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Review Article

A Review on Mass Spectroscopy and Its Fragmentation Rules

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ABSTRACT

Mass spectrometry is a widely utilized analytical technique for determining the mass of molecules, elucidating molecular structures, and identifying unknown compounds. The method operates by ionizing molecules, separating the ions based on their mass-to-charge ratio, and detecting them to produce a mass spectrum. The techniques sensitivity and precision make it indispensable in various scientific fields such as proteomics, genomics, forensic analysis, pharmaceutical research, and environmental studies. Fragmentation rules, including homolytic and heterolytic cleavage, as well as specific rearrangements like McLafferty and retro Diels- alder reactions, are critical in interpreting mass spectrometry is often combined with techniques like HPLC and GC to enhance its analytical capabilities, enabling detection of trace-level substances and structural elucidation of complex biomolecules. The continued advancement of instrumentation and fragmentation understanding positions mass spectrometry as a vital tool in modern research and industrial applications.


INTRODUCTION

The technique of mass spectrometry was first introduced by J.J. Thomson in 1960. Mass spectrometry (MS) is a powerful analytical technique for identifying unknown compounds, determine molecular structures, and studying chemical principles. Mass spectroscopy measures molecular mass by converting samples (solid or liquid) into gas, ionizing them, and separating ions by their mass to charge ratio(m/z). The resulting

mass spectrum shows the types and quantities of ions present. For a pure substance, the graph will show a strong peak at a specific mass to charge (m/z) value. Mass spectrometry is one of the most advanced and widely applied analytical methods in educational and research laboratories worldwide. It plays a crucial role in discovering organic molecules and becomes even more powerful when paired with techniques like HPLC etc... allowing the detection extremely small amounts of

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substances. It is also used to study the structure and mass of organic, bio-organic and organometallic compounds. one of its important roles is in proteomics – the study of proteins structures.

Some of its most common uses are related to:

- Performing doping tests in athletes
- Locating petroleum reservoirs through the use of precursors in the rocks.
- Controlling fermentation of products in biotechnology processes.
- Determining genetic damage.
- Determining the presence of contaminants in food.
- Identifying the structure of biomolecules, such as nucleic acid

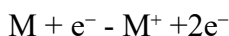
- Analyzing the biodegradation of medication.
- Establishing the age of geochemical and archeological sample.

Principle

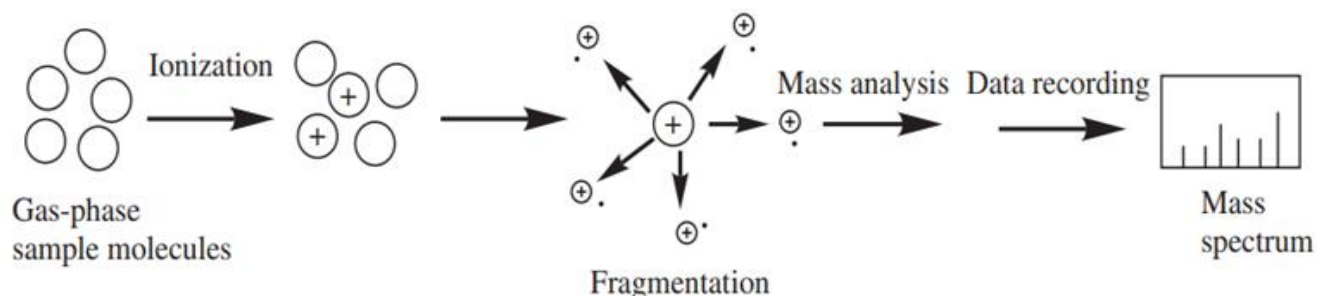
Mass spectrometry detects ions rather than neutral molecules, since ions can be easily manipulated and measured using electric or magnetic fields.

The analysis involves three main steps:

- 1) The sample molecules are converted into gas phase ions. For example, in electron ionization



This process involves adding or removing electrons or protons. The extra energy from ionization can also break the molecules into characteristic fragments.



- 2) Based on the mass to charge ratio (m/z) the ions are separated
- 3) The ion current from these separated ions is measured, amplified, and finally displayed as a mass spectrum.

