MASS SPECTROMETRY (MS) & SECONDARY ION MASS SPECTROMETRY (SIMS)

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Outline

- Historical Background
- Applications of MS
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- MS Components
- MS Principles
- MS Ionization Methods
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- MALDI
- Tandem MS
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Historical Background

- The work of Thomson and Aston at the Cavendish Laboratories, Cambridge University
- J.J. Thomson built MS prototype to measure m/z of electron, awarded Nobel Prize in 1906
- Design to measure mass of elements
- Aston awarded Nobel Prize in 1922
- 1948-52 Time-of-flight (TOF)
- 1955 Quadrupole ion filters – W. Paul (1989 Nobel Prize)
- 1968-Tandem Mass Spectrometry
An analytical technique;

1) It’s objective to ionize a gas phase molecule and then analyze the masses of it’s produced ions

- Ions, an atom or a group of atoms, electrically charged. They lose or gain electrons, thus they are negatively or positively charged.

- 2) The produced ions are separated according to their mass-to-charge ratio (m/z) and then the relative abundance of each ionic species present is recorded (measured).
3) Quantitative analysis of atoms and molecules because of m/z ratio.

**MS Applications**

In industry and academia. Major mass spectrometric applications:

- **Biotechnology:** The analysis of proteins, peptides, oligonucleotides
- **Pharmaceutical:** Drug discovery, combinatorial chemistry, pharmacokinetics, drug metabolism
- **Clinical:** Newborn screening, hemoglobin analysis, drug testing
- **Environmental:** PAHs, PCBs, water quality, food contamination
- **Geological:** Oil composition
For what purposes MS is used?

- For measuring the molecular mass of a sample
- For identification of the unknown compounds
- Little as $10^{-12}$g, $10^{-15}$ moles; compounds identified at very low concentrations (one part in $10^{12}$) in chemically complex mixtures
- For quantification of known compounds
- Used to discover the number of isotopes, determine the relative abundance of the isotopes and measure of their exact masses
For what purposes MS is used?

- Within an accuracy of 0.01% of total weight of sample and within 5 ppm for small organic molecules
- Helps to determine the chemical and structural information about molecules
- Fragment sample & analyse products
- Uses a machine called Mass Spectrometer.
MS COMPONENTS

- Depend on the used ionization method, the type and the complexity of the sample

- 3 major components;
  1. An ion source,
  2. A mass analyzer and
  3. A detector

- A sample introduction system (inlet system)
Schematic Diagram of a MS

1. Sample
2. Inlet system
3. Ion source
4. Mass analyzer
5. Detector
6. Vacuum system
7. Signal processor
8. Readout

Flow: Sample → Inlet system → Ion source → Mass analyzer → Detector → Vacuum system → Signal processor → Readout
1) **The ion source**;
   - produces gaseous ions from the sample;
   - Samples easier to manipulate if ionised because ions are easier to manipulate than neutral molecules
   - a small sample of compound is ionized especially cations are produced by losing electrons

2) **The mass analyzer**;
   - separates ions according to m/z
   - These ions are extracted into the analyser region of the mass spectrometer

3) **The detector**

4) **The sample introduction**;
   - by introduction into the ion source
   - by ejecting of charged molecular species from a solid surface or solution
Why operating in a high-vacuum system?

- Because of forming and manipulating gas-phase ions
- Ions are very reactive and short-lived
- Atmospheric pressure, 1 atm = 760 Torr. Ions are handled in the pressure of $10^{-5}$ to $10^{-8}$ torr
- Allow unhindered movements of ions
- Mechanical and diffusion pump
Typical Mass Spectrum

Relative Abundance

120 m/z - For singly charged ion, this is the mass
The ability of a spectrometer to separate two adjacent peaks in a spectrum

- For large samples such as **biomolecules**, an accuracy of 0.01% of the total molecular mass of the sample
- For small **organic molecules**, an accuracy of 5 ppm or less
- For the suitable instrument
- Showed by width of the peak
- The increase in resolution or resolving power, the more qualified instrument, mass accuracy increases

\[ R = \frac{M}{\Delta M} \]

- \( M \) = the mass number
- \( \Delta M \) = the difference between two masses
Resolution in MS

\[ \Delta m \text{ at 50\% height} \]

\[ \Delta m \text{ at 5\% height} \]

Two peaks resolved to 10\% valley

Two peaks resolved to 50\% valley
Mass Spectrometry Ionization Methods

- Chemical ionization (CI)
- Plasma and glow discharge
- Electron impact (EI)
- Electrospray ionization (ESI)
- Fast-atom bombardment (FAB)
- Field ionization
- Laser ionization (LIMS)
- Matrix-assisted laser desorption ionization (MALDI)
- Plasma-desorption ionization (PD)
- Resonance ionization (RIMS)
- Secondary ionization (SIMS)
- Spark source
- Thermal ionization (TIMS)
Electron Impact (EI)

