Mass Spectroscopy
Lecture 3

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Mass Spectrometer
• Several Types. Only two will be described:
• Quadrupole spectrometer and the time-of-flight spectrometer.
• General description of instrument components:
**Inlet System:**
To introduce a very small amount of sample (µmol or less) into the mass spectrometer that converted to gaseous ions. (a mean for volatilizing solid or liquid samples is presents).

**Ion sources:**
Convert the components of a sample into ions.
(generally the inlet system and the ion source are combined into a single component). The output is a stream of +ve or –ve ions are accelerated into the mass analyzer.

**Mass analyzer**
Analogous to grating in an optical spectrometer. Dispersion is based upon the mass/charge ratios of the analyte ions rather than upon the wavelength of photons. (Different categories of MS according to mass analyzer).

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**Detectors:**
Convert the beam of ions into an electrical signal that can then be processed, stored in the memory of a computer and displayed or recorded in a variety ways.

**Vacuum System:** To create low pressure (10^{-4} to 10^{-8} torr) in all the instrument components except the signal processor and readout. To prevent interaction of components with atmosphere so destroyed.
Sample Inlet System

For permitting introduction of a representative sample into the ion source with minimal loss of vacuum. Various types of inlets equipped to accommodate different samples: (Batch inlets, direct probe inlets, chromatographic inlets and capillary electrophoretic inlets).

Batch Inlet Systems:

Classical and simplest type.

Sample is volatilized externally and then allowed to leak into evacuated ionization region.

The figure shown is for a one that applicable to gaseous and liquid samples having PB up to 500°C.

Gaseous samples:

- A small measured volume of a gas is trapped between the two valves and then expanded into reservoir flask.

For liquids:

- A small quantity of sample is introduced into a reservoir usually with a micrometer syringe.

In either case vacuum system is used to achieve sample P $10^{-4} - 10^{-5}$ torr.

For samples with boiling points $> 150^\circ$C:

- T must be maintained at elevated T by oven and heating tapes of maximum T of about 350 0°C.
- Sample is now in the gas phase is leaked into the ionization area of the spectrometer via a metal or glass diaphragm containing one or more pinholes.
The Direct Probe Inlet.

- Solids and non volatile liquids can be introduced into the ionization region by means of a sample holder or probe which is inserted through a vacuum lock.

The lock system:

- Designed to limit V of air pumped after probe insertion.
- Probes also used for limited quantity samples (few nanograms)(less wasted than batch system).
- Probe: Sample is held on the surface of a glass or Al capillary tube, a fine wire or a small cup.

- The probe is positioned within a few mm of the ionization source and the slit leading to spectrometer.
- Vacuum used to maintain thermally unstable compounds for spectrum measurements before major decomposition occurs. And to elevate nonvolatile conc. in the ionization area (carbohydrates, steroids, metal-organic species and low molecular weight polymeric substances.
- Partial pressure attained is at least of 10⁻⁸ torr before onset decomposition.

Chromatographic and capillary electrophoretic inlet System.

- MS are often coupled with GC and HPLC or capillary electrophoresis columns.
- Separation and determination of the components of complex mixture is obtained.
- Specialized inlet systems is needed.
Several devices are available for separating ions with different m/z ratios.

**Mass analyzer should be:**
- Capable of distinguishing between minute mass differences.
- Allows passage of sufficient number of ions to yield measurable currents.

**Resolution:** A measure of how well a mass spectrometer separates ions of different mass.

- **Low resolution:** Refers to instruments capable of separating only ions that differ in nominal mass; that is, ions that differ by at least 1 or more atomic mass units.
High resolution: Refers to instruments capable of separating ions that differ in mass by as little as 0.0001 atomic mass unit.

**Resolution of mass spectrometer: R**

\[ R = \frac{m}{\Delta m} = \frac{M_1}{M_1 - M_2} \]

Where: \( m \) : mass of the first peak (or mean mass of the two peaks).
\( \Delta m \) : mass difference between two adjacent peaks.

**\( \Delta M \) = full width at half maximum (FWHM)**

**Example:**

What \( R \) is needed to separate \( \text{C}_2\text{H}_4^+ \) and \( \text{CH}_2\text{N}^+ \) ions?