Mass spectrometer is an instrument that forms ions, separates them based on their mass to charge ratio (m/z), detects them, and shows the results as a mass spectrum. These instruments come in many types with differences in size, resolution, flexibility, and cost.

Instrumentation

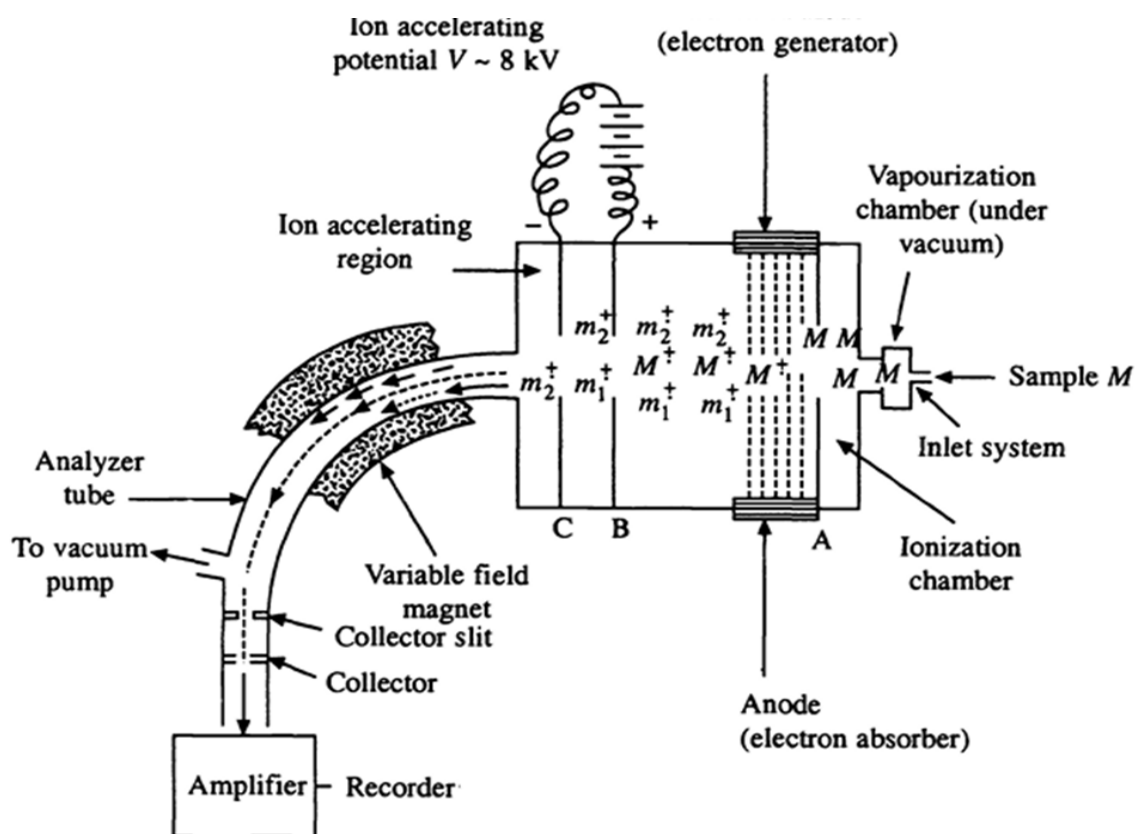
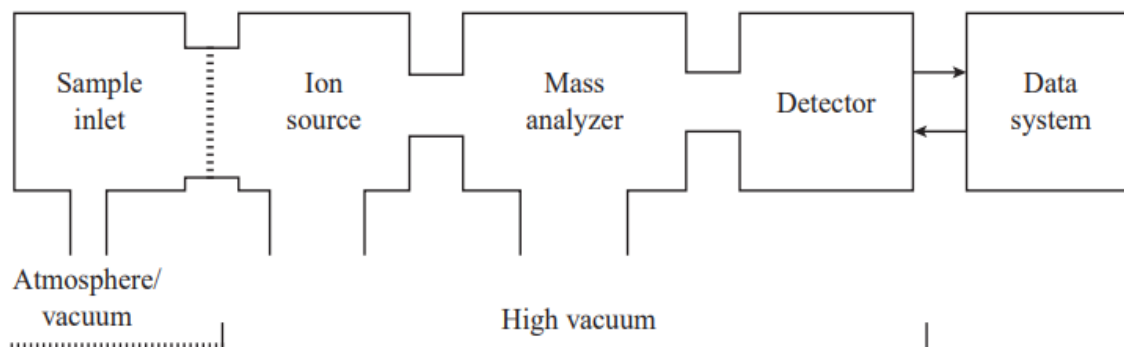


