (magnetic sector mass spectrometer). The quadrupole's mass resolving properties are instead a result of the ion's stability/trajectory within the oscillating electrical field.

A quadrupole system consists of four rods that are arranged an equal distance from each other in a parallel manner. Paul and Steinwegen theorized in 1953 that hyperbolic cross-sections were necessary. In practice, it has been found that circular cross sections are both effective and easier to manufacture. Each rod is less than a cm in diameter and usually less then 15 cm long. Ions are accelerated by a negative voltage plate before they enter the quadrupole and travel down the center of the rods (in the z direction). The ions' trajectory in the z direction is not altered by the quadrupole's electric field.

The various ions are separated by applying a time independent dc potential as well as a time dependent ac potential. The four rods are divided up into pairs where the diagonal rods have an identical potential. The positive dc potential is applied to the rods in the X-Z plane and the negative dc potential is applied to the rods in the Y-Z plane. The subsequent ac potential is applied to both pairs of rods but the potential on one pair is the opposite sign of the other, and is commonly referred to as being 180˚ out of phase (Figure 5.9).

Mathematically the potentials that ions are subjected to are described by the following equations:

$$\Phi_{X\text{-}Z} = +(U + V\cos\omega t)$$

and *Eqn 5.7*

$$\Phi_{Y\text{-}Z} = -(U + V\cos\omega t)$$

where Φ is the potential applied to the X-Z and Y-Z rods respectively, ω is the angular frequency (in rad/s) and is equal to 2πv where v is the radio frequency of the field, U is the dc potential and V is the zero-to-peak amplitude of the radio frequency voltage (ac potential). The positive and negative signs in the two equations reflect the change in polarity of the opposing rods (electrodes). The values of U range from 500 to 2000 volts and V in the above equation ranges from 0 to 3000 volts.

1

2

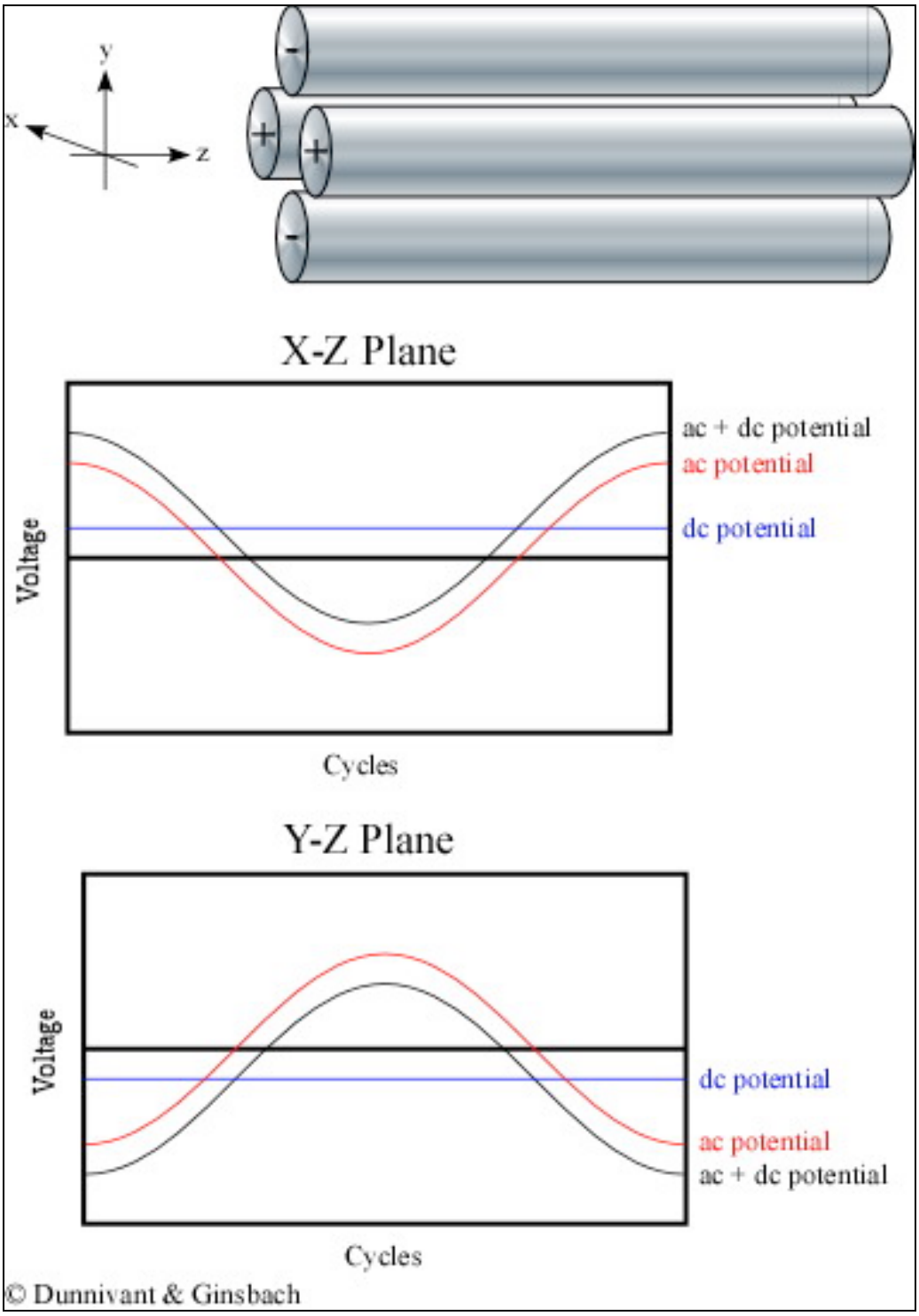


Figure 5.10 AC and DC Potentials in the Quadrupole MS.

To understand the function of each pair, consider the rods in the X-Z plane in isolation. For now, imagine that only an ac potential is applied to the rods. Half the time when the potential was positive, ions (cations) would be repelled by the rod's charge and would consequently move towards the center of the rods. Likewise, when the potential was negative, ions would accelerate towards the rods in response to an attractive force. If during the negative ac potential, an ion comes into contact with the rod, it is neutralized and is removed by the vacuum. The factors that influence whether or not a particle strikes the rod during the negative cycle include the magnitude of the potential (its amplitude), the duration of time the ions are accelerated towards the rod (the frequency of the ac potential), the mass of the particular ion, the charge of the ion, and its position within the quadrupole.

Now imagine that a positive dc potential (at a fraction of the magnitude of the ac potential) is applied to the rod in the X-Z plane. This positive dc potential alone would focus all of the ions towards the center of the pair of rods. When the ac and dc potentials are applied at the same time to the pair of rods in the X-Z plane, ions of different masses respond differently to the resulting potential. Heavy ions are largely unaffected by the alternating current and as a result respond to the average potential of the rods. This results in heavy ions being focused towards the center of the rods. Light ions, on the other hand, will respond more readily to the alternating ac current. Ions that are sufficiently light will have an unstable trajectory in the X-Z plane and will not reach the detector. Only ions heaver than a selected mass will not be filtered out by the X-Z electrodes. As a result, the X-Z plane electrodes only filter light ions and form a high pass mass filter (Figure 5.11).

Now look at the other pair of rods and the converse of the ac/dc potentials. The rods in the Y-Z plane have a negative dc voltage and the ac potential is the same magnitude but the oppose sign as the potential applied to the X-Z plane. Heavy ions are still mostly affected by the dc potential, but since it is negative,

they strike the electrode and are unable to reach the detector. The lighter ions respond to the ac potential and are focused towards the center of the quadrupole. The ac potential can be thought of as correcting the trajectories of the lighter ions, preventing them from striking the electrodes in the Y-Z plane. These electrical parameters result in the construction of a low pass mass filter.

When both the electrodes are combined into the same system, they are able to selectively allow a single mass to charge ratio to have a stable trajectory through the quadrupole. *Altering the magnitude of the ac and dc potential changes the mass to charge ratio that has a stable trajectory resulting in the construction of mass spectra.* Different ions possess a stable trajectory at different magnitudes and reach the detector at different times during a sweep of the ac/dc magnitude range. The graph of the combined effect, shown in Figure 5.10c, is actually a simplification of the actual stability diagram.

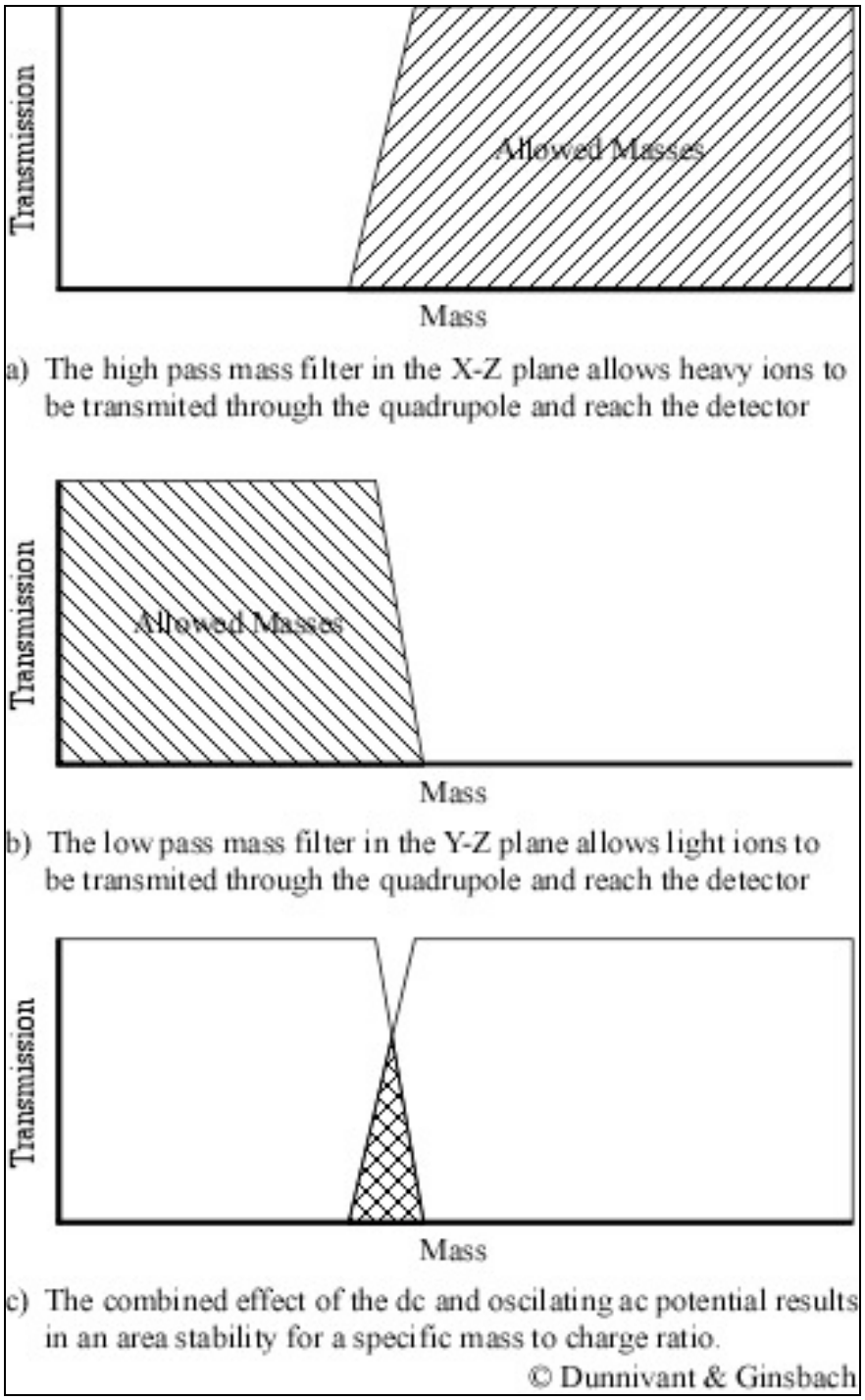


Figure 5.11 A “Conceptual” Stability Diagram

One way to generate an actual stability diagram is to perform a series of experiments where a single mass ion is introduced into the quadrupole. The dc

and ac voltages are allowed to vary and the stability of the ion is mapped. After performing a great number of experiments the resulting plot would look like Figure 5.12. The shaded area under the curve represents values of ac and dc voltages where the ion has a stable trajectory through the potential and would reach the detector. The white space outside the stability diagram indicates ac and dc voltages where the ion would not reach the detector.

While any ac and dc voltages that fall inside the stability diagram could be utilized, in practice, quadrupoles keep the ratio of the dc to ac potential constant, while the scan is performed by changing the magnitude of the ac and dc potential. The result of this is illustrated as the mass scan line intersecting the stability diagram in Figure 5.12. The graphs below the stability diagram correspond to specific points along the scan and help to illustrate the ions' trajectories in the X-Z and Y-Z plane (Figure 5.12). While the mass to charge ratio of the ion remains constant in each pair of horizontal figures, the magnitude of the applied voltages are changing while their ratio stays constant. As a result, examining points along the mass scan line in Figure 5.12 is equivalent to shifting the position of the high and low pass mass filters with respect to the x axis illustrated in Figure 5.11. Even though the mass is not changing for the stability diagram discussed here, the mass that has a stable trajectory is altered.