\( \Delta m = 28.0313 - 28.0187 = 0.0126 \)

\[ R = \frac{m}{\Delta m} = \frac{28.025}{0.0126} = 2.22 \times 10^3 \]

Where 28.025 is the mean mass for the two species.
**High Resolution MS**

- High resolution data reports include ppm estimate
  - ppm = parts per million (1 ppm = 0.0001%)
- 5 ppm @ m/z 300 = 300 * (5/10^6) = ±0.0015 Da
- 5 ppm @ m/z 3,000 = 3,000 * (5/10^6) = ±0.015 Da
- A molecule with mass of 44 could be C_3H_8, C_2H_4O, CO_2, or CN_2H_4.
- If a more exact mass is 44.029, pick the correct structure from the table:

<table>
<thead>
<tr>
<th></th>
<th>C_3H_8</th>
<th>C_2H_4O</th>
<th>CO_2</th>
<th>CN_2H_4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>44.06260</td>
<td>44.02620</td>
<td>43.98983</td>
<td>44.03740</td>
</tr>
</tbody>
</table>
**Resolution**

– $C_3H_6O$ and $C_3H_8O$ have nominal masses of 58 and 60, and can be distinguished by low-resolution MS.
– $C_3H_8O$ and $C_2H_4O_2$ both have nominal masses of 60.
– Distinguish between them by high-resolution MS.

<table>
<thead>
<tr>
<th>Molecular Formula</th>
<th>Nominal Mass</th>
<th>Precise Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_3H_6O$</td>
<td>60</td>
<td>60.05754</td>
</tr>
<tr>
<td>$C_2H_4O_2$</td>
<td>60</td>
<td>60.02112</td>
</tr>
</tbody>
</table>

High resolution MS can replace elemental analysis for chemical formula confirmation

R needed in MS depends upon its application.

**Example 1:** same nominal mass ions: $C_2H_4^+$, $CH_2N^+$, $N_2^+$ and $CO^+$

(All ions of nominal mass 28 Da).

Exact masses: 28.0313, 28.0187, 28.0061 and 27.9949 Da respectively.

These requires an instrument with a resolution of several thousands.

**Example 2:** Low MWt ions with a unit mass difference or more: $NH_3^+$ ($m = 17$) and $CH_4^+$ ($m = 16$) R instrument of 50 or less is sufficient.

Commercial MS are available with R range of 500 - 500,000.
Types of Mass Analyzers

Magnetic Sector Analyzers

- Employ a permanent magnet or an electromagnet.
- Cause beam from the ion source to travel in a circular path most commonly of (180, 90 or 60 deg)
- Schematic of a 90-deg magnetic sector spectrometer

Magnetic Sector: Single Focusing: A magnetic field is used to focus ions based on their momentum as they are ejected from an ion source at high energy. Resolution <2000
Operation:

- Ions formed by Electron impact are accelerated through slit B into the metal analyzer tube internal $P = 10^{-7}$ torr.
- Ions of different mass can be scanned across the exit slit by varying the field strength of the magnet or the accelerating potential between slits A and B.
- Ions passing through the exit slit fall on a collector electrode, ion current resulted that amplified and recorded.
- Translation or KE of an ion of mass m bearing z exiting slit B is given by:

$$KE = ZeV = \frac{1}{2} mv^2$$

Where $V$ is voltage between A and B
$v$ is velocity of the ion after acceleration
$e$ is the electronic charge $= 1.60 \times 10^{-19}$ C

Note: all ions having the same number of charges are assumed to have the same KE after accelerating regardless of their mass. (approximately true).

All ions leaving the slit have approximately same KE, the heavier ions must travel at lower velocities.

The path in sector by ions of a given m/z represents a balance between two forces acting upon them.

**The magnetic force $F_M$:**

$$F_M = BzeV$$

Where $B$ is MF strength

**The balancing centripetal force:**

$$F_C = \frac{mv^2}{r}$$

Where $r$ is the radius of curvature of the magnetic sector.
For ion to traverse the circular path to the collector, $F_M$ and $F_C$ must be equal.
\( F_M = BzeV = F_c = \frac{mv^2}{r} \)
\( v = \frac{Bzer}{m} \)

Substituting the previous equation in \( ZeV = \frac{1}{2} mv^2 \)
\( m/z = \frac{B^2 r^2 e}{2V} \)

**Mass Spectra:**

- Varying one of three variables (B, V or r) while holding the other two constant.
- Modern MS ions are sorted by holding V and r constant while varying the current in the magnet and thus B.
- In sector MS (using photographic recording) B and V are constants, r is the variable.

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What accelerating potential will be required to direct a singly charged water molecule through the exit slit of a magnetic mass spectrometer if \( B = 0.240 \text{ T} \) and \( r \) of curvature of the ion through the magnetic field is 12.7 cm?

