INTRODUCTION TO MASS SPECTROMETRY

PAPER 9: TECHNIQUES USED IN MOLECULAR BIOPHYSICS I
MODULE NO. 25

Learning objectives:

This module is prepared with the learning objective of the introductory part of the mass spectrometry for beginners. The main emphasis is given only for basic information and instrumentation. More information of each subset will be provided in the later modules of the mass spectrometry section.

1. **Introduction and basic principle of Mass spectrometry (MS)**
2. **Mass spectrometer and its major components**
    1. Methods of sample ionization
    2. Types of mass analyzers and separation of sample ions
    3. Detection and recording of sample ions
3. **Applications of Mass spectrometry**

1. What is mass spectrometry (MS)?

Mass spectrometry (MS) is an analytical technique that can identify a substance, its chemical nature and quantity present in a sample by measuring its mass-to-charge ratio. In this technique sample is first ionized into gaseous ions by ionizing energy on molecule, further, these ions get characterized by their mass to charge ratios (m/z).

Mass spectrometry can identify compounds on the basis of the atomic sample composition of the molecules and their charge state. This feature leads to a prominent benefit of the mass spectrometry over other identification techniques and "blind" analysis of unknown samples is possible since MS does not require comprehensive prior information of the sample. Typically, mass spectrometry is employed to identify unknown compounds via molecular weight determination, to quantify known compounds, and to determine structure and chemical properties of molecules.

1.1 The Mass Spectrometer

Mass spectrometer is an instrument which converts the molecules into ions so that they can be moved about and manipulated by external electric and magnetic fields. The mass spectrometer consists of following three fundamental components.

I. **Ion Source:** An ion source is the first component of mass spectrometer that creates gaseous ions from the substance being studied. A small sample is ionized, generally to cations by loss of an electron. The two most common ion source devices are Matrix-assisted laser desorption/ionization (MALDI) and Electrospray ionization (ESI).

II. **Mass Analyzer:** A mass analyzer is the second component of the mass spectrometer that receives ionized masses and resolved them on the basis of charge to mass ratios and outputs them to the detector. The most common mass analyzers are Time of Flight Mass Analyzer, Quadrupole Mass Analyzer, and Quadrupole Ion Trap Mass Analyzers etc.

III. **Detector System:** It is the last component of mass spectrometer which is required for detecting the ions and recording the relative abundance of each of the resolved ionic species. The results are displayed on a chart as mass spectrum. A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds.

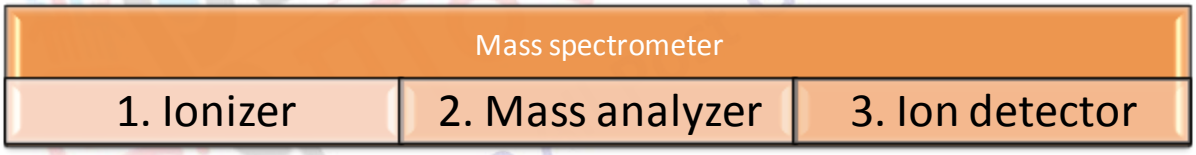

Figure1: Three major components of a Mass Spectrometer

In general, all the major components of mass spectrometer are maintained in vacuum which protects possible hindrance from air to molecular ions which are generated from ion source of the instrument.

1.2 Basic Principle

For mass spectrometric analysis of a compound, the sample has to be introduced into the ionization source of the instrument and the production of gas phase ions of the compound is done by electron ionization. The sample passes through an electron beam which knocks off some electrons from the molecules and turns them into ions. A mass spectrometer generates multiple ions from the single sample. Ionic forms of molecules of sample are advantageous as ions are easier to manipulate than neutral molecules. These molecular ions undergo further fragmentation. These ions are separated according to their **mass (m)/charge (z) ratios i.e. (m/z)** in the analyzer region. The resulted mass spectrum is displayed in the form of a plot of ion abundance versus mass-to-charge ratio. Thus the structure and nature of pre precursor molecule is elucidated. The separated ions are detected and this signal sent to a data system where the m/z ratios are stored together with their relative abundance for presentation in the format of an **m/z spectrum.**

2. Components and their functions in a mass spectrometer

2.1 Sample introduction

Pure samples can be inserted directly into the ionization source. While, complex mixture of sample are first processed through various chromatography like high pressure liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE) separation column to separate them into a series of components which can then finally enter the mass spectrometer sequentially for individual analysis.