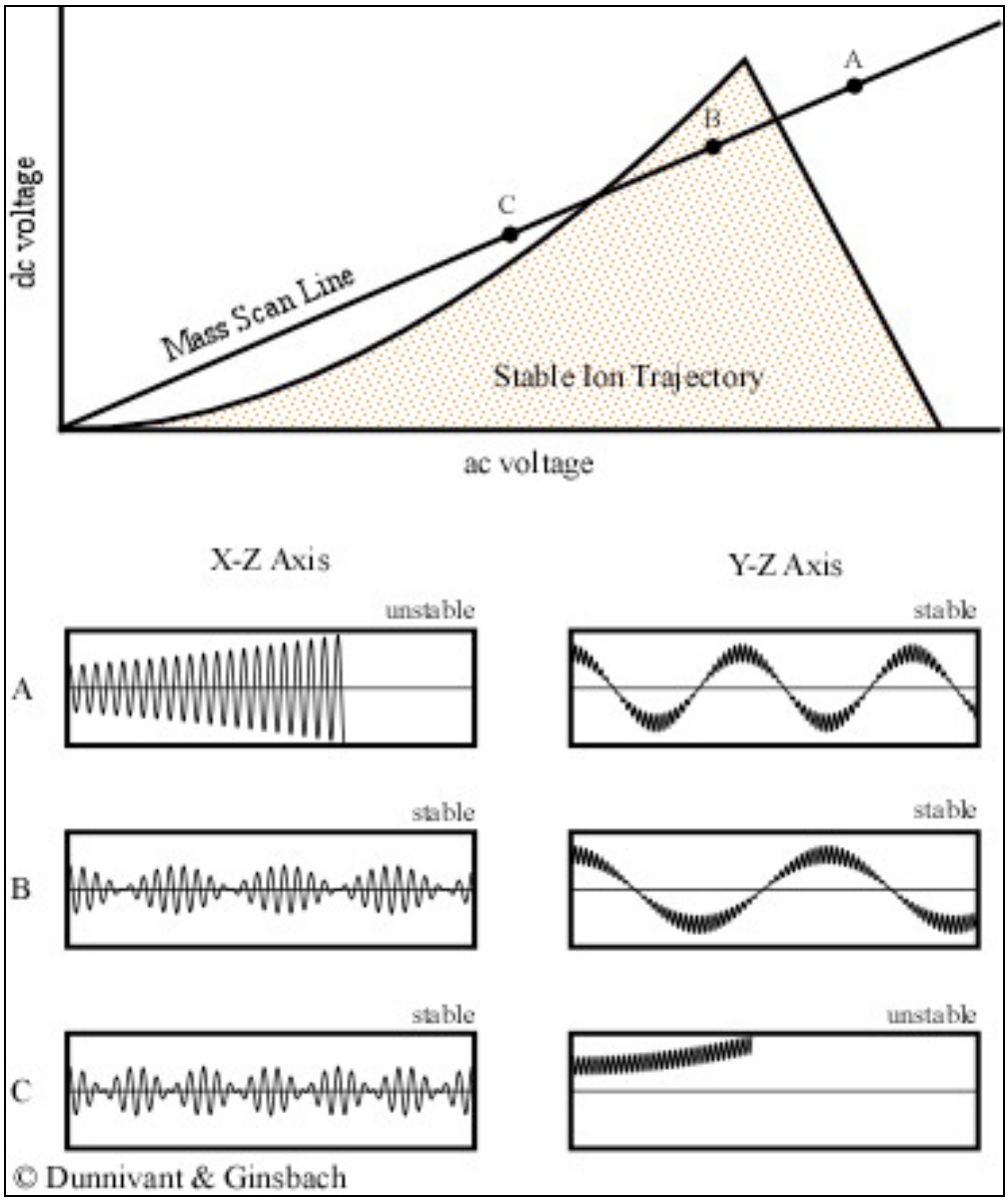


Figure 5.12 Stability Diagram for a Single Ion Mass. Used with permission from the Journal of Chemical Education, Vol. 75, No. 8, 1998, p. 1051; copyright © 1998, Division of Chemical Education, Inc.

In the above figure, the graph corresponding to point A indicates that the ion is too light to pass through the X-Z plane because of the high magnitude of the ac and dc potentials. As a result, its oscillation is unstable, and it eventually impacts the electrode/rod. The motion of the particle in the Y axis is stable because the combination of the ac potential as well as the negative dc potential

yields a stable trajectory. This is the graphical representation of the ac potential correcting the trajectory of the light ions in the Y-Z plane. At point B the magnitude of voltages has been altered so the trajectories of the ion in both the X-Z and Y-Z plane are stable and the ion successfully reaches the detector. At point C, the ion has been eliminated by the low mass pass filter. In this case, the ac potential is too low to allow the ion to pass through the detector and it strikes the rod. This is caused by the ions increased response to the negative dc potential in the Y-Z plane. The trajectory in the X-Z axis is stable since the dc potential focusing the ion towards the center of the poles overwhelms the ac potential.

Until this point, the stability diagram shown above is only applicable to a single mass. If a similar experiment were to be performed using a different mass, the positions of the ac and dc potential on the x and y axes would be altered but the overall shape of the curve would remain the same. Fortunately, there is a less time consuming way to generate the general stability diagram for a quadrupole mass filter using a force balance approach. This derivation requires a complex understanding of differential equations and is beyond the scope of an introductory text, but it can be explained graphically (Figure 5.13). The parameters in the axes are explained below the figure.

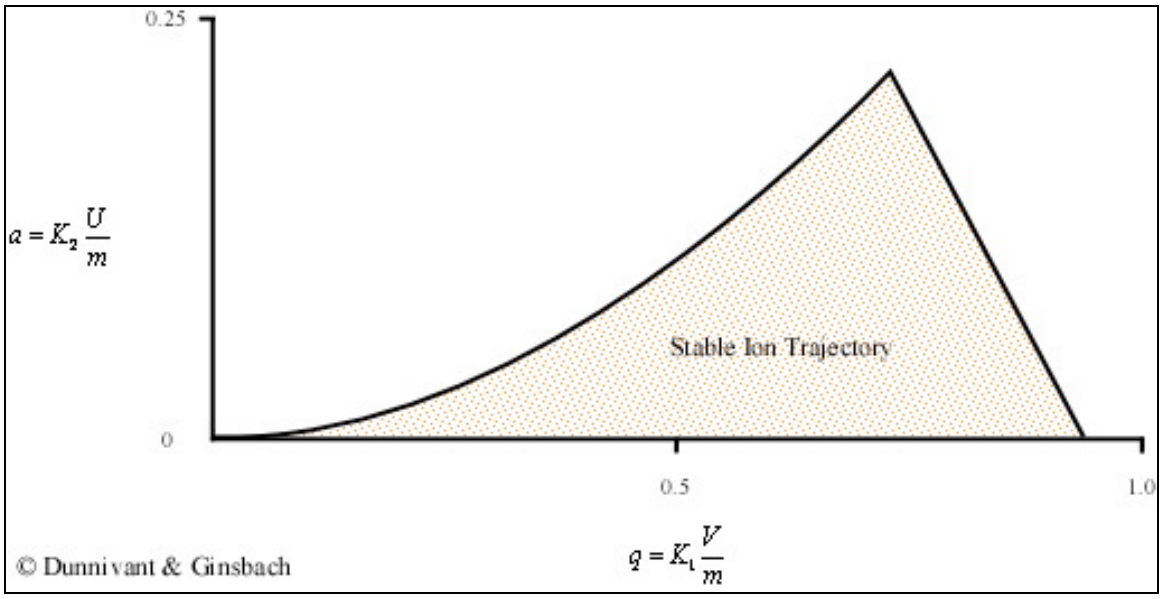


Figure 5.13 The General Stability Diagram

While this derivation is particularly complex, the physical interpretation of the result helps explain how a quadrupole is able to perform a scan. The final solution is dependent on six variables, but the simplified two-variable problem is shown above. Utilizing the reduced parameters, a and q, the problem becomes a more manageable two-dimensional problem. While the complete derivation allows researchers to perform scans in multiple ways, we will focus only on the basic mode that makes up the majority of mass spectrometers. For the majority of commercially available mass spectrometers, *the magnitude of the ac potential (V) and the dc potential (U) are the only parameters that are altered during run time* and allows a sweep of mass to charge ranges. The rest of the parameters that describe K_1 and K_2 are held constant. The values for K_1 and K_2 in the general stability diagram can be attributed to the following equations:

$$K_1 = \frac{2e}{r^2\omega^2} \qquad \textit{Eqn 5.8}$$

$$K_2 = \frac{4e}{r^2\omega^2} \qquad \textit{Eqn 5.9}$$

The parameters that make up K_1 and K_2 are exactly what we predicted when listing the variables earlier that would affect the point charge. Both K terms depend upon the charge of the ion *e*, its position within the quadrupole *r*, and the

frequency of the ac oscillation ω. These parameters can be altered, but for the majority of applications remain constant. The charge of the ion (e) can be assumed to be equivalent to positive one for almost all cases. The distance from the center of the quadrupole (r) is carefully controlled by the manufacturing process and an Einzel lens that focuses the ions into the center of the quadrupole and is also a constant. Also the angular frequency (ω) of the applied ac waveform can be assumed to be a constant for the purposes of most spectrometers and for this discussion.

The first important note for the general stability diagram is the relationship between potential and mass. The general stability diagram (Figure 5.13) is illustrated where there is an inverse relationship between the two. Figure 5.12 shows the lighter ions (m-1) are higher on the mass scan line and the heavy ions (m+1) are lower on the line. This is why in Figure 5.12 at point A, the molecule was too light for the selected frequencies, and it was too heavy at point C.

From the general stability diagram, it is also possible to explain how an instrument's resolution can be altered. The resolution is improved when the mass scan line intersects the smallest area at the top of the stability diagram (Figure 5.14). The resolution can be improved when the slope of the mass line is increased. The resolution will subsequently increase until the line no longer intersects the stability diagram. While it would be best for the line to intersect at the apex of the stability diagram, this is impractical due to fluctuations in the ac (V) and dc (U) voltage. As a result, the line intersects a little below this point allowing the quadrupole to obtain unit resolution.

Once the resolution has been determined, the ratio of the ac to dc potential is left unchanged throughout the scan process. Again, to perform a scan, the magnitude of the ac and dc voltages is altered while their ratio stays constant. This places a different mass to charge inside the stability diagram. For example, if the ac and dc voltages are doubled, the mass to charge ratio of the

selected ion would also be doubled as illustrated in the second part of Figure 5.14. By scanning throughout a voltage range, the quadrupole is able to create the majority of mass spectra produced in today's chemical laboratories. But it should be noted that quadrupole mass filters have a upper limit of approximately 650 amus.

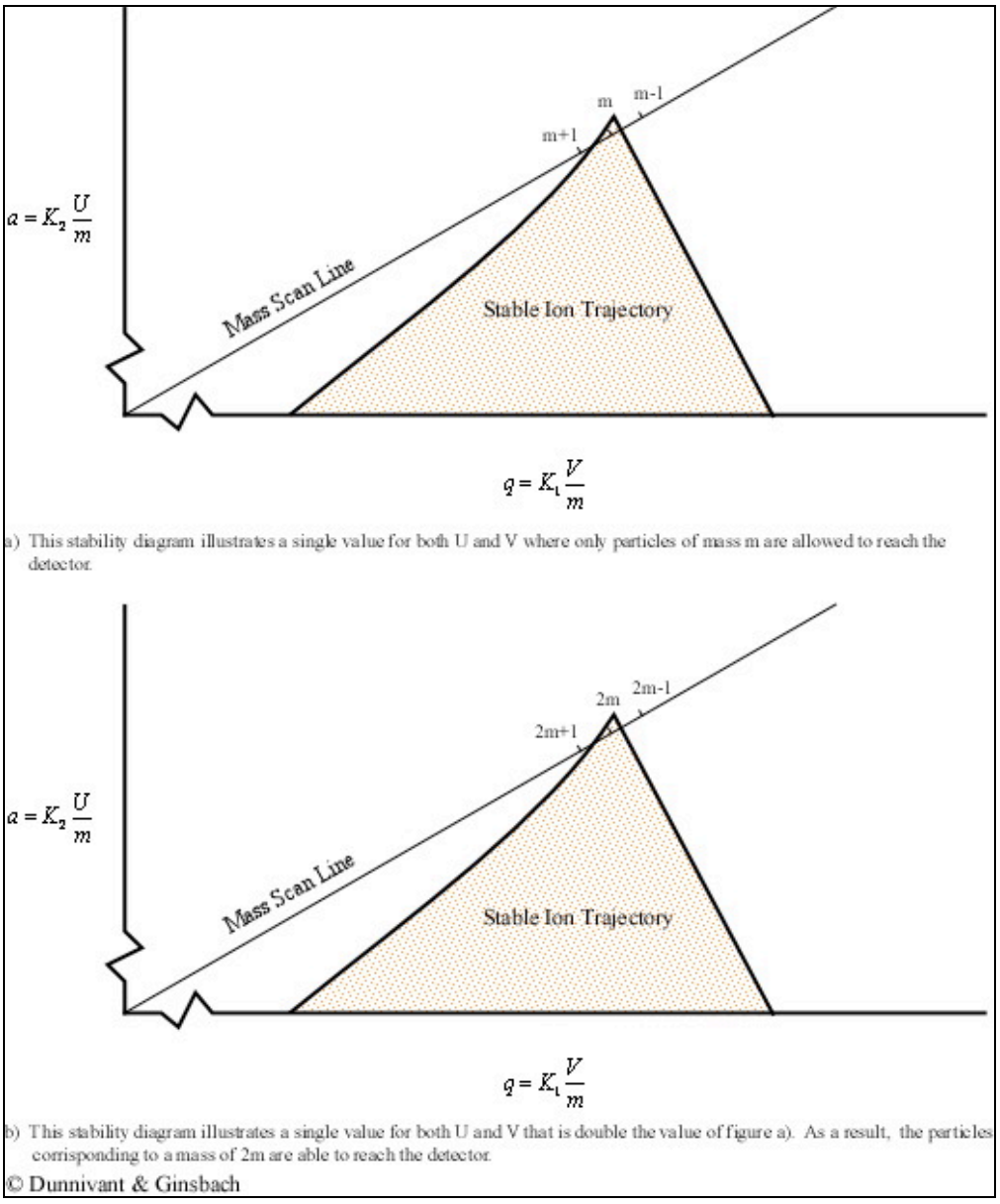


Figure 5.14 Quadrupole Mass Scan. Used with permission from the Journal of Chemical Education, Vol. 63, No. 7, 1998, p. 621; copyright © 1986, Division of Chemical Education, Inc.

