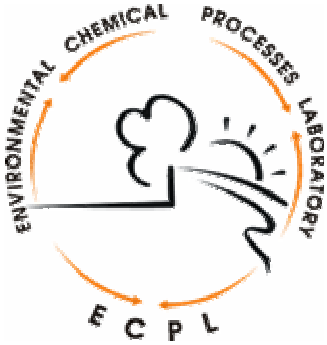
University of Crete

Department of Chemistry



Environmental Chemical Processes Laboratory

ENVIRONMENTAL SCIENCE AND ENGINEERING PROGRAM

Environmental Analytical Chemistry Course

I. Introduction to Modern Mass Spectrometry

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Important notice:

The notes of this course are exclusively for the Graduate Program "Environmental Science and Engineering" (ESE).

The printed notes of this course are distributed to the ESE program students for free.

I. Introduction

History

Modern mass spectrometer is based on the work performed by **J. J. Thomson** (1906 Nobel Prize in Physics), who studied the effects of electric and magnetic fields on ions generated in a cathode ray tube. Thomson observed that ions move through parabolic trajectories proportional to their "mass-to-charge" ratios. From 1930 to 1970 important advances in mass spectrometry were achieved. The most important works to cite are those by **F. W. Aston** (1920 Nobel Prize in Chemistry) and **A. J. Dempster** (higher accuracy mass spectrometers), **A. Neir** (advances in vacuum technology and electronics), **Wiley and McLaren** (1955, time-of-flight analyzers), **W. Paul** (quadrupole analyzer, 1989 Nobel Prize in Physics). These works triggered greater discoveries in the late 1980s and early 1990s. Two ionization techniques namely, electrospray ionization (ESI, developed by **J. Fenn**, 2001 Nobel Prize in Chemistry and **M. Dole**) and matrix assisted laser desorption/ionization (MALDI, developed by **Tanaka**, 2001 Nobel Prize in Chemistry), have had a significant impact on the capabilities of mass spectrometry especially for sophisticated applications of mass spectrometry to the fields of biology and medicine. Applications (once inconceivable) are in wide used today: Sequencing of peptides and proteins, studies of non covalent complexes and immunological molecules, DNA sequencing, and the analysis of intact viruses.

Mass spectrometry can be defined as an instrumental approach that allows for the mass measurement of molecules in very low quantities (as low as 10^{-18} moles). The five basic parts of any mass spectrometer are (see also Fig. 1):

1) A vacuum system.
2) A sample introduction device.
3) An ionization system.
4) A mass analyzer.
5) An ion detector.

Combining these parts a mass spectrometer determines the molecular weight of chemical compounds by **ionizing**, **separating**, and **measuring molecular** and **fragment ions** according to their **mass-to-charge ratio** (**m/z**). The ions are generated in the **ionization source** by inducing either the **loss** or the **gain** of a **charge** (e.g. **electron ejection, protonation,** or **deprotonation**).

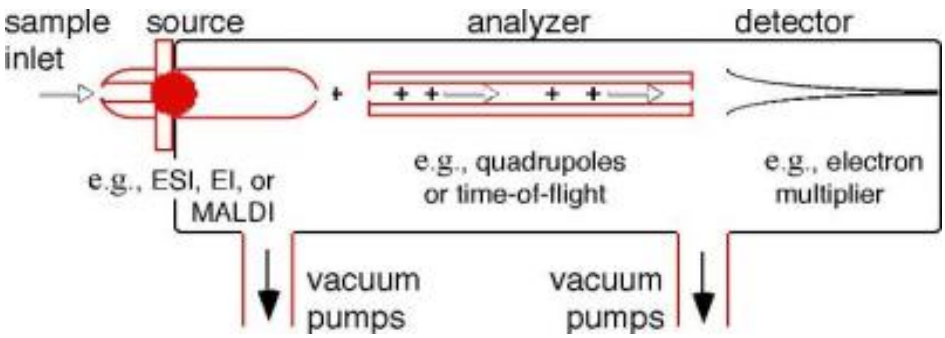


Figure 1. *Components of a Mass Spectrometer*

Once the ions are formed in the gas phase they can be electrostatically directed into a mass analyzer, separated according to mass and finally detected. The result of ionization, ion

separation, and detection is a mass spectrum that can provide molecular weight or even structural information.

Mass spectrometers have become essential instruments for a wide range of applications in the analysis of inorganic, organic, and bio-organic chemicals. Examples include dating of geologic samples, drug testing and drug discovery, process monitoring in the petroleum, chemical, and pharmaceutical industries, surface analysis and the structural identification of unknowns. Further, mass spectrometry is being continually improved and has recently had significant advances in its application to molecular biology, where it is now possible to analyze proteins, DNA, and even viruses.

II. Theory and Practice of Mass Spectrometry

The evolution of mass spectrometry occurred conjointly with the increasing demand for its application to difficult problems (e.g. analysis of biomolecules) and the development of computer technology. New developments in the technology have created a great variety of instruments. However, the indispensable components of all mass spectrometers are the same. The components of an MS instrument may be better understood if we trace the way of ions through them:

1) An **ion source ionizes** the molecule of interest.

2) A **mass analyzer** differentiates the ions according to their **mass-to-charge ratio (m/z).**

3) A **detector** measures the **ion current** produced by the ionization of the molecules of the analyte.

1. Vacuum System

Mass spectrometry requires a low pressure to operate.

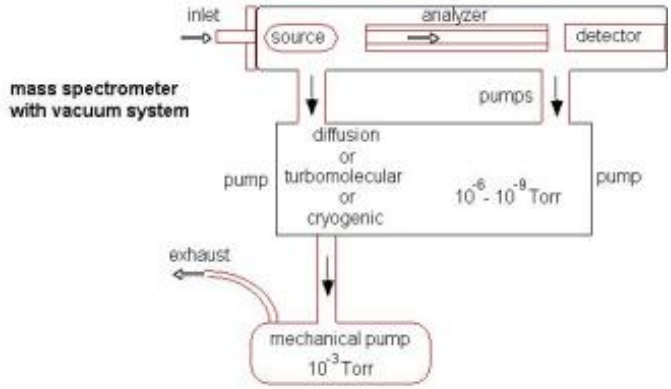


Figure 2. *A diagram of a Mass Spectrometer outlining the vacuum system.*

[Note: Pressure (pounds per square inch -PSI, or neutons per square meter -N m^{-2}) is usually reported in atmospheres (atm) or torr (1.0 mm of Hg in the appropriate pressure gauge: 1 atm = 760 torr).]

Table 1. *Examples of common pressure gauges*

Gauge	Pressure Range	Typical Use
Manometer	760 - 1 torr	systems near atmospheric pressure
Thermocouple gauge	1 - 10^{-3} torr	monitoring mechanical pumps
Ionization gauge	10^{-3} - 10^{-9} torr	high-vacuum systems

Thermocouple gauges operate on the dependence of thermal-conductivity on gas pressure. In these gauges, a constant current is applied to a metal filament to heat the filament. The temperature of the filament depends on the heat transfer to gas molecules, which depends on the pressure. The temperature of the filament is measured by making a thermocouple

junction with the filament. The pressure reading is based on a calibration, which depends on the gas present in the vacuum system.
The most common type of ionization gauge is a thermionic, or hot-cathode gauge. It consists of an electrically heated filament and two electrodes. The filament (at ground voltage) emits electrons that are accelerated to the positively electrode. If the electrons collide with gas atoms or molecules they produce positive ions. Positive ions are collected at the negative electrode, creating an ion current which can be measured.
The two important parameters of a vacuum pump are its lowest attainable pressure, and its pumping speed (liters per minute - l/min or lpm; cubic feet per minute - cfm).

Table 2. *Examples of common vacuum pumps*

Pump	Lowest Attainable Pressure	Typical Use
Mechanical pump	10^{-3} - 10^{-4} torr	roughing or backing pump
Diffusion pump	10^{-6} torr	vacuum lines
Turbomolecular pump	10^{-9} torr	high-vacuum systems

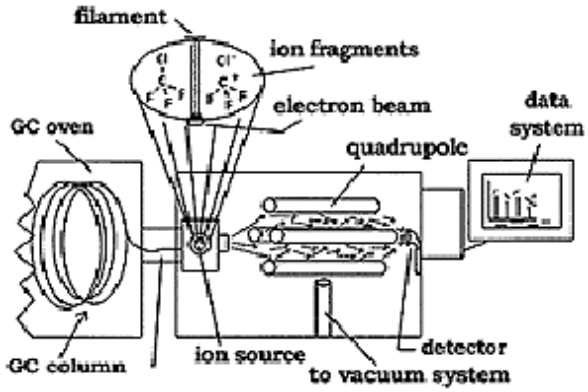
A **diffusion pump** consists of a bath of boiling oil that streams through a jet-shaped volume. The oil entrains gas molecules and transports them in the direction of the oil flow. A **mechanical pump** can then pump away the exhaust from the diffusion pump.
A **turbomolecular pump** contains a turbine that is spinning at a very high rate of revolution, typically tens of thousands revolutions per second. The turbine blades are spinning faster than the average speed of gas atoms or molecules, so that any gas-phase species that enter the turbo pump are physically forced out of the pump by the turbine blades. A **mechanical pump** is required to maintain a low pressure and pump away the exhaust from a turbo pump.

2. Sample Introduction Device

The sample inlet is the interface between the sample and the mass spectrometer. To introduce pure compounds, the sample is placed on a **probe** which is then inserted, usually through a vacuum lock, into the ionization source of the mass spectrometer. The sample can then be heated to facilitate thermal desorption or undergo any number of high-energy desorption processes used to achieve vaporization and ionization.

Capillary infusion is often used because it can efficiently introduce small quantities of a sample into a mass spectrometer without destroying the vacuum. Capillary columns are routinely used to interface the ionization source of a mass spectrometer with other separation techniques, including **gas chromatography** (**GC**) and **liquid chromatography** (**LC**). Gas chromatography and liquid chromatography can serve to separate a mixture of compounds into its different components prior to mass analysis. Prior to the 1980's, interfacing liquid chromatography with the available ionization techniques was unsuitable because of the low sample concentrations and relatively high flow rates (milliliter/minute) of liquid chromatography. However, new ionization techniques such as electrospray were developed that now allow liquid chromatography mass spectrometry to be routinely performed. When mass spectrometry is used as a detector for these chromatography techniques the extra information that mass analysis provides can be very valuable for compound identification.

A



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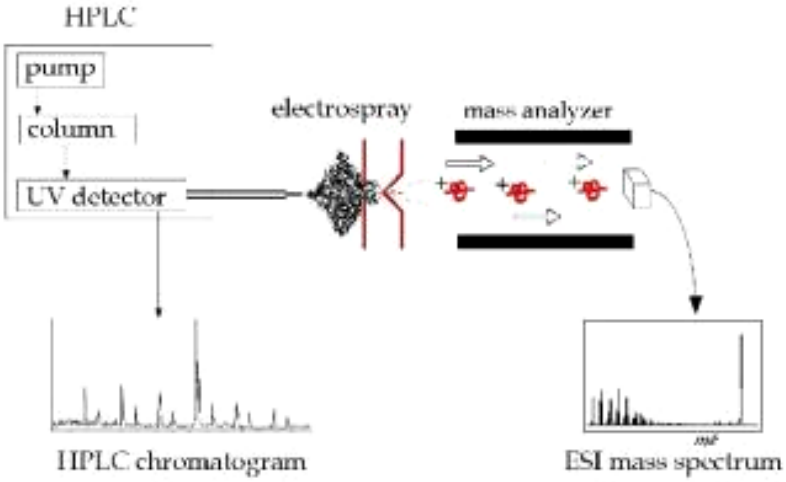


Figure 3. *Interfacing A) gas chromatography with mass spectrometry and B) liquid chromatography with electrospray ionization mass spectrometry.*

3. Ionization Techniques

3.1 Electron Ionization – Electron Impact (EI)

Electrons are produced by **thermionic emission** from a tungsten or rhenium filament (filament current ca. $1*10^{-4}$Amps). The electrons leave the filament surface and are accelerated towards the **ion source** chamber which is held at a positive potential (equal to the **accelerating voltage**). The electrons acquire energy equal to the voltage between the filament and the source chamber - typically **70 electron volts** (**70 eV**). The **electron trap** is held at a fixed positive potential with respect to the source chamber. A proportion of the **electron beam** will strike the electron trap producing the **trap current**. This is used as a feedback circuit to stabilize the electron beam.

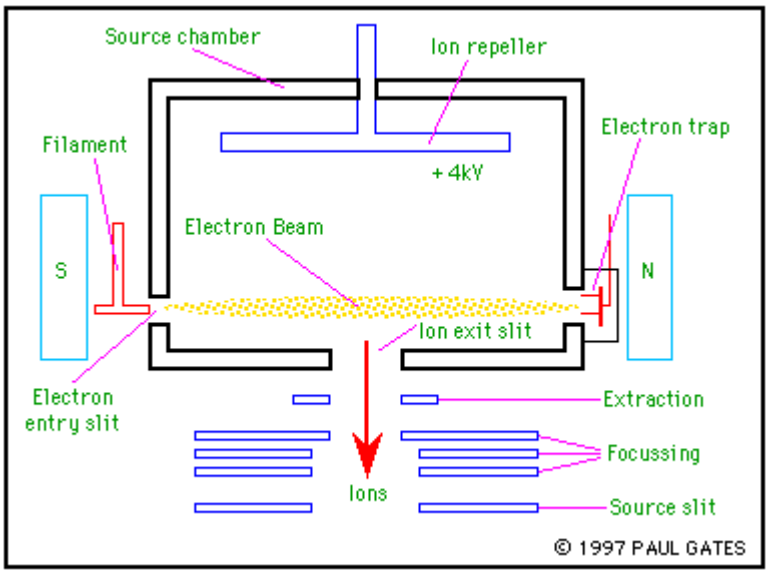


Figure 4 *Schematic of an Electron Ionization (EI) Source*

A permanent magnet is positioned across the ion chamber to produce a magnetic flux in parallel to the electron beam (Fig. 4). This causes the electron beam to spiral from the filament to the trap, increasing the chance and efficiency of the molecule ionization. Gaseous molecules are introduced into the path of the electron beam where they may be ionized by electronic interactions with this electron beam. Ionization can also be brought about by direct impaction of an electron with a molecule.
The positive **ion repelling** voltage and the negative **excitation voltage** work together to produce an **electric field** in the source chamber such that ions will leave the source through the ion exit slit. The ions are directed through the various focusing and centering lenses and are focused onto the source exit slit. The ions can then be separated in the mass analyzer.

Mechanisms of ion formation

The ionization of the analyte molecules AB can be considered as follows:

1. $AB + e^{-*} \text{-----> } A^{+} + B^{-} + e^{-}$

2. $AB + e^{-*} \text{-----> } A^{+} + B^{\circ} + 2e^{-}$

3. $AB + e^{-*} \text{-----> } [AB^{+\circ *}] + 2e^{-}$

Followed by: **$[AB^{+\circ *}] \text{-----> } AB^{+\circ}$**

4. $AB + e^{-*}$ -----> $[AB^{2+*}]" + 3e^-$

Followed by:

$[AB^{2+*}]"$ -----> $A^+ + B^+$ - very low abundance

5. $AH + e^{-*}$ -----> $AH^* + e^-$

Followed by

$AH^* + AH$ -----> $[AH+H]^+ + A^-$

'Self chemical ionization'

Explanations for signs and ionization mechanism:

Species with a **** superscript*** *are in high energy sates.*
Species with a ***° superscript*** *are radicals.*
Species with a ***"superscript*** *are short lived intermediates which are not seen in the spectra.*

1 *and* ***2*** *are the highest abundance and are termed instantaneous fragmentation. This is the reason why EI is considered a "****hard"*** *ionization process.*

3 *is fairly high abundance and is the process responsible for the molecular ion formation. Unfortunately the highly energetic radical intermediate* ***[$AB^{+\circ *}$]*** *tends to undergo fragmentation (or rearrangement) as a stabilizing process, this is responsible for the lower mass fragment ions present in the spectra.*

4 *is a very low abundance process, but theoretically it can occur.*

5 *is a process which can occur at higher pressures (self Chemical Ionization), this is especially problematic in the ionization of alcohols and amines where you may find that the dominant ionization process is proton exchange between two analyte molecules, leading to the formation of the* ***[M+H]***$^{+}$ *pseudo-molecular ion.]*

3.2 Chemical ionization (CI)

In analytical applications the reliable determination of molecular weights, along with correlation of fragmentation with structural features, are often the main objectives. In conventional ionization by EI, limitations may arise because the sample has to be ionized in the gas phase, yielding molecular ions $M^{+\circ}$ often insufficiently stable to permit detection. A quite different, than EI, approach is used in chemical ionization (CI): Ions characteristic of the sample molecule are formed in ion-molecule reactions between reagent ions and the sample. A reagent gas is ionized at a pressure of 0.3-1 torr to produce a high yield of reagent ions which may be positively or negatively charged and react with the molecules to form ions which constitute the CI spectrum of the compound. The reactions between the sample molecule and the reagent gas ions can be of three types in the positive CI:

I) Acid-base reaction type:

M (molecule of sample) + XH^+ (reagent ions) --> MH^+ ("molecular ions") + X (reagent gas)

II) Complex formation reaction type:

M (molecule of sample) + XH^+ (reagent ions) --> MXH^+ ("molecular ions")

III) Charge transfer reaction type (redox):

M (molecule of sample) + $G^{+\bullet}$ (reagent ions) --> $M^{+\bullet}$ ("molecular ions") + G (reagent gas)

The reagent gases in positive CI

The most utilized reagent gases are methane (CH_4), ammonia (NH_3), isobutane (i-C_4H_{10}) and noble gases for the charge transfer reactions.