2.2 Sample ionization

There are several methods of ionization used in mass spectrometry as given in Table 1. Among these, the electron impact (EI) and Fast Atom Bombardment (FAB) are older methods and usually required in specific needs e.g. EI for environmental work using GC-MS. The most common ionization methods employed in biological systems are Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption Ionization (MALDI), and atmospheric pressure chemical Ionization (APCI) etc. Both positively and negatively charged sample ions are generated to the proton affinity of the sample. The ionization method to be used depends on the nature of sample and the type of mass spectrometer.

Table 1: List and specifications of different Ionization methods.

Ionization method	Analyte type	Mass range	Sample introduction
Chemical Ionization **(CI)**	Small and volatile	Upto 1000 Da	GC or liquid/solid probe
Electron Impact **(EI)**	Small and volatile	Upto 1000 Da	GC or liquid/solid probe
Fast Atom Bombardment **(FAB)**	carbohydrates, organometalics peptides, non-volatlite	Upto 6000 Da	Sample mixed with viscous matrix
Electrospray Ionization **(ESI)**	Biomolecules,proteins, peptides, non-volatlite	> 500,000 Da	Liquid chromatography or capillary
Atmospheric Pressure Chemical Ionization **(APCI)**	Biomolecules, proteins, peptides etc., non-volatlite	> 500,000 Da	Liquid chromatography or capillary
Matrix Assisted Laser Desorption Ionization **(MALDI**	Biomolecules, proteins, peptides, etc.,	Upto 500,000 Da	Sample mixed with solid matrix

2.2.1 Electron Impact ionization (EI) – This method is mostly used for GC-MS and considered as hard ionization technique, because it causes the fragmentation of ions. Ionization of gaseous and volatile substances can be done by energetic electrons. In this method a beam of electrons is formed by heating a filament bias at a negative voltage compared to the source (-70 volts). The electrons are further used for bombardment to the gas phase molecules. The following reaction describes the electron ionization process:

$$M + e^- \longrightarrow M^{+\bullet} + 2\,e^-$$

where M is the analyte molecule, e^- is the electron and $M^{+\cdot}$ is termed the molecular ion.

2.2.2 Fast Atom Bombardment (FAB) - FAB is one of the first techniques used for ionization of non-volatile compounds. In FAB, the sample is mixed in a matrices like glycerol, 1-thioglycerol, a mixture of dithiothreitol and dithioerythritol, 3-nitrobenzyl alcohol, and triethanolamine to keep the sample in a liquid state as it enters the high vacuum ion source. This matrix also protects the analyte from being damaged by the high energy bombarding particle. Ionization is done by bombarding a sample with a beam of atoms, typically Ar or Xe, accelerated to 8-10 keV kinetic energy. The ions formed by FAB are adducts to the molecule, where adducts could be protons, sodium, potassium or ammonium ions etc.

2.2.3 Electro-spray ionization (ESI) – Currently, ESI is one of the most popular ionization techniques and generally employed in the mass analysis of polar molecules. ESI has an advantage in its easy compatibility with LC.

In ESI, the sample is dissolved in a polar, volatile solvent and introduced by a narrow capillary **(Figure 2.)**. The electro-spray is produced by applying a high voltage (3- 4 kV) on a flow of liquid at atmospheric pressure. The concurrent flow of nebulising gas **(**nitrogen) is used to direct the spray emerging from the capillary towards the mass spectrometer. The spray enters into the vacuum system and the droplets are de-solvated by heat, vacuum and high voltage. Further, the ions are ejected from the droplets and pushed into the mass analyzer **(Figure 2.)**. Ions may contain multiple charges, allowing the detection of very large molecules on analyzers.