SI units:
\( E_z = 1.60 \times 10^{-19} \text{ C} \times 1 \quad r = 0.127 \text{ m} \)

\[
m = \frac{18.02 \text{ g} \text{ H}_2\text{O}^+ / \text{ mol}}{6.02 \times 10^{23} \text{ H}_2\text{O}^+ / \text{ mol}} \times 10^{-3} \text{ kg / g}
\]

\[B = 0.240 \text{ T} = 0.240 \text{ W/m}^2\]

\[V = \frac{B^2 r^2 e z}{2m}\]
\[= \left[0.240 \text{ W/m}^2\right]^2 \left[0.127\text{ m}\right]^2 \left[1.60 \times 10^{-19}\text{ C}\right] \times 2 \times 2.99 \times 10^{-26}\text{ kg}\]
\[= 2.49 \times 10^2 \text{ W}^2\text{C/m}^2\text{kg} \text{ or volts}\]
Double focusing spectrometers

Previous are single-focusing spectrometers.

Limitation: Low precision

1. Directional distribution of ions
2. Energy distribution of ions

- Same m/z ratio but with small diverging directional distribution are focused so limiting the resolution of magnetic sector instruments ($R \leq 2000$).
- This because of the translational $E$ distribution of ions leaving a source (Boltzmann dist.) arises from energies of original molecules and source field inhomogeneities.
- Spread of KE causes a broadening of the beam reaching the transducer and a loss of resolution.

Double Focusing Spectrometers:

- Correction for both the directional and $E$ distribution of ions.
- Both directional and $E$ aberrations of a population of ions are simultaneously minimized by use of carefully selected combinations of electrostatic and magnetic fields.

Magnetic sector double focusing: An electrostatic analyzer is used serially with a magnet to select monoenergetic ions. High resolution >10,000 mass up to 100,000Da
• The ion beam is first passed through an electrostatic analyzer (ESA).
• Consists of two smooth curved metallic plates across which a dc potential is applied which limiting the KE of the ions reaching the magnetic sector to a closely defined range.
• Ions with E greater than average strike the upper side of ESA slit and lost to the ground.
• Ions with E less than average strike the lower side of the ESA slit and are thus removed.
• Directional focusing occurs along the focal plane (d).
• Energy focusing takes place along the plane e.
• Only ions of one m/z are double focused at the intersection of d and e for any given V and B.
• The collector slit is located at this locus of double focus.
Quadrupole Mass Spectrometer
Comparing with magnetic sector instrument it is:
• Less expensive and more rugged.
• More compact
• Found in commercial benchtop ms
• Low scan times (<100 ms) which is useful for chromatography.
• Most common mass analyzers in use today.
Quadrupole mass analyzer is responsible for filtering the samples ions.
• Consists of four parallel metal rods.
• Each rod pair is connected together electrically.
• A radio frequency voltage is applied between one pair of rods then the other.
Operation:

- Ions travel down the quadrupole in between the rods
- Electric field separates ions
- Ions are subjected to complex forces
- Only ions of a particular m/z reaches the detector

Advantages
- Inexpensive
- Easily Interfaced to Many Ionization Methods

Disadvantages
- Low Resolution (<4000)
- Low Accuracy (>100ppm)
- MS/MS requires multiple analyzers
- Low Mass Range (<4000)
- Slow Scanning
**Time-of-Flight MS (TOF)**

- **TOF:**
  - +ve ions are produced periodically by bombardment of the sample with brief pulses of electrons, secondary ions or laser generated photons.
  - The ions are then accelerated into a field-free drift tube by an electric field pulse of $10^3 - 10^4$ V.
  - Separation of ions on the basis of mass occurs during the transit of the ions to the detector located at the end of the tube.
  - All ions have same KE but their velocities are inversely proportional to their masses.
  - Lighter particles arrived earlier.
  - Typical flight times are 1 to 30 µs.
Improved mass resolution in MALDI TOF-MS has been obtained by the utilization of a single-stage or a dual-stage reflectron (RETOF-MS). The reflectron, located at the end of the flight tube, is used to compensate for the difference in flight times of the same m/z ions of slightly different kinetic energies by means of an ion reflector. This results in focusing the ion packets in space and time at the detector.
Linear Time-of-Flight (TOF)

**Advantages**
- Simplicity and ruggedness.
- Ease of accessibility of the ion source.
- Extremely High Mass Range (>1 MDa)
- Fast Scanning

**Disadvantages**
- Low Resolution (4000)
- Low sensitivity
- Low Accuracy (>200ppm)
- MS/MS not possible

Reflectron Time-of-Flight (TOF)

