An Introduction to Mass Spectrometry

by

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INTRODUCTION:

Mass Spectrometry is a powerful technique for identifying unknowns, studying molecular structure, and probing the fundamental principles of chemistry. Applications of mass spectrometry include identifying and quantitating pesticides in water samples, it identifying steroids in athletes, determining metals at ppq (Parts Per Quadrillion) levels in water samples, carbon-14 dating the Shroud of Turin using only 40 mg of sample (*1*), looking for life on Mars, determining the mass of an ^{28}Si atom with an accuracy of 70 ppt(*2*), and studying the effect of molecular collision angle on reaction mechanisms.

Mass spectrometry is essentially a technique for "weighing" molecules.* Obviously, this is not done with a conventional balance or scale. Instead, mass spectrometry is based upon the motion of a charged particle, called an ion, in an electric or magnetic field. The mass to charge ratio (m/z)** of the ion effects this motion. Since the charge of an electron is known, the mass to charge ratio a measurement of an ion's mass. Typical mass spectrometry research focuses on the formation of gas phase ions, the chemistry of ions, and applications of mass spectrometry.

This paper covers the basics of mass spectrometry instrumentation and introduces the interpretation of mass spectra. It is only an introduction and interested readers are encouraged to consult more specialized books and journal articles for additional details. The articles and books referenced in this paper should be available at most college and university libraries.

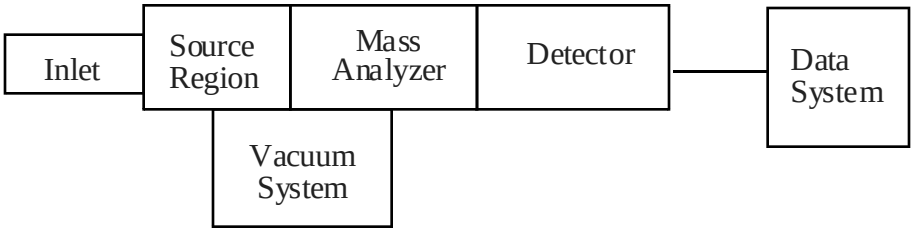

Figure 1. Mass Spectrometer Block Diagram

Figure 1 is a block diagram that shows the basic parts of a mass spectrometer. The inlet transfers the sample into the vacuum of the mass spectrometer. In the source region, neutral sample molecules are ionized and then accelerated into the mass analyzer. The mass analyzer is the heart of the mass spectrometer. This section separates ions, either in space or in time, according to their mass to charge ratio. After the ions are separated, they are detected and the

*More precisely mass spectrometry determines the mass of a molecule.

**The mass to charge ratio (m/z) is used to describe ions observed in mass spectrometry. By convention, m is the numerical value for the mass of the ion and z is the numerical value for the charge of the ion. The unified atomic mass (u) and the elementary charge units (e) are used for these values. The unified atomic mass is defined as 1/12 the mass of an atom of ^{12}C. Older terms still in use but not accepted as SI units include the atomic mass unit (amu) and the dalton (Da). The amu is no longer acceptable because there are conflicting definitions. The dalton is frequently used for polymers, peptides and other large molecules. The elementary charge unit is defined as z is an integer equal to the number of electrons lost (or gained for negative ions). For most experiments one electron is lost during ionization so z is +1 and the m/z value is equivalent to the relative molecular mass of the ion. Because the unified atomic mass and the charge number are pure numbers the mass-to-charge ratio is a number and does not have any units. For calculations of the physical behavior of ions it is often necessary to use the actual mass (SI units of kilogram) and charge (SI units of coulomb).

signal is transferred to a data system for analysis. All mass spectrometers also have a vacuum system to maintain the low pressure, which is also called high vacuum, required for operation. High vacuum minimizes ion-molecule reactions, scattering, and neutralization of the ions. In some experiments, the pressure in the source region or a part of the mass spectrometer is intentionally increased to study these ion-molecule reactions. Under normal operation, however, any collisions will interfere with the analysis.

SAMPLE INTRODUCTION:

The selection of a sample inlet depends upon the sample and the sample matrix. Most ionization techniques are designed for gas phase molecules so the inlet must transfer the analyte into the source as a gas phase molecule. If the analyte is sufficiently volatile and thermally stable, a variety of inlets are available. Gases and samples with high vapor pressure are introduced directly into the source region. Liquids and solids are usually heated to increase the vapor pressure for analysis. If the analyte is thermally labile (it decomposes at high temperatures) or if it does not have a sufficient vapor pressure, the sample must be directly ionized from the condensed phase. These direct ionization techniques require special instrumentation and are more difficult to use. However, they greatly extend the range of compounds that may be analyzed by mass spectrometry. Commercial instruments are available that use direct ionization techniques to routinely analyze proteins and polymers with molecular weights greater than 100,000 dalton.

Direct Vapor Inlet. The simplest sample introduction method is a direct vapor inlet. The gas phase analyte is introduced directly into the source region of the mass spectrometer through a needle valve. Pump out lines are usually included to remove air from the sample. This inlet works well for gases, liquids, or solids with a high vapor pressure. Samples with low vapor pressure are heated to increase the vapor pressure. Since this inlet is limited to stable compounds and modest temperatures, it only works for some samples.

Gas Chromatography. Gas chromatography is probably the most common technique for introducing samples into a mass spectrometer. Complex mixtures are routinely separated by gas chromatography and mass spectrometry is used to identify and quantitate the individual components. Several different interface designs are used to connect these two instruments. The most significant characteristics of the inlets are the amount of GC carrier gas that enters the mass spectrometer and the amount of analyte that enters the mass spectrometer. If a large flow of GC carrier gas enters the mass spectrometer it will increase the pressure in the source region. Maintaining the required source pressure will require larger and more expensive vacuum pumps. The amount of analyte that enters the mass spectrometer is important for improving the detection limits of the instrument. Ideally all the analyte and none of the GC carrier gas would enter the source region.

The most common GC/MS interface now uses a capillary GC column. Since the carrier gas flow rate is very small for these columns, the end of the capillary is inserted directly into the source region of the mass spectrometer. The entire flow from the GC enters the mass spectrometer. Since capillary columns are now very common, this inlet is widely used.

However, wide bore capillaries and packed GC columns have higher flow rates. This

significantly increases the pressure in the mass spectrometer. Several inlet designs are available to reduce the gas flow into the source. The simplest design splits the GC effluent so that only a small portion of the total flow enters the mass spectrometer. Although this inlet reduces the gas load on the vacuum system, it also reduces the amount of analyte. Effusive separators and membrane inlets are more selective and transport a higher fraction of the analyte into the source region. Each of these methods has efficiency and resolution drawbacks but they are necessary for some experiments.

Liquid Chromatography. Liquid chromatography inlets are used to introduce thermally labile compounds not easily separated by gas chromatography. These inlets have undergone considerable development and are now fairly routine. Because these inlets are used for temperature sensitive compounds, the sample is ionized directly from the condensed phase. These inlets are discussed in greater detail in the section on ionization techniques.

Direct Insertion Probe. The Direct Insertion Probe (DIP) is widely used to introduce low vapor pressure liquids and solids into the mass spectrometer. The sample is loaded into a short capillary tube at the end of a heated sleeve. This sleeve is then inserted through a vacuum lock so the sample is inside the source region. After the probe is positioned, the temperature of the capillary tube is increased to vaporize the sample. This probe is used at higher temperatures than are possible with a direct vapor inlet. In addition, the sample is under vacuum and located close to the source so that lower temperatures are required for analysis. This is important for analyzing temperature sensitive compounds. Although the direct insertion probe is more cumbersome than the direct vapor inlet, it is useful for a wider range of samples.

Direct Ionization of Sample. Unfortunately, some compounds either decompose when heated or have no significant vapor pressure. These samples may be introduced to the mass spectrometer by direct ionization from the condensed phase. These direct ionization techniques are used for liquid chromatography/mass spectrometry, glow discharge mass spectrometry, fast atom bombardment and laser ablation. The development of new ionization techniques is an active research area and these techniques are rapidly evolving. Direct ionization is discussed in greater detail in the next section.

IONIZATION TECHNIQUES:

A variety of ionization techniques are used for mass spectrometry. Most ionization techniques excite the neutral analyte molecule which then ejects an electron to form a radical cation $(M^{+\bullet})^{*}$. Other ionization techniques involve ion molecule reactions that produce adduct ions (MH^{+}).** The most important considerations are the physical state of the analyte and the ionization energy. Electron ionization and chemical ionization are only suitable for gas phase ionization. Fast atom bombardment, secondary ion mass spectrometry, electrospray, and matrix assisted laser desorption are used to ionize condensed phase samples. The ionization energy is

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* $M^{+\bullet}$ is the molecular ion produced by removing a single electron to form a radical cation. M is the molecule, $^{+}$ is the charge of the cation, and $^{\bullet}$ is the remaining unpaired electron of the radical.

** Adduct ions are produced by a chemical reaction between an ion and a neutral molecule. Many of these reactions cause the addition of a proton (H^{+}) to the molecule (M) and produce an adduct ion (MH^{+}).

significant because it controls the amount of fragmentation observed in the mass spectrum. . Although this fragmentation complicates the mass spectrum, it provides structural information for the identification of unknown compounds. Some ionization techniques are very soft and only produce molecular ions,* other techniques are very energetic and cause ions to undergo extensive fragmentation. Although this fragmentation complicates the mass spectrum, it provides structural information for the identification of unknown compounds.

Electron Ionization. Electron Ionization (EI) is the most common ionization technique used for mass spectrometry.** EI works well for many gas phase molecules, but it does have some limitations. Although the mass spectra are very reproducible and are widely used for spectral libraries, EI causes extensive fragmentation so that the molecular ion is not observed for many compounds. Fragmentation is useful because it provides structural information for interpreting unknown spectra.

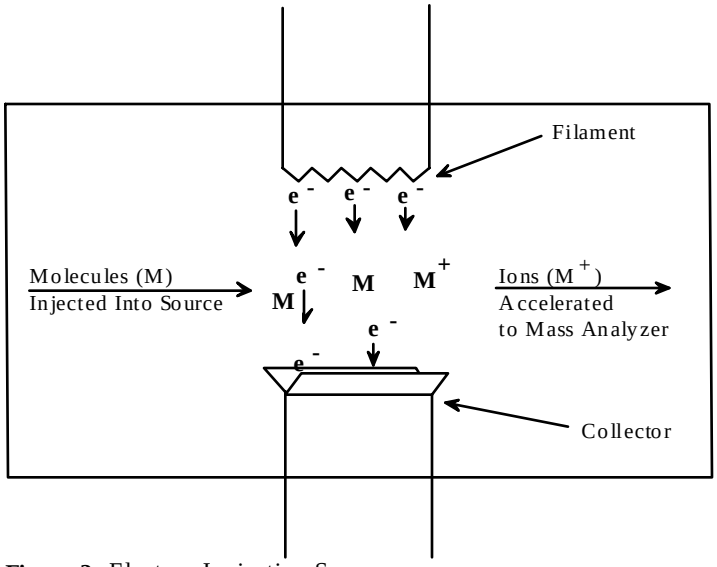

Figure 2. Electron Ionization Source.

The electrons used for ionization are produced by passing a current through a wire filament (Figure 2). The amount of current controls the number of electrons emitted by the filament. An electric field accelerates these electrons across the source region to produce a beam of high energy electrons. When an analyte molecule passes through this electron beam, a valence shell electron can be removed from the molecule to produce an ion.

*Molecular ions are the intact ionized analyte molecule. Fragment ions are formed by subsequent fragmentation of molecular ions.

**Some older literature will refer to EI as electron impact, but this term is not considered accurate. Electron Ionization is the currently accepted term.