Figure 1: schematic diagram of a mass spectrometer

The mass spectrometer consists the following components they are:

- The mass spectrometer consists the following components they are:**
- Sample inlet
 - Ion source
 - Mass analyser
 - Detector
 - Data system
 - Vacuum system



Sample inlet

- Organic compounds with medium vapour pressure are placed in an ampoule at about 300°C.
- The sample turns into vapour and slowly diffuses into the ionization chamber
- If the sample has low vapour pressure, it can be introduced directly into the chamber using a probe.
- Heating at the probe tip helps in controlled vaporization.
- The vapour passes through a small slit into the ion chamber
- Inside, it is bombarded with high energy electron (around 70 eV) from the tungsten filament.
- When these electrons strike the molecule, the molecule lose one electron and become positively charged parent ions.

Ion source

- The first and most important step in getting a mass spectrum is to ionize the sample. The least amount of energy needed to remove an electron from an atom or molecule is called the ionization potential.
- To create ions in a mass spectrometer is by bombarding the sample with electrons. These electrons come from a tungsten filament that is heated electrically.
- The small amount of the sample, just a few milligrams, is vaporized inside the ion source at very low pressure (about 10⁻⁶mm).
- The vapour then passes through a small opening called slit A into the ion chamber. Inside this chamber, the vapor is bombarded with a stream of electrons from a tungsten filament. These electrons have an energy around 70 electron volts (eV).
- When a molecule is hit by these electrons, they usually lose one electron, turning into positively charged ion known as parent ion radical.

Ionization process

Mass analyzer

- Separates and mass analyses the ionic species.
- Based on their mass to charge ratio(m/z) ions are sorted in the device.
- When positively charged ions are accelerated from the ionization chamber, they enter the mass analyser.
- In this analyser they move through a uniform magnetic field that is perpendicular to their direction of motion.
- Each ions have a mass m and move with a velocity v, so its kinetics energy is given by:

$$\frac{1}{2} mv^2$$

- The ions are accelerated by an electric potential difference since each ions carries a single positive charge e, the energy gain from the electric field is:

$$eV$$

- Since this electrical energy is entirely converted into kinetic energy.

$$\frac{1}{2} mv^2=eV$$

- This equation forms the basis for further analysis in the mass spectrometer.



Types of mass analyser used in mass spectroscopy.

- Quadrupole
- Time of flight
- Magnetic sector
- Ion trap and orbitrap

Detector

- Mass detectors are essential components in mass spectrometry. They are responsible for identifying ions that have been separated based on their mass to charge ratio (m/z).
- Detectors are used to measure and amplify the ion current of mass resolved ions.
- Different types of detectors are employed for identifying and measure the ions once they have been separated according to their mass-to-charge ratio.

Types of detectors use in mass spectrometry:

Ion detectors

- Electron multiplier
- Faraday cups
- Photographic plates
- Scintillation counter
- Channel electron multipliers
- Resistive anode encoder image detectors
- High mass detection detectors

Other detectors

- Tandem quadrupole MS detector
- Photonis bipolar maldit of detector
- Fluxar SQ 300 MS Detector

Ion detector

Electron multiplier

- An electron multiplier is a widely used ion detector consisting of 12-24 aluminium oxide (Al_2O_3) dynodes, each set at progressively higher voltages. When ions hit the first dynode, electrons are emitted.
- These electrons strike subsequent dynodes generating more electrons at each stage. This cascade effect produces a large current gain often over a million times.
- A High Energy Dynode (HED) is accelerated ions with an electrostatic field. Boosting their velocity. Since electron multiplier signals depend on ion speed, the HED enhances signal strength and overall sensitivity.