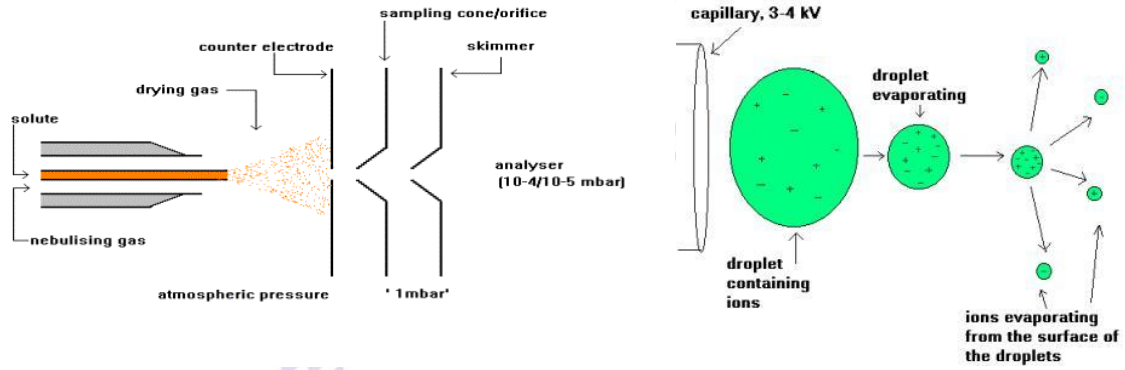

Figure 2. Electrospray ionization source and process (taken from creative commons)

Usually, formic acid is added for protonation of the sample molecules in positive ionization mode; while ammonia solution or a volatile amine may be added for deprotonation of sample in negative mode. Peptides and Proteins are usually analyzed in positive mode whereas nucleic acids and saccharides are investigated in negative ionization conditions.

2.2.4 Atmospheric Pressure Chemical Ionization (APCI)- APCI is a similar method as ESI., In this method, the voltage is applied on a needle that creates a corona discharge of H_3O^+ or water clusters ions at atmospheric pressures. The sample gets volatilizes by a heated gas and injected into the discharge. Ions are formed by proton transfer from the H_3O^+ to the sample. These ions are then accelerated into the vacuum as done in electrospray.

2.2.5 Matrix Assisted Laser Desorption Ionization (MALDI)- MALDI is a soft ionization technique where the sample is embedded e in a solid matrix (e.g. sinapinic acid, 2,5-dihydroxybenzoic acid etc.) which absorbs energy at the wavelength of the laser (337 nm) and transfer a proton to the sample.

Generally, the $[M+H]^+$ ion, or $[M+Na]^+$, $[M+K]^+$, etc., are preferentially formed in the positive ion mode, and $[M-H]^-$ ion in the negative ion mode. MALDI mostly forms single charged ions and preferentially coupled with time-of-flight (TOF) analyzers.

2.3 Mass Analyzers

After ions are formed in the source region they are accelerated into the mass analyzer by an electric field. A mass analyzer is the section in mass spectrometer which receives ionized masses and resolves them on basis of their charge to mass ratios. The choice of a mass analyzer depends upon the mass range, resolution, scan rate and detection limits.

Following are the mass analyzers which are used in mass spectrometry.

1. Time of Flight Mass Analyzer
2. Quadrupole Mass Analyzer
3. Ion Trap Mass Analyzers
4. Magnetic Sector Mass Analyzer
5. Electrostatic Sector Mass Analyzer
6. Ion Cyclotron Resonance

Among all the mass analyzers TOF, Quadrupole and Ion Trap Mass Analyzers are discussed in lower sections. Other mass analyzers will be discussed in details in next chapters at appropriate places.

2.3.1 Time of Flight (TOF) mass analyzer- Time of Flight (TOF) mass analyzer is one of the simplest mass analyzers used in mass spectrometry, often interfaced with MALDI ionization source (figure 3). This is a very simple mass spectrometer that uses fixed voltages and does not require a magnetic field. Mass analysis is rapid with good resolution and sensitivity, due to this TOF instrument is now become an accepted preference in bimolecular identifications e.g. proteins identification.