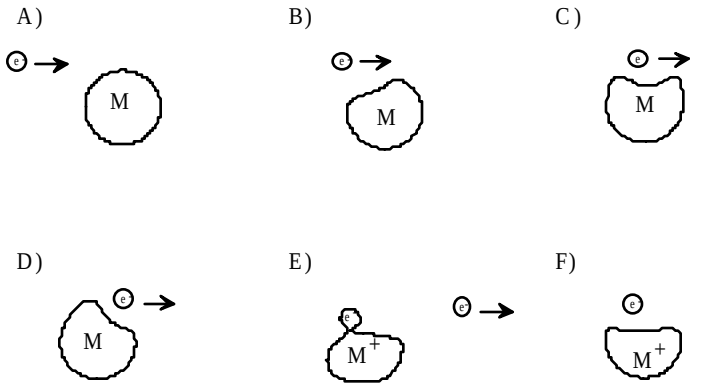

Figure 3 Electron Ionization Process. A) Ionizing electron approaches the electron cloud of a molecule; B) Electron cloud distorted by ionizing electron; C) Electron cloud further distorted by ionizing electron; D) Ionizing electron passes by the molecule; E) Electron cloud of molecule ejecting an electron; F) Molecular ion and ejected electron.

Ionization does not occur by electron capture, which is highly dependent upon molecular structure. Instead, EI produces positive ions by knocking a valence electron off the analyte molecule (Figure 3). As the electron passes close to the molecule the negative charge of the electron repels and distorts the electron cloud surrounding the molecule. This distortion transfers kinetic energy from the fast-moving electron to the electron cloud of the molecule. If enough energy is transferred by the process, the molecule will eject a valence electron and form a radical cation ($M^{+\bullet}$).

Since the ionization is produced by a single electron that is accelerated to 70 V, this is commonly referred to as 70 eV EI. This is enough energy to cause extensive fragmentation, and at this level small changes in the electron energy do not significantly effect the fragmentation patterns. The amount of energy transferred during this process depends upon how fast the electron is traveling and how close it passes to the molecule. In most 70 eV EI experiments, approximately 1400 kJ/mole (15 eV)* of energy is transferred during the ionization process. There is, however, a distribution of energy and as much as 2800 kJ/mole (30 eV) is transferred to some molecules. Since approximately 960 kJ/mole (10 eV) is required to ionize most organic compounds and a typical chemical bond energy is 290 kJ/mole (3 eV), extensive fragmentation is often observed in 70 eV EI mass spectra. The distribution of energy transferred during ionization and the large number of fragmentation pathways results in a variety of products for a given analyte. Other electron voltages may be used to vary the amount of fragmentation produced during ionization. For most organic compounds the threshold energy for EI is about 10 eV.

Because a mass spectrum is produced by ionizing many molecules, the spectrum is a distribution of the possible product ions. Intact molecular ions are observed from ions produced

*The SI unit for energy is the Joule. The energetics of chemical reactions are typically expressed in kJ/mole. In many gas phase experiments (like mass spectrometry), the mole is not a convenient unit. The electron volt is frequently used as an energy unit for single molecules or atoms. 1 eV = 1.602 177 33 (49) x 10^{-19} J. So that: 1 eV (per molecule or atom) = 96.415 220 6 kJ/mole.

with little excess energy. Other molecular ions have more energy and undergo fragmentation in the source region. The abundance of the resulting fragments, often called product ions*, is determined by the kinetics of the fragmentation pathways and the ionization energy. Changing the ionization energy changes the observed distribution of fragment ions. This distribution provides the structural information for interpreting mass spectra and is discussed in detail in the section on interpretation.

Chemical Ionization (3, 4). Chemical Ionization (CI) is a "soft" ionization technique that produces ions with little excess energy. As a result, less fragmentation is observed in the mass spectrum. Since this increases the abundance of the molecular ion, the technique is complimentary to 70 eV EI. CI is often used to verify the molecular mass of an unknown. Only slight modifications of an EI source region are required for CI experiments.

In Chemical Ionization the source is enclosed in a small cell with openings for the electron beam, the reagent gas and the sample. The reagent gas is added to this cell at approximately 10 Pa (0.1 torr) pressure. This is higher than the 10^{-3} Pa (10^{-5} torr) pressure typical for a mass spectrometer source. At 10^{-3} Pa the mean free path between collisions is approximately 2 meters and ion-molecule reactions are unlikely. In the CI source, however, the mean free path between collisions is only 10^{-4} meters and analyte molecules undergo many collisions with the reagent gas. The reagent gas in the CI source is ionized with an electron beam to produce a cloud of ions. The reagent gas ions in this cloud react and produce adduct ions like CH_5^+ (Figure 4), which are excellent proton donors.

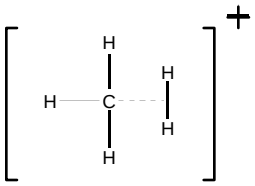

Figure 4. CH_5^+ ion.

When analyte molecules (M) are introduced to a source region with this cloud of ions, the reagent gas ions donate a proton to the analyte molecule and produce MH^+ ions. The energetics of the proton transfer is controlled by using different reagent gases. The most common reagent gases are methane, isobutane and ammonia. Methane is the strongest proton donor commonly used with a proton affinity (PA) of 5.7 eV. For softer ionization, isobutane (PA 8.5 eV) and ammonia (PA 9.0 eV) are frequently used. Acid base chemistry is frequently used to describe the chemical ionization reactions. The reagent gas must be a strong enough Brønsted acid to transfer a proton to the analyte. Fragmentation is minimized in CI by reducing the amount of excess energy produced by the reaction. Because the adduct ions have little excess energy and are relatively stable, CI is very useful for molecular mass determination. Some typical reactions in a CI source are shown in Figure 5.

*Product or fragment ions may be called daughter ions in some literature articles, but the term is considered controversial and is falling into disuse.

A) EI of reagent gas to form ions:

$$CH_4 + e^- \rightarrow CH_4^{+\bullet} + 2\,e^-$$

B) Reaction of reagent gas ions to form adducts:

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

OR

$$CH_4^{+\bullet} \rightarrow CH_3^{+} + H^{\bullet}$$
$$CH_3^{+} + CH_4 \rightarrow C_2H_5^{+} + H_2$$

C) Reaction of Reagent Gas Ions with analyte molecules:

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

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

$$CH_3^{+} + M \rightarrow CH_4 + (M\text{-}H)^{+}$$

Figure 5 Chemical Ionization Reactions

Fast Atom Bombardment and Secondary Ion Mass Spectrometry. (*5*) Fast Atom Bombardment (FAB) and Secondary Ion Mass Spectrometry (SIMS) both use high energy atoms to sputter and ionize the sample in a single step. In these techniques, a beam of rare gas neutrals (FAB) or ions (SIMS) is focused on the liquid or solid sample. The impact of this high energy beam causes the analyte molecules to sputter into the gas phase and ionize in a single step (Figure 6). The exact mechanism of this process is not well understood, but these techniques work well for compounds with molecular weights up to a few thousand dalton. Since no heating is required, sputtering techniques (especially FAB) are useful for studying thermally labile compounds that decompose in conventional inlets (*6*, *7*).

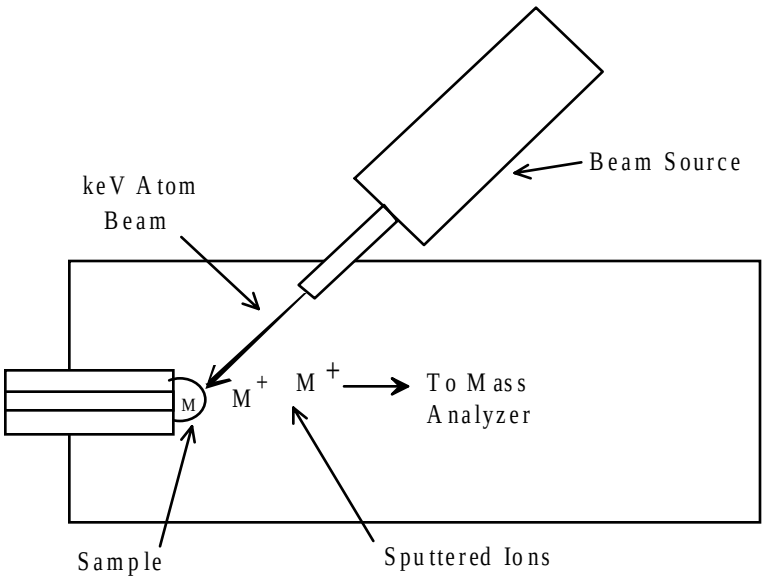

Figure 6. Fast Atom Bombardment Source.

The most significant difference between FAB and SIMS is the sample preparation. In FAB the analyte is dissolved in a liquid matrix. A drop of the sample/matrix mixture is placed at the end of an insertion probe and introduced to the source region. The fast atom beam is focused on this droplet to produce analyte ions. Glycerol or similar low vapor pressure liquids are typically used for the matrix. Ideally, the analyte is soluble in the liquid matrix and a monolayer of analyte forms on the surface of the droplet. According to one theory, this monolayer concentrates the analyte while the dissolved sample provides a reservoir to replenish the monolayer as the analyte is depleted. Without this constant replenishment from the bulk solution, the ionizing beam will rapidly deplete the analyte and the signal is difficult to observe.

SIMS experiments(*8*) are used to study surface species and solid samples.* No matrix is used and the ionizing beam is focused directly on the sample. Although this makes sampling more difficult, it is useful for studying surface chemistry. High resolution chemical maps are produced by scanning a tightly focused ionizing beam across the surface and depth profiles are produced by probing a single location(*9*, *10*). Although SIMS is a very sensitive and powerful technique for surface chemistry and materials analysis, the results are often difficult to quantitate.

Atmospheric Pressure Ionization and Electrospray Ionization. (*11*, *12*, *13*) Atmospheric Pressure Ionization (API) sources ionize the sample at atmospheric pressure and then transfer the ions into the mass spectrometer. These techniques are used to ionize thermally labile samples such as peptides, proteins and polymers directly from the condensed phase. The sample is dissolved in an appropriate solvent and this solution is introduced into the mass spectrometer. With conventional inlets the solvent increases the pressure in the source region of the mass spectrometer. In addition, Joule-Thompson cooling of the liquid as it enters the vacuum causes

* Liquid SIMS (LSIMS) is very similar to FAB except Cesium ions are used for higher energy collisions.

the solvent droplets to freeze. The frozen clusters trap analyte molecules and reduce the sensitivity of the experiment.

API sources introduce the sample through a series of differentially pumped stages. This maintains the large pressure difference between the ion source and the mass spectrometer (Figure 7) without using extremely large vacuum pumps. In addition a drying gas is used to break up the clusters that form as the solvent evaporates. Because the analyte molecules have more momentum than the solvent and air molecules, they travel through the pumping stages to the mass analyzer.

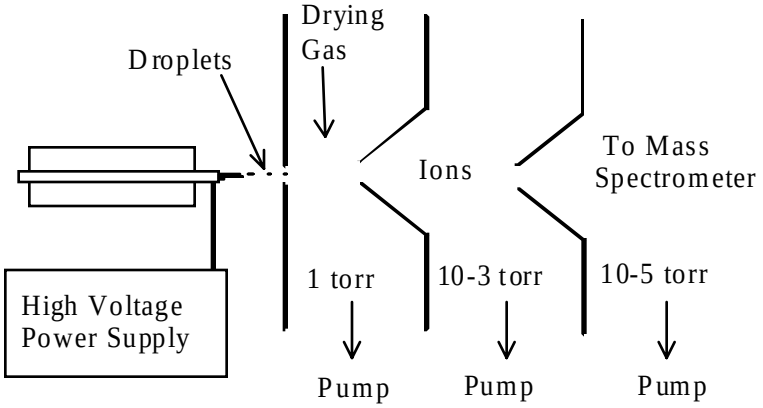

Figure 7. Electrospray Ionization Source.

ElectroSpray Ionization (ESI) is the most common API application. It has undergone remarkable growth in recent years and is frequently used for LC/MS of thermally labile and high molecular weight compounds. The electrospray is created by applying a large potential between the metal inlet needle and the first skimmer in an API source (Figure 7). The mechanism for the ionization process is not well understood and there are several different theories that explain this ionization process. One theory is that as the liquid leaves the nozzle, the electric field induces a net charge on the small droplets. As the solvent evaporates, the droplet shrinks and the charge density at the surface of the droplet increases. The droplet finally reaches a point where the coulombic repulsion from this electric charge is greater than the surface tension holding it together. This causes the droplet to explode and produce multiply charged analyte ions. A typical ESI spectrum shows a distribution of molecular ions with different charge numbers.