The reagent ions produced by methane CI are described below. The reagent ions are produced by introducing a large excess of methane (relative to the analyte) into an electron

impact (EI) ion source. Electron collisions produce CH_4^+ and CH_3^+ which further react with methane to form CH_5^+ and $C_2H_5^+$:

CH_4 (EI ionization) --> CH_4^+, CH_3^+, CH_2^+, CH^+, C^+

$CH_4^+ + CH_4^{+\bullet}$ --> CH_5^+ (47%) + CH_3

$CH_3^+ + CH_4$ --> $C_2H_5^+$ (41%) + H_2

$CH_4 + C_2H_3^+$ --> $C_3H_5^+$ (6%) + H_2

The reagent ions react with the molecules like Brønsted or like Lewis acids:

$CH_5^+ + M$ --> $MH^+ + CH_4\uparrow$ ("molecular ion": at [m/z]+1)

$C_2H_5^+ + M$ --> $MH^+ + C_2H_4\uparrow$ ("molecular ion": at [m/z]+1)

$C_2H_5^+ + M$ --> $[M\ C_2H_5]^+$ ("molecular ion": at [m/z]+29)

$C_3H_5^+ + M$ --> $[M\ C_3H_5]^+$ ("molecular ion": at [m/z]+41)

The reagent ions produced by ammonia (NH_3) CI are described below:

NH_3 (EI ionization) --> $NH_3^{+\bullet}$

$NH_3 + NH_3^{+\bullet}$ --> $NH_4^+ + NH_2^\bullet$

$NH_3 + NH_4^+$ --> $(NH_3)_2H^+$

The reagent ions react with the molecules like Brønsted or like Lewis acids:

$NH_4^+ + M$ --> $MH^+ + NH_3\uparrow$ ("molecular ion": at [m/z]+1)

$NH_4^+ + M$ --> $[M\ NH_4]^+$ ("molecular ion": at [m/z]+18)

The reagent ions ($C_4H_9^+$ and $C_3H_3^+$) of isobutane (i-C_4H_{10}) react with the molecules like Brønsted or like Lewis acids:

$C_4H_9^+ + M \rightarrow MH^+$ ("molecular ion"[m/z]+1) or/and $[M\ C_4H_9]^+$ ([m/z]+57)

$C_3H_3^+ + M \rightarrow MH^+$ ("molecular ion"[m/z]+1) or/and $[M\ C_3H_3]^+$ ([m/z]+39)

The reagent gases in negative CI

Like EI, acid/base and also redox-type ionization find a counterpart in the formation of negative ions. Whereas "negative charge exchange" occurs by resonance capture of thermal electrons, such as are present in nitrogen plasma (electron attachment) in negative CI of the acid/base type, protons transfer occurs in analogy to the positive mode (but in opposite direction), together with the formation of addition complexes. Reagent ions such as $\{CH_3O]^-$ (from CH_3ONO) or $[Cl]^-$ (from R-Cl by dissociative electron addition) now function as Brønsted or like Lewis bases respectively:

$CH_3O^- + MH \rightarrow M^- + CH_3OH$ ("molecular ion": at [m/z]-1)

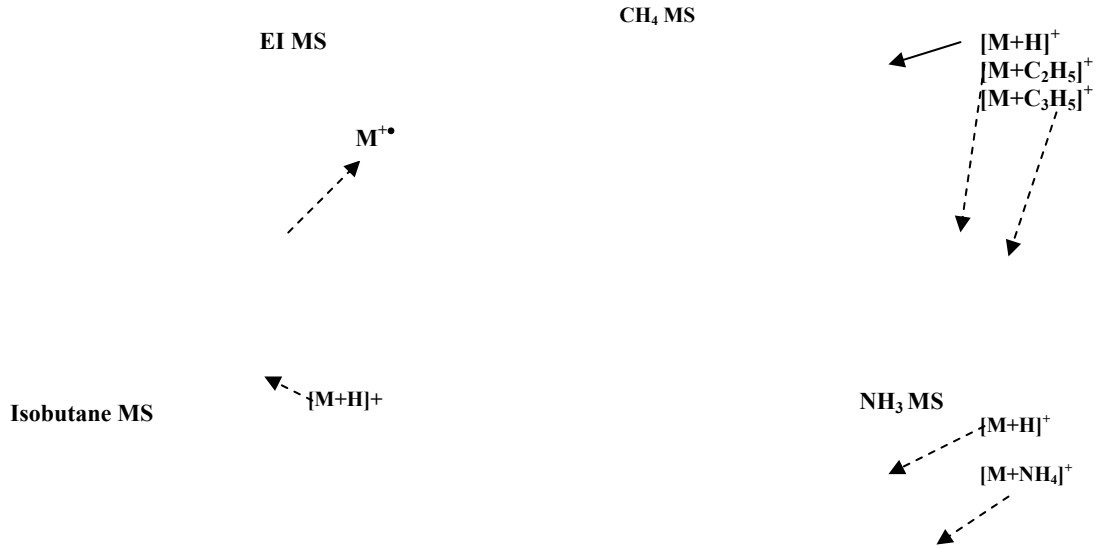
$e^- + R\text{-}Cl \rightarrow Cl^- + R^\bullet$

$Cl^- + R\text{-}Cl \rightarrow [RCl_2]^-$ ("molecular ion": at [m/z]+35)

Example of positive CI mass spectra:

Compound: Nopinone (MW 138)

Nopinone is one of the photo-oxidation products of *β*-pinene, a monoterpene, emitted by plants (I. Kavouras, N. Mihalopoulos, **E. G. Stephanou***, "Secondary aerosol formation vs. primary organic aerosol emission: In situ evidence for the chemical coupling between monoterpene acidic photo-oxidation products and new particle formation over forests" *Environ. Sci. Technol.* 33, 1028-1037, 1999). In the figure below are presented the mass spectra of nopinone measured under EI ionization conditions, and CI conditions with methane, isobutane and ammonia. The differences in the molecular ion region are very obvious in relation to the above mechanisms: In EI the molecular ion at m/z 138 is very low. The pseudo molecular ions foreseen ($[M+H]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$) are present in the CI-methane spectrum. The $[M+H]^+$ is present in very high abundance in the CI-isobutane spectrum, while both $[M+H]^+$ and $[M+NH_4]^+$ ions are present in the CI-ammonia spectrum. The CI spectra allow a confident MW identification, while the EI spectrum offers important information for structural identification. This example shows the complementarity of EI and CI spectra.



***Example**: Electron Ionization (EI MS) and Chemical Ionization mass spectra of nopinone with methane (CH_4 MS), isobutene (Isobutane MS) and ammonia (NH_3 MS).*

3.3 Fast Atom Bombardment (FAB) and Liquid Secondary Ion Mass Spectrometry (LSIMS)

The techniques of **FAB** and **LSIMS** involve the bombardment of a solid [analyte+matrix] mixture by a fast particle beam (see Fig. 5). The matrix (glycerol or 3-nitrobenzyl alcohol, 3-NBA) is used to keep a homogeneous surface for bombardment, to extend the spectral lifetime and enhance sensitivity.
In FAB, the particle beam is a neutral inert gas, typically Ar or Xe, at bombardment energies of 4-10 KeV.
In LSIMS, the particle beam is an ion, typically CS^+, at bombardment energies of 2-30KeV.
The particle beam is incident at the analyte surface, where it transfers much of its energy to the surroundings, setting up momentary collisions and disruptions.
Some species are ejected off the surface as positive and negative ions by this process, and these secondary ions are then extracted from the source and analyzed by the mass spectrometer.
Both FAB and LSIMS are comparatively **soft** ionization techniques, and are thus well suited to the analysis of low volatility species, typically producing large peaks for the pseudo-molecular ion species $[M+H]^+$ and $[M-H]^-$, along with structurally significant fragment ions and some higher mass cluster ions and dimers.

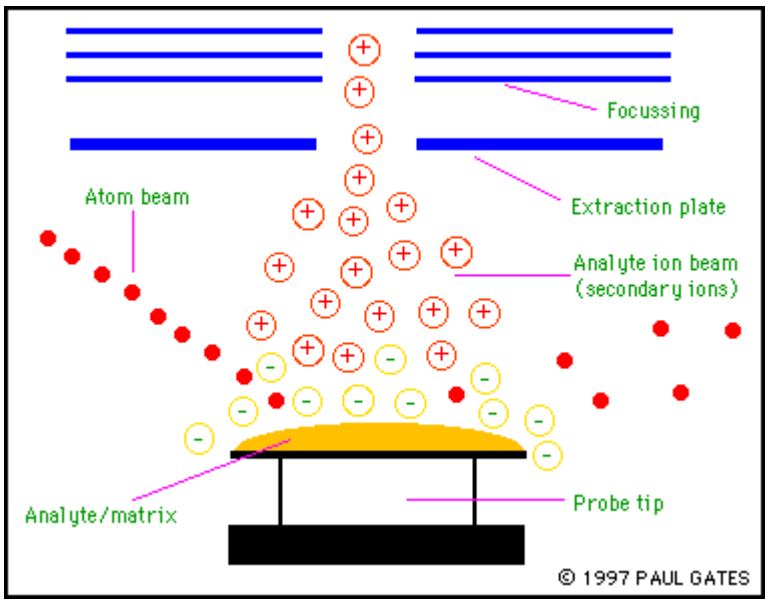


Figure 5. *Schematic of a Fast Atom Bombardment source (positive mode).*

The polarity of the source extraction can be switched depending on what species are to be analyzed. In the figure, the extraction is negative and thus only the positive ions are being analyzed, this is termed +FAB. For LSIMS the atom beam is replaced by an ion beam.

3. 5 Electrospray Ionization (ESI)

The production of ions by evaporation of charged droplets obtained through spraying has been already but it was only recently discovered that these ions may hold more than one charge.
Large charged droplets are produced by forcing of the analyte solution through a needle, at the end of which is applied a potential - the potential used is sufficiently high (e.g. 4,000 V) to disperse the emerging solution into a very fine spray of charged droplets all at the same polarity. The solvent evaporates away, shrinking the droplet size and increasing the charge concentration at the droplet's surface.
The ionized sample molecules in the gas phase are then 'swept' into a MS that is held essentially in vacuum and the ions separated and detected.
The charges are statistically distributed amongst the analyte's available charge sites, leading to the possible formation of multiply charged ions under the correct conditions. Increasing the rate of solvent evaporation, by introducing a drying gas flow counter current to the sprayed ions (see Fig. 6), increases the extent of multiple-charging. Decreasing the capillary diameter and lowering the analyte solution flow rate i.e. in **nanospray ionization**, will create ions with higher $^{m}/_{z}$ ratios (i.e. it is a softer ionization technique) than those produced by 'conventional' ESI and are of much more use in the field of analysis of biomolecules.

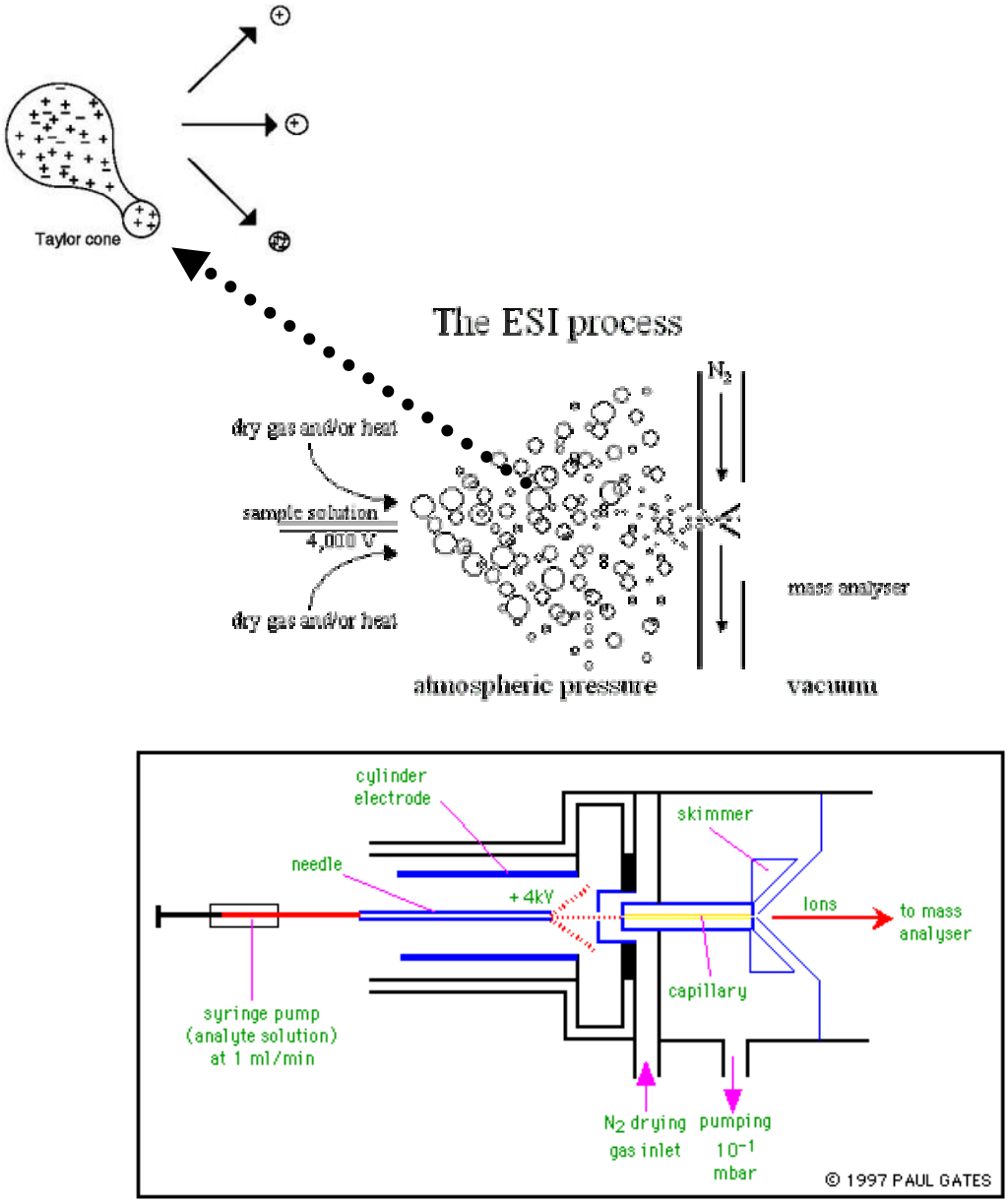


Figure 6. *A schematic diagram of the typical layout of an electrospray source.*

3. 6 Atmospheric Pressure Chemical Ionization

Similar to electrospray ionization, liquid effluent is introduced directly into the **Atmospheric Pressure Chemical Ionization** (**APCI**) source, however the similarity with electrospray stops there. The APCI source contains a heated vaporizer which facilitates rapid desolvation/vaporization of the droplets. Vaporized sample molecules are carried through an ion-molecule reaction region at atmospheric pressure. The ionization occurs through a corona discharge, creating reagent ions from the solvent vapor. Chemical ionization of sample molecules is very efficient at atmospheric pressure due to the high collision frequency. Proton transfer (protonation MH^+ reactions) occurs in the positive mode, and either electron transfer or proton transfer (proton loss, $[M-H]^-$) in the negative

mode. The moderating influence of the solvent clusters on the reagent ions, and of the high gas pressure, reduces fragmentation during ionization and results in primarily molecular ions. APCI is widely used in the pharmaceutical industry to analyze relatively nonpolar, semi volatile samples of less than 1200 Daltons and it is an especially good ionization source for liquid chromatography.

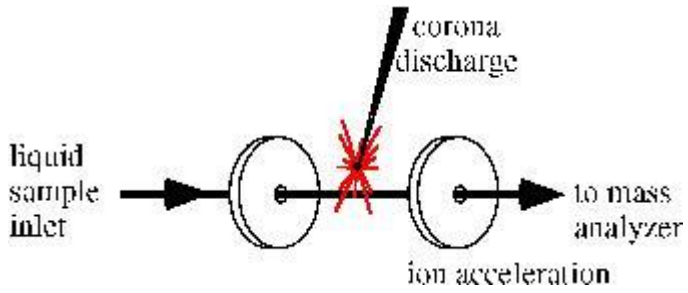


Figure 7. *Atmospheric Pressure Chemical Ionization (APCI) Mass Spectrometry.*

3. 7 Matrix-assisted Laser Desorption/Ionization (MALDI)

The study of polar compounds has always been a problem for mass spectrometry. It was demonstrated in the early 1960s that irradiation of low-mass organic samples with a high-intensity laser pulse, produces ions that could be successfully mass analyzed. The extension of laser desorption (LD) to the analysis of non-volatile polar biological and organic macromolecules and polymers was a groundbreaking step in the development of LD. These experiments, however, revealed an upper mass limit of 5-10 kDa.

The primary requirement for successful LD is that the energy transfer from the laser beam to the analyte should take place in as short a time as possible, to prevent decomposition of the thermally labile analyte molecules. The mass limit is probably due to the energy required to cause the resonant excitation and successful energy transfer being greater than the dissociation energy of the analyte. Thus the analyte is not desorbed as the intact molecule ion in any significant amount resulting in a spectrum of low-mass fragment ions only.

Another major restriction of LD ionization is the short duration of the ion burst following the laser pulse. A consequence is that the technique is unsuitable for scanning analysis on sector or quadrupole instruments meaning that LD is particularly suited to TOF mass spectrometry.

It was discovered that the use of a matrix in LD could circumvent the restrictive mass limitations of the technique. Requirements of the matrix are that it has a strong absorbance at the laser wavelength and was of low enough mass to be sublimable. A low concentration of the analyte is uniformly dispersed throughout the solid or liquid matrix, deposited on the end of a probe or onto a metal plate and introduced into the pulsed laser beam.

The low concentration of analyte used has several important advantages. The efficiency of energy transfer from the laser to the analyte (via matrix) is increased, whereas problems associated with analyte dissociation are greatly reduced. The association of the analyte molecules to form high-mass clusters is also reduced and it is believed that suitable matrices can even enhance ion formation.