Faraday cup

- A Faraday cup works by detecting ions that hit a dynode surface typically made from materials like Beryllium oxide, Copper - Silver-Steel-Braiding. When an ion strikes this surface, it knocks off some secondary electrons. When ions hit the dynode surface, they eject secondary electrons leaving a temporary positive charge. This draws in electrons from the circuit, generating a measurable current. Though not highly sensitive or amplifying, the detector works well at higher pressures.

Data system



- After ionization the release ions are detected in a detector that is connected to a computer.
- The computer display results as a spectrum of ions which is known as the mass spectrum.
- Records, processes, stores and displays data in the digital format.
- ❖ Ensure analyte ions have a long enough mean free path to travel uninterrupted.
- ❖ Allows ions to move along their indented trajectories without collisions.
- ❖ Reduce unwanted reactions between ions and gas molecules.

Vacuum system

- A vacuum system maintains a very low pressure in the mass spectrometer.
- The ion source region is usually maintained at a pressure of 10^{-4} to 10^{-8} torr.
- The mass analyser region required around 10^{-8} torr. Somewhat lower pressure is required in the mass analyser region. Most instruments use a differential pumping system to maintain an optimal vacuum.
- To operate accurately and efficiently, mass analysers require a high level of vacuum.
- This ensures that the analyte ions being studied are effectively controlled and influenced by the instrument's electrostatic components.
- Vacuum systems remove most background gas molecules, allowing ions to travel along precise paths dictated by electric, magnetic, or radiofrequency fields without interference from collisions.
- Modern LC-MS instruments typically use a differentially pumped vacuum system. This includes a foreline pump that creates a rough vacuum and one or more high vacuum pumps mounted on the analyser to achieve the extremely low pressure necessary for accurate mass to charge (m/z) measurements.

- ❖ Minimizes background interference, improving signal clarity.

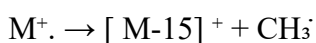
Examples of vacuum pumps

- ❖ Rotary pumps
- ❖ Fireline pumps
- ❖ Turbo molecular pumps
- ❖ Diffusion pumps

Fragmentation process

When molecular ions lose a methyl radical (CH_3), in mass spectrometry, the resulting ion will appear 15 mass units below the molecular ion peak. This happens because a methyl group has a mass of 15.

This process can be written as:



Where:

M^+ is the molecular ion,

$[\text{M}-15]^+$ is the fragment ion observed in the mass spectrum,

$\text{CH}_3\cdot$ Is the lost methyl radical (neutral, not detected).

General Fragmentation Modes

Maintain a high vacuum is crucial because it:



The relative abundance of the fragment ion formed depends upon

- the stability of the ion an
- the stability of the radical lost.

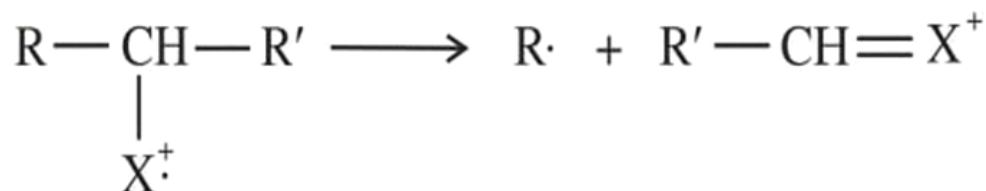
The stability of the ion can be judged by stabilization of the charge which depends upon

- Resonance
- Inductive effect,
- Polarizability and so on.

The radical site is reactive and can form a new bond. The formation of a new bond is a powerful driving force for ion decompositions. The energy released during bond formation is available for the cleavage of some other bonds in the ion. Some important fragmentation modes are described below:

Simple cleavage.

This process involves homolytic or heterolytic cleavage of a single covalent bond. The homolytic cleavage is initiated by a radical site.



- More abundant peaks are formed by the cleavage of carbon-carbon bond which is in position to the hetero atom in the mass spectra of alcohols, amines, ethers etc. The mass spectra of three isomeric butyl alcohols are different. Secondary and tertiary butanol's undergo this type of simple cleavage.

Homolytic cleavage.