Now that we have given a detailed description of the factors influencing the movement of a charged particle through the quadrupole, it is advantageous to summarize the entire process as a physicist would do in the form of a force

balance. This is the origin of the governing equation where the French scientist E. Mathieu balanced the equations for the motion of ionized particles to the potential forces (electrical potentials) encountered in a quadrupole mass analyzer. This six-parameter differential equation, known as the Mathieu equation, is represented by

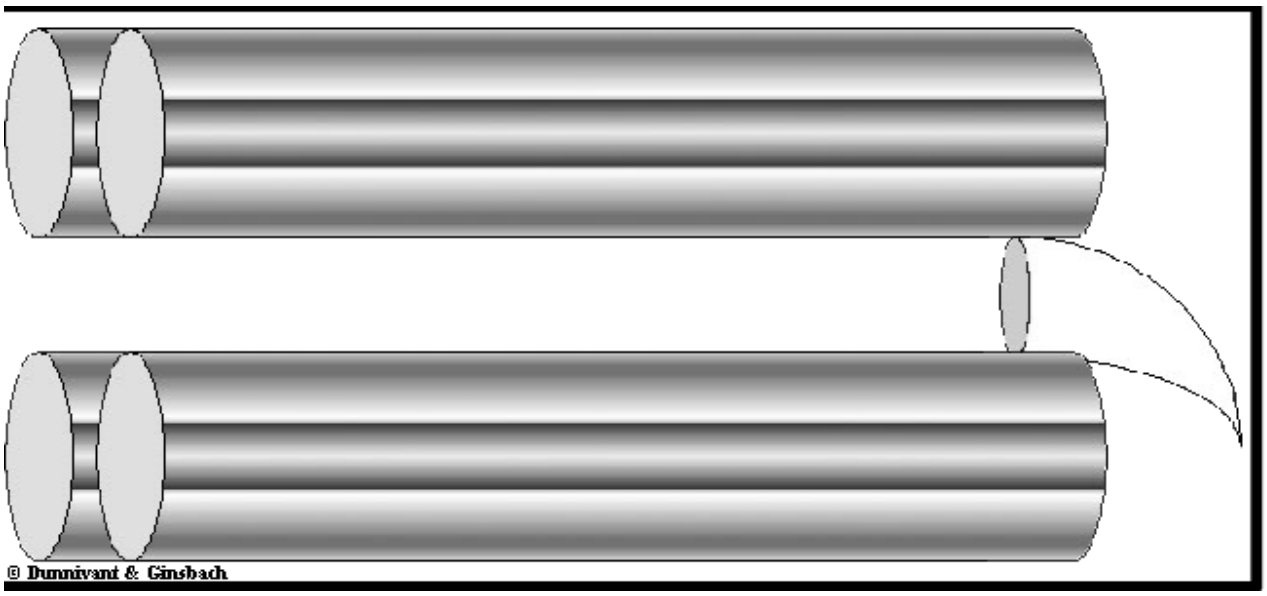
$$\frac{d^2u}{d\xi^2} + [a_u + 2qu\mathrm{Cos}2\xi]u = 0 \qquad \text{Eqn 5.10}$$

where

$$a = \frac{4eU}{\omega^2 r_0^2 m} \quad \text{and} \quad q = \frac{2eV}{\omega^2 r_0^2 m}$$

and where *u* is either the x or y directional coordinate, ξ is the redefining of time (t/2), *e* is the charge of the ion, U is the magnitude of the dc potential, ω is the angular frequency (2pf) of the applied ac waveform, r_o is the distance from the center axis (the z axis) to the surface of any electrode (rod), *m* is the mass of the ion, and V is the magnitude of the applied ac or radio frequency waveform. By using the reduced terms, *a* and *q*, the six-parameter equation (*e, w,* r_o*, m, U, and V*) can be simplified to a two-parameter equation (involving *a* and *q*). Thus, when the two opposing forces are balanced, the movement of a charged particle in an electrical field, the particle will pass through the quadrupole and strike the detector.

View Animation 5.6 for an illustration of how the trajectory of ions of different masses are changed during a mass scan.



Animation 5.6. Illustration of a Quadrupole Mass Filter.

5.5.3 *Quadrupole ion trap mass filter:* While the operation of the ion trap was characterized shortly after the linear quadrupole in 1960 by Paul and Steinwedel, its application in the chemical laboratory was severely limited. This was due to difficulties associated with manufacturing a circular electrode and performance problems. These performance problems were overcome when a group at Finnigan MAT lead by Stafford discovered two breakthroughs that lead to the production of a commercially available ion trap mass filter. The first ion trap developed used a mode of operation where a single mass could be stored in the trap when previously all of the ions had to be stored. Their next important discovery was the ability for 1 mtorr of helium gas to improve the instruments resolution. The helium molecules' collisions with the ions reduced their kinetic energy and subsequently focused them towards the center of the trap.

After these initial hurdles were cleared, many new techniques were developed for a diverse set of applications especially in biochemistry. This is a

result of its comparative advantage over the quadrupole when analyzing high molecular mass compounds (up to several thousand m/z units) to unit resolution in commonly encountered instruments. The ion trap is also an extremely sensitive instrument which allows a molecular weight to be determined with a small number of molecules. The ion trap is also the only mass filter that can contain ions that need to be analyzed for any significant duration of time. This allows the instrument to be particularly useful in monitoring the kinetics of a given reaction. The most powerful application of the ion trap is its ability to be used in tandem mass spectrometry (section 5.5.7).

The ion trap is made up of a single ring electrode that is placed in the X-Y plane between two end cap electrodes (Figure 5.15). Both an ac and dc voltage can be applied to the ring electrode while only an ac voltage can be applied to the end cap electrodes. The two end cap electrodes and the ring electrode ideally have a hyperbolic shape to establish an ideal field however in practice, non-ideal fields can operate effectively. While the ion trap is applying force to the charged ions in three directions, the problem can be simplified into a two-dimensional problem. Since the ring is symmetrical, the force in any direction is always the same. As a result of this symmetry, movement of the molecules can be expressed in terms of r and z where $r = \sqrt{x^2 + y^2}$ where x and y are coordinates. For commercially available instruments, r_0 (the distance from the center of the trap to the ring electrode is either 1.00 or 0.707 cm.

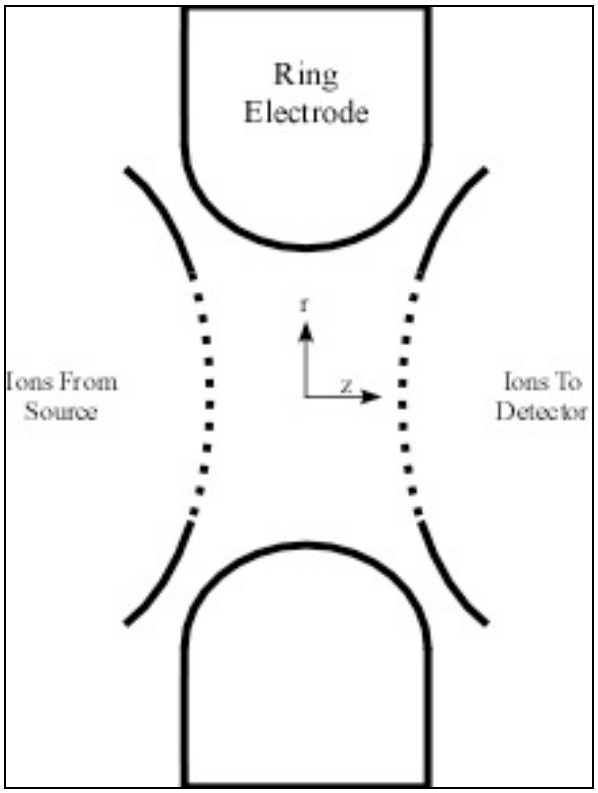


Figure 5.15 A Cross Section of the Ion Trap

After the sample molecules have been ionized by the source, they enter into the ion trap through an electric gate located on a single end cap electrode. This gate functions in the same fashion as the one that is utilized in time of flight mass spectrometry (Section 5.5.4). The gate's purpose is to prevent a large number of molecules from entering into the trap. If too many sample molecules enter into the trap, the interaction with other molecules becomes significant resulting in space-charge effects, a distortion of the electrical field that minimizes the ion trap's performance. Once the ions enter the trap, their collisions with the helium gas focus the ions towards the center of the trap. An ac frequency is also applied to the ring electrode to assist in focusing the ions towards the center of the trap.

In the ion trap, the ring electrode oscillates with a very high frequency (typically 1.1 MHz) while both the end cap electrodes are kept at a ground

potential (U equals 0 Volts). This frequency causes the ion to oscillate in both the r and z direction (Figure 5.16). The oscillation in the r direction is an expected response to the force generated by the ring electrode. The oscillation in the z direction, on the other hand, may seem counter intuitive. This is a response to both the grounded end cap electrodes and the shape of the ring electrode. When the ac potential increases, the trajectory of the ion becomes unstable in the z direction. The theoretical basis for this motion will be discussed later. While it would be convenient to describe the ion trap's function as a point charge responding to an electrical field, the complexity of the generated field makes this impractical.

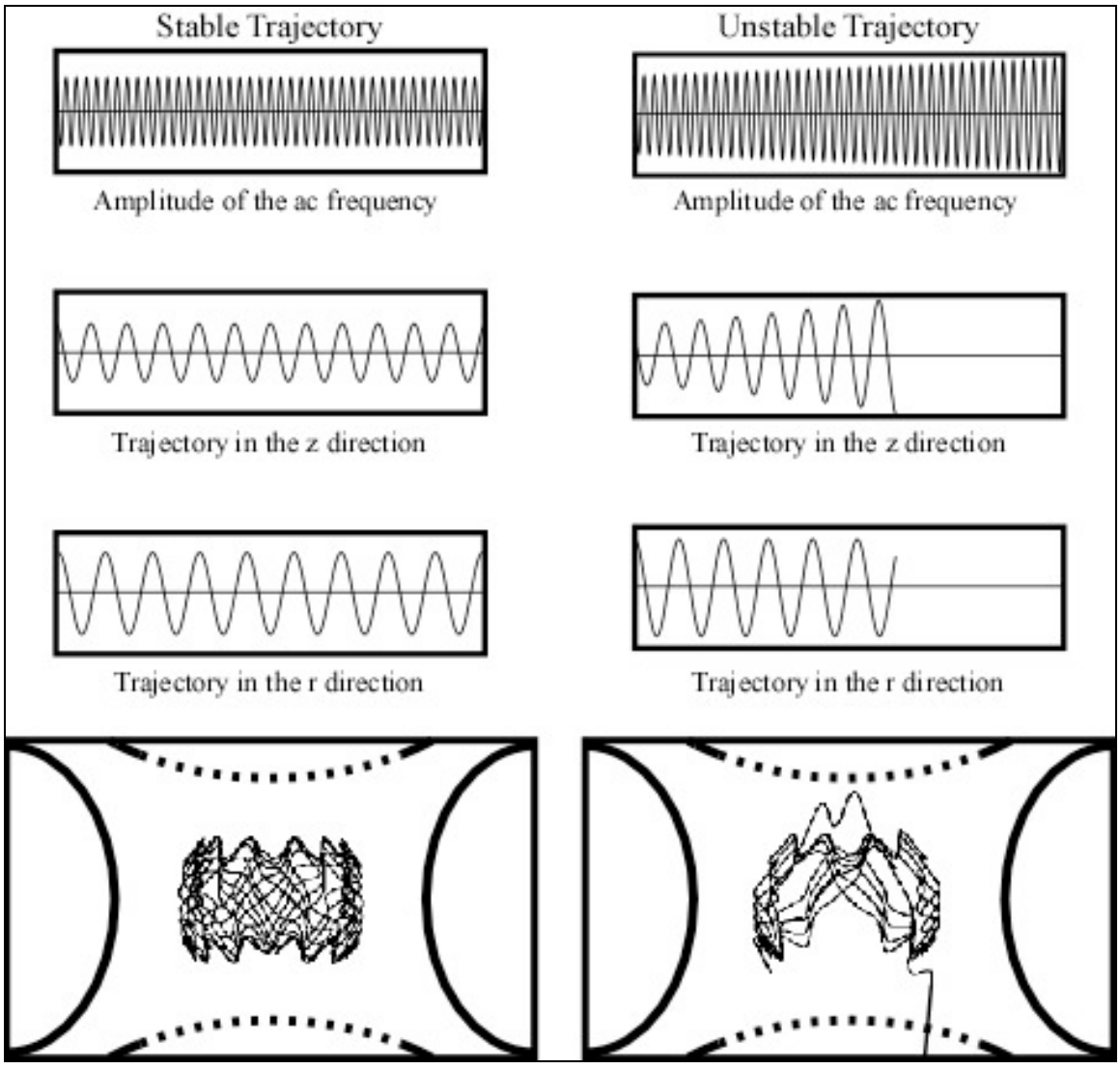


Figure 5.16 The Trajectories of a Single Mass Within the Electrical Field. Figure 6 from Wong and Cooks, 1997. Reprinted with permission of Bioanalytical Systems, Inc., West Lafayette, IN.