The Mechanism of Ion Desorption: The mechanism of MALDI is not totally understood, but it is believed to work along the following lines (see also Fig. 8):
(i) The Formation of a 'Solid Solution'. The analyte molecules are distributed throughout the matrix so that they are completely isolated from one other. This is necessary if the matrix is to form a homogenous 'solid solution' (any liquid solvent(s) used in preparation of the solution are removed when the mixture is dried before analysis).
(ii) Matrix Excitation. Some of the laser energy incident on the solid solution is absorbed by the matrix, causing rapid vibrational excitation, bringing about localized disintegration of the solid solution, forming clusters made up of a single analyte molecule surrounded by neutral and excited matrix molecules. The matrix molecules evaporate away from these clusters to leave the excited analyte molecule.
(iii) Analyte Ionization. The analyte molecules can become ionized by simple protonation by the photo-excited matrix, leading to the formation of the typical $[M+X]^+$ type species (where X= H, Li, Na, K, etc.). Some multiply charged species, dimers and trimers can also be formed. Negative ions are formed from reactions involving deprotonation of the analyte by the matrix to form $[M-H]^-$ and from interactions with photoelectrons to form the $[M]^{\cdot}$ radical molecular ions.

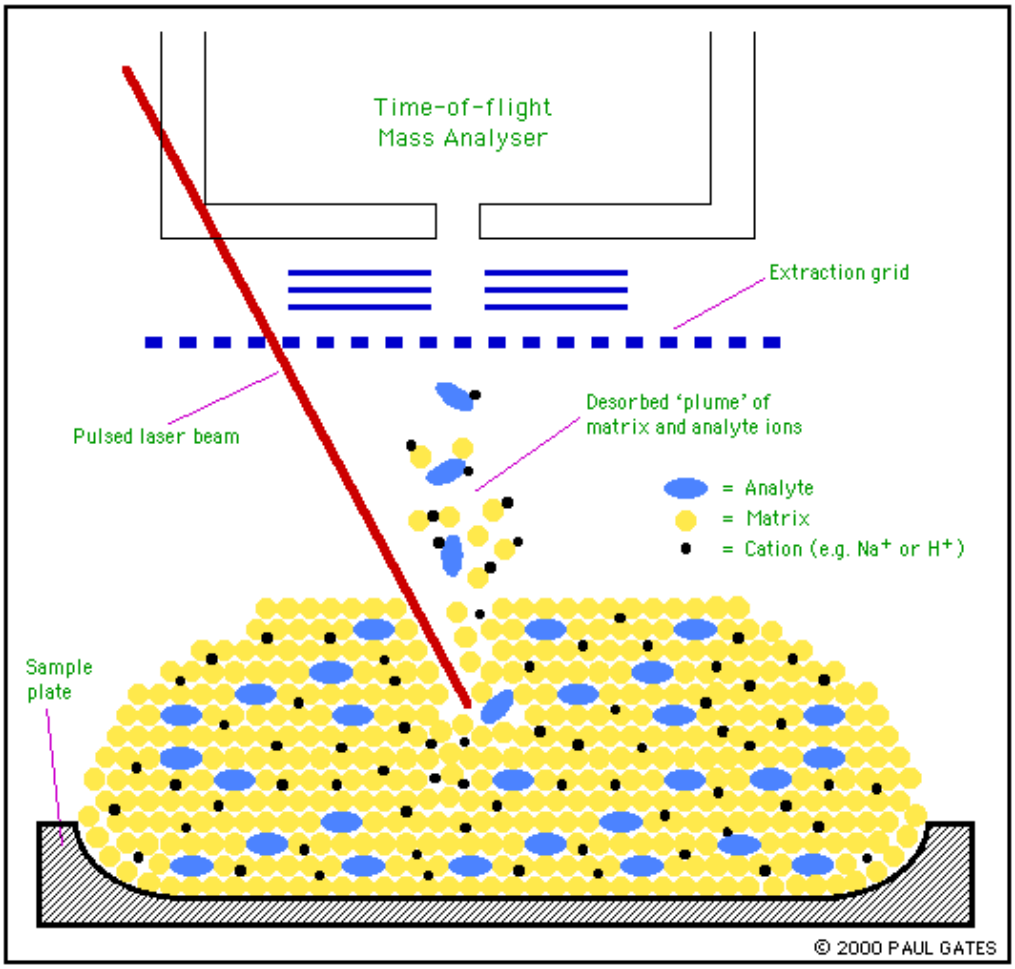


Figure 8. *Schematic of MALDI*

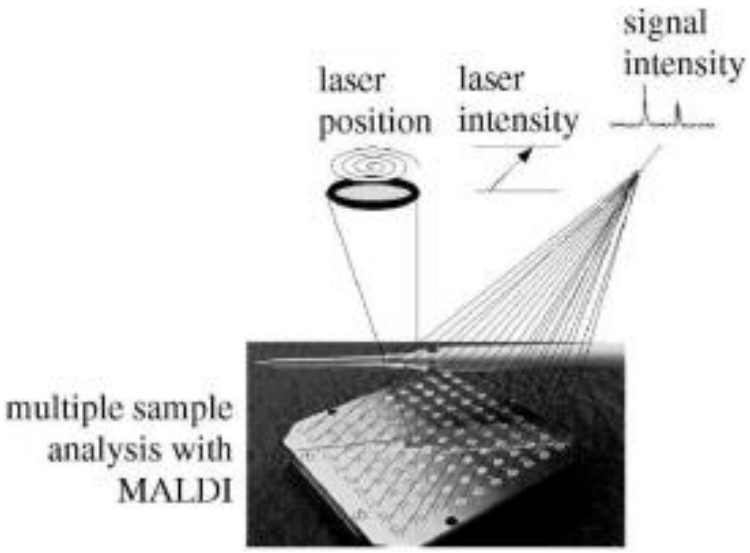


Figure 9*. The MALDI sample plate can be used for multi-sample preparation and automated sample analysis. Three different parameters can be used to adjust and monitor MALDI auto-sampling including: laser position, laser intensity, signal intensity and mass range.*

These ionization reactions occur in the first tens of nanoseconds after irradiance, and within the initial desorbing matrix/analyte cloud. It is in this way that the characteristic MALDI spectra are created, typically giving large signals for species of the type $[NM+X]^{n+}$ (N * n).

3.8 Techniques elemental analysis: Inductively-Coupled Plasma (ICP) Excitation Source

Inductively coupled plasma (ICP) is a very high temperature (7000-8000K) excitation source that efficiently desolvates, vaporizes, excites, and ionizes atoms. Molecular interferences are greatly reduced with this excitation source but are not eliminated completely. ICP sources are used to excite atoms for atomic-emission spectroscopy and to ionize atoms for mass spectrometry.
The sample is nebulized and entrained in the flow of plasma support gas, which is typically Ar. The plasma torch consists of concentric quartz tubes, with the inner tube containing the sample aerosol and Ar support gas and the outer tube containing an Ar gas flow to cool the tubes (see schematic). A radiofrequency (RF) generator (typically 1-5 kW @ 27 MHz or 41 MHz) produces an oscillating current in an induction coil that wraps around the tubes. The induction coil creates an oscillating magnetic field, which produces an oscillating magnetic field. The magnetic field in turn sets up an oscillating current in the ions and electrons of the support gas. These ions and electrons transfer energy to other atoms in the support gas by collisions to create very high temperature plasma.

Figure 10. *Schematic cross-section of an ICP-source*

Table3. *Overview of ionization methods in Mass Spectroscopy*

Ionization method	Typical Analytes	Sample Introduction	Mass Range	Method Capability
Electron Impact (EI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Hard method versatile provides structure info
Chemical Ionization (CI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Soft method molecular ion peak $[M+H]^+$
Electrospray (ESI)	Peptides Proteins nonvolatile	Liquid Chromatography or syringe	to 200,000 Daltons	Soft method ions often multiply charged
Fast Atom Bombardment (FAB)	Carbohydrates Organometallics Peptides nonvolatile	Sample mixed in viscous matrix	to 6,000 Daltons	Soft method but harder than ESI or MALDI
Matrix Assisted Laser Desorption (MALDI)	Peptides Proteins Nucleotides	Sample mixed in solid matrix	to 500,000 Daltons	Soft method very high mass
Inductively-Coupled Plasma (ICP) Excitation Source	Elements and organometallics	Probe, GC, LC		Multi-elemental analysis in one run

4. Mass Analyzer

4.1 Double Focusing (Sector) Analysis

The sector mass spectrometer is generally considered to be the 'classical' method for mass separation and analysis and is the technique most often taught in schools. Up until very recently, it was the technique most often encountered in general applications laboratories because the other techniques had more specialized applications. In the last ten years however, with the development of the newer softer ionization techniques like MALDI and ESI, sector mass analysis had begun to be marginalized in favor of the cheaper quadrupole (for ESI) or time-of-flight (for MALDI) mass analysis techniques or the more versatile FT-ICR-MS. Sector analysis does, however, still have a very important role to play in mass spectrometry, that of high resolution mass analysis of singularly charged high-mass ions, something that is, so far, not as easy with the other techniques.

Ions are accelerated from the source, under action of the accelerating voltage, V_a, and enter the electrostatic analyzer (see Fig. 11) which acts as an energy focusing device. Because of the physical nature of many types of ionization, ions of the same mass and charge are often produced with an energy distribution which if not corrected for, will drastically reduce the observed resolution and mass accuracy. The electrostatic analyzer focuses ions of the same mass-to-charge ratio into a more coherent path through to the magnetic sector.

The magnetic sector consists of a broad flight tube through a variable magnet of field strength B and radius r (typically 60-120^{O}). The change in the magnetic field strength will focus ions of different mass-to-charge ratios at the 'double focusing point' (see Fig. 11) and subsequently to the ion detector (usually an electron multiplier).

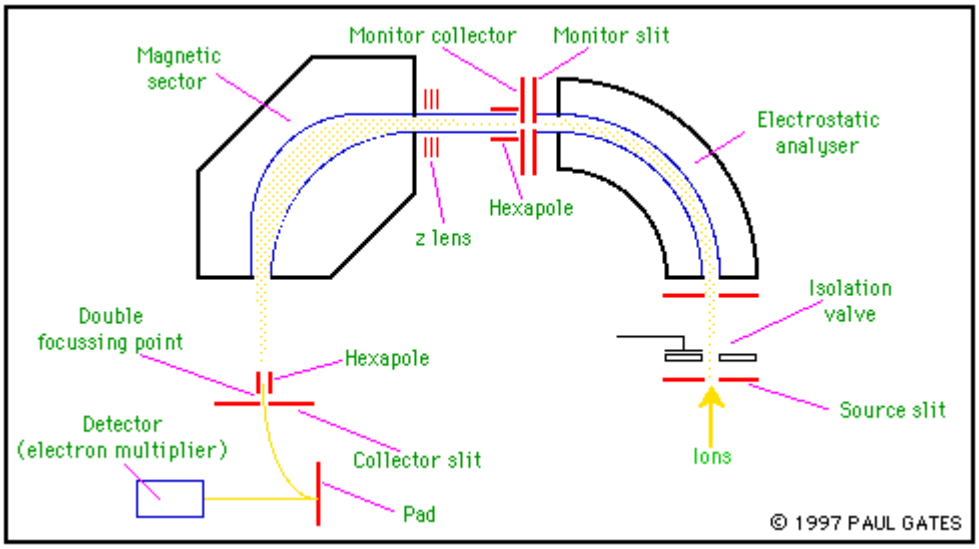


Figure 11. *A schematic diagram of two-sector mass spectrometer.*

Ions of mass, m_i, charge, z_i and velocity v, enter the magnetic field with the kinetic energy, as defined by equation 1, which they had on leaving the source slit.

Equation 1:

$$\text{kinetic energy} = \frac{m_i v^2}{2} = z_i V_a$$

The centrifugal force experienced by the ions must balance the Lorentz force exerted by the magnet field (strength B, radius r) for it to be focused onto the double focusing point, this is shown by equation 2.

Equation 2:

$$\frac{m_i v^2}{r} = B z_i v$$

Combining equation 1 and 2 leads to the equation for focusing each mass-to-charge ratio (equation 3). It can be seen that by varying either the accelerating potential, V_a, or the magnetic field strength, B, ions are successively brought into focus onto the collector slit. It is most usual for the magnet to be scanned and for the accelerating potential to be fixed.

Equation 3:

$$\frac{m_i}{z_i} = \frac{B^2 r^2}{2 V_a}$$

The addition of an electric sector allows only ions of uniform kinetic energy to reach the detector, thereby increasing the resolution of the two-sector instrument to 100,000. Magnetic double-focusing instrumentation is commonly used with FAB and EI ionization however they are not widely used for electrospray and MALDI ionization sources primarily because of the much higher cost of these instruments.

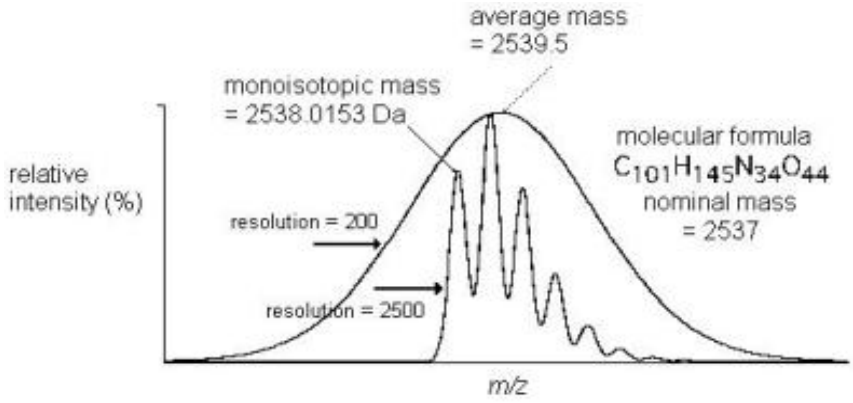


Figure 12. *An increase in resolution allows for better peak distinction within a spectrum.*

4.2 Time-of-Flight (TOF)

Time-of-flight mass spectrometry (TOF-MS) was developed around fifty years ago. TOF-MS has only recently begun to fulfill its true potential with the development of higher resolution instruments. The inherent characteristics of the TOF mass analyzer, lead to spectra of virtually unlimited mass range being obtained in a few microseconds with relative ease.

Recently, along with the introduction of matrix-assisted laser desorption/ionization in 1988 there has been a large increase in interest in TOF-MS, especially in the fields of biological and polymer chemistry.

The TOF mass spectrometer (see Fig. 13) is the simplest type of common mass analyzer and has a very high sensitivity at a virtually unlimited mass range. The sample ions are generated in a source zone, s, of the instrument, by whatever ionization method is being employed. A potential, (V_s - the source extraction) is applied across the source to extract and accelerate the ions from the source into the field-free 'drift' zone of the instrument, d.

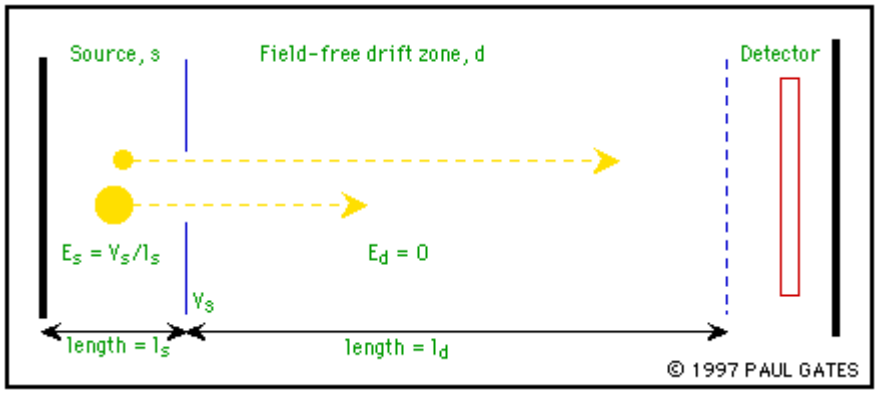


Figure 13. *Schematic diagram of the process of time-of-flight mass spectrometry: The larger the ion, the slower its velocity and thus the longer it takes to traverse the field-free drift zone. The field in the field-free zone, E_d, is zero at all times.*

In the ideal case, all ions produced will leave the source at the same time with the same kinetic energy, due to their having been accelerated through the same potential difference. In this case the time-of-flight of the ions produced will only be dependent on the mass and the charge of the produced ion. Neglecting the extraction time from the source, the basic formula for TOF mass analysis is given by the equation:

$$\frac{m_i}{z_i} = 2eE\, l_s \left(\frac{t_i}{l_d}\right)^2$$

Where:

m_i = mass of analyte ion, z_i = charge on analyte ion, E = extraction field, t_i = time-of-flight of ion, l_s = length of the source, l_d = length of the field-free drift region, e = electronic charge ($1.6022*10^{-19}$ C).

For a reliable mass spectrum to be obtained, the time of ion extraction must be known to a high degree of accuracy. This problem is usually addressed by using a pulsed ionization technique like laser desorption or MALDI. There are a number of problems with the technique which cause a time-of-flight distribution at each mass, thus lowering the resolution. These factors must be corrected or allowed for if a high-resolution spectrum is required. Achieving high resolution normally involves using the more complex reflectron instruments, long flight tubes and/or delayed ion extraction.

4.3 Quadrupole Mass Analyzer

In a quadrupole mass analyzer, only electric fields are used to separate ions according to their m/z values. A quadrupole consists of four parallel rods or poles through which the ions being separated are passed. The poles have a fixed DC and alternating RF voltages applied to them. Depending on the produced electric field, only ions of a particular m/z will be focused on the detector, all the other ions will be deflected into the rods. By varying the strengths and frequencies of electric fields, different ions will be detected thus making the mass spectrum. The trajectory of an ion through a quadrupole is very complex (see Fig. 14).