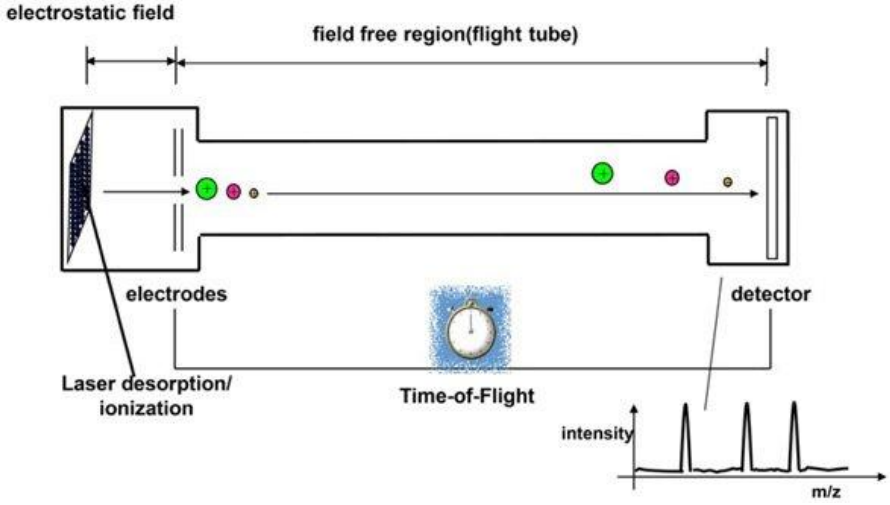

Figure3: A representation of MALDI and TOF-analyser
Ref: Application of MALDI-TOF MS for the Identification of Food Borne Bacteria **DOI:** 10.2174/1874285801307010135 (taken from creative commons)

In TOF analyzer, the separation is based on the kinetic energy and velocity of the ions. The TOF analyzer works by measuring the time required for ions generated in the source to fly through the analyzer and strike the detector at the other side. The principle is based on an ion of mass *m* leaving the ionization source with a charge *z* and accelerating potential *V*, thus having energy *zV* equal to the kinetic energy of the ion:

$$\mathbf{K = z.V = mv^2/2} \quad \text{..... (1)}$$

If the time taken (**t**), for the ion to fly the distance (**d**) of the flight tube at velocity **(v)** is given by:

$$\mathbf{t = d/v} \quad \text{..... (2)}$$

$$\mathbf{v = d/t} \quad \text{..... (3)}$$

Therefore, **equation 1** can be rewritten as

$$t^2 = \frac{m}{z}\left(\frac{d^2}{2V}\right) \quad \text{..... (4)}$$

Fixed distance (d) and accelerating potential (V) remain constant and m/z can be determined from t^2. Single charged ions with a more mass will travel with a lower velocity down the tube as compared to those with a lesser mass and hence separated according to their velocities.

2.3.2 Quadrupole Mass Analyzer- As the name suggests, this analyzer consists of four circular parallel metal rods and electric field between them is quadrupolar (figure 4). An electrical field is generated by applying a radio frequency (r.f) which is 180 degrees out of phase to the opposite pairs of rods. Thus, the shape of one waveform is the equal and opposite of the other. Direct current (DC) of equal magnitude but opposite polarities with respect to ground is supplied to each pair of opposing rods. The electrical field created in the mass analyzer acts as a filter in that it only allows ions of certain masses ('resonant ions') to pass down the gap between the four rods, and therefore, the quadrupole acts a mass-to-charge *filter*. A narrow band filter is produced due to the relation of the radio frequency to DC voltage. This band filter allows the ions of one m/z value, to take a stable trajectory between the alternating rods to the detector. While, ions of different m/z ratios (non-resonant ions) collide with the rods and become neutralized.

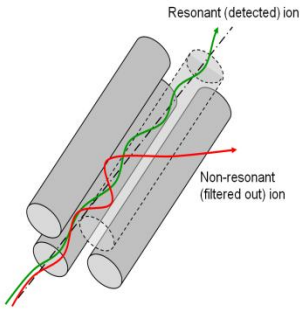

Figure 4: Schematic representation of a Quadrupole Mass Analyzer

Ref: http://www-mtl.mit.edu/wpmu/lfv/research/miniaturized-analytical-components-and-systems/quadrupole-mass-filters (taken from creative commons)

2.3.3 Ion Trap Mass Analyzers- This analyzer also works on similar principles as the quadrupole analyzer mentioned above. It uses an electric field for the separation of the ions. The analyzer is madeup of a ring electrode with grounded end cap with a specific voltage. The ions enter into the cavity between the electrodes through one of the end caps and there the electric field causes the ions of certain m/z values to orbit in the space (figure 5). On increasing the radio frequency voltage, heavier mass ion orbits become more stabilized. While light mass ions become less stabilized and collide with the wall do not detected by the detector. An increasing mass selective ejection of trapped ions occurs in ion-trap mass analyzers by gradually increasing the applied radio frequency voltage.