Because electrospray produces multiply charged ions, high molecular weight compounds are observed at lower *m/z* value. This increases the mass range of the analyzer so that higher molecular weight compounds may be analyzed with a less expensive mass spectrometer. An ion with a mass of 5000 u and a charge of +10 is observed at *m/z* 500 and is easily analyzed with an inexpensive quadrupole analyzer.

API Sources are also used for Inductively Coupled Plasma Mass Spectrometry (ICP/MS) and glow discharge experiments (*14*, *15*, *16*). In ICP/MS a nebulizer is used to introduce liquid samples into a high temperature plasma. The temperature of the plasma is high enough to efficiently ionize most elements. These ions are introduced to the mass spectrometer using an series of differentially pumped regions similar to the electrospray source discussed above. Glow discharge experiments are similar, but used for solid samples. The high sensitivity and

selectivity of the mass spectrometer provides rapid multielement detection at very low levels. Because the high temperature of the plasma destroys any chemical bonds, these techniques are used for elemental analysis.

Matrix Assisted Laser Desorption/Ionization. (*17*, *18*) Matrix Assisted Laser Desorption/Ionization (MALDI) is used to analyze extremely large molecules. This technique directly ionizes and vaporizes the analyte from the condensed phase. MALDI is often used for the analysis of synthetic and natural polymers, proteins, and peptides. Analysis of compounds with molecular weights up to 200,000 dalton is possible and this high mass limit is continually increasing.

In MALDI, both desorption and ionization are induced by a single laser pulse (Figure 8). The sample is prepared by mixing the analyte and a matrix compound chosen to absorb the laser wavelength. This is placed on a probe tip and dried. A vacuum lock is used to insert the probe into the source region of the mass spectrometer. A laser beam is then focused on this dried mixture and the energy from a laser pulse is absorbed by the matrix. This energy ejects analyte ions from the surface so that a mass spectrum is acquired for each laser pulse. The mechanism for this process is not well understood and is the subject of much controversy in the literature. This technique is more universal (works with more compounds) than other laser ionization techniques because the matrix absorbs the laser pulse. With other laser ionization techniques, the analyte must absorb at the laser wavelength. Typical MALDI spectra include the molecular ion, some multiply charged ions, and very few fragments.

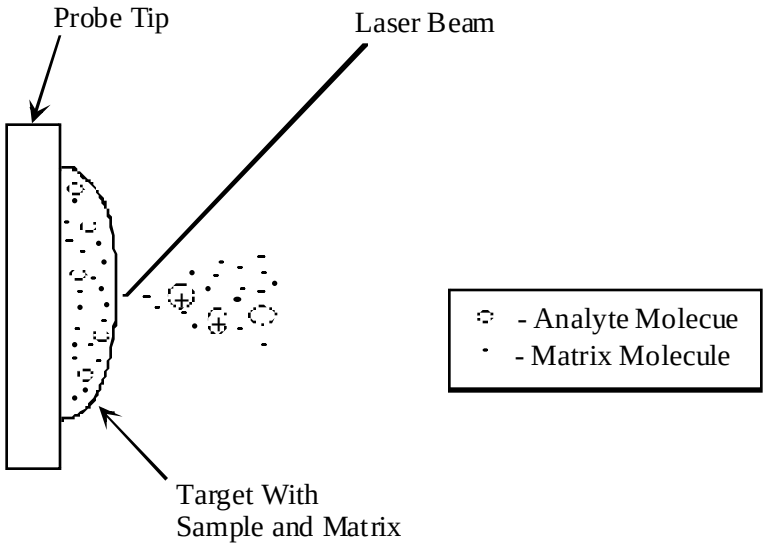

Figure 8. MALDI Ionization

Other Ionization Methods. There are several other ionization methods used for mass spectrometry and interested readers are referred to the chemical literature for additional information about other techniques. Field Desorption (*19*) was used for ionization and vaporization of moderate sized molecules before the development of FAB, electrospray, and MALDI. It is still an important technique for some analysis and is typically used for non-polar

polymers and petroleum samples. Plasma Desorption (PD) (*20*, *21*) is a technique used to analyze high molecular weight compounds before the development of MALDI and electrospray. However, it is very complex and has not found widespread application. Resonance Ionization Mass Spectrometry (RIMS) is used for selective atomic and molecular ionization. (*22*) Photoionization with lasers, lamps, and synchrotron sources is used to study the photochemistry and energetics of many compounds. (*23*) Lasers are used to ionize surface samples with Laser Microprobe Mass Analysis (LAMMA). (*24*, *25*)

Self-Test #1:* What ionization technique would be appropriate for analyzing the following compounds: a) gasoline fractions, b) pesticide residue, c) ibuprofen and acetaminophen, d) insulin, e) tripeptides, f) heavy metals in water.

MASS ANALYZERS:

After ions are formed in the source region they are accelerated into the mass analyzer by an electric field. The mass analyzer separates these ions according to their *m/z* value. The selection of a mass analyzer depends upon the resolution,** (*26*) mass range,*** scan rate**** and detection limits required for an application. Each analyzer has very different operating characteristics and the selection of an instrument involves important tradeoffs.

Analyzers are typically described as either continuous or pulsed. Continuous analyzers include quadrupole filters and magnetic sectors. These analyzers are similar to a filter or monochromator used for optical spectroscopy. They transmit a single selected *m/z* to the detector and the mass spectrum is obtained by scanning the analyzer so that different mass to charge ratio ions are detected. While a certain *m/z* is selected, any ions at other *m/z* ratios are lost, reducing the S/N for continuous analyzers. Single Ion Monitoring (SIM) enhances the S/N by setting the mass spectrometer at the *m/z* for an ion of interest. Since the instrument is not scanned the S/N improves, but any information about other ions is lost. Pulsed mass analyzers are the other major class of mass analyzer. These are less common but they have some distinct advantages. These

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*Answers to Self-Test questions are given at the end of the paper.

** Resolution in mass spectrometry refers to the separation of two ions where $R=m/\Delta m$. These terms are defined several different ways. The most common are the 10% valley definition "Let two peaks of equal height in a mass spectrum at masses m and m-Δm be separated by a valley that at its lowest point is just 10% of the height of either peak." and the peak width definition "For a single peak made up of singly charged ions at mass m in a mass spectrum, the resolution may be expressed as m/Δm, where Δm is the width of the peak at a height that is a specified fraction of the maximum peak height. It is recommended that one of three values 50 %, 5 % or 0.5 % be used."(*34*)

***Mass range refers to the highest mass to charge ratio transmitted by the mass spectrometer.

****The scan rate of a mass spectrometer refers to how fast it scans a mass spectrum. This is important for chromatography applications where the entire mass spectrum must be scanned faster than the elution time of the chromatographic peak. Ideally, a minimum of ten complete mass spectra are acquired for a single chromatographic peak

instruments collect an entire mass spectrum from a single pulse of ions. This results in a signal to noise advantage similar to Fourier transform or multichannel spectroscopic techniques. Pulsed analyzers include time-of-flight, ion cyclotron resonance, and quadrupole ion trap mass spectrometers.

Quadrupole27. The quadrupole mass spectrometer is the most common mass analyzer. Its compact size, fast scan rate, high transmission efficiency,* and modest vacuum requirements are ideal for small inexpensive instruments. Most quadrupole instruments are limited to unit *m/z* resolution** and have a mass range of *m/z* 1000. Many benchtop instruments have a mass range of *m/z* 500 but research instruments are available with mass range up to *m/z* 4000.

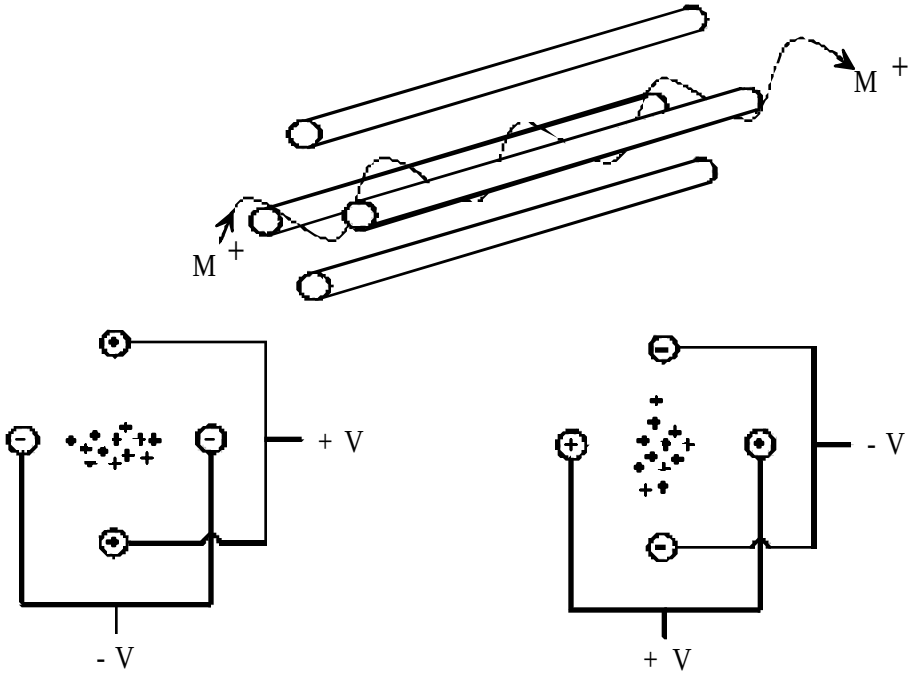

Figure 9. Quadrupole Mass Analyzer. A) Ion trajectory through the quadrupole, B) Ion focusing during first half of RF cycle, C) Ion focusing during second half of RF cycle.

In the mass spectrometer, an electric field accelerates ions out of the source region and into the quadrupole analyzer. The analyzer consists of four rods or electrodes arranged across from each other (Figure 9). As the ions travel through the quadrupole they are filtered according

*Transmission efficiency refers to how many of the ions produced in the source region actually reach the detector. This is an important measure of sensitivity for mass spectrometers.

**Unit resolution (or low resolution) mass spectra distinguish between ions separated by 1 *m/z* unit. The spectra, like those presented here, are commonly displayed as histograms. This is a common method for presenting spectra because it results in much smaller data file size. Some mass analyzers can obtain spectra at much higher resolution. This is discussed in detail in the interpretation section.

to their *m/z* value so that only a single *m/z* value ion can strike the detector. The *m/z* value transmitted by the quadrupole is determined by the Radio Frequency (RF) and Direct Current (DC) voltages applied to the electrodes. These voltages produce an oscillating electric field that functions as a bandpass filter to transmit the selected *m/z* value.

The RF voltage rejects or transmits ions according to their *m/z* value by alternately focusing them in different planes (Figure 9). The four electrodes are connected in pairs and the RF potential is applied between these two pairs of electrodes. During the first part of the RF cycle the top and bottom rods are at a positive potential and the left and right rods are at a negative potential. This squeezes positive ions into the horizontal plane. During the second half of the RF cycle the polarity of the rods is reversed. This changes the electric field and focuses the ions in the vertical plane. The quadrupole field continues to alternate as the ions travel through the mass analyzer. This causes the ions to undergo a complex set of motions that produces a three-dimensional wave.

The quadrupole field transmits selected ions because the amplitude of this three-dimensional wave depends upon the *m/z* value of the ion, the potentials applied, and the RF frequency. By selecting an appropriate RF frequency and potential, the quadrupole acts like a high pass filter, transmitting high *m/z* ions and rejecting low *m/z* ions. The low *m/z* ions have a greater acceleration rate so the wave for these ions has a greater amplitude. If this amplitude is great enough the ions will collide with the electrodes and can not reach the detector. The low *m/z* value cutoff of the quadrupole is changed by adjusting the RF potential or the RF frequency. Any ions with a *m/z* greater than this cutoff are transmitted by the quadrupole.