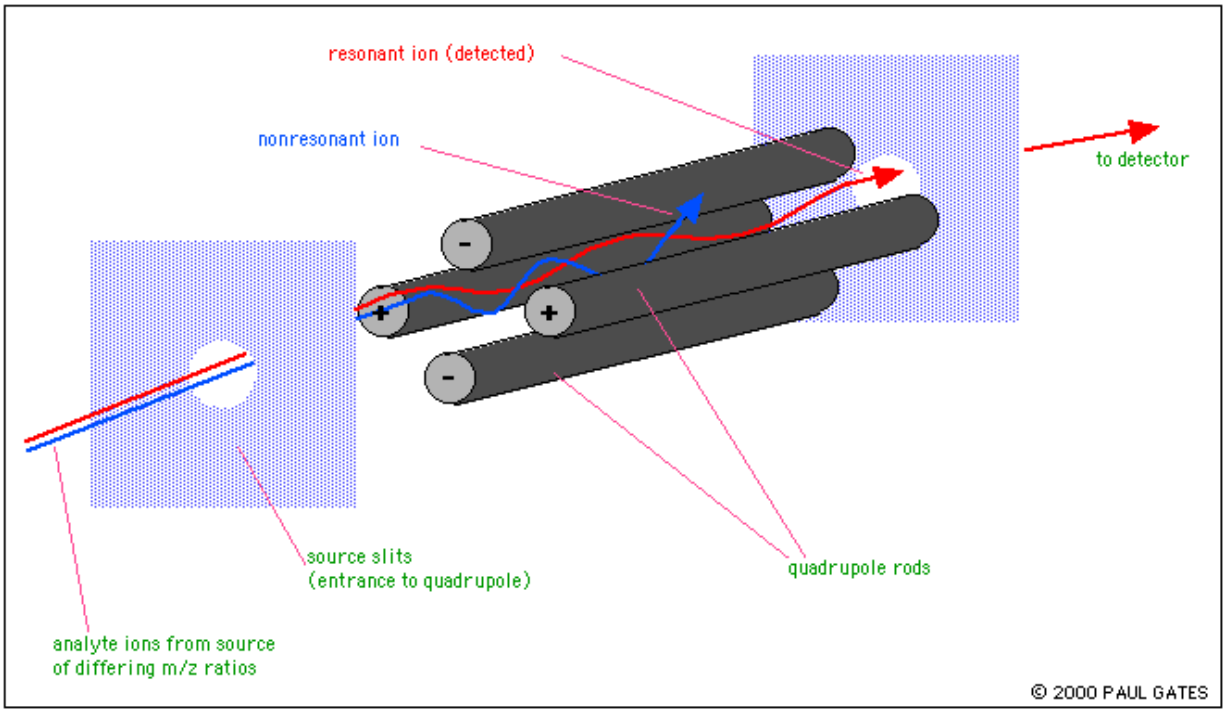


Figure 14. *Schematic diagram of a quadrupole mass analyser: The four rods are shown as being circular in the diagram but in practice they have a hyperbolic cross-section.*

Two opposite rods will have a potential of **+(U+Vcos(ωt))** and the other two of **-(U+Vcos(ωt))** where **U** is a fixed potential and **Vcos(ωt)** represents a radio frequency (**RF**) field of amplitude **V** and frequency **ω**.
When cos(ωt) cycles with time, t, the applied voltages on opposed pairs of rods will vary in a sinusoidal manner but in opposite polarity (due to them being offset). Along the central axis of the quadrupole assembly and also the axis between each adjoining rod the resultant electric field is zero. In the transverse direction of the quadrupoles, an ion will oscillate amongst the poles in a complex fashion, depending on its m/z, the voltages U and V and the frequency, ω, of the alternating RF potential. By suitable choices of U, V and ω, only ions of one m/z will oscillate stably through the quadrupole mass analyser to the detector. All other ions will have greater amplitude of oscillation causing them to strike one of the rods. In practice, the typical frequency values are from 1 to 2 MHz.
The length and diameter of the rods will determine the mass range and ultimate resolution that can be achieved by the quadrupole assembly. However, the maximum mass range that is normally achieved is around 4,000 Da with a resolution of around 2000.

4.4 Fourier Transform Ion Cyclotron Resonance (FT-ICR)

Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry is probably the most complex method of mass analysis. It is the most sensitive of the techniques in common use today, and has almost unlimited mass resolution, >10^6 is observable with most instruments and resolutions in the 10^4 to 10^5 range are routinely available.
The FT-ICR mass spectrometer consists of three main sections. The first is the sample source, which can be practically any of the available techniques, although ESI and MALDI are the most common. The second section is the ion transfer region, where the ions produce in the source are focused, bunched and transferred into the high vacuum region before entering the analyzer cell. The final region is the cell itself. It is possible in some cases (especially with MALDI) to produce ions directly in the cell, this does away with the need for the complex ion focusing and pumping regions.
The standard arrangement for the analyzer region, of the FT-ICR instrument, is an ion-trap located within a spatially uniform static magnetic field of strength, $\mathbf{B}_0$. The effect of the magnetic field is to constrain the incident ions in a circular orbit (see figure), the frequency of which is determined by the mass, m_i, charge, z_i, and velocity, v, of the ion, by action of the Lorentz force, defined in equation 1. The analyte ion is bent into a circular path in a plane perpendicular to the magnetic field. The ion's angular frequency, ω_c, is defined by equation 2. The opposite sense of rotation is experienced by ions of opposite charge.

Equation 1:	**Equation 2:**	**Equation 3:**
$\mathbf{F} = z_i \mathbf{v} \times \mathbf{B}_0$	$\omega_c = \frac{z_i \mathbf{B}_0}{2\pi m_i}$	$\frac{m_i}{z_i} = \frac{\mathbf{B}_0}{2\pi\omega_c}$

Where:
F = Lorentz force (observed by the incident ion), $\mathbf{B}_0$ = ICR magnetic field, $\mathbf{m}_i$ = mass of the ion, $\mathbf{z}_i$ = charge on the ion, **v** = incident velocity of the ion, $\boldsymbol{\omega}_c$ = induced rotational (cyclotron) frequency

The presence of ions between a pair of detector electrodes (in the trapping cell) will not actually produce any measurable signal. It is necessary to excite the ions of a given $^{m}/_{z}$ as a coherent package to a larger orbital radius, by applying an RF sweep of a few milliseconds across the cell (see Fig. 15). One frequency will excite one particular $^{m}/_{z}$.

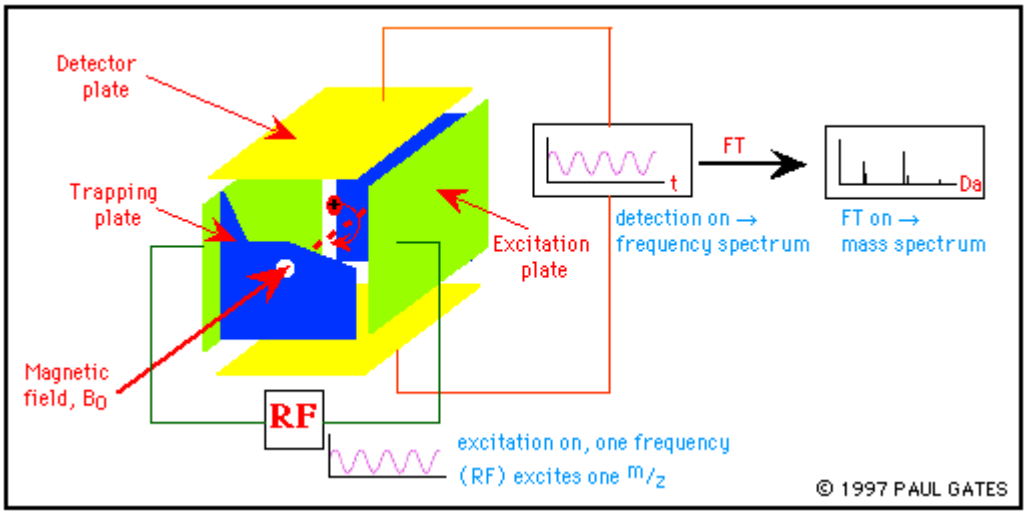


Figure 15. *A schematic diagram of the trapping, excitation and detection of a ions, to produce a mass spectrum, in FT-ICR mass spectrometry.*

The principle of Fourier transformation is: a signal whose intensity is measured as a time-dependent function is made up of many frequencies superposed one over the other, each with its own intensity. The Fourier transformation allows one to find again the individual frequencies and their intensities. Measurement of the angular frequency (equation 2) leads to values for $^{m}/_{z}$ (equation 3) and thus to the mass spectrum. Because frequency can be measured more accurately than any other physical property, the technique has a very high mass resolution. After excitation, the ions are allowed to relax back to their natural ICR motion for later re-measurement, if required.

4.5 Ion Trap Analysis

The quadrupole ion trap mass analyzer (see Fig. 16) consists of **three hyperbolic electrodes**: the **ring** electrode, the entrance **endcap** electrode and the **exit endcap** electrode. These electrodes form a cavity in which it is possible to trap and analyze ions. Both endcap electrodes have a small hole in their centers through which the ions can travel. The ring electrode is located halfway between the two endcap electrodes.
Ions produced from the source enter the trap through the inlet focusing system and the entrance endcap electrode. Various voltages are applied to the electrodes to trap and eject ions according to their mass-to-charge ratios. The ring electrode RF potential, an a.c. potential of constant frequency and variable amplitude, is applied to the ring electrode to produce a 3D quadrupolar potential field within the trapping cavity. This will trap ions in a stable oscillating trajectory confined within the trapping cell. The nature of the trajectory is dependent on the trapping potential and the mass-to-charge ratio of the ions. During detection, the electrode system potentials are altered to produce instabilities in the ion trajectories and thus eject the ions in the axial direction. The ions are ejected in order of

increasing mass-to-charge ratio, focused by the exit lens and detected by the ion detector system.

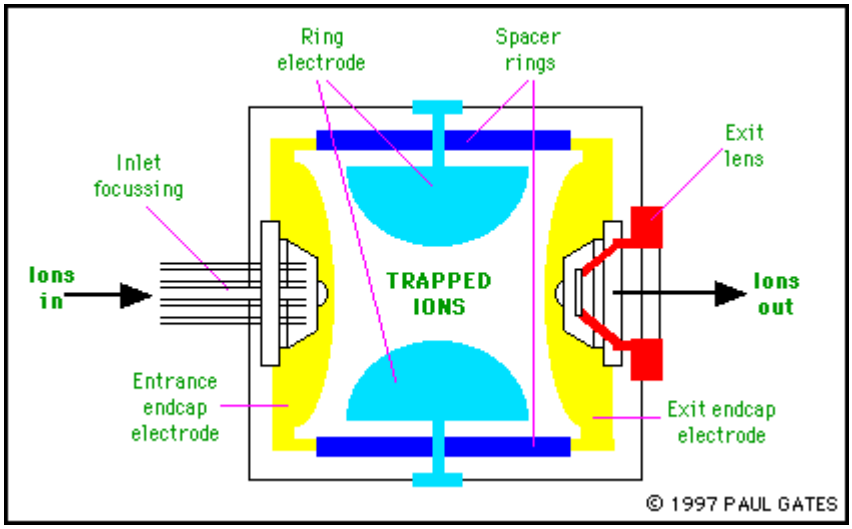


Figure 16. *A schematic diagram of an ion trap mass spectrometer.*

4.6 Tandem Mass Spectrometry

In contrast to electron ionization (EI) which produces many fragment ions, the new ionization techniques are relatively gentle and do not produce a significant amount of fragment ions. To obtain more information on the molecular ions generated in the electrospray ionization and MALDI ionization sources, it has been necessary to apply techniques such as tandem mass spectrometry (MS/MS) to induce fragmentation. Tandem mass spectrometry (abbreviated MS^n - where n refers to the number of generations of fragment ions being analyzed) allows one to induce fragmentation and mass analyze the fragment ions. This is accomplished by collisionally generating fragments from a selected ion and then mass analyzing the fragment ions. Fragmentation can be achieved by inducing ion/molecule collisions by a process known as collision-induced dissociation (CID) (also known as collision-activated dissociation (CAD)). Collision-induced dissociation is accomplished by selecting an ion of interest with a mass analyzer and introducing that ion into a collision cell. The selected ion then collides with a collision gas (typically argon or helium) resulting in fragmentation.

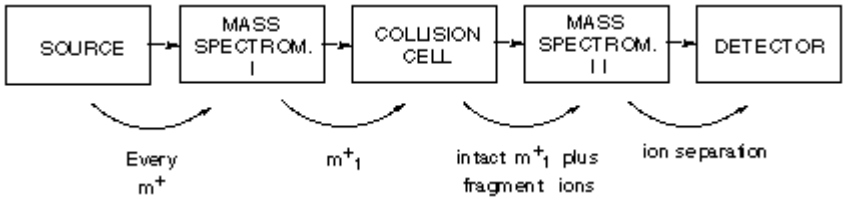


Figure 17. *Illustration of MS/MS instruments*

The fragments are then analyzed to obtain a fragment ion spectrum. The abbreviation MS^n is applied to processes which analyze beyond the initial ions (MS) to the fragment ions (MS^2) and subsequent generations of fragment ions (MS^3, MS^4 and …). Tandem mass analysis is primarily used to obtain structural information.

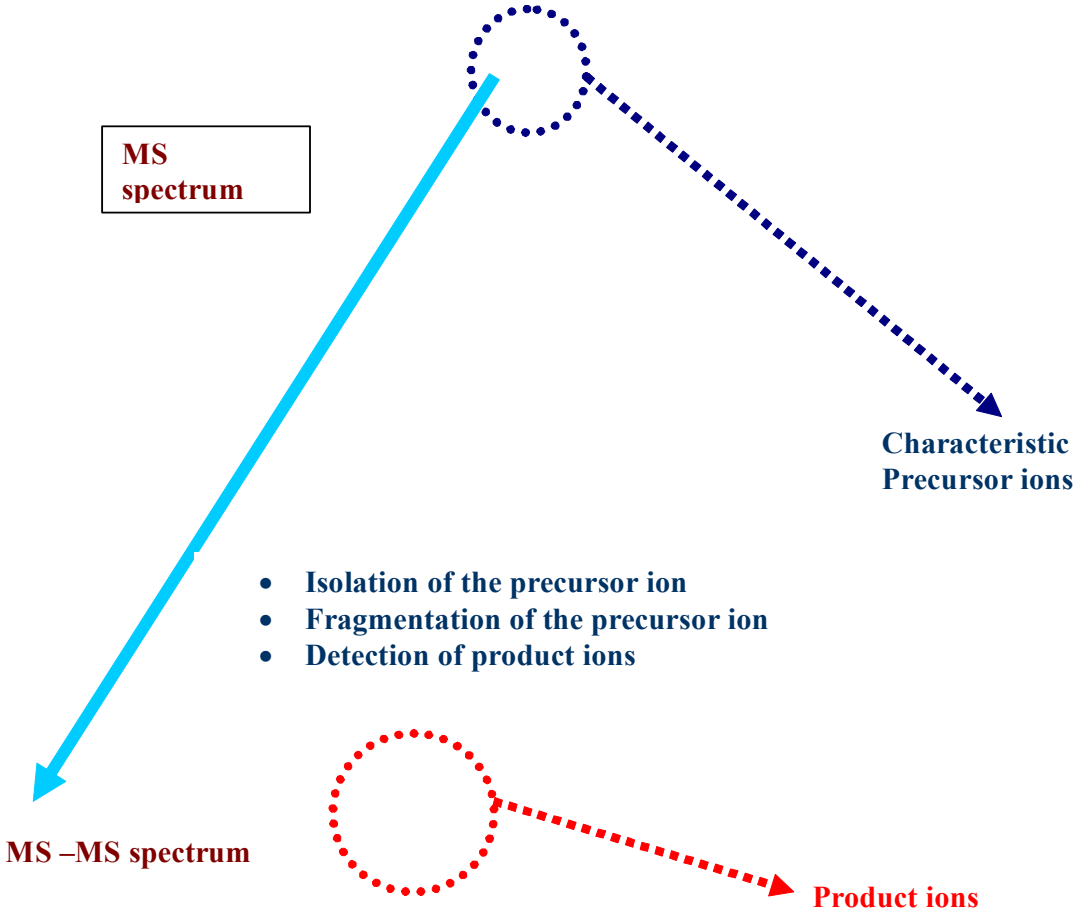


Figure 18. *Fragments are analyzed to obtain a fragment ion spectrum. The abbreviation MS^n is applied to processes which analyze beyond the initial ions (MS) to the fragment ions (MS^2) and subsequent generations of fragment ions (MS^3, MS^4 and …).*

Table 4. *Overview of the most used mass analyzers*

Analyzer	System Highlights
Quadrupole	Unit mass resolution, fast scan, low cost
Sector (Magnetic and/or Electrostatic)	High resolution, exact mass
Time-of-Flight (TOF)	Theoretically, no limitation for m/z maximum, high throughput
Ion Cyclotron Resonance (ICR)	Very high resolution, exact mass, perform ion chemistry

5. Ion Detection

Once the ion passes through the mass analyzer it is then detected by the ion detector, the final element of the mass spectrometer. The detector allows a mass spectrometer to generate a signal current from incident ions by generating secondary electrons, which are further amplified. Alternatively, some detectors operate by inducing a current generated by a moving charge. Among the detectors described, the electron multiplier and scintillation counter are the most commonly used and convert the kinetic energy of incident ions into a cascade of secondary electrons.