The simplest way to understand how the ion trap creates mass spectra is to study how ions respond to the electrical field. It is necessary to begin by constructing a stability diagram for a single ion. Imagine a single mass to charge ratio being introduced into the ion trap. Then, the ac and dc voltages of the ring electrode are altered and the ions stability in both the z and r directions are determined simultaneously. If this experiment was performed multiple times, the stability diagram for that single mass would look similar to Figure 5.17.

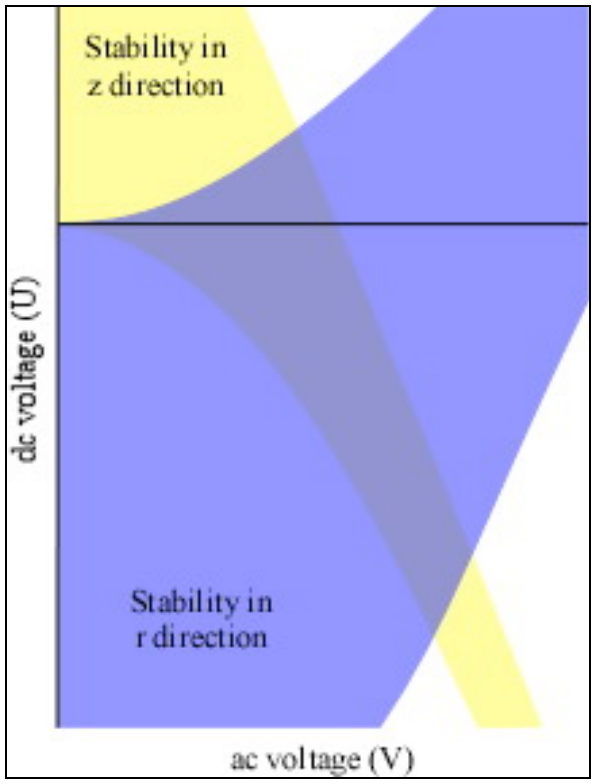


Figure 5.17 A Single Mass Stability Diagram for an Ion Trap. Adapted from Figure 5 from Wong and Cooks, 1997. Reprinted with permission of Bioanalytical Systems, Inc., West Lafayette, IN.

The yellow area indicates the values of the ac and dc voltages where the given mass has a stable trajectory in the z direction but the ion's trajectory in the r direction is unstable. As a result, the ion strikes the ring electrode, is neutralized, and removed by the vacuum. The blue area is voltages where the ion has a stable trajectory in the r direction, but not in the z direction. At these voltages, the ion exits the trap through the slits in the end cap electrode towards a detector. The detector is on if the analyst is attempting to generate a mass spectrum, and can be left off if the goal is to isolate a particular mass to charge ratio of interest. The gray-purple area is where the stability in both the r and z direction overlap. For these voltages, the ion has a stable trajectory and remains inside the trap.

Similar to the quadrupole mass filter, differential equations are able to expand the single mass stability diagram to a general stability diagram. The derivation of this result requires an in depth understanding of differential equations, so only the graphical result will be presented here (Figure 5.18). As with the linear quadrupole mass filter, the solution here is simplified from a six-variable problem to a simpler two-variable problem.

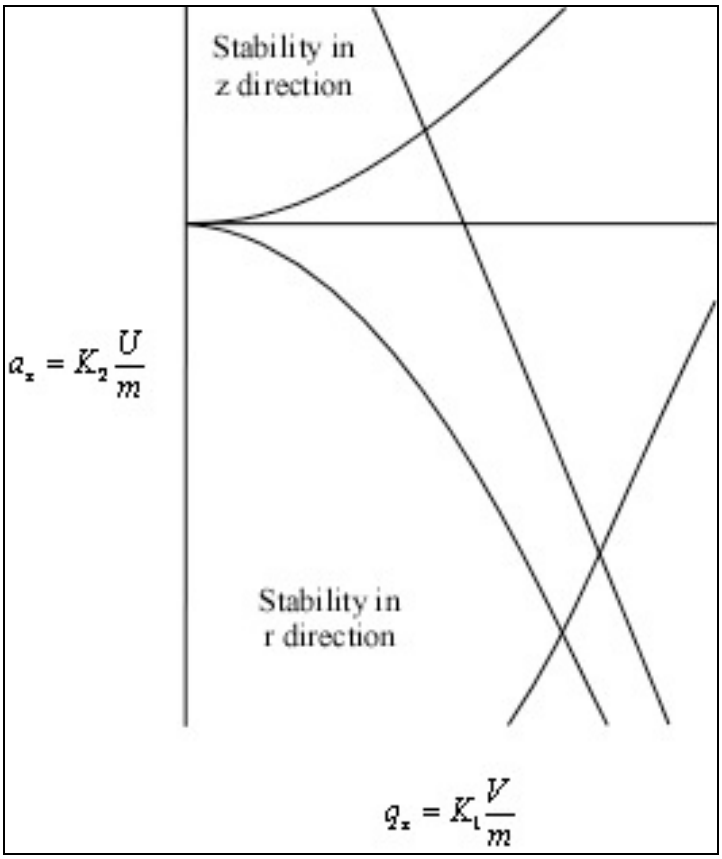


Figure 5.18 A General Stability Diagram. Adapted from Figure 5 from Wong and Cooks, 1997. Reprinted with permission of Bioanalytical Systems, Inc., West Lafayette, IN.

From the general stability diagram it becomes visible how scans can be performed by just altering the ac voltage on the ring electrode. But before we discuss the ion trap's operation it is necessary to understand the parameters that affect ions stability within the field. The terms K_1 and K_2 are characterized by the following equations:

$$K_1 = \frac{4e}{r_0^2 \omega^2} \qquad \textit{Eqn 5.11}$$

$$K_2 = \frac{-8e}{r_0^2 \omega^2} \qquad \textit{Eqn 5.12}$$

As expected, these parameters are very similar to the ones that resulted from the general stability diagram for the quadrupole mass filter. These parameters, like in the quadrupole, are also kept constant during a scan. The charge of the particle (*e*), the distance from the center of the trap to the ring electrode (r_0), and the radial frequency of the ac voltage (ω) are all kept constant during the run. While it would be possible to alter both the ac and dc voltages, in practice it is only necessary to alter the ac voltage (V) of the ring electrode. The dc voltage (U) on the other hand, is kept at zero. If the dc voltage is kept at a ground potential, increasing the ac voltage will eventually result in an unstable trajectory in the z direction. When ac voltage creates a q_z value that is greater than 0.908, the particle will be ejected from the trap towards a detector through the end cap electrode. As illustrated below, the q_z value is dependent on the mass to charge ratio of the particle, each different mass has a unique ac voltage that causes them to exit the trap.

For example, let's place four different ion masses into the ion trap where each has a single positive charge. The general stability diagram in Figure 5.19 is identical to Figure 5.18 except that it is focused around a dc voltage (U) of zero and the scale is enlarged; thus, a_x is equal to zero through a scan. A mass scan is performed by starting the ring electrode out at a low ac voltage. Each distinct mass has a unique q_z value, which is visually illustrated by placing these particles on the stability diagram. As the ac frequency begins to increase, the q_z values for these masses also increases. Once the q_z value becomes greater than 0.908, the ions still have a stable trajectory in the r direction but now have an unstable trajectory in the z direction. As a result, they are ejected out of the trap through the end cap electrode towards the detector.

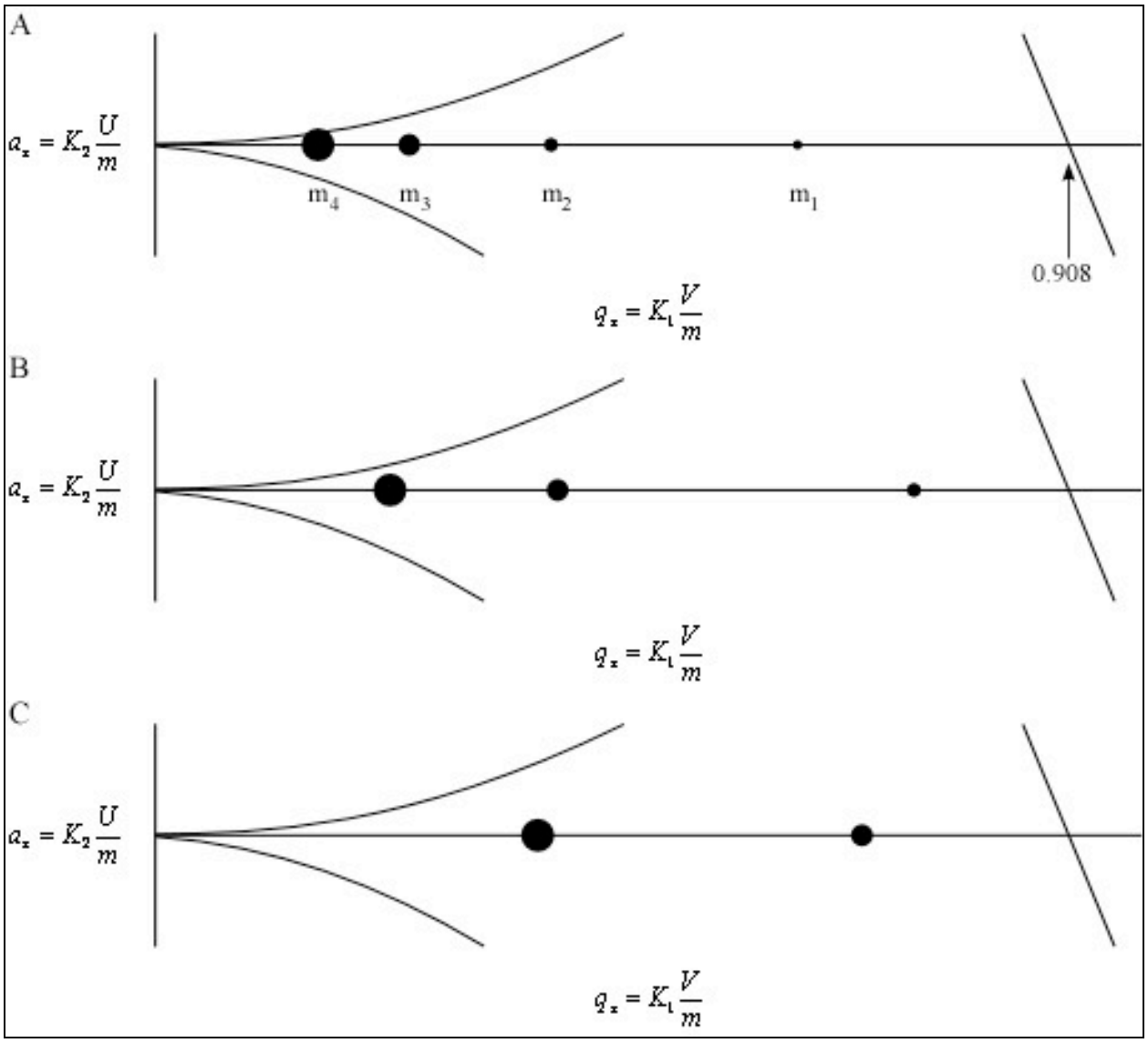


Figure 5.19 A Stability Diagram During a Sample Scan

The stability diagram above at A, B, and C was the result of taking a snapshot of the ac voltage during the scan and placing each mass at its corresponding q_z values for that particular voltage. In this mode of operation, the lightest masses (m_1) are always ejected from the trap (Figure 15.19 B) before the heaver masses (m_2). The heaviest masses (m_3 and m_4) still remain in the trap at point C. To eject these ions, a very large ac voltage is necessary. This voltage is so high that it becomes extremely difficulty to eject ions over a m/z value of 650. Since it is impractical to apply such high voltages to the electrode and its

circuits, a new method of operation needed to be discovered so the ion trap could analyze more massive molecules.

As a result, resonance ejection was developed to extend the mass range of the ion trap to a m/z value of several thousand. Under normal scanning conditions, ions oscillate at a given frequency depending on their q_z value which is a function of its mass, charge, and the amplitude of the ac voltage. This frequency is referred to as the ion's secular frequency. It was discovered that an ac voltage applied to the end cap electrodes would only affect one ion's secular frequency. The effected ion's oscillation in the z direction would increase linearly until it was ejected from the trap. Resonance ejection can be conceptualized as a "hole" inside the stability diagram at any chosen q_z value. Then the ac voltage of the ring electrode can be altered so any mass can have the same q_z value as the "hole" and exit the trap in the z direction (Figure 5.20). This mode of operation not only extended the mass analyzer's mass range, but it also made it possible to eject ions from the trap in any order. Before this mode of operation existed, it was only possible to eject the ions in order from lightest to heaviest. Figure 5.20 illustrates how it is possible to eject the heaviest ion (m_4) before the lighter ion (m_3).