A DC voltage is also applied across the rods of the analyzer. This potential combined with the RF potential acts like a low pass filter to reject high *m/z* ions. Because they respond quickly to the changing RF field the motion of the low *m/z* ions is dominated by the RF potential. This motion stabilizes their trajectory by refocusing each time the RF potential changes polarity. Because low *m/z* ions are quickly refocused, the DC potential does not affect these ions. High *m/z* ions, however, do not refocus as quickly during the RF cycle. The DC potential has a greater influence on their trajectory and they slowly drift away from the center of the quadrupole. At the end of the analyzer, they are too far off-axis to strike the detector.

The combination of high and low pass filters produced by the RF and DC potentials is adjusted to only transmit the selected *m/z* value. All ions above or below the set *m/z* value are rejected by the quadrupole filter. The RF and DC fields are scanned (either by potential or frequency) to collect a complete mass spectrum. Quadrupole mass analyzers are often called mass filters because of the similarity between *m/z* selection by a quadrupole and wavelength selection by an optical filter or frequency selection by an electronic filter.

Magnetic Sector. The first mass spectrometer, built by J.J. Thompson in 1897, used a magnet to measure the *m/z* value of an electron. Magnetic sector instruments have evolved from this same concept. Sector instruments have higher resolution and greater mass range than quadrupole instruments, but they require larger vacuum pumps and often scan more slowly. The typical mass range is to *m/z* 5000, but this may be extended to *m/z* 30,000. Magnetic sector instruments are often used in series with an electric sector, described below, for high resolution and tandem mass spectrometry experiments.

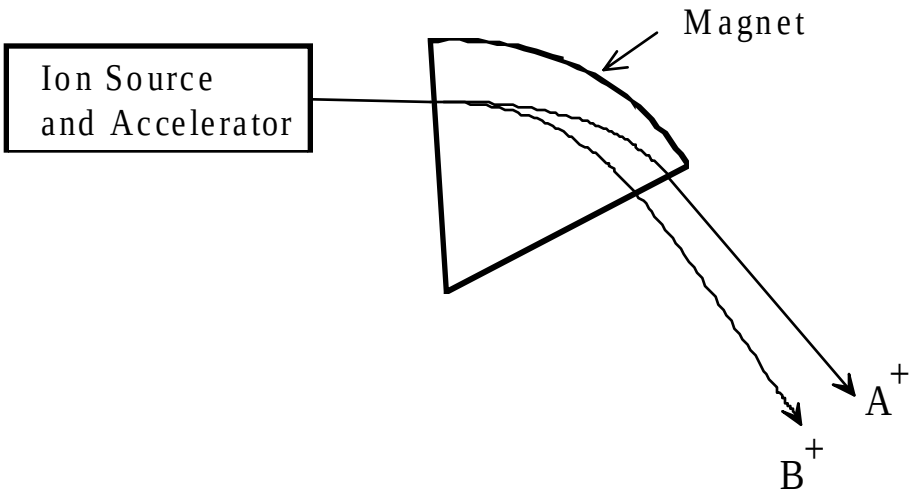

Figure 10. Magnetic Sector Mass Spectrometer. The low m/z ion (B^+) is separated from the high m/z ion (A^+).

Magnetic sector instruments (Figure 10) separate ions in a magnetic field according to the momentum and charge of the ion. Ions are accelerated from the source region into the magnetic sector by a 1 to 10 kV electric field. This acceleration is significantly greater than the 100 V acceleration typical for a quadrupole instrument. Since the ions are charged, as they move through the magnetic sector, the magnetic field bends the ion beam in an arc. This is the same principal that causes electric motors to turn. The radius of this arc (r) depends upon the momentum of the ion (μ), the charge of the ion (C) and the magnetic field strength (B).

$$r = \frac{\mu}{C \times B} \qquad \textbf{Equation 1}$$

Ions with greater momentum will follow an arc with a larger radius. This separates ions according to their momentum, so magnetic sectors are often called momentum analyzers. The momentum of the ion is the product of the mass (m) and the velocity (v). The charge of the ion is the product of the charge number of the ion (z) and the charge of an electron (e). Substituting these variables into eq. 1 yields:

$$r = \frac{m/z \times v}{B \times e} \qquad \textbf{Equation 2}$$

The velocity of an ion is determined by the acceleration voltage in the source region (V) and the mass to charge ratio (m/z) of the ion. Equation 2 rearranges to give the m/z ion transmitted for a given radius, magnetic field, and acceleration voltage as:

$$m/z = \frac{r^2 B^2 e}{2V} \qquad \textbf{Equation 3}$$

Only one *m/z* value will satisfy Equation 3 for a given radius, magnetic field and acceleration voltage. Other *m/z* ions will travel a different radius in the magnetic sector.

Older magnetic sector instruments use a photographic plate to simultaneously detect ions at different radii. Since each *m/z* has a different radius, they strike the photographic plate at a different location. Modern instruments have a set of slits at a fixed radius to transmit a single *m/z* to the detector. The mass spectrum is scanned by changing the magnetic field or the acceleration voltage to transmit different *m/z* ions. Some new instruments use multichannel diode array detectors to simultaneously detect ions over a range of *m/z* values.

Electric Sector/Double Focusing Mass Spectrometers(28). An electric sector consists of two concentric curved plates. A voltage is applied across these plates to bend the ion beam as it travels through the analyzer. The voltage is set so that the beam follows the curve of the analyzer. The radius of the ion trajectory (r) depends upon the kinetic energy of the ion (V) and the potential field (E) applied across the plates.

$$r = \frac{2V}{E} \qquad \textbf{Equation 4}$$

Equation 4 shows that an electric sector will not separate ions accelerated to a uniform kinetic energy. The radius of the ion beam is independent of the ion's mass to charge ratio so the electric sector is not useful as a standalone mass analyzer.* An electric sector is, however, useful in series with a magnetic sector. The mass resolution of a magnetic sector is limited by the kinetic energy distribution (ΔV) of the ion beam (See eq. 3). This kinetic energy distribution results from variations in the acceleration of ions produced at different locations in the source and from the initial kinetic energy distribution of the molecules. An electric sector significantly improves the resolution of the magnetic sector by reducing the kinetic energy distribution of the ions**. These high resolutions experiments are discussed in the section on mass spectral interpretation. The effect of the electric sector is shown in Figure 11 for a reverse geometry (BE) instrument with the magnetic sector (B) located before the electric sector (E).

*The electric sector is a kinetic energy analyzer. In the source region of the mass spectrometer all ions are accelerated to the same kinetic energy. Because they have the same kinetic energy, they are not separated by an electric sector. A magnetic sector will resolve different mass ions accelerated to a uniform kinetic energy because it separates ions based upon their momentum (See eqs 1-3).

**Ion optics are complex and interested readers are referred to the literature for more detail. The model presented here provides a framework for understanding many high resolution and tandem mass spectrometry experiments. The article by Nier (25) provides an excellent introduction, a historical perspective, and many references for the development and theory of these instruments.

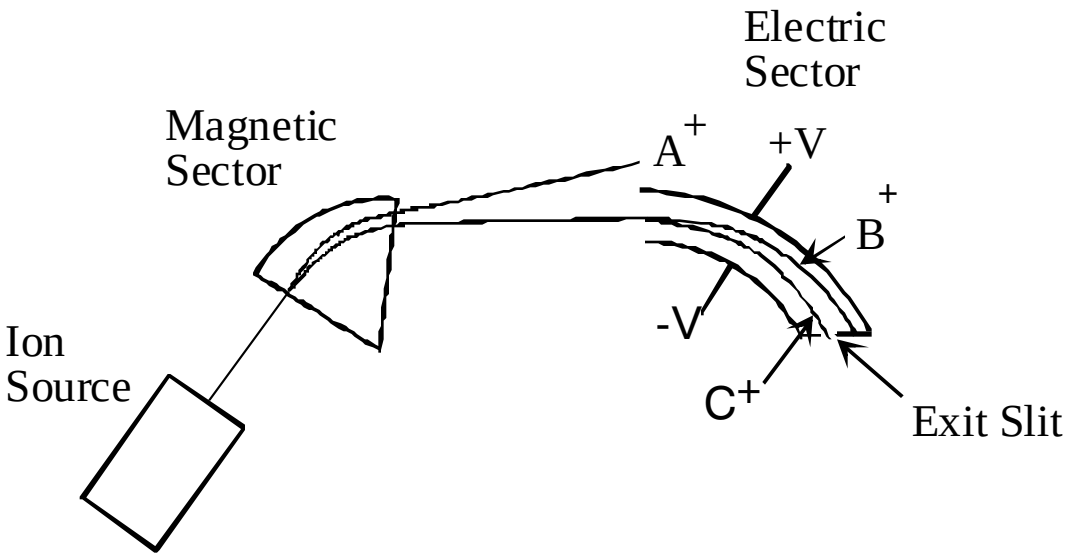

Figure 11. Reverse Geometry Double Focusing Mass Spectrometer. A^+ is a m/z 100.00 ion, B^+ is a m/z of 50.00 and C^+ is a m/z of 50.01. A^+ is rejected by the magnetic sector because of it's greater momentum. B^+ and C^+ are not resolved by the magnetic sector because they have the same momentum. To have the same momentum, however, B^+ must have a more kinetic energy than C^+. As a result the electric sector separates these two ions.

Time-of-Flight. The time-of-flight (TOF) mass analyzer separates ions in time as they travel down a flight tube (Figure 12). This is a very simple mass spectrometer that uses fixed voltages and does not require a magnetic field. The greatest drawback is that TOF instruments have poor mass resolution, usually less than 500. These instruments have high transmission efficiency, no upper *m/z* limit, very low detection limits, and fast scan rates. For some applications these advantages outweigh the low resolution. Recent developments in pulsed ionization techniques and new instrument designs with improved resolution have renewed interest in TOF-MS. (*29*)

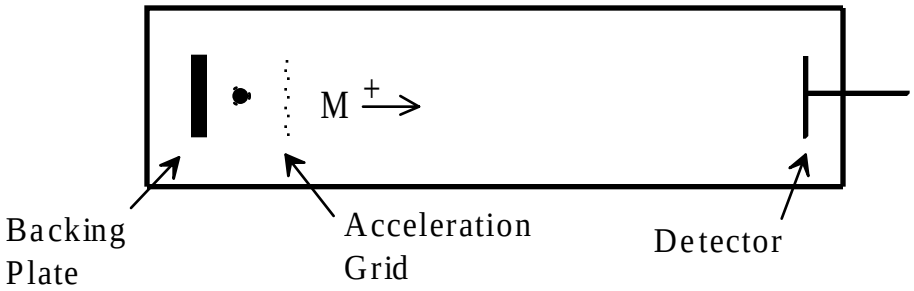

Figure 12. Time-of-Flight Mass Spectrometer.

In the source of a TOF analyzer, a packet of ions is formed by a very fast (ns) ionization pulse. These ions are accelerated into the flight tube by an electric field (typically 2-25 kV) applied between the backing plate and the acceleration grid. Since all the ions are accelerated across the same distance by the same force, they have the same kinetic energy. Because velocity

(v) is dependent upon the kinetic energy ($E_{kinetic}$) and mass (m) lighter ions will travel faster.

$$E_{kinetic} = \frac{1}{2} m v^2 \qquad \textbf{Equation 5}$$

$E_{kinetic}$ is determined by the acceleration voltage of the instrument (V) and the charge of the ion ($e \times z$). Equation 5 rearranges to give the velocity of an ion (v) as a function of acceleration voltage and m/z value.

$$v = \sqrt{\frac{2V \times e}{m/z}} \qquad \textbf{Equation 6}$$

After the ions accelerate, they enter a 1 to 2 meter flight tube. The ions drift through this field free region at the velocity reached during acceleration. At the end of the flight tube they strike a detector. The time delay (t) from the formation of the ions to the time they reach the detector dependents upon the length of the drift region (L), the mass to charge ratio of the ion, and the acceleration voltage in the source.

$$t = \frac{L}{\sqrt{2V \times e}} \sqrt{m/z} \qquad \textbf{Equation 7}$$

Equation 7 shows that low m/z ions will reach the detector first. The mass spectrum is obtained by measuring the detector signal as a function of time for each pulse of ions produced in the source region. Because all the ions are detected, TOF instruments have very high transmission efficiency which increases the S/N level .