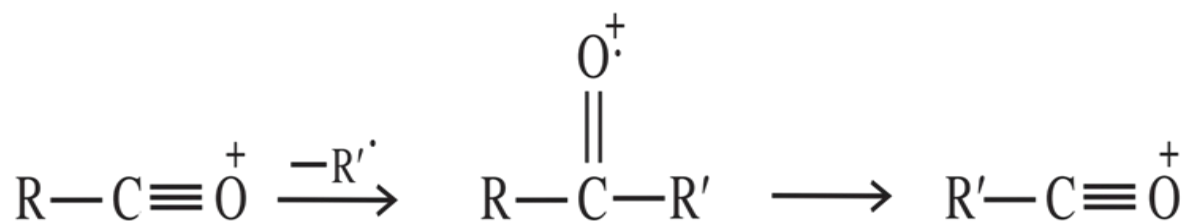
- Odd electron ions have an unpaired electron which is capable of new bond formation. When a bond is formed, energy is released.
- The energy released by bond formation can help offset the energy required for the cleavage of some other bond in the ion.
- Homolytic cleavage reactions are very common and can be classed in the following types:

Type:1

- Fragmentation mode operates in compounds in which a hetero atom is singly bonded to a carbon atom. Parent ion is formed by the removal of one electron from the hetero atom.
- A new bond is formed with the adjacent atom through the donation of the unpaired electron and the transfer of an electron from the adjacent bond.

Type :2

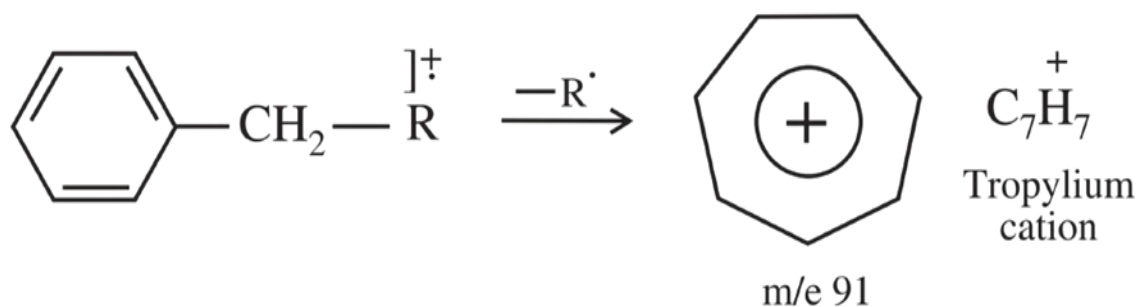
When a hetero atom is attached to a carbon atom by a double bond, α cleavage is the preferred fragmentation mode.



This type of fragmentation is shown by many compounds such as ketones, esters, amides etc. Compounds containing C=S groups do not show this type of fragmentation. In ketones, significant peaks are observed due to the cleavage of carbon-carbon bond which is alpha to the carbonyl group. Unsymmetrical ketones show two types of peaks since either alkyl group can be lost. The elimination of a bigger alkyl radical is preferred. In the same way, the fragmentation mode in aldehydes, esters and amides leads to the cleavage of C—H, C—O and C—N bond respectively.

Type:3

Benzylic cleavage is an energetically preferred fragmentation mode. It involves the cleavage of a carbon-carbon bond which is beta to the aromatic ring. Considerable stabilisation of the tropylium cation is provided by the aromatic-system. Thus, the mass spectrum of ethyl benzene has a very intense (M.+—CH₂) ion at m/e 91.



Heterolytic cleavage.

- In mass spectrometry, heterolytic cleavage involves breaking and bond in such a way that both bonding electrons go to one of the atoms.
- When a C-X bond (where X is a heteroatom like O, N, S, or Cl) undergoes this type of cleavage, the carbon typically retains the positive charge, not the heteroatom.
- Interestingly, it's often harder to break a C-X bond than a C-C bond, but there are exceptions based on the size of the heteroatom.

General Rules of Fragmentation

1. The relative height of the molecular ion peak is greatest for the straight chain compounds and decrease as the degree of branching increase.
2. The relative height of the molecular ion peak usually decreases with increasing molecular weight in a homologous series.
3. Cleavage is favoured at alkyl substituted carbon atom; the more substituted, the more likely is cleavage. This is consequence of the increased stability tertiary carbon atom over a

secondary, which in turn is more stable than the primary.