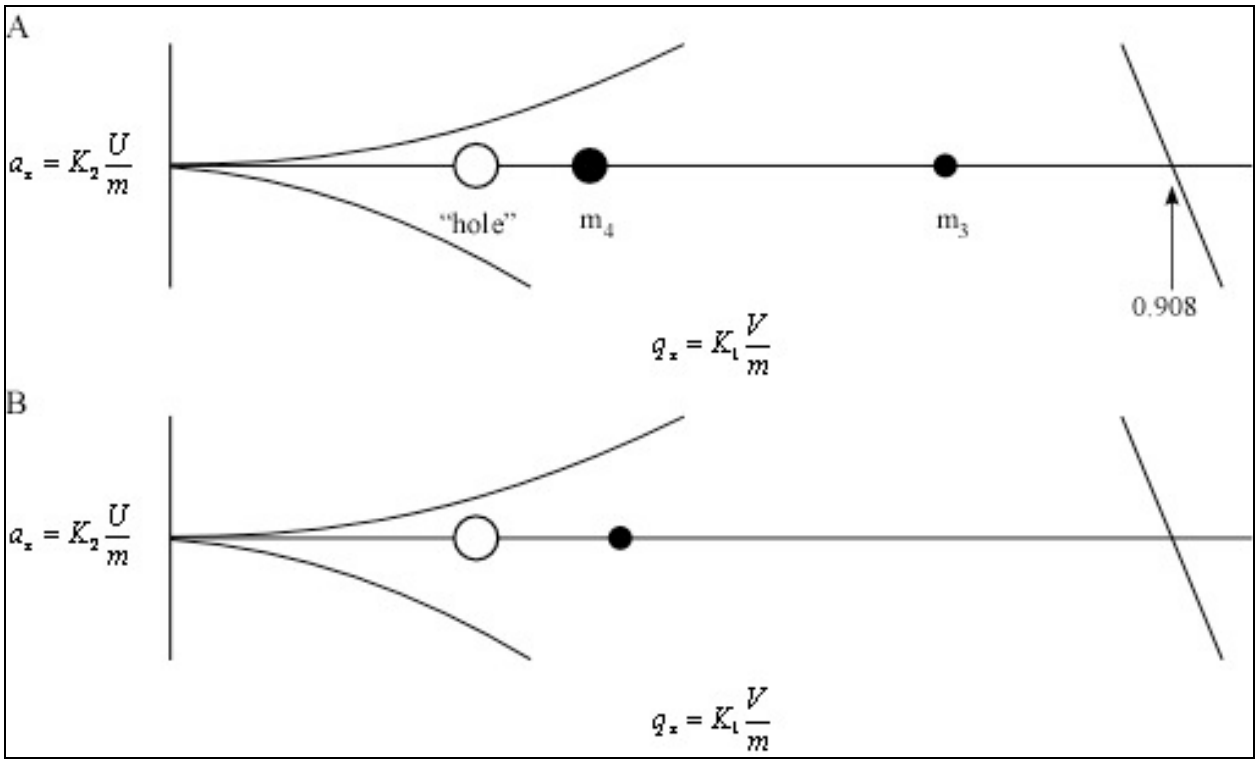
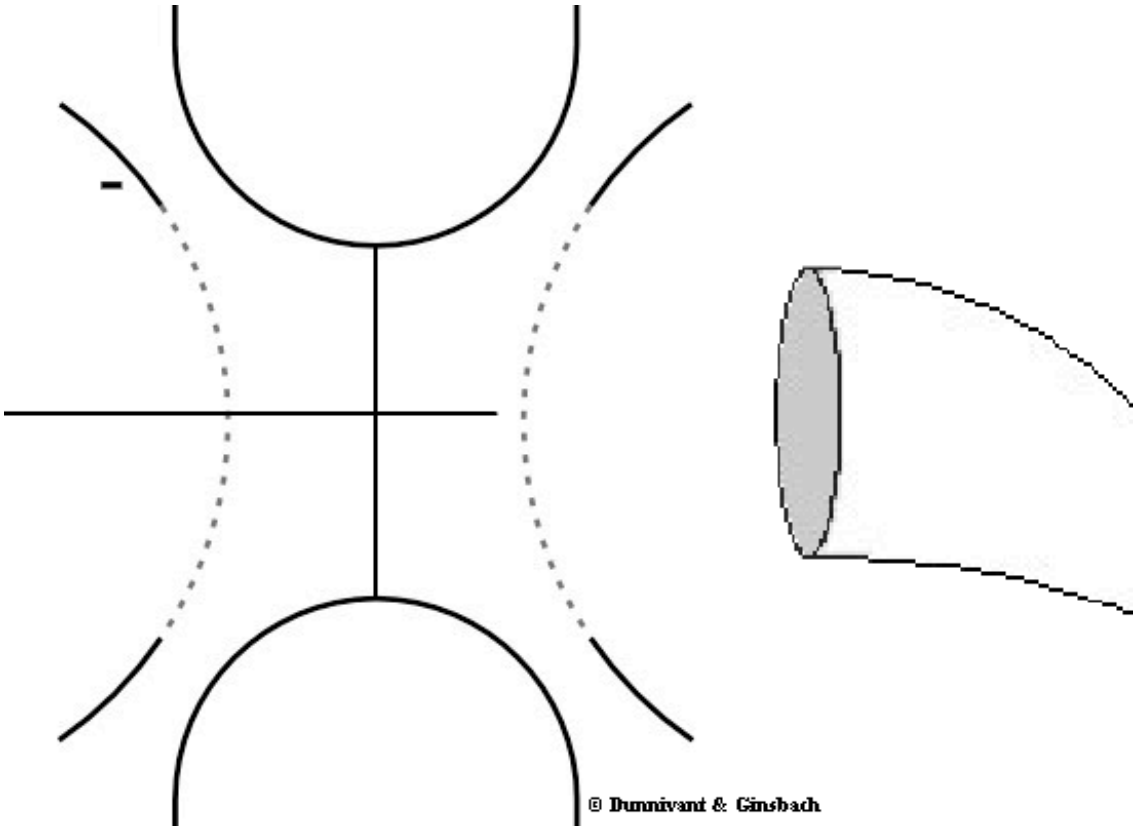


Figure 5.20 A Sample Resonance Ejection Scan

The resonance ejection mode of operation is one reason why the ion trap is such a valuable tool. It not only greatly extends the mass range of the mass analyzer, but it also increased its applications in tandem spectroscopy (section 5.5.8). The ability to isolate any given mass under several thousand amu is an extremely powerful tool. Through the use of both modes of operation, the ion trap has become a valuable tool in performing many specialized mass separations.

View Animation 5.7 at this time for an illustration of how an ion trap mass filter contains and ejects ions of given mass to charge ratios.



Animation 5.7. Illustration of an Ion Trap Mass Filter.

5.5.4 *Time-of-Flight (TOF) mass filter:* While time-of-flight mass filters were one of the first MS systems to be developed, they had limited use due to their need for very fast electronics to process the data. Developments in fast electronics and the need for mass filters capable of resolving high mass ranges (such as in MALDI systems) has renewed interest in time of flight systems. TOF is used exclusively with MALDI systems and also has other applications, as in HPLC where high molecular weight compounds are encountered.

Entry into the TOF mass filter is considerably different than with other mass filters. The entry has to be pulsed or intermittent in order to allow for all of the ions entering the TOF to reach the detector before more ions are created.

With sources that operate in a pulsing fashon such as MALDI or field desorption, the TOF functions easily as a mass analyzer. In sources that continually produce ions such as a GC system or an EI source, the use of a TOF is more difficult. In order to use a TOF system with these continuous sources, an electronic gate must be used to create the necessary pulse of ions. The gate changes the potential on an accelerator plate to only allow ions to enter the TOF mass filter in pulses. When the slit has a positive charge, ions will not approach the entryway to the mass analyzer and are retained in the ionization chamber. When all of the previously admitted ions have reached the detector, the polarity on the accelerator(s) is again changed to negative and ions are accelerated toward the slit(s) and into the TOF mass analyzer. This process is repeated until several scans of each chromatographic peak have been measured. (This type of ionization and slit pulsing will be shown in the animation below). The other way to interface EI with TOFs is to operate the EI source in a pulsing mode. This is achieved by maintaining a constant negative polarity on the accelerator plate/slit, and pulsing the EI source. This method can also periodically introduce packets of ions into the TOF mass filter.

Whichever type of ionization and entry into the TOF mass filter is used the remainder of the process is the same. Prior to developing the mathematics behind TOF separations a simple summary is useful. Mass to charge ratios in the TOF instrument are determined by measuring the time it takes for ions to pass through the “field-free” drift tube to the detector. The term “field-free” is used since there is no electronic or magnetic field affecting the ions. The only force applied to the ions occurs at the repulsion plate and the acceleration plate(s) where ions obtain a similar kinetic energy (KE). All of the ions of the same mass to charge ratio entering the TOF mass analyzers have the same kinetic energy and velocity since they have been exposed to the same voltage on the plates. Ions with different mass to charge ratios will have velocities that will vary inversely to their masses. Lighter ions will have higher velocities and will

arrive at the detector earlier than heavier ones. This is due to the relationship between mass and kinetic energy.

$$KE = mv^2/2 \qquad Eqn\ 5.13$$

The kinetic energy of an ion with a mass m and a total charge of q = ze is described by:

$$mv^2/2 = q\,V_S = z\,e\,V_S \qquad Eqn\ 5.14$$

where V_S is potential difference between the accelerator plates, z is the charge on the ion, and e is the charge of an electron (1.60×10^{-19} C). The length (d) of the drift tube is known and fixed, thus the time (t) required to travel this distance is

$$t = d/v \qquad Eqn\ 5.15$$

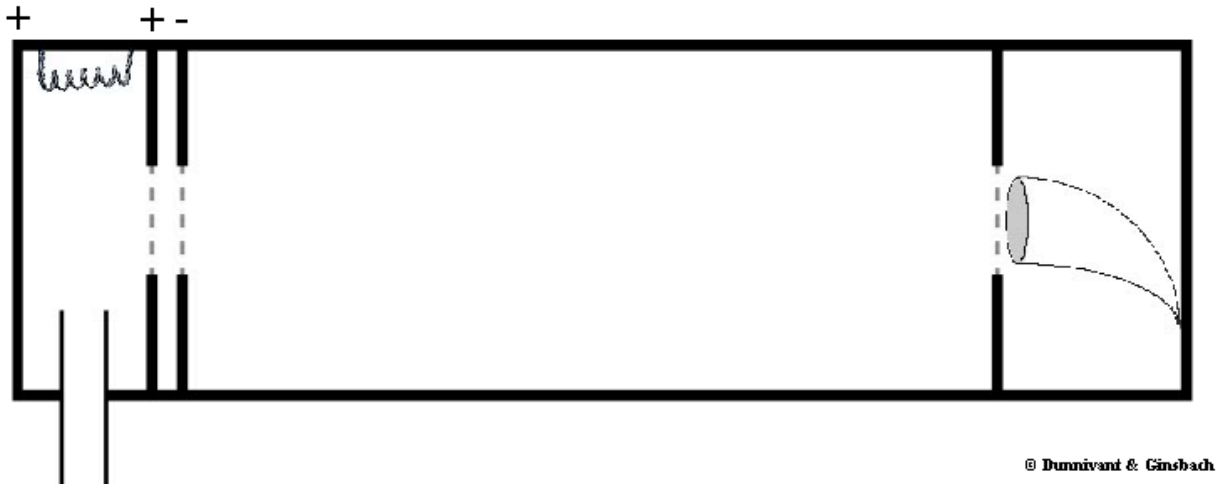
By solving the previous equation for v and substituting it into the above equation we obtain

$$t^2 = \frac{m}{z}\left(\frac{d^2}{2V_S e}\right) \qquad Eqn\ 5.16$$

In a TOF mass analyzer, the terms in parentheses are constant, so the mass to charge of an ion is directly related to the time of travel. Typical times to traverse the field-free drift tube are 1 to 30 ms.

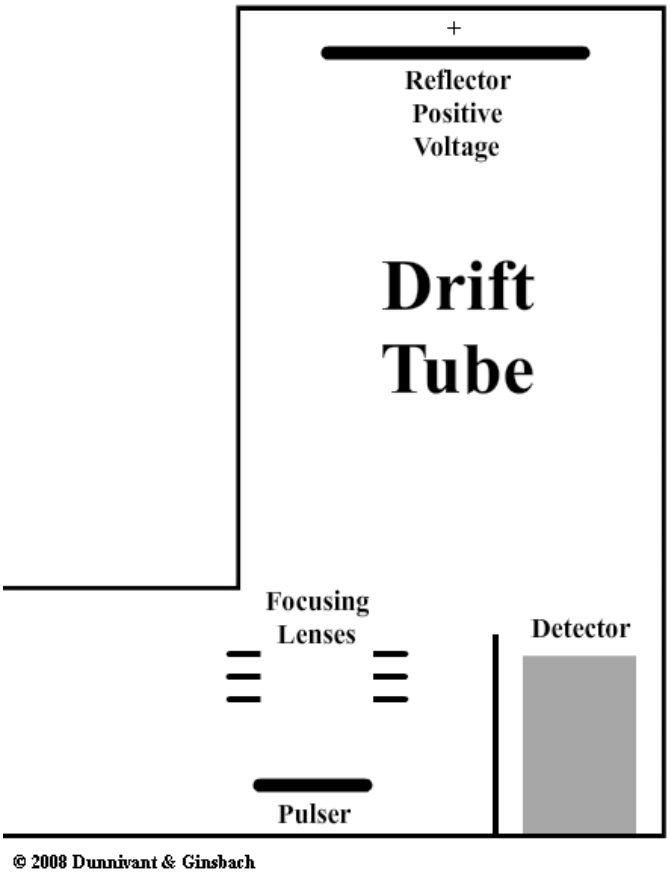
Advantages of a TOF mass filter include their simplicity and ruggedness and a virtually unlimited mass range. Additionally, virtually all ions produced in the ionization chamber enter the TOF mass filter and traverse the drift tube. However, TOF mass filters suffer from limited resolution, related to the relatively

large distribution in flight times among identical ions (resulting from the physical width of the plug of ions entering the mass analyzer). Animation 5.8 illustrates how a pulsed accelerator plate/slit acts as a gate for a TOF mass filter system.