- The original mass spectrometry (MS) ionization method
- The most widely used
- Used for ionization and fragmentation the sample molecules before mass analysis
- Firstly the sample is vaporized and then enters into the ion source
- A beam of electrons help the sample be impacted with sufficient energy to ionize the molecule

\[ \text{M (g)} + \text{e}^- \rightarrow \text{M}^+ (g) + 2 \text{e}^- \]

- \( \text{M}^+ \) is molecular ion molecular weight analytical information
- \( \text{M}_1^+ \rightarrow \text{M}_2 + + \text{M}_n \)
- \( \text{M}_1 \) is the molecular ion (odd)
The fragmentation process

- Structural information about the molecule
- Fragment ions = odd electron or even electron.
  - primary structure,
  - electron energy 70 eV energy
- Ion source temperature
- **The molecular ion is a radical which is** not very stable and tend to fragment.
- EI mass spectra intense fragment ion peaks and much less intense molecular ion peak
- When the molecular ion peak is not observed in the mass spectrum, CI can be used in order to get molecular ion information
- EI ionization requires that the molecules are vaporized before ionization.
- Volatile under the conditions of the ion source
- Sample introduction to the EI source by
  1. a gas chromatography device
  2. a solids probe device.
Fast-Atom Bombardment (FAB)

- Ion production in a mass spectrometer from nonvolatile or thermally fragile organic molecules by bombarding the compound
- A technique for the analysis of protein sequence and structure to various organometallic systems
- A high energy beam of atoms or ions (Cs or Xe) vapourizes, fragments and ionizes a protein solution
- Flow FAB is a variant that analyses the continuous liquid stream from a HPLC or capillary electrophoresis separation
Electrospray Ionization (ESI)

- Soft ionization technique, desorption ionization method
- Solid or liquid samples, nonvolatile or thermally unstable.
- Proteins, peptides, and other biological macromolecules
- No fragmentation of the macromolecules into smaller charged particles; turning the macromolecule being ionized into small droplets
- Large mass molecules are detected in the small mass range of the instrument
- As the m/z value increases, the number of protons attached to the molecular ion decreases
- When mixtures are used as the analyte, this technique is out of use
- Purification methods: HPLC, Capillary Electrophoresis, and Liquid-Solid Column Chromatography
**Advantages:**
- samples with large masses can be handled
- analyzing biological samples that are defined by non-covalent interactions because of soft ionization method

**Disadvantages:**
- cannot analyze mixtures very well
- the apparatus becomes contaminated quickly and hard to clean
- the multiple charges that are attached to the molecular ions can make for confusing spectral data.
Secondary Ionization (SIMS)

- Organic and inorganic analyses; spectrometers quite different
- Surface analysis technique used to characterize the surface and sub-surface region of materials.
- Energetic primary particles (electrons, ions, neutrals or photons)
- Cause bombardment on a solid surface; A primary ion beam; such as $^3\text{He}^+$, $^{16}\text{O}^+$, or $^{40}\text{Ar}^+$
- Thus mass spectrometer of ionised particles which are emitted occur

**The Sputtering Process (bombardment)**

- Binary collisions (elastic and inelastic) of primary ions with single target atoms
- Oxygen increases positive ions and cesium increases negative ion
- Metal-oxygen bonds in an oxygen rich zone help oxygen enhance
- Bonds break in the ion emission process, oxygen negatively charged; metal positively charged
- Oxygen beam sputtering increases the concentration of oxygen in the surface layer.
- More secondary electrons are excited over the surface potential barrier.
- Increased availability of electrons leads to increased negative ion formation.
- The variability in ionization efficiencies for different elements
**Advantages of SIMS**

- has a very low detection limit (ppm to ppt)
- detection ability for all elements
- material can be continually sputtered from a surface to determine analyte concentrations as a function of distance from the original surface (depth profiling).

**Disadvantages of SIMS**

- a very great range of ionization rates for different elements
- matrix effects; the rates vary depending on the other species present
- response factors are different for either a positive and a negative beam; difficult quantification
Matrix-Assisted Laser Desorption Ionization (MALDI)

- A laser-based soft ionization method
- Important for protein analysis.
- Ionization occurs with bombarding the sample with laser light
- The sample is embedded in a chemical matrix (UV absorbant)
- The matrix makes the production of intact gas-phase ions from large, nonvolatile, and thermally decomposed compounds such as proteins, oligonucleotides, synthetic polymers easy
- The matrix absorbs the laser light energy thus small amount is vaporized
The MALDI matrix must meet a number of requirements simultaneously:

- be able to embed and isolate analytes (e.g., by co-crystallization)
- be soluble in solvents compatible with analyte
- be vacuum stable
- absorb the laser wavelength
- promote analyte ionization
Mass Analyzers