**Advantages**
- High Resolution (>20,000 in some models)
- High Accuracy (<5ppm)
- 10,000 Mass Range
- Fast Scanning

**Disadvantages**
- Low Resolution for MS/MS (PSD)
Ion Trap Analyzers

- Gaseous anions or cations can be formed and confined for extended periods by electric and/or magnetic fields.
- Several types, A simpler type of ion trap that used for GC/MS
- Now used to obtain mass spectra of a variety of analytes.
It consists of a central doughnut-shaped ring electrode and a pair of endcap electrodes. A variable RF voltage is applied to the ring electrode while the two end-cap electrodes are grounded. Ions of appropriate m/z value circulate in a stable orbit within the cavity surrounded the ring. By increasing RF voltage the orbit of heavier ions become destabilized, while lighter become destabilized causing them to collide with the wall of the ring electrode. By RF voltage scanning the trapped ions destabilized and leave the ring electrode cavity via openings in the lower end cap the emitted to a transducer. Rugged compact and less costly than sector or quadrupole instruments. Capable of resolving ions that differ in mass by unit in the mass range of 500-1000 Da.

Operation of an Ion Trap MS
**Tandem Mass Spectrometry : MS/MS or MS^n**

Mass spectrometry is a very powerful method to analyse the structure of organic compounds, but suffers from 3 major limitations:

1. **Compounds cannot be characterised without clean samples**
2. **This technique has not the ability to provide sensitive and selective analysis of complex mixtures**
3. **For big molecules like peptides spectra are very complex and very difficult to interpret.**

**Tandem:**

\[ m_\text{p}^+ \xrightarrow{CID} m_f^+ + m_n^- \]

The technique has been used for:

1. **Structure elucidation of unknown**
2. **Analysis of complex mixture with minimum sample clean up.**

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**What is MSMS?**

MS/MS means using two mass analyzers (combined in one instrument) to select an analyte (ion) from a mixture, then generate fragments from it to give structural information.
What is MS/MS?

Peptide mixture

1 peptide selected for MS/MS

Have only masses to start

The masses of all the pieces give an MS/MS spectrum

Interpretation of an MSMS spectrum to derive structural information is analogous to solving a puzzle

Use the fragment ion masses as specific pieces of the puzzle to help piece the intact molecule back together
Cleavages Observed in MS/MS of Peptides

Peptide Fragmentation

E=Glu
G=Gly
S=Ser
F=Phe
N=Asn
P=Pro
V=Val
A=Ala
R=Arg
**MS – MS (TANDEM MS) INSTRUMENTS**

- Employs two or more stages of mass analyzers
- Example is two quadrupoles coupled in series
- First analyzer selects ion (precursor ion) and second analyzer selects the fragments of the precursor ion
- Used to obtain more information about the structure of fragment ions.
- Fragment ions may be dissociated into lighter fragment ions or converted into heavier ions by reaction with neutral molecule

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**Tandem Mass Spectrometry (MS/MS)**

- Mass spectrometers are commonly combined with separation devices such as gas chromatographs (GC) and liquid chromatographs (LC).
- The GC or LC separates the components in a mixture, and the components are introduced, one by one, into the mass spectrometer.
- MS/MS is an analogous technique where the first stage

![Simple Schematic of a Mass Spectrometer Interfaced to a GC](image)

\[ \text{GC separated components} \quad \rightarrow \quad \text{Mass spectrum of eluting component} \]
• Suppose that we analyze a mixture of components by a "soft" ionization method (such as chemical ionization, fast atom bombardment, or electrospary ionization). Each component produces characteristic ionic species such as [M+H]+.
• To keep the discussion simple, let's assume that each component of the mixture has a unique molecular weight.
• The mass spectrum of the mixture contains peaks for each compound present in the mixture.
• Now, suppose that we would like to identify one of the mixture components.
• All the mass spectrum tells us is the molecular weight, but we would really like to see fragment ions that provide structural information for the component of interest.

The simplest form of tandem mass spectrometry combines two mass spectrometers.
• The first mass spectrometer is used to select a single (precursor) mass that is characteristic of a given analyte in a mixture.
• The mass-selected ions pass through a region where they are activated in some way that causes them to fall apart to produce fragment (product) ions.
• This is usually done by colliding the ions with a neutral gas in a process called collisional activation (CA) or collision-induced dissociation (CID).
• The second mass spectrometer is used to separate the fragment ions according to mass.
• The resulting "MS/MS" spectrum consists only of product ions from the selected precursor.
• Chemical background and other mixture components are absent.
Tandem Mass Spectrometry: MS/MS or MS^n

- Enhance selectivity by combining complimentary techniques
- Number of Stages =
  1. GC
  2. GC/MS
  3. GC/MS/MS
  4. GC/MS/MS/MS

Signal
Chemical Noise
S/N

Tandem Mass Spectrometry: MS/MS or MS^n

Intensity vs m/z

Inteometry

m/z

m/z
• The ion selected by the MS1 is the parent ion and can be a molecular ion resulting from the primary fragmentation.