Animation 5.8. Illustration of a Traditional TOF Mass Filter.

Animation 5.9 illustrations how a pulsed accelerator plate/slit acts as a gate for a reflective TOF mass filter system. (The system shown is actually for the analysis of metal isotopes with an Inductively Coupled Plasma (ICP), but the reflective TOF works the same for organic analytes.



Animation 5.9. Illustration of a Reflective TOF Mass Filter

Ion Mobility Mass Spectrometry:

If you have been in an airport recently you have seen or your luggage has been analyzed by an Ion Mobility Spectrometer (IMS). Although originally developed by Earl W. McDaniel of Georgia Institute of Technology in the 1950s, IMS systems have gained popularity recently due to their versatility—they can designed for specific classes of compounds, they have excellent detection limits, and they can be manufactured to be light-weight and mobile.

The basic design is similar to the TOF mass filter. Important differences are that they use an easier ionization source, they can be operated at atmospheric pressure and therefore do not necessarily require pressurized gases or high vacuum pumps, and as a result of their atmospheric pressure sample introduction they have superior detection limits. Samples are introduced at atmospheric pressure and ionized by corona discharge, atmospheric pressure photoionization (APPI), electrospray ionization (ESI), or a radioactive source such as a small piece of ^{63}Ni or ^{241}Am, similar to the thoses used in ionization smoke detectors or GC electron capture detectors. The ionized analytes are then introduced to the drift tube by a gate valve similar to the one described earlier in this section for TOF mass filters. However, the IMS drift tube is different in that it can be operated at atmospheric pressure and is a counter current environment. The analytes travel from left to right in the one-meter drift tube due to a 10-30 kV potential difference between the inlet and exit. As the analytes are mobile due to the potential they travel through a buffer gas that is passed from right to left in the drift tube (and atmospheric gases are commonly used). Separation of different analtyes is achieved due to each ion having a different mass, charge, size and shape (the ion mobility). As the ions are electrically drawn toward the detector, the ion's cross sectional area strikes buffer gases and its velocity is reduced based on its size and shape. Larger ions will collide with more buffer gas and be impeded, travel slower, and arrive at the detector after longer times in the drift tube. Detectors for IMS are usually relatively simple Faraday cups but better detection limits can be obtained with an EM.

The most common use of IMS is for volatile organic molecules. IMS has been expanded for use in gas, liquid, and super critical fluid chromatography.

5.5.5 *Double Focusing Systems:* The magnetic sector MS described earlier is referred to as a single-focusing instrument since it only uses the magnetic component to separate ion mass to charge ratios. This can be

improved by adding a second electrostatic-field based mass filter, and is referred to as double focusing. A magnetic field instrument focuses on the distribution of translational energies imparted on the ions leaving the ionization source as a means of separation. But in doing so, the magnetic sector instruments broaden the range of kinetic energies of the ions, resulting in a loss of resolution. If we combine both separation techniques by passing the ions separately through an electrostatic field (to focus the kinetic energy of the ion packet) and magnetic field (to focus the translational energies of the ion packet), we will greatly improve our resolution. In fact, by doing this we can measure ion masses to within a few parts per million (precision) which results in a resolution of ~2500. Compare this to the unit resolutions (28 versus 29 Daltons) discussed at the beginning of this section (under resolution). On the downside, these instruments can be costly.

5.5.6 Fourier Transform Ion Cyclotron – Mass Spectrometry: (by Nicole James)

Developed by Alan G. Marshall and Melvin B. Comisarow at the University of British Columbia, the use of FT-ICR MS first began in 1974 with approximately 235 instruments in use by 1998. FT-ICR MS has higher mass resolution and accuracy than any other MS system and can detect multiple mass-to-charge ratio ions simultaneously. However, FT-ICR MS can be prohibitively expensive at $1-2 million for a standard instrument.

The general steps of an FT-ICR MS experiment are: (1) ion formation outside of the detector; (2) ion focusing and accumulation; (3) transportation of ions into a Penning trap; (4) selection of ions based on mass-to-charge ratio and ejection of these ions from the Penning trap; (5) excitation; (6) detection; (7) fast Fourier transform of the digital time-domain signal; (8) conversion of frequency to mass-to-charge ratio.

Ion-Cyclotron Motion

If a moving ion is exposed to a uniform magnetic field, it is subject to a force dependent on the mass, charge and velocity of the ion. If an ion does not collide with another particle and hit off its natural course, the magnetic field will bend the ion's path into a circular orbit.

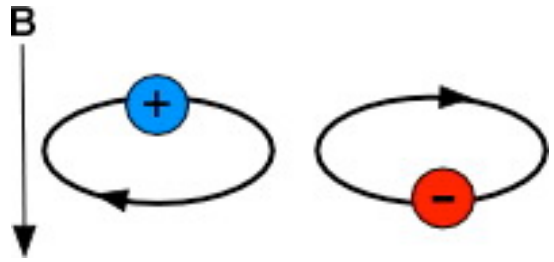


Figure 5.21a Illustration of a particle in a magnetic field.

The motion of the ion can be described by the equation below, where *w* is the unperturbed ion cyclotron frequency, B_0 is the magnetic field in Tesla, q is the charge in Coulombs, and *m* is the mass in micrograms.

$$w = \frac{qB_o}{m}$$

This equation can be rearranged into the following equation where *v* is the velocity, and *z* is the charge of the ion in units of elemental charge (e.g., +1, +2, etc).

$$v = \frac{w}{2\pi} = \frac{1.5356 \times 10^7 \ B_o}{m/z}$$

It is important to note that the above equation is dependent on only the mass-to-charge ratio of the ion and not its velocity. This makes ion-cyclotron resonance especially useful in mass spectrometry, as one does not need to focus translational energy—which requires longer experiment times, larger apparatus and more powerful electronics—in order to obtain high-accuracy results.

The radius of the circle an ion makes when exposed to the magnetic field can be found by the equation below, where *r* is radius in meters, and *T* is the temperature in Kelvin:

$$r = \frac{1.3365 \times 10^{-6}}{zB_0}\sqrt{mT}$$

One can see from the above equation that an ion with a mass of 100 amu and a charge of +1 in a magnetic field of 1 Tesla at room temperature (298 K) would have a radius of 0.2 mm; the same ion with triple the magnetic field (3 T) at room temperature would have a radius of 0.077 mm. Thus, ions can be easily confined to a relatively small orbit by a reasonable magnetic field; this is called ion trapping and is vital to ICR-MS because the longer (approximately 1s) experiment times require one to be able to retain the ions in a designated space. Additionally, a 3T magnetic field is easily attainable for commercially available electronics. The largest FT ICR MS built as of 2010 can attain a magnetic field of 15T, allowing one to confine an ion with an m/z value of 60,000.

Ion Cyclotron Excitation and Detection

A number of ions at a specific mass-to-charge ratio spinning in an ion-cyclotron orbit does not, itself, generate an observable electric signal, because (a) the ions were randomly placed (i.e, incoherent; ions are spread throughout the radio of orbit) as they began orbiting, meaning that an ion at a specific position will have its charge cancelled out by an ion half an orbit away from it, leaving no net electrical current, and (b) the radius of the orbits are generally too small to be detectable, even if all ions were in the same phase. Thus, ions must be excited in order to be detected.

Particles in an ion-cyclotron orbit can be excited by applying an oscillating or rotating uniform electric field at or near the frequency of ions of a given mass-to-charge ratio. This excitation can be used for three purposes: (1) accelerating the ions into a larger orbital radius for detection, (2) accelerating the ions to a larger orbital that is ejected from the ion trap, and (3) increasing the kinetic energy of an ion to the point that it further ionizes or reacts with another molecule. For the purposes of this text, excitation in order to accelerate the ions for detection is most significant.

Applying an oscillating or rotating radio-frequency electric field in resonance with (at the same frequency as) a specific m/z value or range applies a force on the ion(s) that continuously enlarges the circular orbit of the ion(s) at one point—in other words, the orbiting ions begin to spiral outward. Ions of different types will spiral outward at different rates. The post-excitation orbit for an ion excited for a period of time, t, is shown in the following equation, where E_0 is the applied electric field and B_0 is the magnetic field:

$$r = \frac{E_0 t}{2B_o}$$

The fact that the above equation is independent of the mass-to-charge ratio of the ion means that all ions can be excited by a radio-frequency electric field to enlarged ion-cyclotron orbits for detection. This simultaneous detection vastly decreases both the time an experiment will take and the amount of analyte required.

When a group of ions with the same mass-to-charge ratio are excited, they are pushed off-axis due to their spiraling nature. By pushing the ions off axis, not all ions have a “partner” ion half a cycle away—the ions are considered to be “cohered.” A cohered packet of orbiting ions causes a difference in current between opposing detection plates within the ion trap; this differential current can be modeled as an “image” current opposing the current on the detection plates; this image current is proportional to the number of coherent orbiting ions. This is the ICR signal; the ICR signal increases linearly with increasing ion-cyclotron radius after excitation and with increasing ion charge. Throughout most of the frequency range possible on the instrument, the signal-to-noise ratio (S/N) is proportional to the differential current observed. The number of ions required for a S/N ratio of 3:1 on a standard instrument using standard parameters is approximately 190 ions. Other detection processes have been designed to such high accuracy and detection that they are able to detect a single ion and have been used to corroborate the theory that protons and anti-protons do, in fact, have the same mass (Gabrielse et al, 1990).

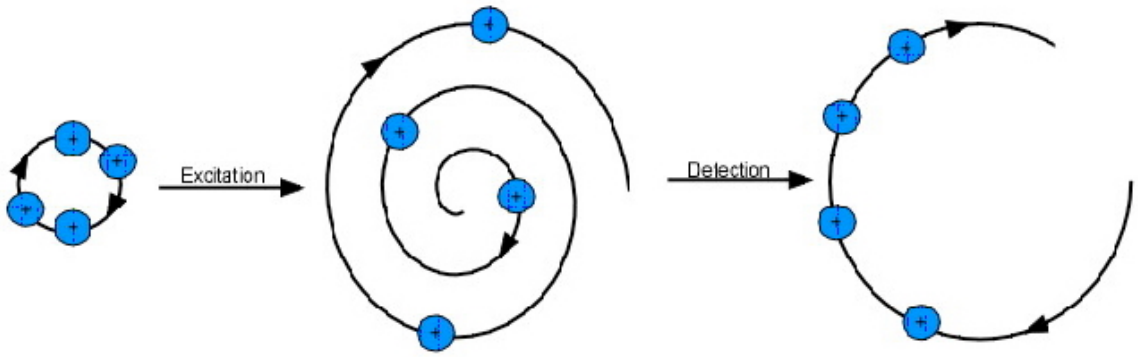


Figure 5.21b Excitation and Detection of an Ion.

The Penning Trap

The most common ion trap used in FT-ICR MS is the Penning trap, designed in the 1950s by Hans Georg Dehmelt, who named it after Frances Michel Penning for his work on the Penning gauge. The ion-cyclotron motion induced by a radial magnetic field contains ions radially, but it is necessary to add an axial electric field in order to trap the ions axially. Thus, the motion of an ion inside a Penning trap is essentially the combination of three distinct motions: the cyclotron, “magnetron” (a component of ion-cyclotron motion), and the axial motion. The axial containment is accomplished by introducing two “end-cap” electrodes. The end-cap electrodes are coupled by capacitance, which allows for a nearly perfect rf electric field to be used for the ion-cyclotron excitation without any negative effects on other electronics. Opposing plates with an electric field applied across them within the Penning trap are used as detector plates.

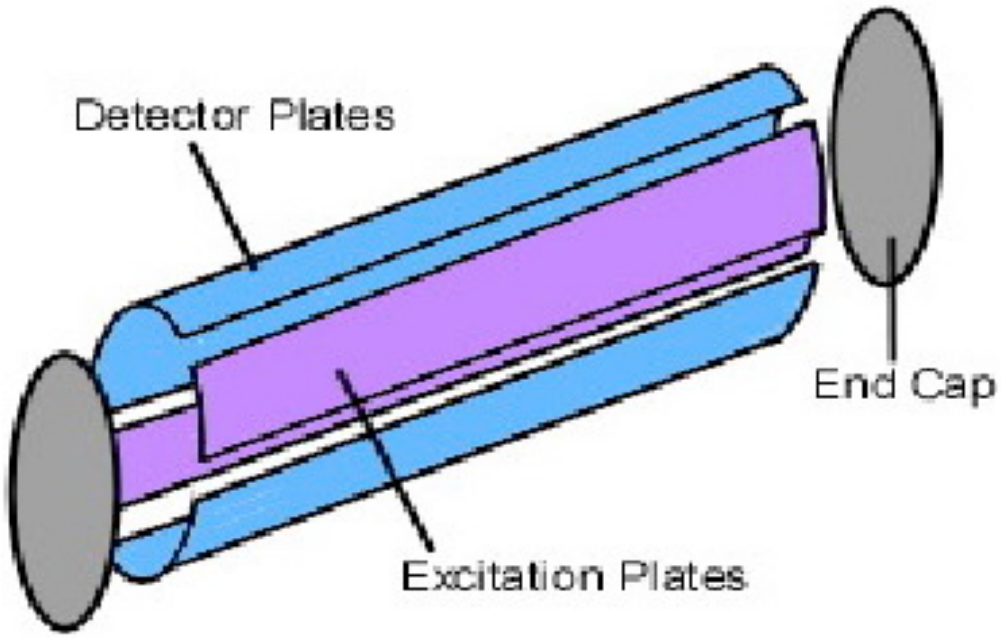


Figure 5.21c Diagram of a Penning Trap.