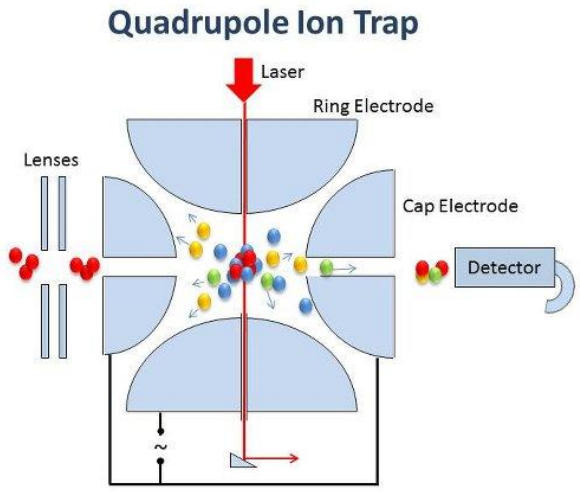

Figure 5: Schematic representation of a Quadrupole Ion trap Mass Analyzer

Ref: http://www.york.ac.uk/chemistry/staff/resfellows/yoshikawan/(taken from creative commons)

2.4 Detection and recording of sample ions.

The detector monitors the ion current, amplifies it and the signal is then transmitted to the data system where it is recorded in the form of mass spectra, which is the most common data representation. The m/z values of the ions are plotted against their intensities to show the number of components in the sample, the molecular mass of each component, and the relative abundance of the various components in the sample. The type of detector is supplied to suit the type of analyzer; the more common ones are the photomultiplier, the electron multiplier and the micro-channel plate-detectors.

3. Applications of mass spectrometers

Mass spectrometry has a wide number of applications in current scenario. Use of mass spectrometers is not limited to routine industrial applications but also required academia for research purposes. Among these following applications are being utilized at a higher level.

- **Characterization and quantification of biomolecules:** sample confirmation, to determine the purity of a sample, to verify amino acid substitutions, to detect post-translational modifications, to calculate the number of disulphide bridges
- **Amino acid sequencing:** Peptide mass fingerprinting, sequence confirmation, *de novo* characterization of peptides, identification of proteins, macromolecular structure
- **Oligonucleotide sequencing:** the characterization or quality control of oligonucleotides
- **Reaction monitoring:** enzyme identification and their subunit modification, protein digestion
- **Pharmaceutical:** medicinal chemistry, pharmacokinetics, drug metabolism
- **Clinical:** screening of specific disease, drug testing, biomarker discovery
- **Environmental:** Polyaromatic hydrocarbons analysis, water quality and food toxicology
- **Geological:** oil composition

4. Summary

1. Mass spectrometry (MS) is an analytical technique used to identify the type and amount of chemicals present in a sample by measuring their mass-to-charge ratio and abundance of gas-phase ions.
2. The mass spectrometer is an instrument capable of producing a beam of ions from sample under investigation, separating these ions according to their mass-to charge (m/z) ratios, recording the relative abundances of the separated ion species as a mass spectrum.
3. The mass spectrometer consists of following fundamental components Ion source, mass analyzer, and detector.
4. Ion source and mass analyzers are generally operated in vacuum with electrical and magnetic components.
5. Commonly used ionization sources are Electron impact (EI), Fast Atom Bombardment (FAB), Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption Ionization (MALDI), and atmospheric pressure chemical Ionization (APCI) etc.
6. A mass analyzer is the section in mass spectrometer which receives ionized masses and resolves them on basis of their charge to mass ratios. Examples of mass analyzers are Time of Flight Mass Analyzer (TOF), Quadrupole and Ion Trap Mass Analyzers etc.
7. The ion-currents corresponding to the different species are amplified and either displayed on an oscilloscope or a chart-recorder, or are stored in a computer
8. Mass spectrometer serves for establishment of the molecular weight, structure of both inorganic and organic compounds, the identification and determination of analytes in complex mixtures.