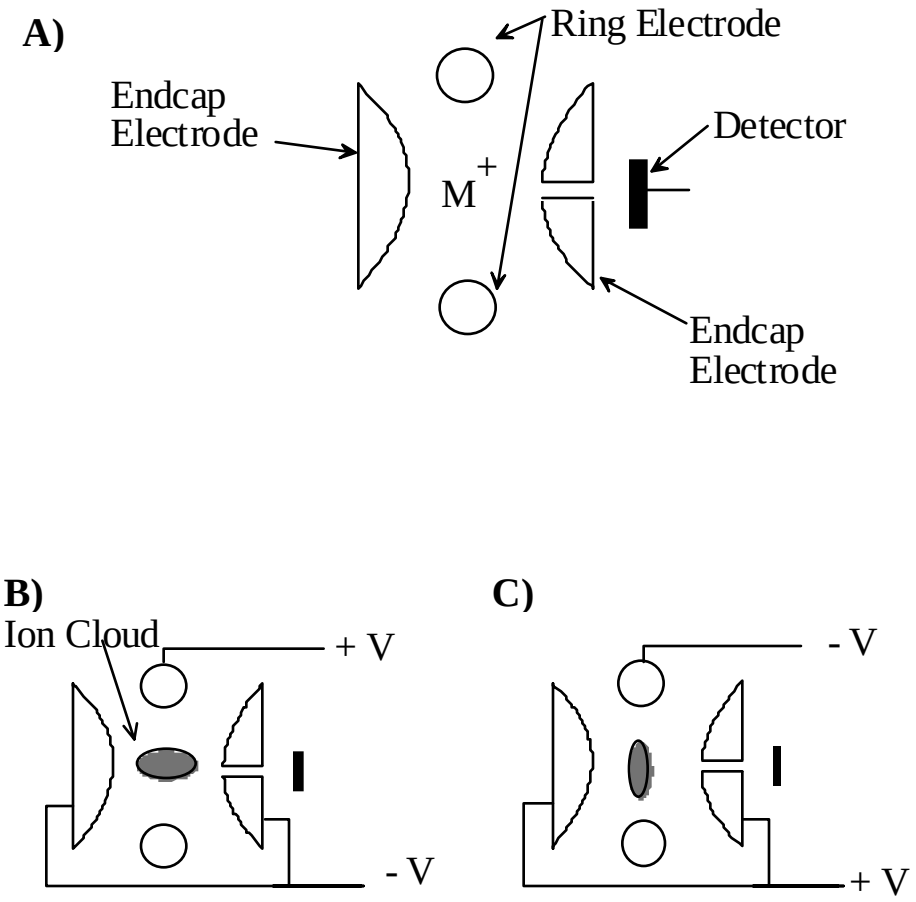

Figure 13. Quadrupole Ion Trap Mass Spectrometer. A) shows the major components. B) and C) ion response to the applied RF field.

Quadrupole Ion Trap. (30, 31, 32) The Quadrupole ion storage trap mass spectrometer (QUISTOR) is a recently developed mass analyzer with some special capabilities. Several commercial instruments are available and this analyzer is becoming more popular. QUISTORs are very sensitive, relatively inexpensive, and scan fast enough for GC/MS experiments. The sensitivity of the QUISTOR results from trapping and then analyzing all the ions produced in the source. Since all the ions are detected, the S/N is high.

The QUISTOR consists of a doughnut shaped ring electrode and two endcap electrodes. A cutaway view of this arrangement is shown in Figure 13. A combination of RF and DC voltages is applied to the electrodes to create a quadrupole electric field similar to the electric field for the quadrupole mass analyzer. This electric field traps ions in a potential energy well at the center of the analyzer. The mass spectrum is acquired by scanning the RF and DC fields to destabilize low mass to charge ions. These destabilized ions are ejected through a hole in one endcap electrode and strike a detector. The mass spectrum is generated by scanning the fields so that ions of increasing *m/z* value are ejected from the cell and detected. The trap is then refilled with a new batch of ions to acquire the next mass spectrum. The mass resolution of the ion trap

is increased by adding a small amount 0.1 Pa (10^{-3} torr) of Helium as a bath gas. Collisions between the analyte ions and the inert bath gas dampen the motion of the ions and increases the trapping efficiency of the analyzer.

Ion Cyclotron Resonance. (30, 33, 34) The Ion Cyclotron Resonance (ICR) mass spectrometer uses a superconducting magnet to trap ions in a small sample cell. This type of mass analyzer has extremely high mass resolution (ca. 10^9) and is also useful for tandem mass spectrometry experiments. These instruments are very expensive and are typically used for specialized research applications. The ICR traps ions in a magnetic field that causes ions travel in a circular path (Figure 14). This is similar to the path of an ion in a magnetic sector, but the ions are not traveling as fast and the magnetic field is stronger. As a result the ions are contained in the small volume of the trap.

The ion's cyclotron frequency (ω), is the angular frequency* of an ion's orbit. This frequency is determined by the magnetic field strength (B) and the *m/z* value of the ion.

$$\omega = \frac{B \times e}{m/z} \qquad \textbf{Equation 8}$$

After ions are trapped in this cell they are detected by measuring the signal at this cyclotron frequency. This signal is measured by placing electrodes on each side of the ions circular orbit. An RF voltage is applied to the transmitter electrodes at the cyclotron frequency of the ion of interest. This RF energy moves ions at the applied frequency to a larger orbit. As these ions travel around the ICR cell they are close enough to the receiver electrodes to induce a capacitive current. This capacitive current oscillates at the cyclotron frequency and is detected as the signal.

The ICR is also used as a Fourier Transform Mass Spectrometer (FT-MS). Instead of using a single excitation frequency, a fast RF pulse is applied to the transmitter electrodes. This simultaneously excites all the ions and produces a signal at the cyclotron frequency of each *m/z* ion present. This signal is similar to the Free Induction Decay (FID) produced in an FT-NMR experiment. A complete mass spectrum is obtained by using the Fourier transform to convert this signal from the time domain to the frequency domain.

*The angular frequency (ω) is in radians per second. The unit Hertz (Hz) is in cycles per second where there are 2π radians per cycle.

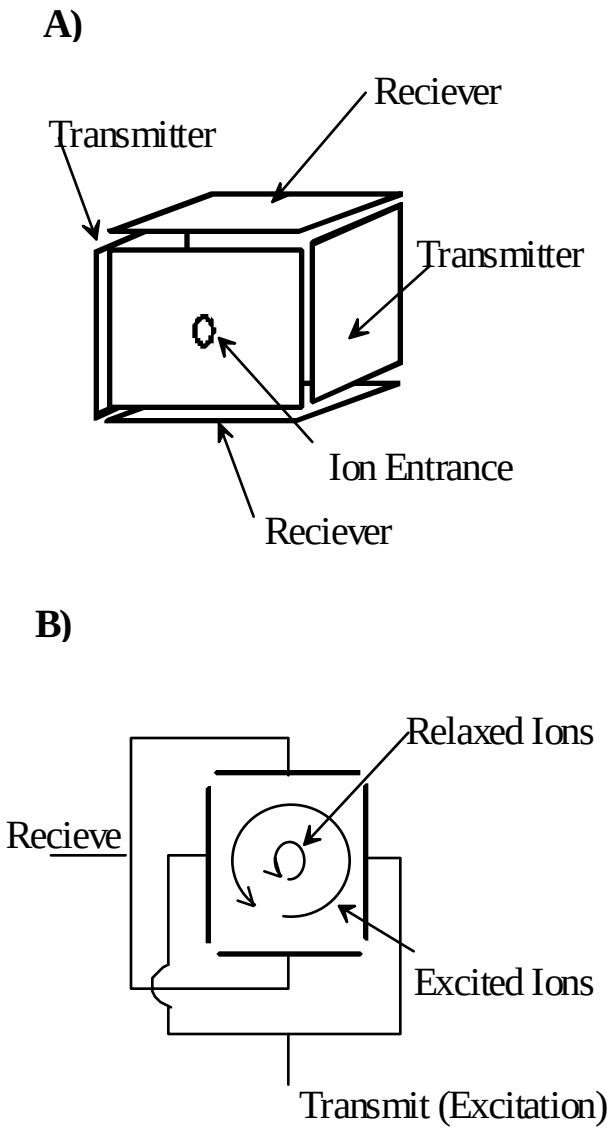

Figure 14. Ion Cyclotron Mass Spectrometer. A) major components, B) ion motion within the trap.

Self-Test #2: Which mass analyzer is appropriate for the following analysis: a) Routine analysis of drug testing samples, b) Analysis of small, 2000 dalton, peptides, c) Analysis of 50,000 dalton polymers, d) High sensitivity for detecting chemical warfare agents, e) High resolution analysis.

DETECTORS:

Detection of ions is based upon their charge or momentum. For large signals a faraday cup is used to collect ions and measure the current. Older instruments used photographic plates to measure the ion abundance at each mass to charge ratio. Most detectors currently used amplify the ion signal using a collector similar to a photomultiplier tube. These amplifying

detectors include: electron multipliers, channeltrons and multichannel plates. The gain is controlled by changing the high voltage applied to the detector. A detector is selected for it's speed, dynamic range, gain, and geometry. Some detectors are sensitive enough to detect single ions.

VACUUM SYSTEM:

All mass spectrometers operate at very low pressure (high vacuum). This reduces the chance of ions colliding with other molecules in the mass analyzer. Any collision can cause the ions to react, neutralize, scatter, or fragment. All these processes will interfere with the mass spectrum. To minimize collisions, experiments are conducted under high vacuum conditions, typically 10^{-2} to 10^{-5} Pa (10^{-4} to 10^{-7} torr) depending upon the geometry of the instrument. This high vacuum requires two pumping stages. The first stage is a mechanical pump that provides rough vacuum down to 0.1 Pa (10^{-3} torr). The second stage uses diffusion pumps or turbomolecular pumps to provide high vacuum. ICR instruments have even higher vacuum requirements and often include a cryogenic pump for a third pumping stage. The pumping system is an important part of any mass spectrometer but a detailed discussion is beyond the scope of this paper.

DATA SYSTEM:

The final component of a mass spectrometer is the data system. This part of the instrument has undergone revolutionary changes in the past twenty years. It has evolved from photographic plates and strip chart recorders to data systems that control the instrument, acquire hundreds of spectra in a minute and search tens of thousands of reference spectra to identify an unknown. Because these systems are evolving so rapidly, a through discussion is not included in this paper. Interested readers should study the manuals for their instrument.

INTERPRETATION:[*]

Although mass spectrometry is a very sensitive instrumental technique, there are other techniques with picogram detection limits. In addition to sensitivity, however, mass spectrometry also is also useful for identifying the chemical structure of this picogram sample. Since the mass spectrum is a fingerprint of the molecular structure, comparison to a computer databases can be used to identify an unknown compound. This is often done using Probability Based Matching (PBM), a popular pattern recognition technique. Although these computer searches are convenient and powerful, it is important to understand how to interpret a mass spectrum. A computer only compares the unknown spectrum to the library spectra and offers a selection of compounds in the database that produce similar spectra. This computer search is very useful and it makes interpretation much easier, but there are limits to the computer search.

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[*]Molecular structure is important for understanding mass spectral interpretation. To get the most from this section, draw out the structures of the molecules discussed. During the discussion find which bonds break and calculate the mass of the fragments. Actively reading this section will result in a much greater understanding of and appreciation for mass spectrometry.

You can interpret the spectrum but it will take some effort. One common mistake made in mass spectrometry is to blindly trust the results of a computer library match. **You need to learn how to interpret and understand the mass spectrum to effectively use these computer searches.** This section should help you get started.