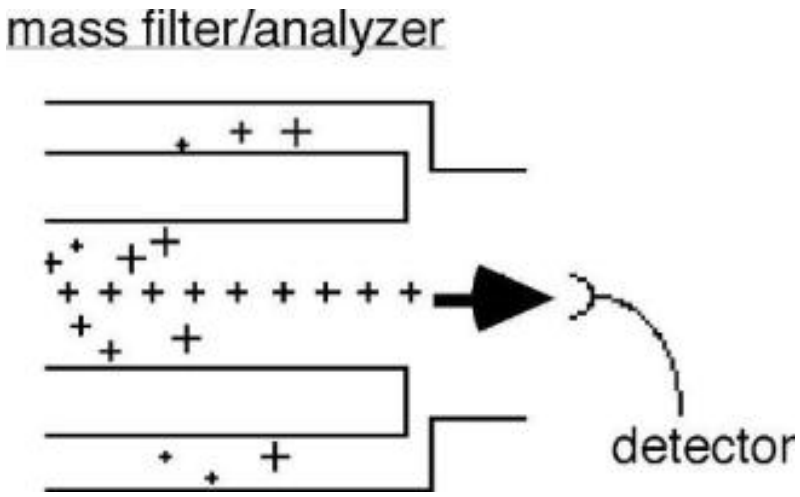


Figure 19. *After an ion passes through the Mass Analyzer a signal is produced by the detector.*

5.1. Faraday Cup

A Faraday cup operates on the basic principle that a change in charge on a metal plate results in a flow of electrons and therefore creates a current. **One ion striking the dynode surface of the Faraday cup (a dynode is a secondary emitting material**, usually BeO, GaP, or CsSb) **induces several secondary electrons to be ejected and temporarily displaced**. This temporary emission of electrons induces a current in the cup and provides for a small

amplification of signal when an ion strikes the cup. This detector is relatively insensitive, yet robust and simple in design.

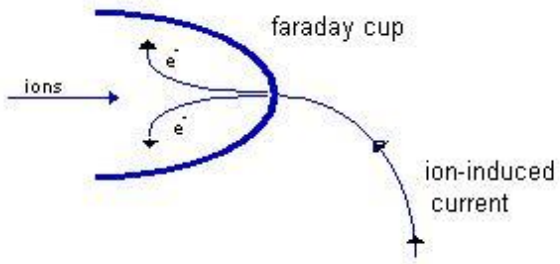


Figure 20. *Faraday cup converts the striking ion into a current.*

5.2 Electron multiplier

An electron multiplier is one of the most common means of detecting ions, achieving high sensitivity by extending the principle used with a Faraday cup. Whereas a Faraday cup uses one dynode, an electron multiplier is made up of a series of dynodes maintained at ever increasing potentials. Ions strike the dynode surface, resulting in the emission of electrons. These secondary electrons are then attracted to the next dynode where more secondary electrons are generated, ultimately resulting in a cascade of electrons. Typical amplification or current gain of an electron multiplier is one million.

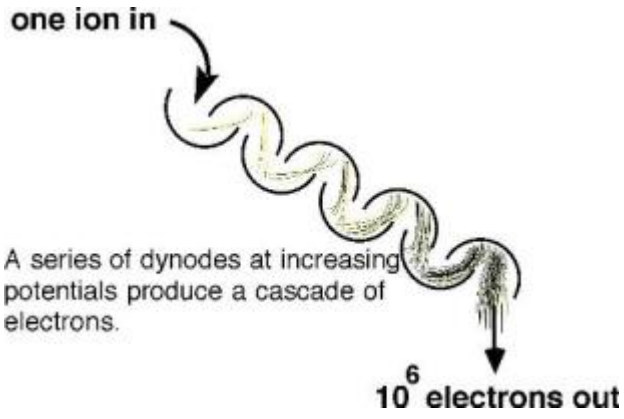


Figure 21. *Electron multiplier and the cascade of electrons that results in a factor of one million signal amplification in a mass spectrometer.*

5.3. The photomultiplier conversion dynode (Scintillation counting or Daly detector)

The photomultiplier conversion dynode detector in Fig. 22 is similar to an electron multiplier where the ions initially strike a dynode, resulting in the emission of electrons. However, with the photomultiplier conversion dynode detector electrons then strike a

phosphorus screen. The phosphorus screen, much like the screen on a television set, releases photons once an electron strikes. These photons are then detected by a photomultiplier, which operates with a cascading action much like an electron multiplier. The primary advantage of the conversion dynode setup is that the photomultiplier tube is sealed in a vacuum (photons pass through sealed glass), unexposed to the internal environment of the mass spectrometer. Thus the possibility of contamination is removed. A five-year or greater lifetime is typical and, with sensitivity similar to electron multipliers, photomultiplier conversion dynode detectors are becoming more widely used in mass spectrometers.

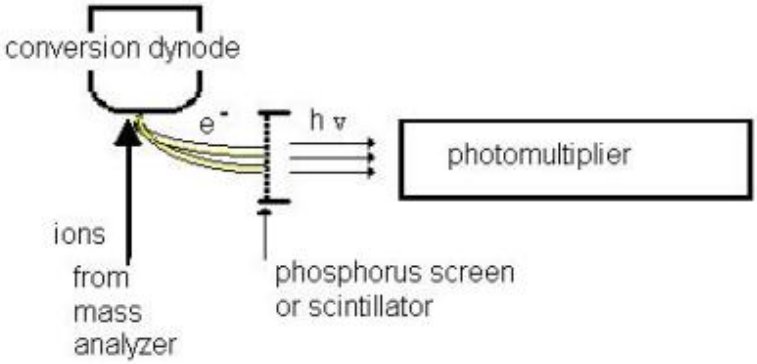


Figure 22. *Scintillation counting relies on the conversion of the ion/electron signal into light. Once the photon(s) are formed, detection is performed with a photomultiplier.*

III. Applications

Mass spectrometry is being applied to a wide variety of questions include protein structure, drug metabolism, flavor and smell chemistry, petroleum and petrochemicals, organic fossils, inherited metabolic diseases, atmospheric chemistry, the analysis of respiratory gases, viral identification, forensics, and many other specialized subjects.

1. Atomic Masses

The discovery of isotopes with the first mass spectrometer answered the question about the integer value of atoms (e.g. carbon-12, nitrogen-14 and oxygen-16). These measurements were originally undertaken by Francis W. Aston and repeated with increasing precision by succeeding generations of scientists. Since those original experiments mass spectroscopy and nuclear physics have combined to determine isotopic masses to a high degree of accuracy. The mass unit now used is defined such that the mass of the carbon-12 isotope is exactly 12 atomic mass units (amu).

2. Geochronology and Geochemistry

The early studies of the radioactive decay of uranium and thorium into lead caused the British physicist Ernest Rutherford to suggest that this process could be used to determine the age of rocks and, consequently, of the Earth by observing the amount of helium retained by a rock relative to its uranium and thorium contents. Mass spectrometers capable of measuring isotopic ratios allow the composition of elements to be determined in which one or more isotopes result from radioactive decay. The age of the rock from which the element has been obtained can be determined if the amount of the parent element can be measured and certain requirements on the environmental history of the rock are met.
The use of isotopes has also proven to be especially valuable in understanding the origin and nature of the solar system. A great body of evidence now suggests that meteorites are objects that solidified very early in the history of the solar system. Extinct radioactivity of elements with various half-lives have been identified that set limits on the time between the synthesis of the elements and their condensation. An example is the excess of magnesium-26 (^{26}Mg) found in primitive meteorites that resulted from the decay of aluminum-26 (^{26}Al), which has a 720,000-year half-life.

3. Accelerator MS and dating materials

The particle accelerators used in nuclear physics can be viewed as mass spectrometers of rather distorted forms, but the three principal elements--the ion source, analyzer, and detector--are always present. L.W. Alvarez and Robert Cornog of the United States first used an accelerator as a mass spectrometer in 1939 when they employed a cyclotron to demonstrate that helium-3 was stable rather than hydrogen-3, an important question in nuclear physics at the time. They also showed that helium-3 was a constituent of natural helium. The method was not employed again for nearly 40 years; however, it has found application in measuring cosmogenic isotopes, the radioisotopes produced by cosmic rays incident on the Earth or planetary objects. These isotopes are exceedingly rare, having abundances on the order of one million millionth of the corresponding terrestrial element, which is an isotopic ratio far beyond the capabilities of normal mass spectrometers. If the half-life of a cosmogenic isotope is relatively short, such as that of beryllium-7 (^{7}Be; 53 days) or carbon-14 (^{14}C; 5,730 years), its concentration in a sample can be determined by

radioactive counting; However, if the half-life is long, such as that of beryllium-10 (^{10}Be; 1.5 million years) or chlorine-36 (^{36}Cl; 0.3 million years), such a course is ineffective. The advantage of the large, high-energy accelerator mass spectrometer is the great detector selectivity that results from ions having 1,000 times more energy than any previously available machine could provide. Conventional mass spectrometers have difficulty measuring abundances less than one hundred-thousandth of the reference isotope, because interfering ions are scattered into the analyzer location where the low-abundance isotope is to be sought. Extremes of high vacuum and antiscattering precautions can improve this by a factor of 10, but not the factor of 100 million that is required. An accelerator suffers from this defect to an even greater degree, and large quantities of "trash" ions are found at the expected analyzer location of the cosmogenic isotope. The ability of certain kinds of nuclear particle detectors to identify the relevant ion unambiguously enables the accelerator mass spectrometer to overcome this shortcoming and function as a powerful analytical tool.
The accelerator method has opened lines of investigation that had previously been inaccessible. A strong motivation for the inventors was the improvement of radiocarbon dating. Scientists are now able to make age determinations from much smaller samples and to make them much more rapidly than by radioactive counting, but carbon-14 proved to be a considerably more difficult problem for instrumental development than the other cosmogenic isotopes. The method was applied almost immediately to analyses involving beryllium-10 and chlorine-36, with aluminum-26 (^{26}Al), calcium-41 (^{41}Ca), and iodine-129 (^{129}I) following soon after; notable achievements resulted from all five. Cosmic rays striking the atmosphere are a strong source of beryllium-10, carbon-14, and chlorine-36, which are deposited in rain and snow, whence their migration may be followed. A question concerning the origin of the lava of island-arc volcanoes, which had been disputed since the general acceptance of the plate tectonic theory of the Earth's structure, was settled from the observation of beryllium-10 in this lava. The presence of beryllium-10 proved that deep-ocean sediment, rich in the isotope, had been carried on the surface of a descending tectonic plate beneath another such plate and some of the sediment incorporated into the magma. The first application of chlorine-36 was the study of the migration of ancient groundwater. Later improvements in instrumental techniques added iodine-129 as a needed tracer for this challenging problem. Nuclear bomb tests at oceanic sites produced huge amounts of chlorine-36 that were injected into the atmosphere. For a few years rain contained this isotope at a level up to 1,000 times higher than the cosmogenic level. This yielded a tracer with a well-defined time of origin that will be useful long into the future for following the course of such water in soils and aquifers (water-bearing layers of rock). The four lightest of these isotopes have proved useful in determining the ages and irradiation histories of meteorites and lunar samples. There have been extensive studies of beryllium-10 in cores of polar ice and ocean sediments that give unique information about the intensity of cosmic rays over the past few million years.

4. Organic Chemistry

Mass spectrometry has played a critical role in organic chemistry. Its utility in chemical analysis was discussed earlier when describing appropriate experimental techniques. The same techniques can be used in determining the structure of complicated molecules, but perhaps of even greater value for such work are high-resolution measurements.
With a high-resolution mass spectrometer it is possible to carry out mass measurements on the molecular ion (or any other ion in the spectrum) to an accuracy of approximately one part in one million. This mass provides the best index for determining ionic formulas. The

accurate masses of the ions $C_6H_{12}^+$ and $C_4H_4O_2^+$ are, for example, 84.0939 and 84.0211, respectively, and these ions can easily be distinguished solely on the basis of their masses. Once the molecular formula is known it is possible to deduce the total number of rings and double bonds making up the molecular structure and to begin to speculate on possible structural formulas. In order to deduce structural formulas from molecular formulas, it is also essential to study the fragment ions in the mass spectrum. It is still not possible to predict definitively the fragmentation patterns for organic molecules, but many semi-empirical rules of fragmentation are known, and it is usually possible to pick out peaks in the spectrum that are characteristic of particular chemical groups. The technique is valuable in that it is generally not necessary to know any details of the composition of the unknown compound in order to deduce a complete or partial structure. Only a small quantity of compound, a hundred micrograms or less is necessary for an analysis.

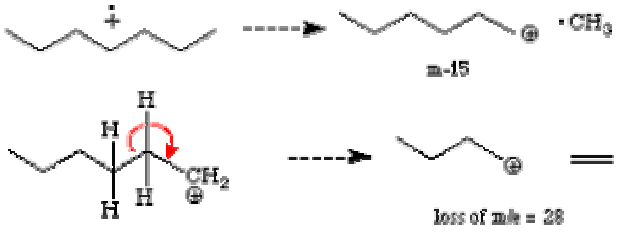
Using a computer coupled to a high-resolution mass spectrometer, about 1,000 mass peaks per minute can be plotted at a resolving power of up to 20,000. Accurate measurements can be made on each peak, and peak heights and ion compositions can be printed out in the form of an "element map" to aid in the interpretation of the spectrum. It is also possible for the computer to carry out many of the logical steps in reducing the data that lead to structural elucidation.

Continuous sampling of the materials contained in a reaction vessel, followed by analysis with a mass spectrometer, has been used to identify and measure the quantity of intermediate species formed during a reaction as a function of time. This kind of analysis is important, both in suggesting the mechanism by which the overall reaction takes place and in enabling the detailed kinetics of reactions to be resolved.

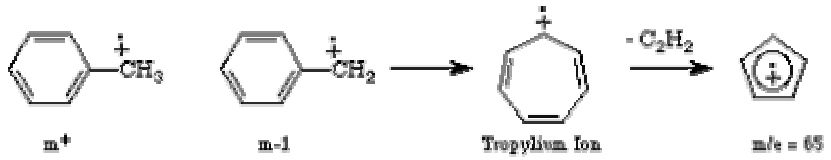
Isotopic labeling is widely used in such studies. It can indicate which particular atoms are involved in the reaction; in rearrangement reactions it can show whether an intramolecular or intermolecular process is involved; in exchange reactions it can show that particular atoms of, for example, hydrogen are exchanging between the reacting species. Labeling is also widely used in mass-spectrometric research to give information about the fragmentation reactions occurring in the mass spectrometer.

Mass Spectrometry of Functional Groups

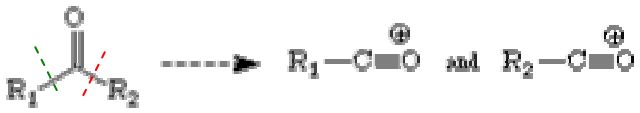
Alkanes: Simple alkanes tend to undergo fragmentation by the initial loss of a methyl group to form a (m-15) species. This carbocation can then undergo stepwise cleavage down the alkyl chain, expelling neutral two-carbon units (ethene). Branched hydrocarbons form more stable secondary and tertiary carbocations, and these peaks will tend to dominate the mass spectrum.



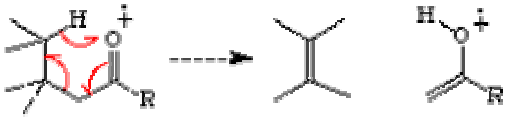
Aromatic Hydrocarbons: The fragmentation of the aromatic nucleus is somewhat complex, generating a series of peaks having m/e = 77, 65, 63, etc. While these peaks are difficult to describe in simple terms, they do form a pattern (the "aromatic cluster") that becomes recognizable with experience. If the molecule contains a benzyl unit, the major cleavage will be to generate the benzyl carbocation, which rearranges to form the tropylium ion. Expulsion of acetylene (ethyne) from this generates a characteristic m/e = 65 peak.



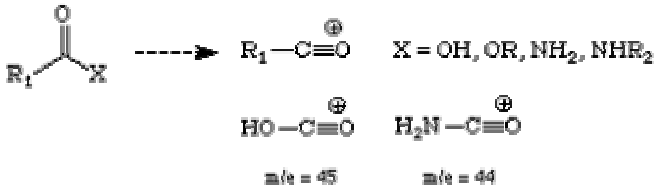
Aldehydes and Ketones: The predominate cleavage in aldehydes and ketones is loss of one of the side-chains to generate the substituted oxonium ion (α-cleavage). This is an extremely favorable cleavage and this ion often represents the base peak in the spectrum. The methyl derivative ($CH_3C{\equiv}O^+$) is commonly referred to as the "acylium ion".



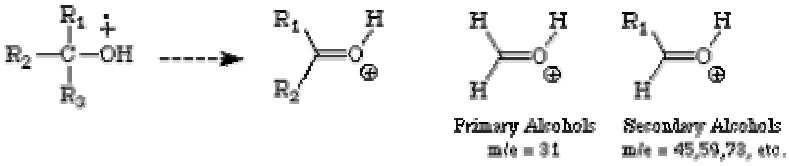
Another common fragmentation observed in carbonyl compounds (and in nitriles, etc.) involves the expulsion of neutral ethene *via* a process known as the *McLafferty rearrangement*, following the general mechanism shown below.



Esters, Acids and Amides: As with aldehydes and ketones, the major cleavage observed for these compounds involves expulsion of the "X" group, as shown below, to form the substituted oxonium ion. For carboxylic acids and unsubstituted amides, characteristic peaks at m/e = 45 and 44 are also often observed.



Alcohols: In addition to losing a proton and hydroxy radical, alcohols tend to lose one of the α-alkyl groups (or hydrogens) to form the oxonium ions shown below. For primary alcohols, this generates a peak at m/e = 31; secondary alcohols generate peaks with m/e = 45, 59, 73, etc., according to substitution.



Ethers: Following the trend of alcohols, ethers will fragment, often by loss of an alkyl radical, to form a substituted oxonium ion, as shown below for diethyl ether.