Analysis of Results

The signal detected by an experiment is in units of current per time. To extract mass-to-charge data, one must apply a Fourier transform. In general, a Fourier transform (FT) takes a time-based signal and converts it into a frequency-based plot. Since the initial function is a function of time, it is typically called the *time domain*; the frequency plot is called the frequency domain, or the *frequency domain representation* of the initial function. More specifically, the Fourier transform uses the fact that almost any function can be degraded into a sum of sine and cosine waves; each component sine and/or cosine wave represents a periodic component of the data. By finding each component sine or cosine wave, one can make a frequency plot by representing a specific sine or cosine wave as a peak on a plot of amplitude versus the frequency of the wave. The sharper the peak, the more “exact” the periodicity is; in most real-life applications, the peak will be somewhat broad—not just a vertical line.

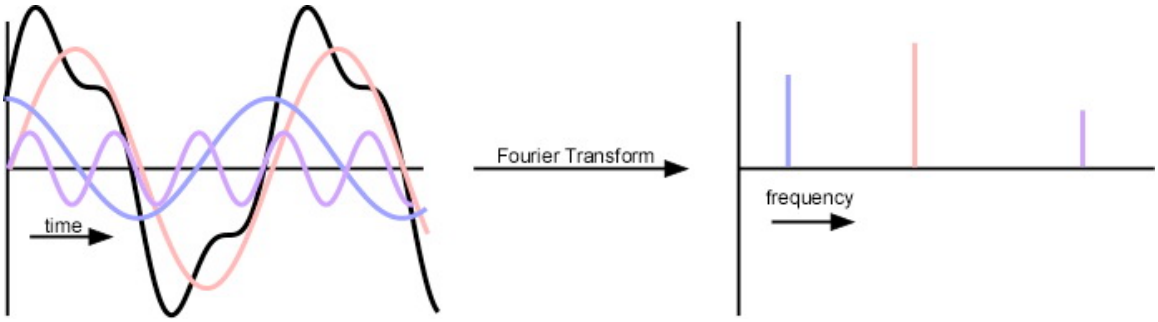


Figure 5.21d Graphic Representation of the Fourier-Transform Process where a time domain signal is transformed to a frequency output.

A Fourier-transform of the (time-domain) ICR response results in a frequency plot that can be mass-corrected to result in a mass spectrum. Obtaining this mass spectrum with most other types of MS would have required sweeping slowly across the entire range of mass-to-charge ratios; being able to quickly and simultaneously detect all mass-to-charge ratios decreases the time, effort and supplies that must be used to test a sample. In addition, the greatly increased resolution means that FTICR-MS will continue being an extremely powerful instrument.

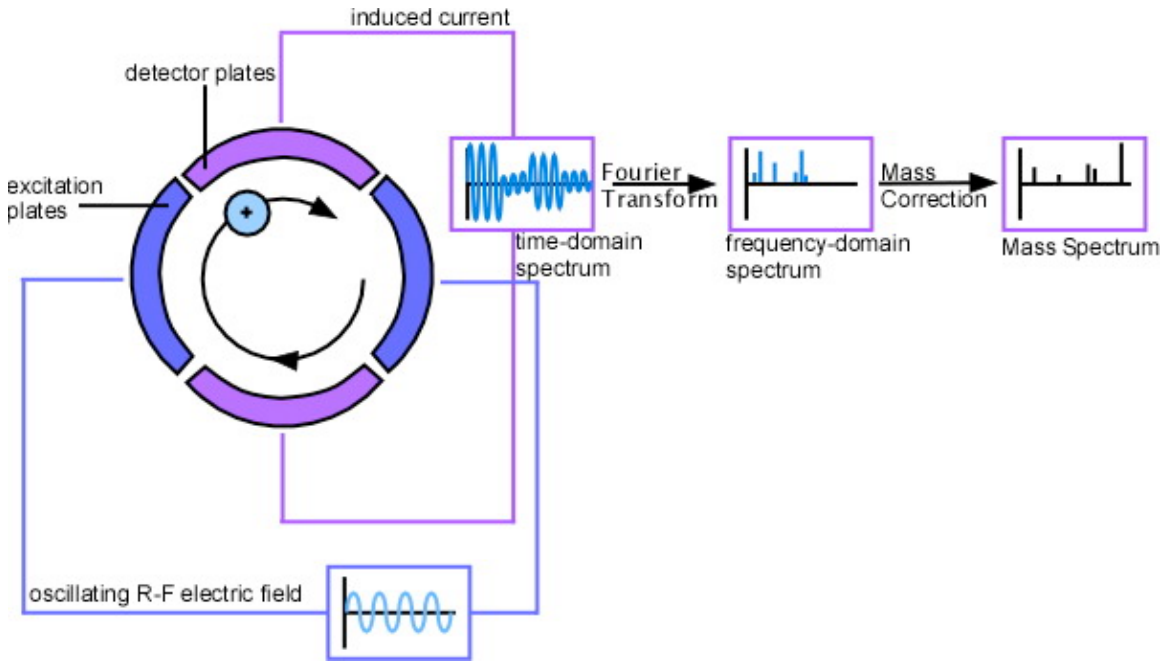


Figure 5.21e Overall Schematic of an Ion Cyclotron Mass Spectrometer.

5.5.7 Orbitrap Analyzers (by Nicole James)

Designed in 2005 by Alexander Makarov, the Orbitrap mass spectrometer features a mass resolution of up to 150,000, high mass accuracy (2-5ppm, compared with approximately 20ppm for quadrapole systems), a mass-to-charge ratio range of 6,000 and a dynamic range larger than 1,000.

The Orbitrap works similarly to an FT ICR-MS: all ions are identified simultaneously by reading an image current of oscillations that are unique to a given mass-to-charge ratio and a Fourier transform is applied to the data to isolate individual signals. However, the Orbitrap requires no magnet, no RF field, and no excitation sequence. Despite this, Orbitrap systems generally cost at least $600,000.

Ions are first ionized by a given source; given the large m/z range, Orbitrap systems are often used to study biological molecules such as proteins, peptides, oligsaccharides—consequently, one of the most common ionization methods is ESI. The ions are then transported to a storage cell, generally a

storage quadrapole, which is kept at a vacuum near 10^{-3} mbar. A series of transfer lenses gradually increases the electric field experienced by the ions until they are at the level of the Orbitrap.

After ions have been transferred into the Orbitrap, the system uses only electrostatic (DC) fields. The Orbitrap itself is composed of an outer “barrel“ electrode, an inner “spindle” electrode, and two endcap electrodes. Upon introduction into the Orbitrap, stable ion trajectories will result in orbiting around the center electrode while also oscillating in the z-direction. The motion in the z-direction can be described as an harmonic oscillator, which is described in equation 5.5.6.1, where ω is oscillation frequency, z is the ion charge, m is the ion mass and k is the field curvature.

$$\omega = \sqrt{\frac{z}{m}k}$$

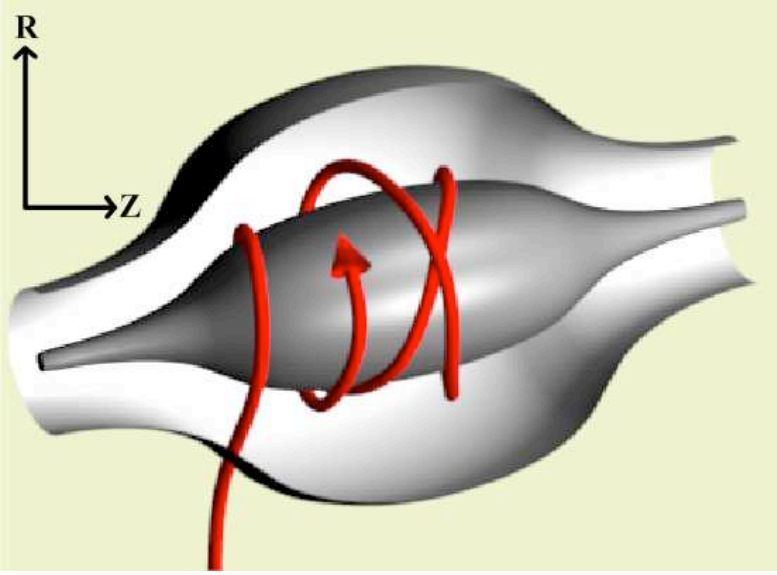


Figure 5.22 The Orbitrap (reprinted from WikiPedia via the *GNU Free Documentation License*)

While the frequency of orbiting the central electrode is also dependent on the ion’s mass-to-charge ratio, this frequency is also dependent on the ion’s energy and when it was introduced into the Orbitrap, whereas oscillations in the z-

direction are independent of energy and any initial parameters. The oscillations in the z-direction are read by the image current produced on the end-cap electrodes. While all ions of a given mass to charge ratio oscillate in phase for hundreds of thousands of oscillations, small imperfections in the Orbitrap or orbital shape, along with occasionally collisions with background gas molecules (despite the 10^{-10}mbar vacuum) can result in the loss or displacement of some ions, ultimately resulting in a slow decrease in the intensity of the signal until it is completely lost in instrument noise. This results in a free induction decay (FID), similar to that which is acquired in NMR analysis. A Fourier Transform of the FID results in a mass spectrum.

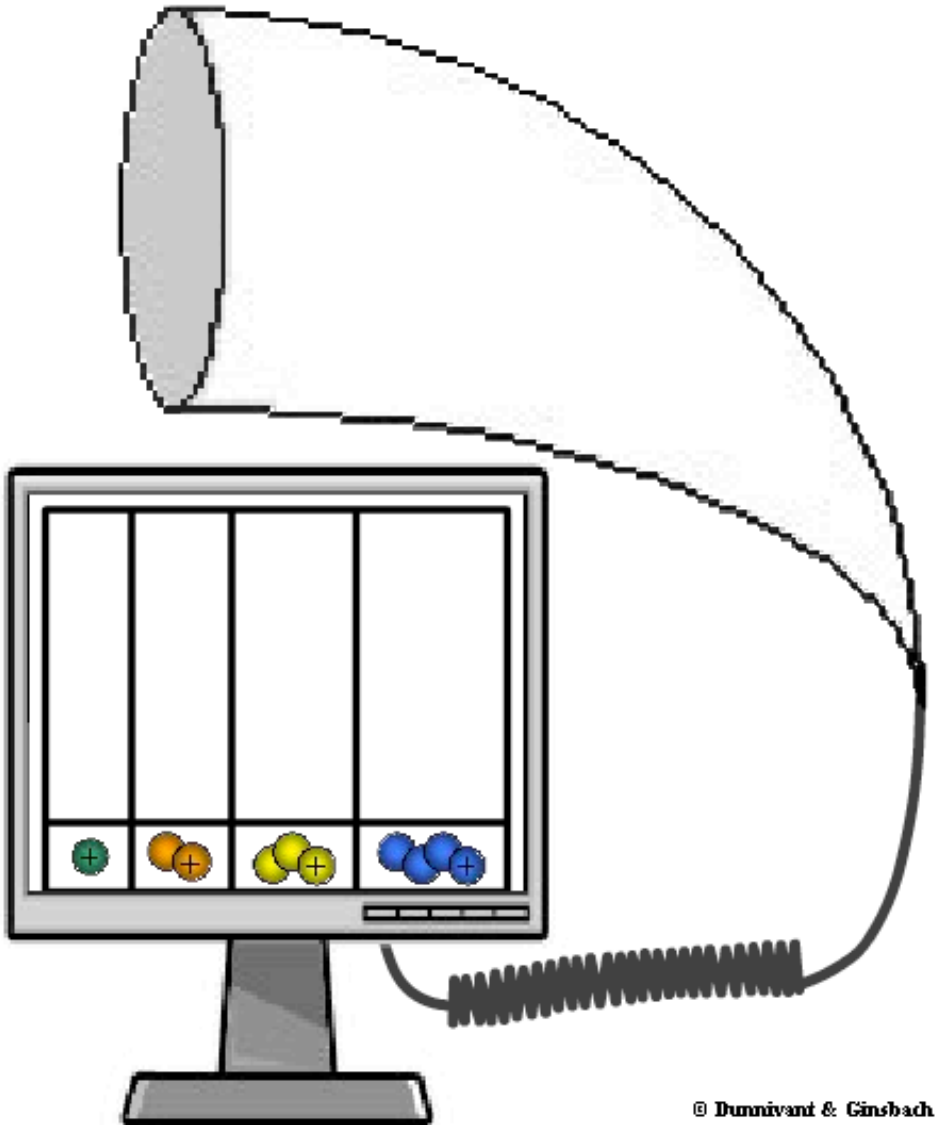
5.5.8 *Tandem Mass Spectroscopy:* Mass spectroscopy is commonly referred to as a confirmatory technique since there is little doubt (error) in the identity of an analyte. To be even more certain of an analyte's identify, two or even three, mass spectrometers can be used in series (the output of one MS is the input of another MS). Most often a soft ionization source, such as chemical ionization, is used in the first MS and allows for selection of the molecular ion in the first MS, while a harder ionization is used in the second MS to create fragments. A subsequent MS will select for a specific ion fragment from the second MS and further fragment it for identification. This technique allows a molecular ion (or ion fragment) to be isolated in the first MS, subsequently fragmented in the second and third MS, and identified based on its final fragment pattern. You should be able to see the confirmatory nature of this technique.