Table 1. Characteristics of Molecular Ions

1) The m/z value must correspond to a reasonable molecular formula with the proper isotope abundance.

2) Most compounds have an even molecular mass. The one common exception to this is the "Nitrogen Rule" discussed below.

3) The Nitrogen Rule: Any compound with an odd number of nitrogen atoms will have an odd molecular mass. Any compound with an even number of nitrogen atoms (including zero) will have an even molecular mass. This is because nitrogen is the only common atom where the most common isotope has an odd valence and an even mass. For example: the molecular ion for CH_4 is m/z 16, the molecular ion for NH_3 is m/z 17, and the molecular ion for N_2H_4 is m/z 32.

4) If a peak is the molecular ion, the next highest mass fragment must correspond to the loss of a possible neutral fragment. For example, a peak that corresponds to loss of 5 u from the molecular ion is highly unlikely.

Molecular Ion. The molecular ion provides the molecular mass of the analyte and is the first clue used to interpret a mass spectrum. The mass of the molecular ion is based upon the mass of the most abundant isotope for each element in the molecule. This is not the atomic weight from the periodic table. Since many mass spectrometers have unit mass resolution, the atomic mass is rounded to the nearest whole number, this is called the nominal mass. For example the molecular ion for $CHBr_3$ is observed at *m/z* 250, not at the formula weight of 253.*

In many mass spectra, the molecular ion is easily identified as the ion with the highest mass to charge ratio. However, this assignment should be made with caution because the highest mass to charge ion be an impurity or an isotope of the molecular ion. In addition, many compounds fragment so easily that no molecular ion is observed in the 70 eV EI spectrum. It is important to clarify that the molecular ion **IS NOT** necessarily the ion with the greatest abundance, the ion with the greatest abundance is the base peak. Table 1 lists several characteristics of molecular ions to help you identify them in a mass spectrum. Low energy EI or

*$CHBr_3$; (12 + 1 + 3×79) = 250. The mass of the molecular ion is based upon the isotope with the highest natural abundance. The most common bromine isotope is ^{79}Br. Do not use the weighted average atomic weight for Br (79.9) which is based upon the natural abundance of different isotopes. The mass spectrum of $CHBr_3$ also includes ions for other naturally occurring isotopes. This includes m/z 252 (one ^{81}Br, 49% natural abundance, and two ^{79}Br), m/z 251 (one ^{13}C, 1.1% natural abundance), m/z 257 (^{13}C and three ^{81}Br at the same time) and various other isotope combinations. The intensity of each peak depends upon the probability for that combination of isotopes.

CI may be used to verify the molecular ion. As the ionization energy is reduced, the molecular ion often increases in intensity. In chemical ionization the adduct ion ($MH^{+\bullet}$) is observed at *m/z* M+1.

Fragmentation. Although the molecular ion is useful for identification, it does not provide any structural information about an unknown. The structural information is obtained from the fragmentation patterns of the mass spectrum. Identifying an unknown without analyzing the fragmentation patterns is like putting together a jigsaw puzzle without the picture. Fragmentation patterns are often complex, but they fit together like pieces of the puzzle to identify the structure of the molecule.

A) Electron Ionization

$$ABCD + e^- \rightarrow ABCD^{+\bullet} + 2\,e^-$$

B) Fragmentation

1) Direct Cleavage

$$ABCD^{+\bullet} \rightarrow AB^+ + CD^\bullet$$

2) Rearrangement

$$ABCD^{+\bullet} \rightarrow AD^{+\bullet} + B{=}C$$

Figure 15. Fragmentation mechanisms.

After a molecule is ionized, the molecular ion retains the excess ionization energy. If this excess energy is greater than the energy required to break a chemical bond, the molecule can fragment. The fragmentation processes are typically categorized as direct cleavage or rearrangement (Figure 15). Cleavage reactions are simply the breaking of a bond to produce two fragments. These reactions usually produce an even electron ion (AB^+). The even electron ion is detected at an odd *m/z* value (assuming no nitrogen) and a neutral odd electron radical.* Since the radical is a neutral fragment it is not observed in the mass spectrum. Rearrangements are more complex reactions that involve both making and breaking bonds. These reactions are thermodynamically favorable because they require less energy. However they also require a concerted mechanism that is not as kinetically favorable when compared to a simple cleavage reaction. Rearrangement ions easily identified because they are observed as odd electron ions with an even *m/z* value. These fragments often provide important clues about the location and identity of functional groups.

*Odd electron ions are radical species with an unpaired electron. They are produced by removing a lone pair electron or a bonding electron from a molecule. For example, water is ionized by removing a non-bonding electron from oxygen to produces $H_2O^{+\bullet}$. This is an example of an odd electron ion. Odd electron ions have an even *m/z* value. The exception to this is if the ion has an odd number of nitrogen atoms. Calculate the *m/z* value for some ions to verify this statement.

Even electron ions have all paired electrons. Even electron ions are usually produced by cleavage reactions that result in loss of a neutral radical. An example of this is the cleavage of CH_3-CH_2-$CH_3^{+\bullet}$ to form $CH_3^\bullet$ and $C_2H_5^+$. $C_2H_5^+$ is an even electron ion observed at an odd *m/z* value.

The mass spectra of five different $C_{10}H_{22}$ isomers (Figure 16) show how cleavage patterns help to identify a compound. The spectrum of *n*-decane includes the molecular ion, $C_{10}H_{22}^{+\bullet}$ (*m/z* 142) and an evenly spaced series of fragments. These fragments, with *m/z* 14 separation, are formed by cleavage of the linear alkyl chain at different locations. You should notice the distribution of the fragments, C_3 and C_4 are the most abundant and very few long alkyl chains remain intact. This distribution depends upon the thermodynamics and kinetics of the fragmentation pathways.

At this point get a piece of scratch paper, draw a lewis dot structure for *n*-decane, break some bonds and find the mass of the fragments. Which of these fragments are observed in the mass spectrum. This exercise will take some time, but the practice will help you interpret mass spectra.

The four different methyl-nonane isomers (Figure 16) have the same molecular ion as *n*-decane. However, they have different fragmentation patterns because the position of the methyl group changes the distribution of the fragmentation products. 2-Methyl-nonane has three terminal CH_3- groups so the loss of $CH_3^{\bullet}$ to produce $C_9H_{19}^{+}$ (*m/z* 127)* is more likely than for other $C_{10}H_{22}$ isomers. Loss of $C_2H_5^{\bullet}$ to form $C_8H_{17}^{+}$ (*m/z* 113), however, is less likely for 2-methyl nonane since this loss is only possible at one end of the molecule. As a result, the peak at *m/z* 113 in the mass spectrum of 2-methyl nonane is only 0.5 % the intensity of the base peak** (*m/z* 43). A similar pattern is observed for the loss of $C_3H_7^{\bullet}$ in the mass spectrum of 3-methyl nonane. The resulting fragment, $C_7H_{15}^{+}$ (*m/z* 99), has a relative abundance of only 0.4 %. In 4-methyl nonane loss of $C_4H_9^{\bullet}$ to produce $C_6H_{13}^{+}$ (*m/z* 85) has an abundance of only 7.1%. Loss of $C_5H_{11}^{\bullet}$ to produce $C_5H_{11}^{+}$ (*m/z* 71) for 5-methyl nonane has an abundance of 8.6%.

These five spectra demonstrate the importance of interpreting spectra. A computer search may not distinguish these spectral features, but someone familiar with interpretation will realize the significance of the small missing peaks. Now spend some time with a pencil and a pile of scratch paper to work out this fragmentation.

*Loss of $CH_3^{\bullet}$ (15 u) from the molecular ion, $C_{10}H_{22}^{+\bullet}$ (*m/z* 142), produces $C_9H_{19}^{+}$ (*m/z* 127).

**The base peak is the peak with the greatest abundance. The mass spectrum is usually normalized so that this peak has an intensity of 100.

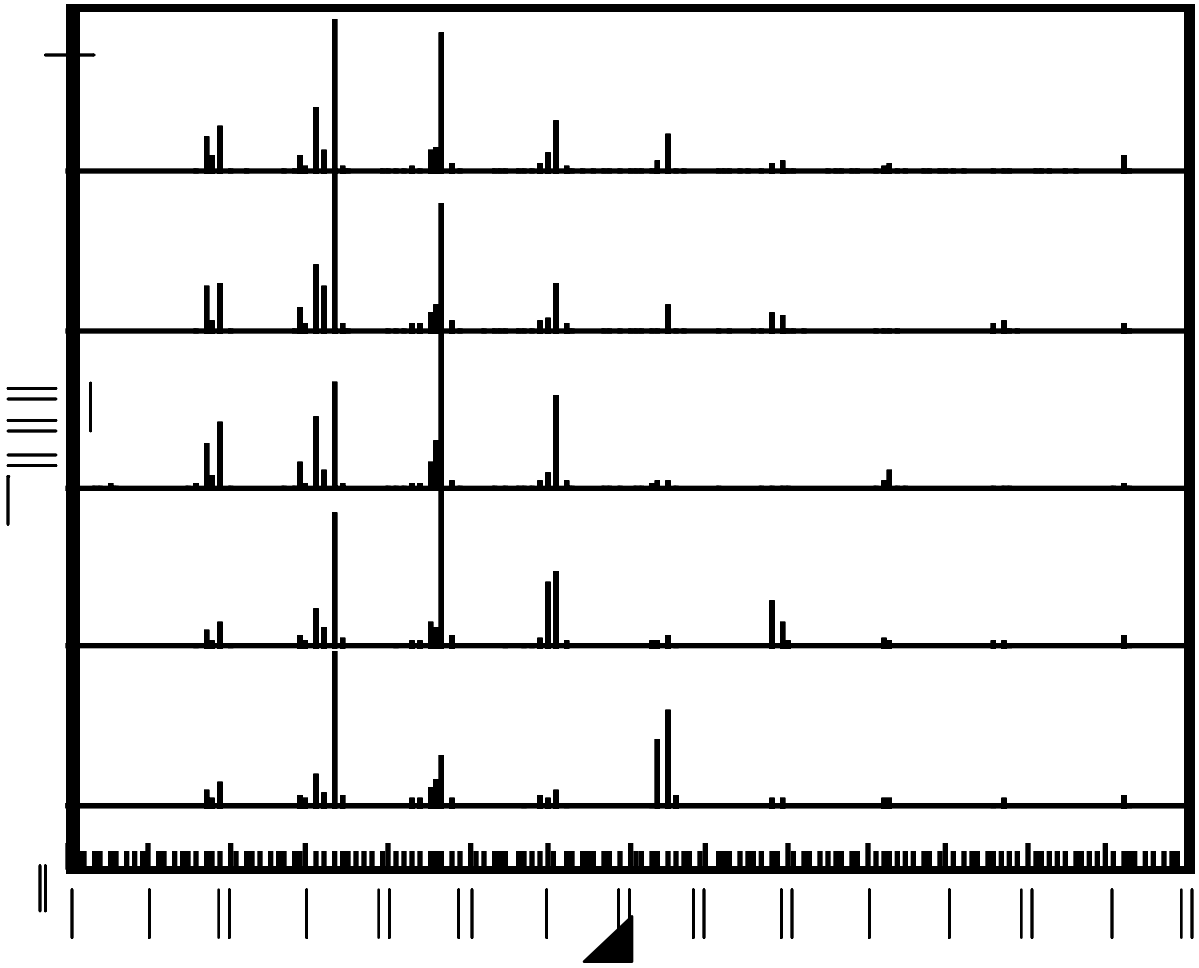

Figure 16. 70 eV EI of $C_{10}H_{22}$ isomers. A) n-Decane, B) 2-methyl-nonane, C) 3-methyl-nonane, D) 4-methyl-nonane, E) 5-methyl-nonane. (NOTE: mass spectra are shown with the m/z along the x-axis and the abundance of each ion shown along the y-axis. The y-axis is normalized to the base peak. This is the largest peak in the spectrum.)