4. Double bonds, cyclic structure, and especially aromatic (or heteroaromatic) ring stabilize the molecular ion and thus increase the probability of its appearance.
5. Double bond favours allylic cleavage and gives the resonance stabilized allylic carbocation. This rule does not hold for simple alkenes because of the ready migration for the double bond but it holds for cycloalkanes.
6. Saturated rings tend to lose alkyl side chain at the alpha bond. This is merely a special case of branching. The positive charge tends to stay with the ring fragment. Unsaturated ring can undergo a retro Diels Alder reaction.
7. In alkyl substituted aromatic compounds, cleavage is very probable at the bond beta to the ring, giving the resonance stabilized benzyl ion or more likely the tropylium ion.
8. The C-C bonds next to hetero atom are frequently cleaved. Leaving the charge on the fragment containing the heteroatom whose non-bonding electrons provide resonance stabilization.
9. Cleavage is often associated with elimination of small, stable, neutral molecules, such as carbon monoxide, olefins, water, ammonia, hydrogen sulphide, hydrogen cyanide, mercaptans, or alcohols, often with rearrangements.

Stevenson's S Rule

In mass spectrometry, fragment ions usually form through unimolecular processes (involving only one molecule), because the ionization chamber as

very low pressure, making collisions between molecules (bimolecular processes) rare.

- According to Stevenson's rule, the most likely fragmentation happens in a way that leaves the positive charge on the more stable fragment the one with the lowest ionization energy.
- This means that more stable ions are favoured, and they appear more often in the mass spectrum.
- This idea is similar to Markovnikov's rule in organic chemistry, where the more stable carbocation forms first and leads to the main product.
- Fragmentation patterns can be predicted using familiar concepts like:
 - Electronegativity
 - Polarizability
 - Resonance
 - Octet rule
- Sometimes a molecule loses a neutral piece doesn't show up in the spectrum. You can figure out what was lost by comparing the masses before and after fragmentation. More stable neutral fragment is more likely to form.

➤ Types of ions:

Odd – electron ion ($OE^{\cdot+}$) can break apart two ways:

Into an even –electron ion (EE^+) and a radical (R^{\cdot}).

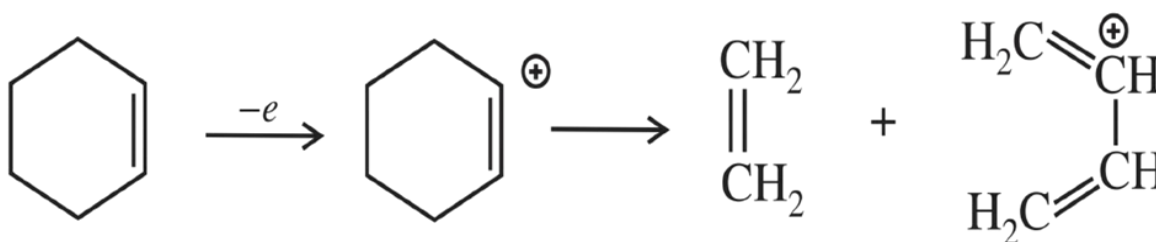
Into another $OE^{\cdot+}$ and a neutral molecule (N).



- An even-electron ion (EE+) usually fragments by forming another EE+ and neutral molecule –this is called even electron rule.
- If there are multiple possible ways to break apart, the molecule usually loses the largest alkyl group (based on Stevenson's rule).

Retro Diels Alder Fragmentation

- Six membered unsaturated rings can split through a process called Retro Diels Alder

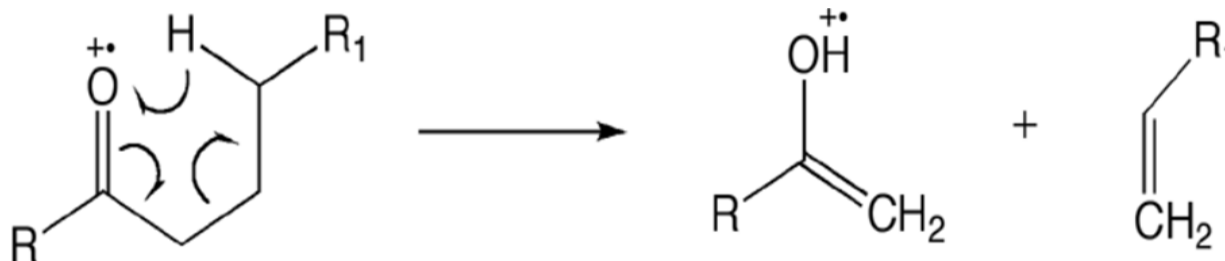


Fragmentation. This produces two fragments: a radical cation (which is a diene) and a neutral alkene.