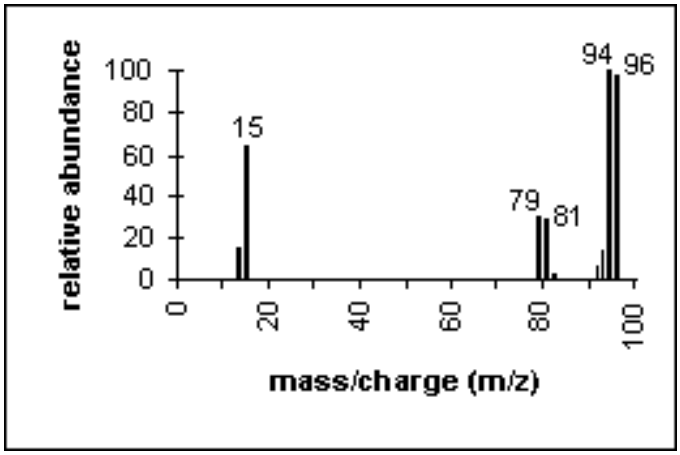
Halides: Organic halides fragment with simple expulsion of the halogen, as shown below. The molecular ions of chlorine and bromine-containing compounds will show multiple peaks due to the fact that each of these exists as two isotopes in relatively high abundance. Thus for chlorine, the $^{35}Cl/^{37}Cl$ ratio is roughly 3.08:1 and for bromine, the $^{79}Br/^{81}Br$ ratio is 1.02:1. The molecular ion of a chlorine-containing compound will have two peaks, separated by two mass units, in the ratio ≈3:1, and a bromine-containing compound will have two peaks, again separated by two mass units, having approximately equal intensities.

Isotopes*:* Isotopes occur in organic compounds analyzed by mass spectrometry in the same abundances that they occur in nature. A few of the isotopes commonly encountered in the analyses of organic compounds are below along with an example of how they can aid in peak identification.

Table 4. *Common isotopes of the most important elements found in organic molecules*

Element	Isotope	Relative Abundance	Isotope	Relative Abundance	Isotope	Relative Abundance
Carbon	^{12}C	100	^{13}C	1.11		
Hydrogen	^{1}H	100	^{2}H	.016		
Nitrogen	^{14}N	100	^{15}N	.38		
Oxygen	^{16}O	100	^{17}O	.04	^{18}O	.20
Sulfur	^{32}S	100	^{33}S	.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0

Methyl Bromide: An example of how isotopes can aid in peak identification.



The ratio of peaks containing ^{79}Br and its isotope ^{81}Br (100/98) confirms the presence of bromine in the compound.

Common Mass Spectrum Fragments

Commonly Lost Fragments

m-15 $\cdot CH_3$

m-17 $\cdot OH$

m-26 $\cdot CN$

m-28 $H_2C{=}CH_2$

m-29 $\cdot CH_2CH_3$ $\cdot CHO$

m-31 $\cdot OCH_3$

m-35 $\cdot Cl$

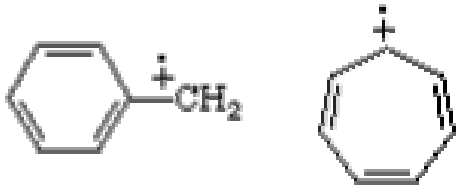
m-43 $CH_3\dot{C}{=}O$

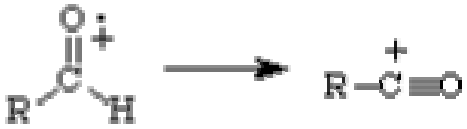
m-45 $\cdot OCH_2CH_3$

m-91 

Common Stable Ions

m/e = 43 $CH_3{-}\overset{+}{C}{\equiv}O$

m/e = 91 

m/e = m-1 

Stages of a mass spectrum interpretation:

1. Search for the molecular ion peak:

- This peak (if it appears) will be the highest mass peak in the spectrum, except for isotope peaks.
- Nominal MW will be an even number for compounds containing only **C, H, O, S, Si**.
- Nominal MW will be an odd number if the compound also contains an odd number of **N** (1,3,...).

2. Calculate the molecular formula:

- The isotope peaks can be very useful, and are best explained with an example.
- **Carbon 12 has an isotope, carbon 13**. Their abundances are **^{12}C=100%, ^{13}C=1.1%.** This means that for every **100 ^{12}C** atoms there are **1.1 ^{13}C** atoms.

Example: *If a compound contains 6 carbons, then each atom has a 1.1% abundance of ^{13}C. Therefore, if the molecular ion peak is 100%, then the isotope peak (1 mass unit higher) would be 6x1.1%=6.6%.*

- If the molecular ion peak is not 100% then calculate the relative abundance of the isotope peak to the ion peak. For example, if the molecular ion peak were 34% and the isotope peak 2.3%: (2.3/34)x100 = 6.8%. 6.8% is the relative abundance of the isotope peak to the ion peak. Next, divide the relative abundance by the isotope abundance: 6.8/1.1=6 carbons.
- Follow this order when looking for information provided by isotopes: (A simplified table of isotopes is provided above, more detailed tables can be found in chemistry texts.)
- Look for A+2 elements: O, Si, S, Cl, Br
- Look for A+1 elements: C, N
- "A" elements: H, F, P, I

3. Calculate the total number of rings plus double bonds:

- For the molecular formula: $C_xH_yN_zO_n$
- Rings + Double Bonds = $x - (1/2)y + (1/2)z + 1$

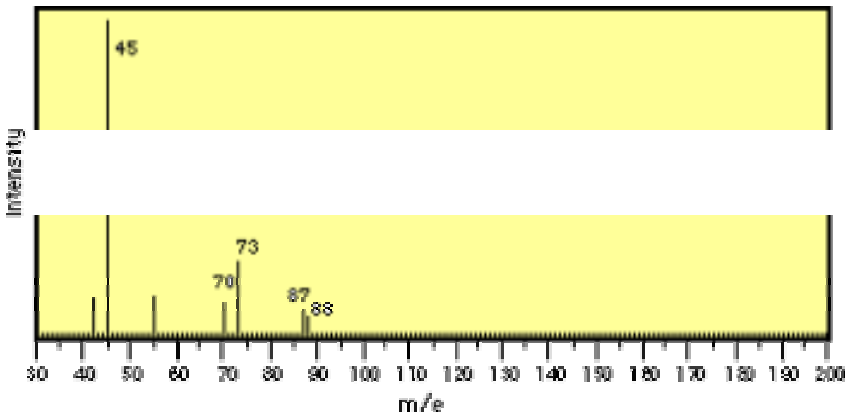
4. Postulate the molecular structure consistent with abundance and m/z of fragments.

- More information on specific fragmentation can be found for each functional group.

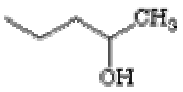
Example #1

Analysis: $C_5H_{12}O$ MW = 88.15

Mass Spectrum

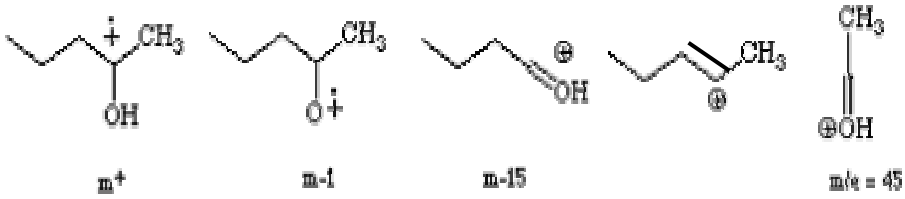


The spectrum shows a small molecular ion and a small m-1 peak, suggesting the presence of an alcohol (it cannot be an aldehyde since there are no degrees of unsaturation). The m-15 peak represents loss of a methyl group and the m-18 is consistent with loss of a H_2O molecule. For an alcohol, the base peak is often formed by expulsion of an alkyl chain to give the simple oxonium ion $R'CR''{=}OH^+$; to generate the observed m/e = 45, R' must be CH_3 and R'' a H.

Structure: 

IUPAC Name: 2-pentanol

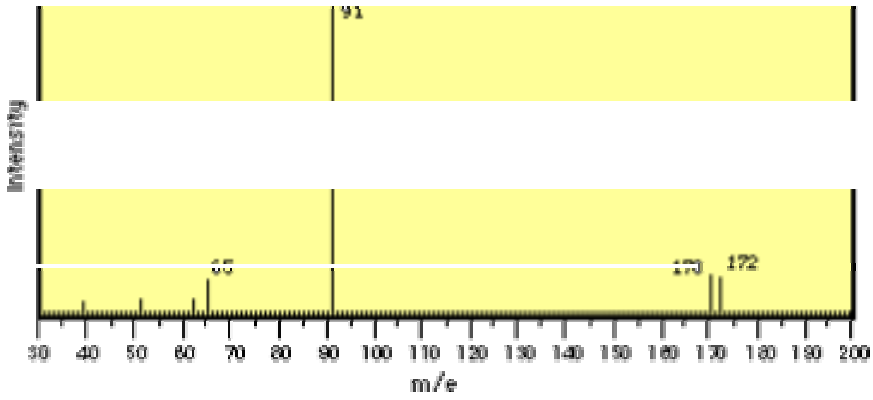
MS Fragments:



Example #2

Analysis: $C_7H_{12}Br$ MW = 171.04

Mass Spectrum

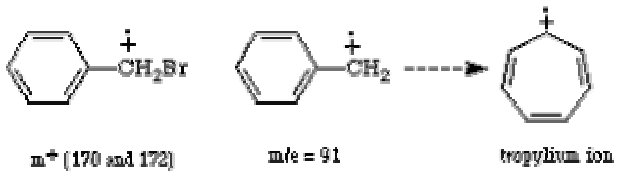


The spectrum shows two small peaks of equal intensity in the molecular ion region, strongly suggesting that the molecule contains bromine (equal concentrations of the ^{79}Br and ^{81}Br isotopes). The base peak represents loss of this bromine to give the peak at m/e = 91, which is highly suggestive of a benzyl fragment.

Structure: 

IUPAC Name: bromomethyl benzene (benzyl bromide)

MS Fragments:

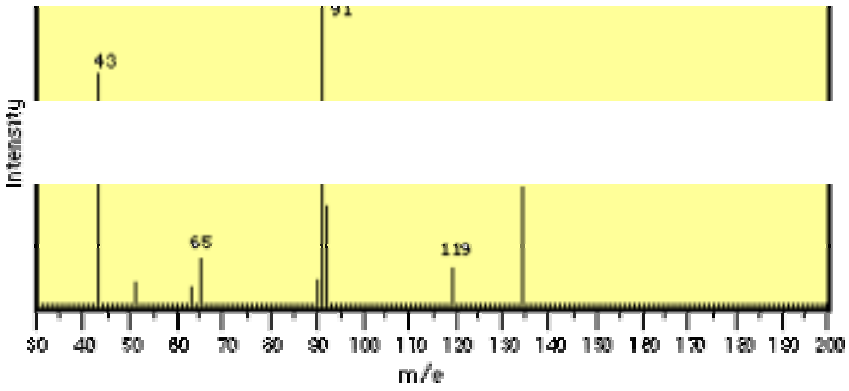


The spectrum shows two small peaks of equal intensity in the molecular ion region, strongly suggesting that the molecule contains bromine (equal concentrations of the ^{79}Br and ^{81}Br isotopes). The base peak represents loss of this bromine to give the peak at m/e = 91, which is highly suggestive of a benzyl fragment, which rearranges to form the tropylium cation.

Example #3

Analysis: $C_9H_{10}O$ MW = 134.18

Mass Spectrum

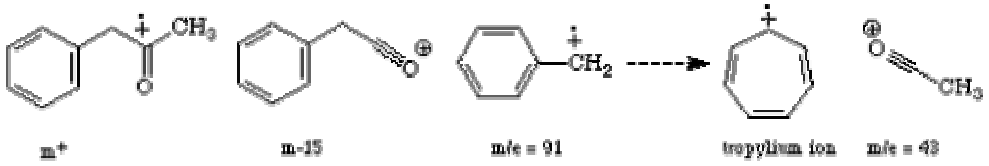


The spectrum shows a moderate molecular ion peak, and a peak at m-15, strongly suggesting the presence of a labile methyl group. The base peak occurs at m/e = 91, which is highly suggestive of a benzyl fragment. The presence of an intense peak at m/e = 43 is also suggestive of the presence of a methyl ketone, which can fragment to form the acylium ion.

Structure: 

IUPAC Name: 3-pentanone (diethyl ketone)

MS Fragments:

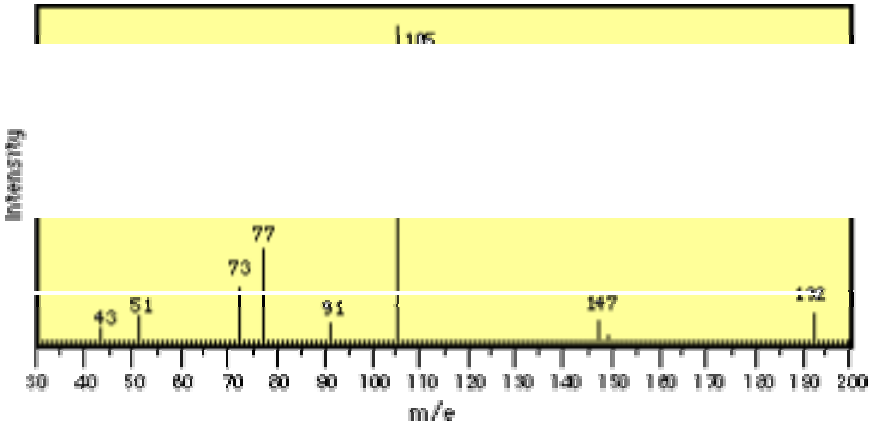


The spectrum shows a moderate molecular ion peak, and a peak at m-15, strongly suggesting the presence of a labile methyl group. The base peak occurs at m/e = 91, which is highly suggestive of a benzyl fragment, which rearranges to form the tropylium cation. The presence of an intense peak at m/e = 43 is also suggestive of the presence of a methyl keton which can fragment to form the acylium ion.

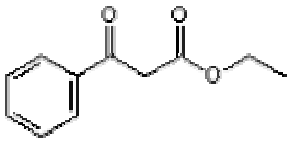
Example #4

Analysis: $C_{11}H_{12}O_3$ MW = 192.21

Mass Spectrum

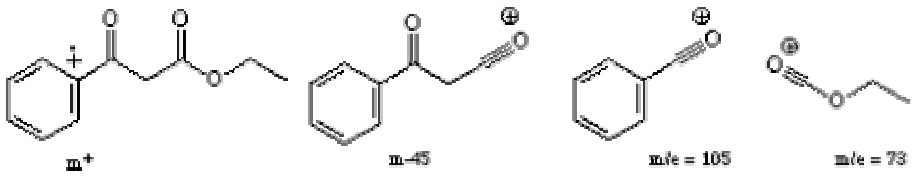


The spectrum shows a small molecular ion peak and a peak at m-45, suggesting the presence of an ethoxy group (-O-CH_2CH_3). The very minor peaks at 91 and 43 suggest that the molecule does not contain a benzyl unit or a methyl keton. The base peak occurs at m/e = 105, and results from loss of a unit of m/e = 73, which is also observed. The molecular ion at 105 is characteristic of a carbonyl bonded directly to an aromatic ring.

Structure: 

IUPAC Name: ethyl 3-oxy-3-phenylpropanoate (ethyl benzoylacetate)

MS Fragments:

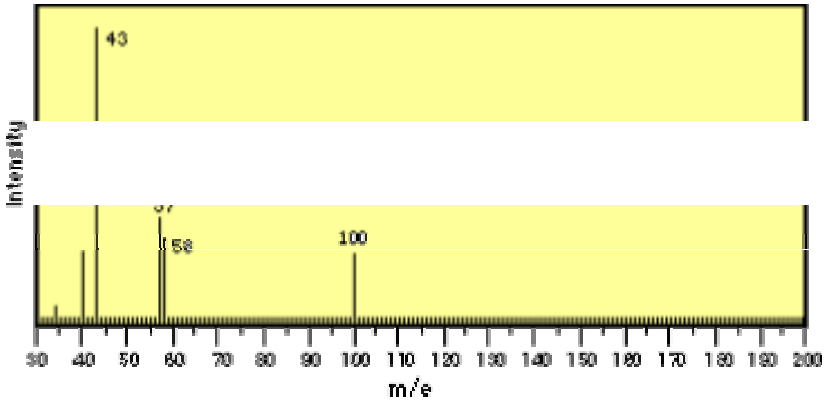


The spectrum shows a small molecular ion peak, and a peak at m-45, suggesting the presence of an ethoxy group (-O-CH_2CH_3). The very minor peaks at 91 and 43 suggest that the molecule does not contain a benzyl unit or a methyl ketone. The base peak occurs at m/e = 105, and results from loss of a unit of m/e = 73, which is also observed. The molecular ion at 105 is characteristic of a carbonyl bonded directly to an aromatic ring.

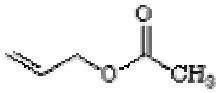
Example #5

Analysis: $C_5H_8O_2$ MW = 100.12

Mass Spectrum

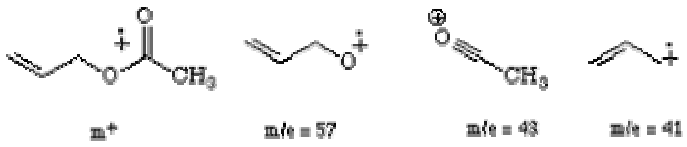


The spectrum shows a small molecular ion peak, and a pair of peaks at m/e = 57 and 58. The peak at m/e = 57 corresponds to loss of m/e = 43, which is the base peak and corresponds to the acylium ion ($CH_3C{\equiv}O^+$). The m/e = 57 fragment corresponds to C_3H_5O, suggesting the original compound was an ester with this molecular formula.