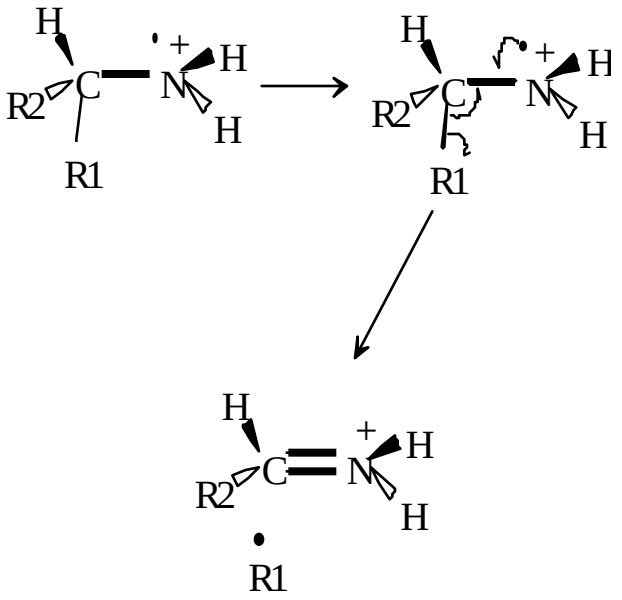

Figure 17. α-Cleavage fragmentation of an amine.

Functional groups can have a significant effect the fragmentation patterns observed in mass spectrometry. For example, aliphatic amines prefer to undergo cleavage at the α C-C bond to produce a relatively stable $CH_2NH_2^+$ ion (Figure 17). The resulting fragments distinguish primary, secondary, and tertiary amines.

The mass spectra of three different *n*-pentaneamine isomers are shown in Figure 18. 1-Pentaneamine has an odd number of nitrogen atoms so the molecular ion (*m/z* 87) has an odd mass to charge ratio and the cleavage fragment (*m/z* 30) has an even mass to charge ratio. α-Cleavage of 1-pentaneamine produces $CH_2NH_2^+$ (*m/z* 30) and $C_4H_9^{\bullet}$. The $C_4H_9^{\bullet}$ fragment is not observed in the mass spectrum because since this is a neutral fragment. 2-Pentaneamine has two α-cleavage sites. Loss of $CH_3^{\bullet}$ produces $C_4H_8NH_2^+$ (*m/z* 72) and loss of $C_3H_7^{\bullet}$ produces $C_2H_4NH_2^+$ (*m/z* 44). Both of these ions are observed but the greater abundance of the *m/z* 44 signal indicates that loss of $C_3H_7^{\bullet}$ is favored. The additional peak at *m/z* 58 corresponds to $C_3H_6NH_2^+$ which could be formed by β-cleavage loss of $C_2H_5^{\bullet}$. 3-Pentaneamine has two α-cleavage sites but they are symmetric so cleavage at either site results in loss of $C_2H_5^{\bullet}$. Loss of $C_2H_5^{\bullet}$ produces $C_3H_6NH_2^+$ (*m/z* 58), the base peak in the mass spectrum. Now spend some time with a piece of scratch paper and interpret some other peaks in these spectra.

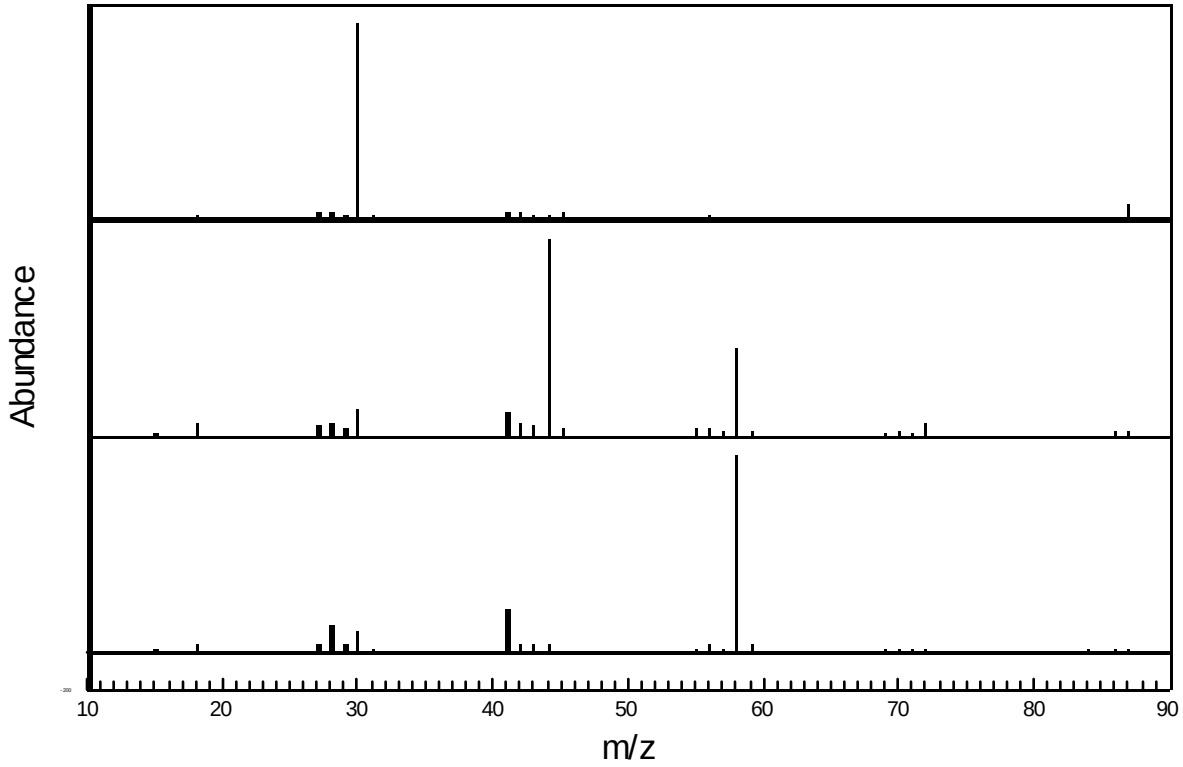

Figure 18. 70 eV EI spectra of pentaneamine isomers. A) 1-Pentaneamine, B) 2-Pentaneamine, C) 3-Pentaneamine.

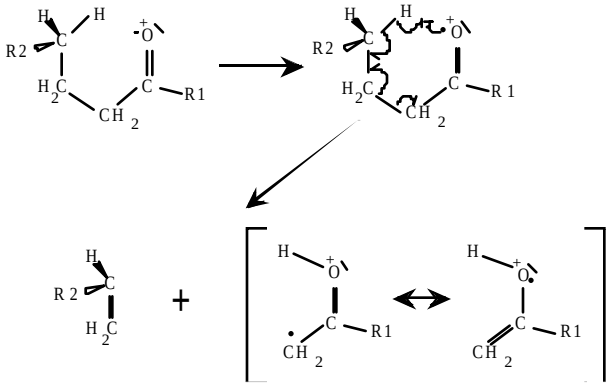

Figure 19. McLafferty rearrangement mechanism.

The McLafferty rearrangement (Figure 19), is a classic example of a rearrangement reaction. This rearrangement results in formation of an intact neutral molecule and a radical ion with an even mass to charge ratio. This reaction is significantly different from the cleavage reactions discussed previously. The McLafferty rearrangement is often observed for carbonyl compounds that contain a linear alkyl chain. If this alkyl chain is long enough, a six-membered

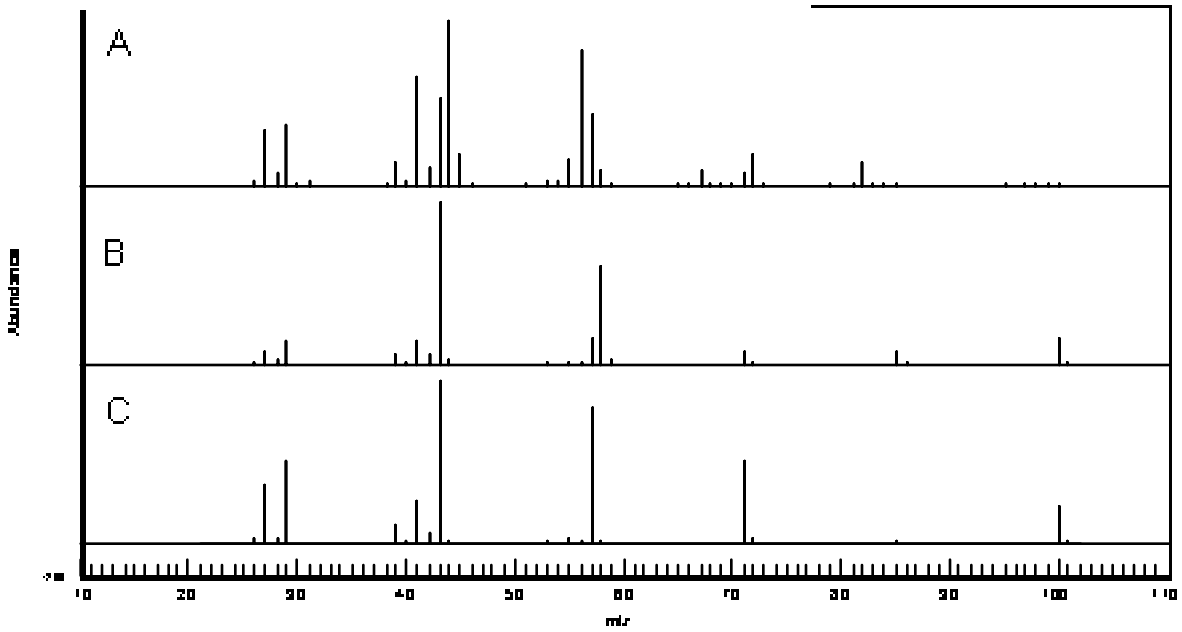

Figure 20. 70 eV EI of $C_6H_{12}O$ isomers. A) *n*-hexanal, B) 2-hexanone, C) 3-hexanone.

ring forms from the carbonyl oxygen to the hydrogen on the fourth carbon. This spacing allows the hydrogen to transfer to the carbonyl oxygen via a six membered ring. The McLafferty rearrangement is energetically favorable because it results in loss of a neutral alkene and formation of a resonance stabilized radical cation.

The products from the McLafferty rearrangement are observed in the mass spectra of $C_6H_{12}O$ isomers (Figure 20). The mass spectrum of *n*-hexanal contains two even mass ions. $C_2H_4O^{+\bullet}$ (*m/z* 44) is produced by the McLafferty rearrangement and $C_4H_8^{+\bullet}$ (*m/z* 56) is the McLafferty compliment. The McLafferty compliment is produced when the charge is transferred to the alkene fragment during the rearrangement. The mass spectrum of 2-hexanone is easily distinguished from *n*-hexanal because the McLafferty rearrangement breaks a different C-C bond. This results in loss of C_3H_6 and produces $C_3H_6O^{+\bullet}$ (*m/z* 58). The mass spectrum of 3-hexanone does not have any major even mass fragment ions so apparently the McLafferty rearrangement is not favorable. If the McLafferty rearrangement did occur, where would the ion be observed in this mass spectrum? What fragmentation would produce the major ions observed in the mass spectrum of 3-hexanone (*m/z* 29, 43, 57, and 71)? Based upon the discussion so far you should be able to identify many of the other fragments in these three mass spectra. Spend some time with a piece of scratch paper and see what you come up with.

Isotope Abundance. The existence of isotopes was first observed by Aston using a mass spectrometer to study neon ions. When interpreting mass spectra it is important to remember that the atomic weight of an element is a weighted average of the naturally occurring isotopes. Mass spectrometers separate these isotopes and are even used to measure their relative abundance. Although this complicates the mas spectrum, it also provides useful information for identifying the elements in an ion.

Chlorine is an excellent example of how isotope distributions are useful for interpretation. The molecular weight of chlorine is 35.45 u. This is calculated from the natural abundance of ^{35}Cl (75%) and ^{37}Cl (25%). The natural abundance of these two isotopes is observed in the mass spectrum as two peaks separated by *m/z* 2 with a relative intensity of 3:1. The mass spectrum of CH_3Cl (Figure 21) clearly shows two peaks with the isotope distribution pattern for an ion with a single chlorine atom. $CH_3{}^{35}Cl^{+\bullet}$ (*m/z* 50) and $CH_3{}^{37}Cl^{+\bullet}$ (*m/z* 52)* are separated by *m/z* 2 and have the 3:1 abundance ratio characteristic of an ion with a single chlorine atom. Can you identify the other peaks in the mass spectrum of CH_3Cl?