- Separation ions depending on m/z ratio
- Magnetic-sector analyzer
- Quadrupole analyzer
- Time-of-flight analyzer
- FT-Ion cyclotron analyzer
- Ion-trap analyzer
Time-of-Flight Analyzer

- Consists of 3 parts: an ion-accelerating region, a flight tube, and a detector
- Used with the MALDI technique for analysis of large biomolecules
- All ions are created in the ion-accelerating region
- Ions are pulsed into the flight tube in short, well defined packets. All ions have the same kinetic energy, same potential difference and a constant homogeneous electrostatic field subjection during acceleration.
- Thus having same kinetic energy leads different velocities depending on their masses
- Masses affect their arrival time at the detector and within order of increasing mass they reach the detector
- The differences of flight times are the basis for resolving ions of different $m/z$ and the mass resolution depends on the flight time differences
- Its insufficient mass resolution, because flight time variations of ions of the same $m/z$
ADVANTAGES:

- Unlimited mass range
- Ideal for Pulsed ionization or spatially confined
- For each ionization complete mass spectrum
- High transmission
- Valid for extremely small sample amounts
- Relatively low cost
- Fast mass scanning

DISADVANTAGES:

- The pulse is not felt by all ions to the same intensity and so a kinetic energy distribution for each discrete \( m/z \) exists. This lowers the resolution by creating a time-of-flight distribution for each \( m/z \).
Quadrupole Analyzer

- four parallel metal rods
- an ion source,
- ion optics ,
- the quadrupole filter itself with control voltage supplies,
- an exit aperture,
- an ion detector,
- detection electronics,
- and a high-vacuum system.
- \((U + V \cos(wt))\) = the potential of two opposite rods
- \(- (U + V \cos(wt))\) = the potential of other rods
- \(U = \) a dc voltage and \(V \cos(wt) = \) an ac voltage
- ions travel down the flight path which is centered between the four rods, and because of the applied voltages the trajectory of ions is affected.
- This filter is selective of ions depending on their certain \(m/z\) and \(V_{ac}/V_{dc}\) ratios: some of them pass and the others are thrown out.
- Voltages on the rod vary and mass spectrum is obtained
- There are two methods: varying \(w\) and holding \(U\) and \(V\) constant, or varying \(U\) and \(V\) \((U/V)\) fixed for a constant \(w\).
Ion-Trap Analyzer

- Three electrodes with hyperbolic surfaces to trap ions in a small volume
- A central ring electrode two adjacent endcap electrodes
- After changing the electrode voltages for ejection of ions from the trap, a mass spectrum is obtained.

**ADVANTAGES**
- Compactness
- The ability to trap and accumulate ions to increase the signal-to-noise ratio of a measurement.
- High sensitivity
- Available for tandem mass spectrometry
- Ion/molecule reactions can be studied for mass-selected ions
- High resolution

**DISADVANTAGES**
- Mass measurement accuracy is relatively poor
FT-Ion Cyclotron Analyzer

- A high-frequency mass spectrometer
- Because of a high-frequency field, the ions absorb maximum energy with a selected m/z ratio
- The cyclotron resonance condition is satisfied by ions after max energy is gained
- And then separation from ions of different m/z
- Cyclotron motion of ions (having different m/z ratios) in a constant B
- This motion is excited simultaneously by a pulse of a RF electric field applied perpendicularly to B
- Has a higher mass resolution than any other mass analyzer available
- Superconducting magnets are used in FT-ICR MS
Magnetic-Sector Analyzer

- 'B' sector and 'E' sector
- The path of a *moving* charged particle (ion) is curved in the sector by a magnetic field.
- A *stationary*, charged, non-magnetic particle is not attracted or repelled by a magnetic field.
- The ions different speed, but have the same kinetic energy.
- They enter the magnetic sector through the source slit where they are deflected according to the left-hand rule.
- Detection of ions that have different m/z consecutively
- **Constant magnetic field, kinetic energy sweep** larger B : larger m/z
- **Constant kinetic energy, magnetic field sweep** larger V : smaller m/z
- Higher-mass ions are deflected less than lower-mass ions
- High-resolution capabilities.
- Can not scan the field of a superconducting magnet, it has a fixed field strength.
MS Detectors

1. Photomultiplier
2. Microchannel plate
3. Electron multiplier
Tandem Mass Spectrometry

- The simplest MS/MS method
- analysis according to the mass/charge ratio.
- Two mass analyzers or filters between them a collision cell filled with Argon or Xenon
- The sample sorted and weighed in the first mass spectrometer, then broken into pieces in the collision cell, and a piece or pieces sorted and weighed in the second mass spectrometer
- Separation and identification of compounds in complex molecules
- Cause fragmentation and mass analyze of fragment ions
- in **newborn screening** to detect molecules such as amino acids and **fatty acids**.
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