Structure: 

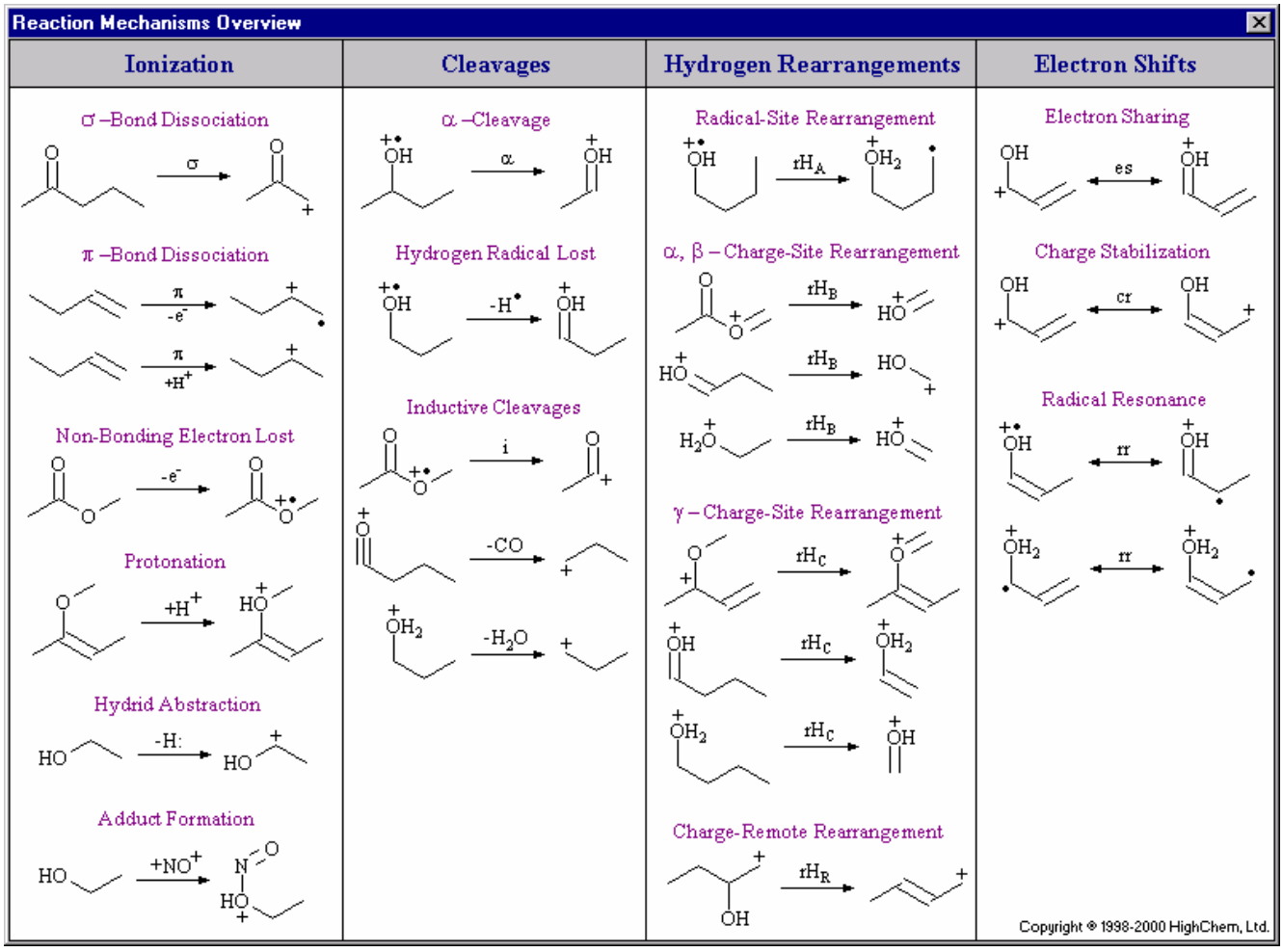
IUPAC Name: 2-propenyl ethanoate (allyl acetate)

MS Fragments:



The spectrum shows a small molecular ion peak, and a pair of peaks at m/e = 57 and 58. The peak at m/e = 57 corresponds to loss of m/e = 43, which is the base peak and corresponds to the acylium ion ($CH_3C{\equiv}O^+$). The m/e = 57 fragment corresponds to C_3H_5O, suggesting the original compound was an allyl (-O-$CH_2CH{=}CH$) or methylvinyl (-O-$CH{=}CHCH_3$) ester. Either of these would generate the peak observed at m/e = 41. The peak at m/e = 58 corresponds to the protonated m/e = 57 cation radical. Proton or ^{13}C NMR would readily distinguish between the two possible structures.

Overview of Reaction mechanisms in EI



5. Combinatorial Chemistry

Combinatorial chemistry is used to create large populations of molecules, or libraries, whereby the generation of huge numbers of compounds increases the probability that they will find novel compounds of significant therapeutic or commercial value. While many fields of research have been influenced by this approach the largest investment has come from the pharmaceutical, biotechnology, and agrochemical arena. In the field of drug development combinatorial chemistry represents a convergence of chemistry and biology, made possible by fundamental advances in automation, such as that of mass spectrometry.
Mass spectrometry is playing an increasingly important role in the molecular characterization and activity of combinatorial libraries. Crucial to distinguishing the most active component or obtaining structure-activity relationships of compounds in a library is

an efficient qualitative and quantitative assay. Toward this end, electrospray ionization and MALDI-MS have been useful for the qualitative, and more recently, the quantitative screening of combinatorial libraries. Moreover, the development of these two techniques has significantly extended MS application toward a wide variety of challenging problems in drug discovery and toward the identification of effective ligand-receptor binding, new catalysts, and enzyme inhibitors. In addition, because mass spectrometry does not involve chromophores or radiolabelling, it provides a viable alternative to existing analytical techniques which typically require extensive sample preparation and optimization time, the disposal of biohazardous waste, or require a significant amount of sample

6. Biochemistry

Mass spectrometry has emerged as an important tool capable of analyzing samples ranging in size from small molecules to whole viruses. Moreover, since matrix-assisted laser desorption/ionization (MALDI) mass spectrometry was added to electrospray ionization (ESI) the repertoire of research methods and the demand for this instrumentation has exploded. Now, the commercial availability of MS instruments which offer picomole (10^{-12} mole) to attomole (10^{-18} mole) sensitivity and enable the analysis of biological fluids with a minimum amount of sample preparation has made routine the analysis of a large variety of compounds, including: proteins, peptides, carbohydrates, oligonucleotides, natural products, drugs and drug metabolites.

Perhaps most exciting is that the developmental stage of mass spectrometry has not stopped; innovations such as nanoelectrospray, curved reflectrons and electrospray with orthogonal spraying continue to expand its capability. Extending beyond simple molecular weight characterization, these "mild" ionization methods can be applied to many new applications, including: protein-protein interactions, dynamic viral analysis, high sensitivity protein sequencing, routine DNA sequencing, protein folding, high throughput analysis in combinatorial chemistry, and drug discovery.

Both electrospray and MALDI-MS are sensitive tools for mass measurement and can provide surprisingly large amounts of other information as well. Initially, both methods were used to obtain accurate molecular weight information on molecules that were traditionally difficult or impossible to analyze (i.e.: proteins, oligonucleotides, and carbohydrates). However, as a result of their improved sensitivity and accuracy, electrospray and MALDI were quickly applied to even more interesting problems.

The ability to analyze complex mixtures has made electrospray and MALDI very useful for the examination of proteolytic digests, an application otherwise known as protein mass mapping. Through the application of sequence specific proteases, protein mass mapping allows for the identification of protein primary structure. Performing mass analysis on the resulting proteolytic fragments thus yields information on fragment masses with accuracy approaching ±5 ppm, or ±0.005 Da for a 1,000 Da peptide. The protease fragmentation pattern is then compared with the patterns predicted for all proteins within a database and matches are statistically evaluated. Since the occurrence of Arg and Lys residues in proteins is statistically high, trypsin cleavage (specific for Arg and Lys) generally produces a large number of fragments, which in turn offer a reasonable probability for unambiguously identifying the target protein.

The characterization of genomic proteins for proteomics is arguably the most important application of modern mass spectrometry where the primary tools are proteases and computer-facilitated data analysis. As a result of generating intact ions, the molecular weight information on the peptides/proteins is quite unambiguous. Sequence specific

enzymes can then provide protein fragments that can be associated with proteins within a database by correlating observed and predicted fragment masses. The success of this strategy, however, relies on the existence of the protein sequence within the database. With the availability of the human genome sequence (which indirectly contains the sequence information of all the proteins in the human body), identification of the proteins can be quickly determined simply by measuring the mass of proteolytic fragments.

Protein mass mapping has also been used for studying higher order protein structure by combining limited proteolytic digestion, mass analysis, and computer-facilitated data analysis. In the analysis of protein structure, enzymes are used to initially cleave surface accessible regions of the protein or protein complex. These initial cleavage sites are then identified using accurate mass measurements combined with the protein's known structure and the known specificity of the enzyme. Computer-based sequence searching programs allow for the identification of each proteolytic fragment, which in turn can be used to map the protein's structure.

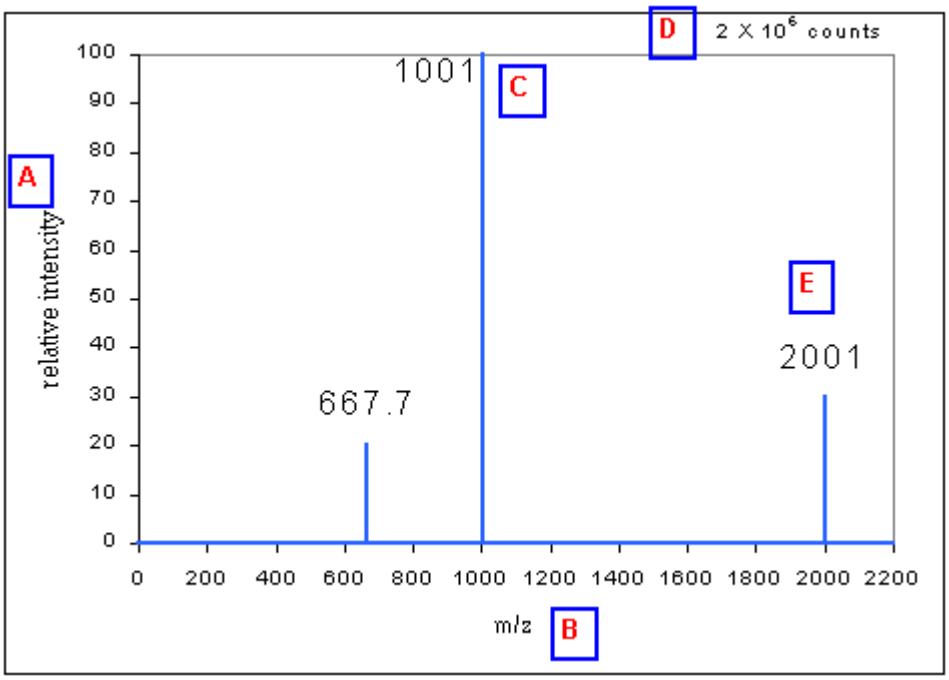
Another application that has generated a great deal of excitement involves electrospray's ability to produce and mass analyze biological noncovalent complexes in the gas phase. Since ESI is a tool for the observation of noncovalent complexes, the possibility of correlating condensed phase intermolecular interactions in the gas phase using mass spectrometry has captured the attention of many researchers. For the first time, mass spectrometry can be used as a tool to observe complexes in the gas phase taken from an aqueous environment, thereby providing insights into specific noncovalent associations in solution. The selectivity of mass spectrometry may eventually help avoid impurity problems associated with immunoaffinity procedures and also facilitate drug screening. Examples include the observation of the hemoglobin complex, DNA duplex, cell-surface carbohydrate association, catalytic antibody-inhibitor interactions, and the analysis of whole viruses.

Until now, small molecule quantitative analysis has been left to traditional techniques such as electron ionization mass spectrometry, however many compounds are too thermally fragile to survive its ionization process. Fortunately, in addition to being useful for large molecules, ESI is an important tool for qualitative and quantitative analysis of small biomolecules. ESI-based methods have recently been developed to quantitatively examine small molecules (steroids) at the attomole level (100×10^{-18} moles).

Interpreting Electrospray Mass Spectra

Interpretation of mass spectra containing multiply charged molecules generated by the electrospray ionization process.

We can use peptides to demonstrate the complexity of ESI spectra.



A: The Y axis is labeled relative intensity. This is the intensity relative to the tallest peak in the spectrum with the tallest peak set to 100%.

B: The X axis is mass divided by charge, m/z. For example if the mass of a molecule is 2000 u and the molecule posses two proton adducts its m/z value is equal to(2000+2)/ 2, the m/z value read on the spectrum is 1001.

C: This is the tallest peak in the spectrum also known as the "base peak"

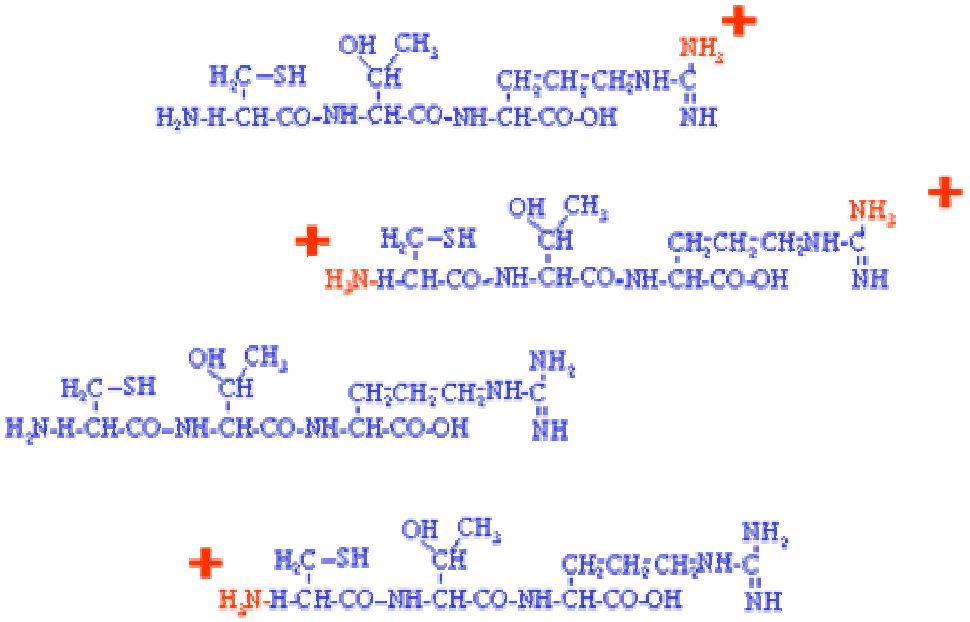
D: A spectrum will have a certain number of counts associated with the tallest peak in the spectrum. This number can be used to gauge the relative intensity or concentration of the analyte. One should be forewarned that the count number is relative and can be adjusted with the multiplier gain and strictly speaking cannot be related to concentration without an internal standard. Counts will also be affected by spray needle and over all source maintenance.

E: All of the peaks in a spectrum should <u>not</u> be referred to as ions.

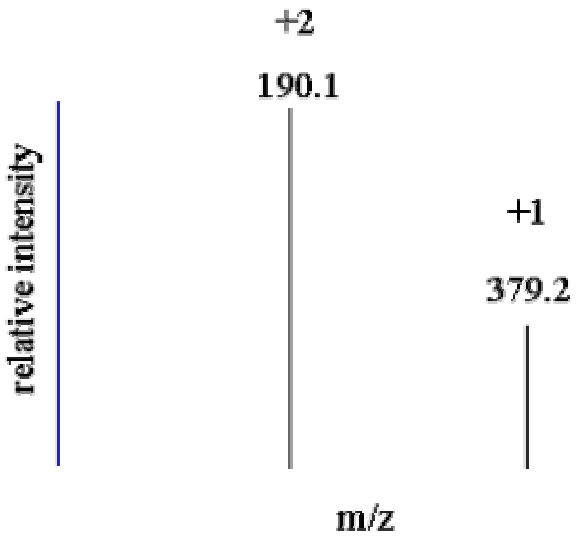
Note:
It is common talking about the "molecular ion" while pointing at an electrospray peak. ***Molecular ions are not generally observed in the electospray ionization process****. A molecular ion is formed by the loss of an electron. In the electrospray process, ionization is accomplished by the loss or gain of a proton (or other adduct), some refer to this ion as the "pseudo molecular ion."*
The electrospray process usually produces a population of multiply charged molecules and this population is accurately reflected in the intensity of the peaks in the spectrum.
In positive ion mode the number of charged species normally observed in an electospray spectrum is reflected in the number of basic sites on a molecule that can be protonated at low pH.

Example:
A single peptide is ionized to produce a population of charged and uncharged peptides. The number of positive charges that a molecule can support is generally related to the number of basic sites on the molecule. In positive ion mode the analyte is sprayed at low pH to encourage positive ion formation. In negative ion the analysis is normally carried out well above a molecules isoelectric point to deprotonate the molecule. The basic principle of all mass spectrometers is that a molecule must be charged (ionized) before the mass spectrometer can influence it in an electric field. See how this population of molecules might appear on a mass spectrum.

<u>Note</u>: *Most peptides obtained from a trypsin digest have* ***two potential sites for protonation****, the* ***amino terminus*** *and* ***the basic C-terminal residue, lysine*** *or* ***arginine***

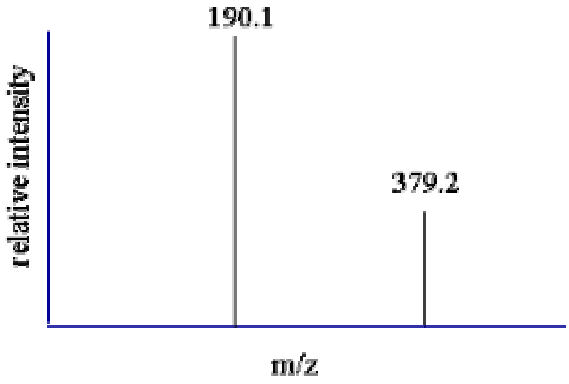


<u>From Gas Phase Ions to Peaks on a Mass Spectrum</u>
A population of variably charged ions is generated in the electrospray process. In this example the population will contain peptides having 1, 2 and 0 sites of protonation.