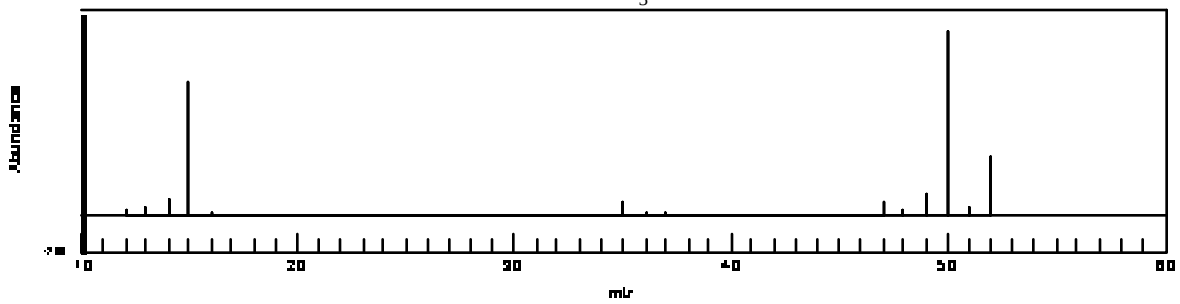

Figure 21. 70 eV EI of CH_3Cl.

If more than one chlorine atom is present, the isotope abundance is more complex. An ion with two chlorine atoms has three possible isotope combinations. This pattern is apparent in the mass spectrum of CH_2Cl_2 (Figure 22). Ions are observed for $CH_2{}^{35}Cl_2{}^{+\bullet}$ (*m/z* 84), $CH_2{}^{35}Cl^{37}Cl^{+\bullet}$ (*m/z* 86), and $CH_2{}^{37}Cl_2{}^{+\bullet}$ (*m/z* 88). Based upon the probability of each combination of isotopes, the relative intensity of these peaks is 10:6:1. The 3:1 isotope ratio for an ion with a single chlorine atom is observed at *m/z* 49 and *m/z* 51. This corresponds to $CH_2{}^{35}Cl^+$ and $CH_2{}^{37}Cl^+$ fragments formed by loss of Cl from the molecular ion. Careful examination of the spectrum also shows ions produced by loss of $H^{\bullet}$ and H_2.

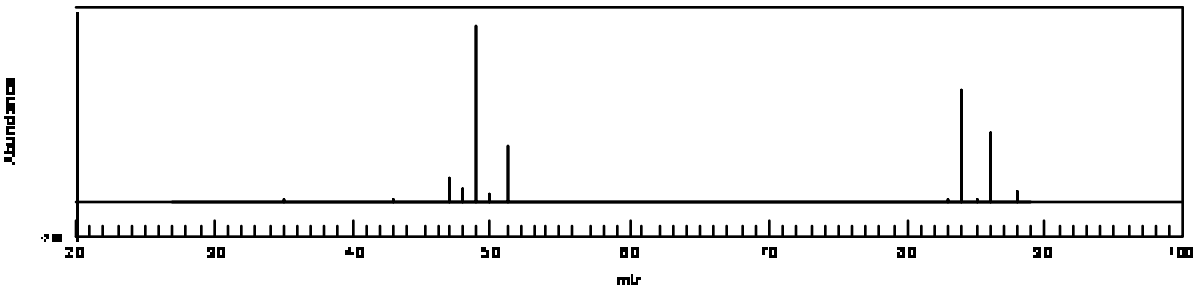

Figure 22. 70 eV EI of CH_2Cl_2.

The 1.1% of natural abundance of ^{13}C is another useful tool for interpreting mass spectra. The abundance of a peak one *m/z* value higher, where a single ^{12}C is replaced by a ^{13}C, is determined by the number of carbons in the ion. The rule of thumb for small compounds is that

*To avoid ambiguity the molecular ion is defined as the ion with the most commonly occurring isotopes. For $CHCl_3$ the molecular ion is $^{12}C^1H_3{}^{35}Cl$ at *m/z* 50.

each carbon atom in the ion increases the abundance of the M+1 peak by 1.1%. This effect is seen in all the spectra discussed in this paper. For example, in the *n*-decane mass spectrum (Figure 16) compare the peak for $^{12}C_9{}^{13}C^1H_{22}{}^{+\bullet}$ at *m/z* 143 (0.9 % relative abundance) to the peak for $^{12}C_{10}{}^1H_{22}$ at *m/z* 142 (9.0 % relative abundance) . The abundance of the ^{13}C peak is 10 % the abundance of the ^{12}C peak. Now look at some previous spectra to find more examples of this pattern.

Because all atoms have several naturally occurring isotopes, the patterns discussed here become more complex. Fortunately, most elements common in organic mass spectrometry have one predominant isotope. The high abundance of the two chlorine isotopes is unusual, so they are easy to identify. The relative abundances for isotopes of frequently encountered elements are given in Table 2.

Table 2. Abundance of Isotopes for Some Common Elements.*

Atom	Isotope A		Isotope A+1		Isotope A+2	
	mass	%**	mass	%	mass	%
H	1	100	2	0.015		
C	12	100	13	1.1		
N	14	100	15	0.37		
O	16	100	17	0.04	18	0.20
F	19	100				
Si	28	100	29	5.1	30	3.4
P	31	100				
S	32	100	33	0.80	34	4.4
Cl	35	100			37	32.5
Br	79	100			81	98.0
I	127	100				

*adapted from McLafferty, F. *Interpretation of Mass Spectra* (University Science, Mill Valley CA: 1980.

**By convention mass spectra are normalized so that the most intense peak has an abundance of 100%.

Exact Mass. In most mass spectrometry experiments the nominal mass is used and the mass to charge ratio of an ion is rounded to the nearest whole number. High resolution instruments, including double focusing and FT-ICR mass spectrometers, are capable of determining the "exact mass" of an ion. This is useful for interpretation because each element has a slightly different mass defect. This “mass defect” is the difference between the mass of the isotope and the nominal mass (which is equivalent to the number of protons and neutrons). Recall that the atomic mass scale is defined by carbon-12 with a mass of exactly 12.0000 u. The exact mass of a specific isotope is determined relative to ^{12}C by high resolution mass spectrometry (see Table 3). High resolution mass spectrometry can distinguish compounds with the same nominal mass but different exact mass caused by different elemental composition.

For example, C_2H_6, CH_2O, and NO all have a nominal mass of 30 u. Because they have the same nominal mass, a mass spectrometer with unit mass resolution can not distinguish these

three ions. However, the exact masses for C_2H_6 (30.04695039), CH_2O (30.01056487) and NO (29.99798882) are different and a high resolution mass spectrometer can distinguish these three compounds.

Table 3 Exact mass of some common isotopes.

Element	Isotope	mass*	
H		^{1}H	1.007 825 031 6 (5)
		^{2}H	2.014 101 777 9 (5)
He		^{4}He	4.002 603 36
		^{3}He	3.016 0
C		^{12}C	12.000 000 000 0 (0)
		^{13}C	13.003 354 838 1 (10)
N		^{14}N	14.003 074 004 0 (12)
		^{15}N	15.000 108 897 7 (11)
O		^{16}O	15.994 914 619 5 (21)
		^{18}O	17.999 2
P		^{31}P	30.973 763 3
S		^{32}S	31.972 072 8
		^{34}S	33.967 9

Values in parentheses indicate error in last digit.

* DiFlippo, F.; et. al. *Phys Rev Lett.* **1994**, *73*, 1482.
McLafferty, F.W. *Interpretation of Mass Spectra*; University Science Books: Mill Valley, 1993.

This section is only an introduction to the interpretation of mass spectra. A full analysis of fragmentation patterns is beyond the scope of this text but with practice interpretation becomes much easier. Several excellent references include McLafferty's book (*35*) and the ACOL book on mass spectrometry (*36*). These contain additional information on mass spectral interpretation and many more practice problems.

ACKNOWLEDGMENTS:

I would like to thank all the people who helped to review this paper. Including: Nate Bower, Murray Johnston, Gordon Nicol, Gary Kinsel, Phil Ross, Pat McKeown, Curt Mowry, and the anonymous reviewer. Their comments and suggestions have been invaluable. Mass Spectra are from the NIST mass spectral database.

END OF PAPER QUESTIONS:[*]

1. You work for a mass spectrometer vendor who sells all the instruments described here. Make a product table that shows all the different options for each major part of the mass spectrometer.

2. Using thermochemical data, find the energy required to remove an electron from the following species: H_2, Na, C, CH_4, and Fe. Express this energy in kJ/mole and eV (per atom).

3. Using thermochemical data, determine the energy required to break the following bonds: H-H, C-C, C=C, C-H, and C-O. Express this energy in kJ/mole and eV (per atom).

4. Determine the kinetic energy, velocity, and momentum of the following ions: *m/z* 10, 50, 100, and 1000. The ions are formed in the center of the source region, which is 10.0 mm long and has a 5000 V potential applied across the two ends.

5. How long would it take each of the ions from question 4 to travel through a 1 meter flight tube in a TOF mass spectrometer?

6. What magnetic field is required to select each of the ions from question 4 in a magnetic sector with a radius of 1 meter?

7. What is the cyclotron frequency of each ion from question 4 in an ICR with a 3 T magnetic field?

8. What electric field strength is required for each of the ions from question 4 to be selected by an electric sector with a radius of 0.5 meter?

9. What is the *m/z* value for the molecular ion produced by EI of the following molecules: A) benzene, B) octane, C) trinitrotoluene, D) acetone, E) t-butyl amine.

10. The highest mass ion observed in a mass spectrum is at *m/z* 127. If the compound contains a single N atom, could this be the molecular ion? If the compound contains four N atoms, could this be the molecular ion? Why?

11. The molecular ion in a high resolution mass spectrometry experiment is observed at *m/z* 58.0055. What is the molecular formula for this compound? If this was a low resolution mass spectrometer, what other molecular formula's are possible?

[*]Solutions to these questions are available from the author.

ANSWERS TO SELF-TEST QUESTIONS:

Self-Test #1: What ionization technique would be appropriate for analyzing each of the following.

a) Gasoline fractions. Since these are very volatile, EI would be very easy to use and would provide abundant fragment information. CI may help to identify the molecular ions.

b) Pesticide residue. These are usually volatile enough to use with EI. Once again CI may provide some useful information that would compliment the fragmentation in the EI spectrum. If the pesticide is thermally labile it may be appropriate to use electrospray to avoid sample decomposition.

c) Ibuprofen and acetaminophen. These pharmaceutics are often analyzed by liquid chromatography, so electrospray would be an ideal interface for ionization.

d) Insulin. This is a large protein molecule. MALDI is probably required.

e) Tripeptides. These are generally small enough to be readily ionized by FAB.

f) Heavy metals in water. Atmospheric pressure ionization in a ICP torch will provide very low limits of detection.

Self-Test #2: Which mass analyzer would be appropriate for the following analysis:

a) Routine analysis of drug testing samples. A quadrupole mass analyzer would provide the necessary mass range and resolution. It is also fast enough for use with high resolution chromatography.

b) Analysis of small, 2000 dalton, proteins. This will push the limits of a quadrupole (unless electrospray ionization is used to create multiply charged ions). A sector instrument with FAB ionization would work well.

c) Analysis of polymers up to 50,000 dalton. The *m/z* value of singly charged ions is probably to high for a sector instrument (It might work with electrospray ionization to form multiply charged ions). A TOF analyzer does not have any mass limit so it would be ideal for this analysis.

d) High sensitivity testing for chemical warfare agents. For this experiment the high sensitivity of a QUISTOR would be beneficial.

e) High resolution analysis. This is usually done with a double focusing sector instrument, although even higher resolution is possible with an ICR.

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