• DISSOCIATION occurs in the fragmentation region.

• The daughter ions are analysed in the Second Spectrometer (MS2).

• In fact, the MS1 can be viewed as an ion source for MS2.
Ion Trap Mass Spectrometer

Fourier Transform (FT) instruments

Mass analysis performed by detecting cyclotron frequencies of ions (depend on m/z) in uniform B, in time domain. Then F.T. to frequency domain (mass spectrum).

FTMS provide:
- improved signal to noise ratios.
- Greater speed.
- Higher sensitivity.
- Higher resolution.

The heart of FTMS is an ion trap within which ions can circulate in a well-defined orbits for extended periods.
The Ion Cyclotron Resonance (ICR)

- Gaseous ion drifts into or formed in a strong MF.
- The motion becomes circular in a plane that is \( \perp \) to the direction of the field.
- The angular frequency of this motion is called cyclotron frequency \( \omega_c \). \( \omega_c = \frac{v}{r} = \frac{zeB}{m} \)
- In fixed field the \( \omega_c \) depends only upon the inverse of the m/z value.
- Increases the velocity of an ion will be accompanied by increase of rotation of the ion.
- This circulated trapped ion in the MF is capable of absorbing E from an ac EF.
- So the EF frequency matches the \( \omega_c \).
- The absorbed E increases \( v \) of the ion and \( r \) of travel without disturbing \( \omega_c \)

MS – Fourier Transform Analyzer

[Diagram of MS – Fourier Transform Analyzer]
• Inner solid line represents the original path to the ion.
• Dashed line shows spiral path when switch is moved briefly to position 1.
• Outer solid line is new circular path when switch again opened.
• For ensemble ions of the same m/z ratio between the two plates; when ac signal is applied the cyclotron resonance frequency sets all the particles into coherent motion that is in phase with the field.
• Ions of different m/z ratios (different \( \omega_c \)) are unaffected by the ac field.

Measurement of the ICR signal:
• Coherent circular motion of resonant ions creates image current observed current after termination of the frequency sweep signal (from position 1 to 2).
• The current decreases exponentially with time.
• It is a capacitor current induced by circular movement of a packet of ions with the same m/z ratios.

Example:
• +ve ions approaches the upper plate electrons attracted from circuit common to this plate causing a momentary current.
• This current reversed at the other plate as ions reach the other plate.
• An ac current produced depends on number of ions in the packet.
• Frequency of ac current is characteristic of m/z value of the ions in the packet.
• This current measures conc. of ions.
• The induced image current decays with time (few tenth of s to several s) by losing energy with collisions between ions (ions reach the thermal equilibrium) (time domain signal).
Diagram of the cell used in pulsed ICR & in FT-MS

- Generally equipped with a trapped ion analyzer cell.
- Gaseous sample molecules are ionized in the center of the cell by electrons that are accelerated from the filament through the cell to a collector plate.
- A pulsed voltage applied at the grid serves as a gate to switch the electron beam on and off periodically.
- The ions are held in the cell by a 1 to 5 V potential applied to the trap plate.
- The ions are accelerated by a radio-frequency signal applied to a transmitter plate.
- The receiver plate is connected to a preamplifier to amplify the image current.
- Ions could be stored for several minutes.
- The dimensions of the cell are not critical (usually a few cm) on a side.
FT-ICR-MS

**Advantages**
- No slits or ion optic lenses to adjust.
- All ions detected simultaneously for a single ionizing pulse.
- Detection sensitivity independent of m.
- Extremely High Mass Resolution (>500,000) high
- e.g. m/r m ~ 220000 for m/z = 84 at B = 1.2T
- Very Good Accuracy (<1 ppm)
- MS/MS in one analyzer

**Disadvantages**
- Expensive
- Requires Superconducting Magnet
- Slow MS/MS

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**what is B/z range?**

**FT-ICR**

Ion image current generation

Rapid scan ICR

Ions subjected to slower scan. When v matches cyclotron resonance frequency of ion it is excited and detected.

**wide-range ms**

**few ms; v varied linearly**

**0.1 sec**

**0.5 kHz**

**2 MHz**

**100 kHz**

**2 MHz**

**5 msec**

**Resonance**

**Resonances**

**Transmit signal**

Electrode 1

Electrode 2

Amp