The resulting spectrum contains singly and doubly charged species. The intensity of the peaks is a reflection of the population generated in the electrospray process. Peak abundance information can be used in protein folding studies. The principles that they apply are: The more tightly folded a protein the more difficult will be to protonate it and then follows that as a protein unfolds the peak distribution may change across a spectrum possibly favoring the more highly charged species.
Note: *The uncharged peptide is not observed because the mass spectrometer can not influence it and thus it never reaches the detector.*

Calculating Mass
With the multiple charging phenomena displayed in electrospray spectra how does one calculate the mass of a compound?
How does one determine if the ions are even related?



Determining the charge state of the peaks:
Before the mass of the compound is determined from the peaks in the spectrum a mathematical relationship must be established. We may assume that the peaks observed in a spectrum are related and then proceed to derive the relationship. The first assumption is that the two adjacent peaks are from the same compound and differ by only one charge.

Before we can calculate the mass of the compound we must determine the charge state of the two peaks.

Step 1 Understand that the peak value observed in the spectrum equals mass divided by charge and that the mass is the mass of the molecule plus any adducts (protons):

190.1 = Mass/Charge and **379.2 = Mass/Charge**

These two equations can be rewritten:

Mass = 190.1 (Charge) and **Mass = 379.2 (Charge)**

Step 2 Assume that the two peaks are related, (charged variants of the same compound), and assume that they differ by a single charge. Since we have assumed that the base mass for these two peaks are equal we can set these two equations equal to each other and solve for the charge state of the peaks.
If the two peaks are related and differ by one proton then more specifically we can write.

m+1=190.1(z+1) and **m = 379.2(z)**

m = [190.1(z + 1)] - 1 and **m = 379.2(z)**

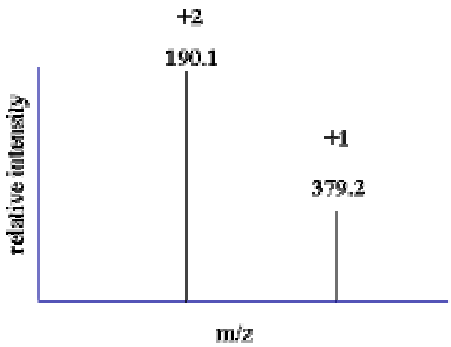
Since "m" is assumed to be the same for both peaks we can set the two equations equal to each other and solve for "z".

190.1z + 189.1 = 379.2z
"z" equals **z = 1**

This tells us that apparently the two peaks in the spectrum are mathematically related and that the charge state of the 379.2 peak is +1 and the charge state for the 190.1 peak is therefore +2.

Step 4 Use the determined charged states to calculate the mass of the compound. One can think of the different peaks in the spectrum as separate mass measurements of the same compound. You can then average the two answers to get the final mass.

Calculating Mass



Once the charge state of a peak is determined a mass can be calculated. An accurate assessment of the mass is obtained by averaging the calculated mass for each peak. Most electrospray software packages are set up to do this calculation, but it is important to know

in order to avoid a black-box approach and in order to be able carrying out a software validation.

Charge State	Calculation	Unprotonated Mass
+1	(379.2 - 1)∗1 =	378.2
+2	(190.1 - 1) ∗2 =	378.2
	average	**378.2**

Study of an Electrospray Mass Spectrum

Search for the tallest peak in the spectrum above 200 mass units. (The low end of a spectrum can often be confounded with solvent noise.) Then search for peaks that are roughly double or half the mass. This may indicate whether there are multiply charged species present which can help with mass determination.

Counts: Avoid to interprete meager spectra. If on a particular day $1.0X10^6$ is a respectable, reliable signal then a spectrum that tops out at $1.0X10^4$ counts (or at background) may not be a reliable spectrum to interpret.

The quality of the spectrum is important: If the quality of the spectrum is very low try averaging several or many low level spectra to obtain a better quality "averaged mass spectrum." Compare this to a background spectrum to see if the peaks really stand out.

Reproducibility is important: The peak must be consistent to be considered a relevant peak. Determining the charge state of a peak when only one peak is obvious
A molecule will often have adduct ions associated with it other than hydrogen . Search for sodium or ammonium adducts. These adducts can often give a hint as to the charge state of a peak. For example if there is only one major species in a spectrum then look for the sodium adduct following that peak. If it is a singly charged species the sodium adduct will be found at +22 mass units. If the peak is doubly charged, the adduct will appear at +11 mass units.

Isotopes: If the mass spectrometer has sufficient resolution search at the isotopes, a singly charged ion will show isotopic peaks that differ by 1 mass unit, a doubly charged ion will show peaks that differ by 0.5 mass units. This is another way to deduce the charge state of a peak and thus the mass.

Important considerations

When a peak is real?

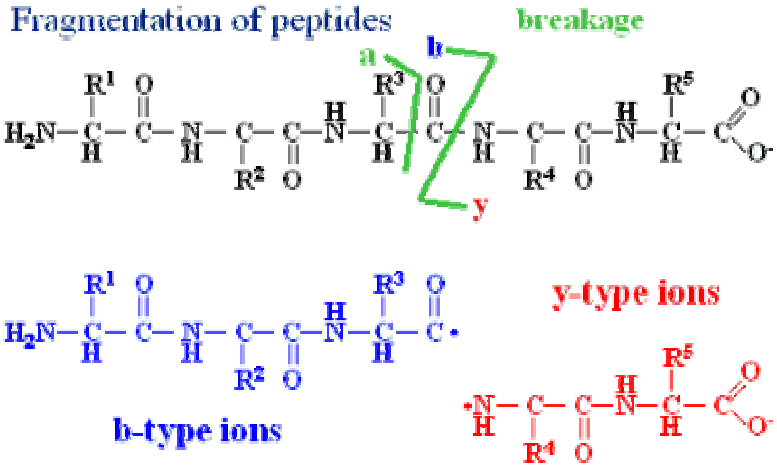
All peaks are real. In an LC/MS run we look for peaks that reoccur in multiple adjacent scans (spectrums) but not in every scan. If the peak occurs in every scan it may be a background peak. It is possible to get system noise or spikes that only occur in one scan or sporadically these are most likely electronic or some other form of system noise.

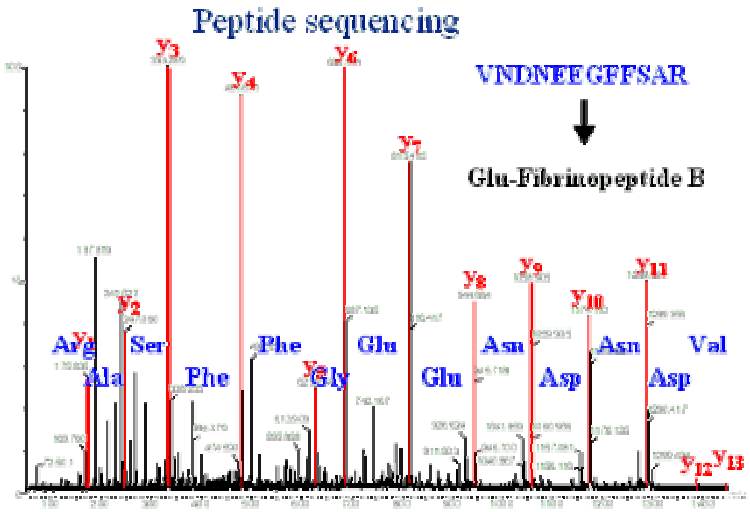
How can we be sure of the identity of a peak?

A mass is just a mass and many compounds have isobaric mass so you can't be sure from just a mass. In the old days we would perform an enzymatic digest on a protein, run an LC/MS peptide map and match up the mass with the theoretical fragments. Today the bar is rightly higher and we go one step further in the identification, we take the peak through a fragmentation and match up the fragment masses with the theoretical CID fragment masses for that peptide. This gives us a positive ID. Another overlooked component in LC/MS is part of what makes LC/MS so powerful and that is the correlation of mass and LC retention time. If the retention time of a molecule has been previously characterized this information can be linked with the mass information for a positive ID.
If you are characterizing a new molecule try modifying the molecule to see if you can modify the mass. Try an enzyme digest if the unknown is a protein or try chemical modification if it is a small molecule and see if the mass of the unknown changes as predicted by the mass ID.
How can we differentiate a compound at one mass from another at twice the mass?
For example a compound with mass 1000 will display peaks at m/z 1001 and 501, and a compound with mass 2000 may display peaks at m/z 2001, 1001, 666.7 and 501. The mass determination can further be confounded if the peptide at 1000 forms dimers during the electrospray process.
1) The peak envelope does not skip peaks, for example the 2000 mass even if it does not have an obvious peak at 2001 it should have the 666.7 peak between the 1001 and 501 peaks.
2) Also try to determine the charge state of the ions from the adducts or from the isotopes. This will tell you what the mass of the compound is.
3) Dimer formation can be a major problem in some analyses. Try to reduce the concentration of the analyte. Often if the concentration is too high dimers will be observed in the spectrum. Also dimers can be reduced by changing some of the setting on the mass spectrometer.
4) With the peak envelope of larger molecules (10kDa+) look for smooth peak distributions. The peak distribution should have a smooth bell shaped curve appearance, sometimes trailing off to the right. The peak to peak relationship should be predictable, if one observes an alternating pattern of peak intensities this may be a clue to a coeluting dimer.

7. Peptide and DNA sequencing

An important goal of mass spectrometry is the routine acquisition of complete sequence information from biopolymers. The realization of this goal has significantly progressed with the development of both electrospray ionization and MALDI. More specifically, electrospray ionization tandem mass spectrometry has been routinely used to generate fragment ions from a selected precursor ion by initiating ion/molecule which can then be mass analyzed and used to obtain sequence information. MALDI-MS is playing an equally exciting role in biopolymer sequencing, used in conjunction with enzymatic or chemical digestion to generate sequence-specific ladders for proteins and oligonucleotides. Protein ladder sequencing involves the analysis of a peptide/protein that has undergone a stepwise degradation in which ladder-generating chemistry produces a family of sequence-defining peptide fragments that differ from the next by one amino acid. Once the mixture of peptides is obtained, MALDI-MS analysis is performed to generate a mass spectral sequence ladder. The implementation of MALDI ladder sequencing to oligonucleotides offers significant advantages over the current, widely used radiolabelling method.





8. Small biomolecule characterization with sleep-inducing potential

The concept that there are endogenous compounds associated with sleep has a long and complicated history, during which a large variety of substances have been proposed. Applying the sensitivity of modern mass spectrometry to the study of sleep, a fresh, purely chemical approach was taken in attempting to identify molecules of the central nervous system.

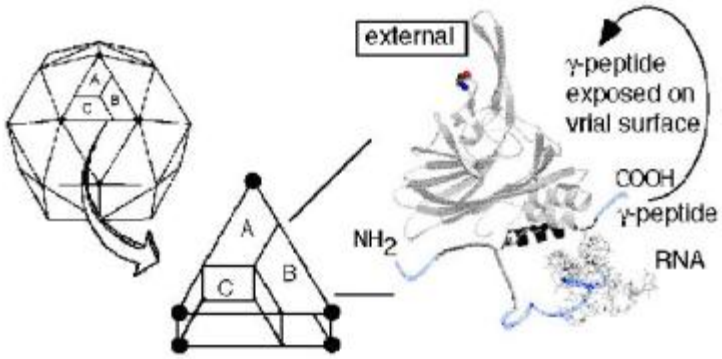
Specifically, the sleep-wake states of subjects were examined in the following study. Cerebrospinal fluid was obtained and then, using liquid chromatography, its components were separated. Electrospray tandem mass spectrometry, gas chromatography mass spectrometry (GCMS), and thin layer chromatography (TLC) were also employed in the analysis of the cerebrospinal fluid, along with infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR), and chemical degradation procedures. The goal was to identify new molecules associated with the sleep-wake cycle.

Cerebrospinal fluid analysis began with preparative liquid chromatography fraction collection. These experiments produced UV data on each fraction to determine any

differences between subjects at various points in their sleep cycle. Even though the compound associated with this absorbance was only present in small amounts, partial characterization was initially obtained by performing exact mass measurements and tandem mass analysis. Using a tandem mass spectrometry, electrospray mass analysis on the fractions associated with the differences in the chromatogram produced a significant ion at m/z 282. That was determined to be the MH^+ ion, and an exact mass determination on the unknown compound by fast atom bombardment (FAB) was consistent with the molecular formula $C_{18}H_{35}NO$.
Collision-induced dissociation (CID) was used to perform MS^2 and MS^3 experiments on the molecular ion m/z 282 revealed a distinct fragmentation pattern in the low molecular mass range consistent with other long chain alkanes. Neutral losses of 17 and 35 Da from the parent ion indicated a loss of ammonia followed by water. Performing additional MS^3 experiments on the daughter ions at m/z 265 and 247 revealed that the daughter ion at 265 fragmented to form the granddaughter ion at 247. This suggested that the ion at 247 was the result of sequential losses (loss of 17 Da {NH_3} followed by 18 Da {H_2O}), as opposed to a neutral loss independent of the daughter ion at 265. Additional deuterium exchange experiments were consistent with at least two protons on this molecule being exchangeable. On the basis of these experiments, compounds that best corresponded to the data were synthesized and the results obtained on these compounds eventually led to the identification of the unknown as cis-9,10-octadecenoamide.
The most interesting aspect of these studies is how analytical technology such as mass spectrometry is allowing scientists to identify compounds associated with our basic biological functions. Without a doubt, mass spectrometry will play and increasing large role in studying other attributes of human behavior such as hunger, pain, and even love.

9 Viruses

Mass spectrometry is offering a new perspective on the solution and gas-phase properties of viruses. Viruses are particles designed to transport genes between hosts and the cells of a host. Most viruses are composed of two parts: genetic information and packaging material. The nucleic acid stores genetic information encoded in either RNA or DNA and the packaging material is made up of a protein capsid that can be enveloped by a combination of lipids, proteins, and carbohydrates. The broad application of mass spectrometry to viral structure provides unique insights into many biological processes, including viral-antibody binding, protein-protein interactions, and protein dynamics. Mass measuring viral proteins is now a routine technique and since viruses are typically well-characterized, in that the capsid proteins and DNA (or RNA) sequences are known, identifying a virus based on the mass of the protein and enzymatic digestion fragments is relatively easy. Using mass spectrometry, it is also possible to identify viral protein post-translational modifications and has even been recently used to study viral protein dynamics. Mass spectrometry has also been applied on a global scale via the mass measurement of entire intact viruses (7 million Daltons).



Crystal structure of the Flock House virus shows that the gamma-peptide and the N- and C-terminus of beta-proteins are localized internal to the virus. Yet, proteolytic time-course experiments demonstrated that these domains are transiently exposed on the viral surface.

Identification of viral mutants typically requires sequencing part or all of the genome to determine the nature of the mutation. Automated DNA sequencing is a well-established method for identifying mutant proteins and is used to pinpoint specific regions that undergo mutation. In fact, high-throughput DNA sequencing often allows for expeditious identification of viral mutants and offers complete and unambiguous sequence information. However, some disadvantages of this approach include technical limitations in sequencing viral RNAs, and the inability to map post-translational modifications. A complementary approach to nucleic acid sequencing of viral mutants is protein mass mapping using MALDI and/or electrospray ionization mass spectrometry. The mass spectrometry approach used to identify mutant proteins consists of enzymatic digestion of a protein(s) followed by mass analysis of the resulting peptide mixture. By comparing differences in the mass of peptides that are released by such treatment, one is able to identify peptides in which amino acid differences occur. This information defines the region containing a mutation and, in cases when nucleotide sequencing is required, significantly narrows the region of the genome that must be sequenced. Accurate mass measurements and tandem mass spectrometry can then be used to definitively identify the amino acid substitution.

10. Forensics

Electrospray ionization and MALDI mass spectrometry have been used to examine evidence in a wide variety of criminal cases. One case that it has recently been helpful was that of a sexual assault. Because condoms are being used increasingly by sexual assailants and some condom brands include the spermicide nonoxynol-9 (nonylphenoxy polyethoxyethanol) in the lubricant formulation the recovery and identification of nonoxynol-9 from evidence items has assisted in proving corpus delicti. A method was developed for the recovery of nonoxynol-9 from internal swabs and for its identification by liquid chromatography electrospray ionization mass spectrometry, and high resolution MALDI Fourier transform mass spectrometry (MALDI-FTMS). The method was tested on extracts from precoitus, immediate postcoitus, and four-hour postcoitus swabs provided by a volunteer, where a condom having a water-soluble gel-type lubricant that includes 5% nonoxynol-9 in its formulation was used. Subsequently, liquid chromatography electrospray ionization mass spectrometry was used to identify traces of nonoxynol-9 from

the internal vaginal swab of a victim of a sexual assault. This evidence was helpful in the conviction of the assailant.

11. Space probes

Future space exploration, addressing the question of whether life exists elsewhere in the solar system, will rely on the mass spectrometer to produce spectra of those molecules characteristic of life, such as amino acids. An unmanned spacecraft equipped with a mass spectrometer has already been used and more missions are being planned to Mars to help learn about its surface and atmosphere, and to determine whether life once existed there.

12. Small Mass Spectrometers

Just as mass spectrometers measure incredibly small molecules allowing them to be called "the smallest scale in the world" the instruments themselves are also getting smaller. Many research groups are actively pursuing the miniaturization of mass spectrometers for a new field known as microfluidics. Smaller instrumentation will help to facilitate a wide variety of tasks such as in space exploration, forensics, environmental studies, explosives detection, combinatorial chemistry, as well as biochemistry.

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