Mass filters of choice for use in tandem include magnetic sector, electrostatic, quadrupole, and ion trap systems. In the absence of HPLC or GC introduction, tandem MS offers many of the same advantages of a single GC-MS or HPLC-MS system but it is much faster since the analyst does not have to wait on the chromatography portion of the analysis. For example, chromatograph separations take from minutes to hours prior to entry into a MS, while tandem MS

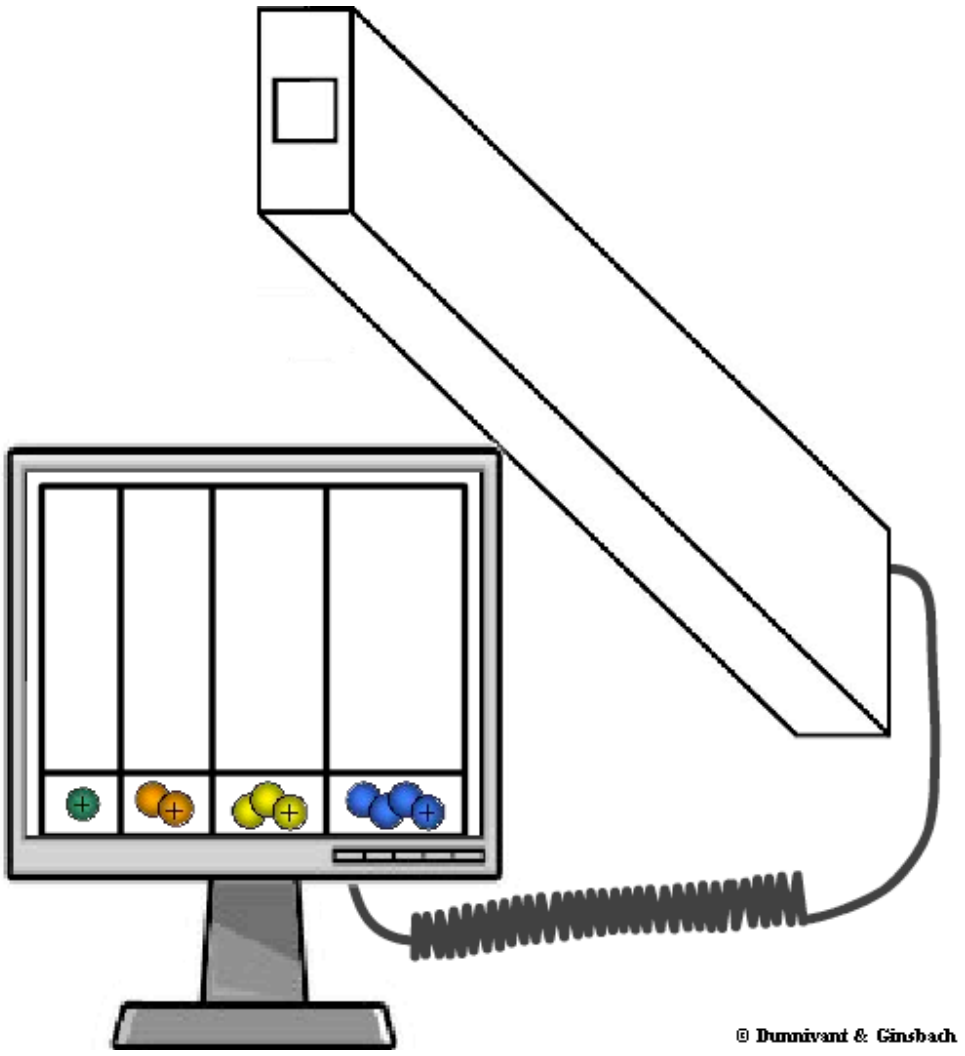
systems (without GC) require only milliseconds. But of course, this saving in time is considerably more expensive than simple chromatographic-based MS systems.

5.6 Ion Detectors

Once the analytes have been ionized, accelerated, and separated in the mass filter, they must be detected. This is most commonly completed with an electron multiplier (EM), much like the ones used in optical spectroscopy. In MS systems, the electron multiplier is insensitive to ion charge, ion mass, or chemical nature of the ion (as a photomultiplier is relatively insensitive to the wavelength of a photon). EMs for MS systems can be a series of discrete dynodes as in the photomultiplier or they can be continuous in design. Most commonly, continuous EMs are used. Continuous EMs are horn shaped and are typically made of glass that is heavily doped with lead oxide. When a potential is placed along the length of the horn, electrons are ejected as ions strike the surface. Ions usually strike at the entrance of the horn and the resulting electrons are directed inward (by the shape of the horn), colliding sequentially with the walls and generating more and more electrons with each collision. Electrical potentials across the horn can range from high hundreds of volts to 3000 V. Signal amplifications are in the 10 000 fold range with nanosecond response times. Animation 1.10 illustrates the response of a continuous electron multiplier as ions, separated in a mass filter, strike its surface.



Animation 5.10. Illustration of a Continuous-Dynode Electron Multiplier.



Animation 5.11. Illustration of a Discrete-Dynode Electron Multiplier.

Another form of MS detector is the Faraday Cup that counts each ion entering the detector zone. These detectors are less expensive but provide no amplification of the signal and are not used in typical instruments due to their poor detection limits.

One of the latest detectors to reach the market is a microchannel plate, a form of an array transducer also called an electrooptical ion detector (EOID). The EOID is a circular disk that contains numerous continuous electron

multipliers (channels). Each channel has a potential applied across it and each cation reaching the detector will generate typically up to 1000 electrons. The electrons produce light as they impinge on a phosphorescent screen behind the disk containing the channels. An optical array detector using fiber optic technology records the flashes of light and produces a two dimensional resolution of the ions. The advantage of an EOID is their ability to greatly increase the speed of mass determinations by detecting a limited range of masses simultaneously, thus reducing the number of discrete magnetic field adjustments required over a large range of masses. EOIDs have not been readily incorporated into instruments as initially anticipated.

5.7 Three-Dimensional Aspects of GC-MS

Typical chromatographic peaks were illustrated in earlier chapters. But as each chromatographic peak enters the MS it is fragmented and separated into a series of ion fragments. When graphed together on an x, y, and z plot, the x-axis represents time and traces the arrival of each compound at the chromatographic detector and the z-axis represents the total detector response that is related to analyte concentration. The mass-to-charge spectrum of each chromatographic peak is represented by a series of lines that are parallel to the y-axis and show the arrival of molecular fragments at the MS detector. Again, detector response and concentration are represented by the height of each peak. This is illustrated for one chromatographic peak in Figure 5.22.

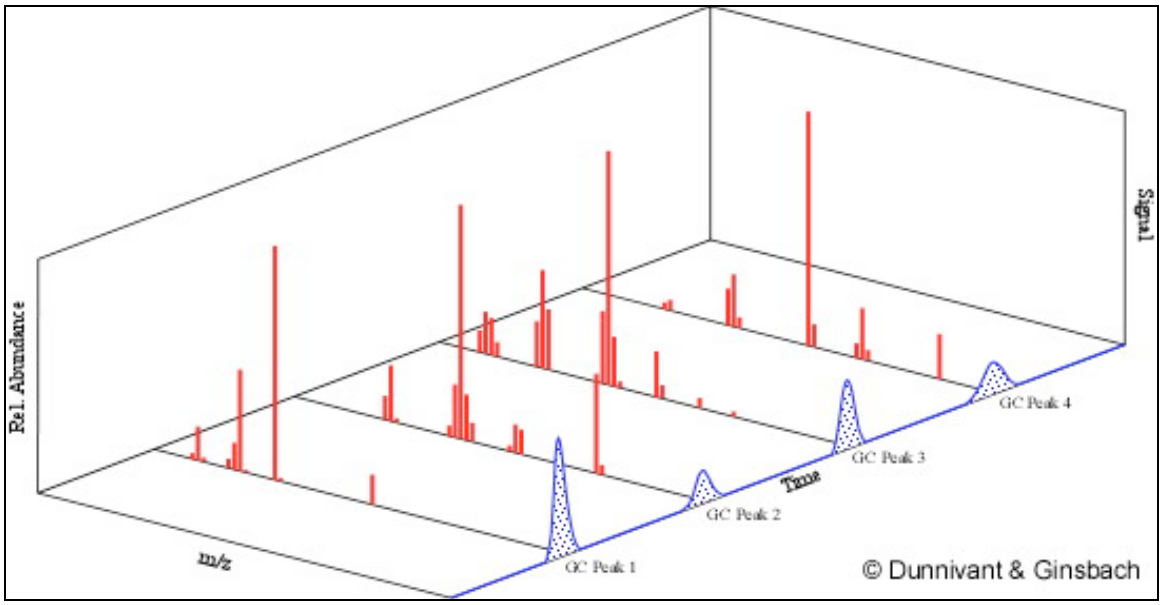


Figure 5.23. The Three-Dimensional Nature of a GC-MS Analysis.

5.8 Summary

In this chapter we illustrated the utility of combining chromatography and MS systems. A variety of possible components provide for interesting instruments that can be used to analyze a broad range of analytes. Hard and soft ionizations techniques provide for the determination of the molecular weight of the analyte, as well as unique fragmentation patterns for confirmational identification of an unknown chemical structure. More inexpensive instruments, such as quadrupole and time of flight mass spectrometers, allow only unit resolution of ions while double focusing instruments yield the determination of differences with resolution of four decimal points in masses. Mass spectrometry, like NMR, is one of the most powerful techniques available to chemists and it is becoming more and more important. While most of the instruments presented in the chapter have detection limits in the sub parts per million range, extremely lower detection limits (10^{-15} moles) have been obtained in research-grade instruments.

A summary of mass filters and their characteristics is given below in Table 5.2.

Table 4.2 Summary of Mass Filter Features. Source: Company Literature and Personal Communiqué David Koppenaal, Thermal Scientific & EMSL, Pacific National Laboratory.

Type of Mass Filter	**Resolution**	**Detection Limit**	**Approximate Instrument Price**
Routine Mass Filters Coupled with ICP			
Single Quadrupole	250-500	low ppb – high ppt	$80 000 - $100 000
Ion Trap	1 000 – 10 000	ppb	$250 000 - $300 000
Time of Flight	3000 – 10 000	high ppt	$300 000 - $400 000
Double Focusing	10 000 – 20 000	mid to high ppt	$750 000 - $1 000 000
Fourier Transform Ion Cyclotron	200 000 – 1 000 000	ppb	$1 000 000 +
New Mass Filters			
Magnetic Sector / Multi-collector with the Mattauch-Herzog Geometry	~500	high ppb	$350 000 - $400 000
Proton Transfer Reaction Ionization Chamber	Depends on type of mass filter	ppt	$120 000
Orbital Trap (Electrostatic Ion Trap)	150 000 – 200 000	ppb	$600 000 (currently only available with HPLC)

5.9 Questions

1. Why are most mass filters maintained at a low pressure?

2. List the common ways samples are introduced into a MS system.

3. How can solid samples be introduced into a MS?

4. Draw and explain how the interface between a GC and a MS works.

5. Why do capillary columns, versus packed columns, work best for MS interfaces?

6. Explain the difference between hard and soft ionization in GC-MS.

7. Why does soft ionization reduce the fragmentation of analytes in GC-MS?

8. Write the chemical reactions occurring when methane is used in soft ionization.

9. Draw and explain how the interface between a LC and a MS works.

10. What is the major problem with interfacing LC (ESI) to MS?

11. Explain how MALDI works. What types of samples is it commonly used for. What type of MS is it commonly coupled with?

12. Draw and explain how the interface between a CE (ESI) and a MS works.

13. Explain resolution with respect to mass filters. Give relevant resolution numbers.

14. Draw and explain how a magnetic sector mass filter works.

15. Draw and explain (in detail) how a quadrupole mass filter works.

16. The governing equation of the quadrupole mass filter consists of a six-parameter differential equation. Which two parameters are used to control the mass filter?

17. What is the purpose of the dc voltage in the quadrupole MS?

18. What is the purpose of the ac cycle in the quadrupole MS?

19. How does the low mass and high mass filters work to create a stable cation region in the quadrupole MS?

20. Explain the mass scan line in the quadrupole MS figures.

21. What is the purpose of sweeping the dc-ac voltages?

22. Extend the concepts of a linear quadrupole mass filter, explained above, to explain how the quadrupole ion trap mass filter works.

23. How is the mass range of a quadrupole ion trap mass filter extended?

24. Explain the concept of resonance ejection in ion trap mass filters.

25. Draw and explain how a time-of-flight mass filter works.

26. Contrast traditional TOF and ion mobility MS.

27. Draw and explain how a PTR-MS works.

28. Give a brief explanation of how an Ion Cyclotron works.

29. Draw and explain how a double focusing mass filter works. What are its advantages?

30. What is tandem mass spectrometry?

31. What types of detectors are used in mass spectrometry?

32. Use the date in Table 5.2 to contrast the various types of mass filters. Which is the most economical? Which has the best mass resolution?

5.10 References

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