- These are the same two components that normally come together in the regular Diels Alder reaction (a $[4\pi+2\pi]$ cycloaddition) between a diene and a dienophile. In these reverse reactions the dienes carries both the positive charge and the unpaired electron, as explained by Stevenson's rule.

McLafferty Rearrangement

- The McLafferty rearrangement was introduced by Fred McLafferty in 1956.
- In this process a hydrogen atom that is three carbon atoms away from the ionized site (called a γ -hydrogen) shifts towards the ionized center.



- This hydrogen transfer takes place through a six membered ring like transition state. At the same time, the bond between the first (α) and second (β) carbon atoms breaks leading to fragmentation.
- This rearrangement usually occurs in compounds like alkenes, ketones, esters, and amides. It forms a new radical cation and an alkene with a double bond between the original α and β carbons.

ADVANTAGES

- ❖ It gives details about how a molecule breaks apart during ionization and also helps determine its molecular weight.
- ❖ It has wide range applications in quantitative analysis. It is easily to detect the materials present in concentrations less than one part per millions (ppm).

- ❖ It gives basic details about the physical nature of atoms and molecules.
- ❖ It quickly reacted when operating conditions are adjusted.
- ❖ I can measure very accurately.
- ❖ Highly sensitive.
- ❖ Purity of the sample is not important.
- ❖ Provides molecular weight of peptides and proteins with high accuracy-(0.1-0.01%)

Applications

Phytochemical Analysis:

- Mass spectrometry is commonly used in phytochemical analysis because it can detect and measure metabolites that have small molecular weights, even when they are present in extremely low concentrations, nanograms per millilitre.

Proteomics:

- Mass spectrometry plays a crucial role in identifying and analysing proteins.
- It often uses gentle ionization techniques, such as electrospray ionization and MALDI, which are well suited for studying proteins.
- MS is widely applied in several areas, including determining peptide sequences, detecting proteins with varying molecular weights, and analysing protein expression under different biological conditions.
- It also helps in identifying proteins that undergo post translational modifications in response to various stimuli and is variable for exploring molecular interactions, such as those

between proteins and ligands, other proteins, or DNA.

Genomics:

- MS is used in the genomic analysis of nucleic acids, proteins, and peptides.
- These are separated by methods like MALDI which separates the molecules using ionization based on size followed by the detection which is accurate and consumes less time.
- MS helps to perform DNA sequencing and the short oligonucleotides sequences can be distinguished, which otherwise would require gel electrophoresis.
- Single nucleotide polymorphism (SNP) can be detected using MS, wherein a large number of SNPs can be precisely identified in a genome.

Structure Elucidation:

- Mass spectroscopy has major use in structure elucidation of compounds. Mass spectrum is produced in the form of bar graph which is interpreted by using the following peaks. There are two types of peaks:
- Molecular ion peak and Fragment ion peak

Peptide And Protein Sequences / Structure Analysis:

- Peptides are converted into amino alcohol which are volatile in nature. The peptide sequence was analysed using combination of gas phase ion and tandem mass spectrometry.

Forensic Science:



- It helps in confirming drug abuse, identifying explosives, arson investigation, etc.

Protein Identification:

- The most commonly used MS to identify proteins is a combination of peptide mass fingerprinting and amino acids sequencing via tandem mass spectrometry.

Monitoring Volatile Anesthetic in Patients' Breath During Surgery:

- The anaesthetic drug propofol is used as an intravenous sedative during major surgeries. The metabolites of propofol can be detected even in low concentrations.
- The surgeons can evaluate the patient's condition regarding the loss of consciousness by estimating the drug present in the expired air using a portable proton transfer reaction (PTR) time of flight (TOF) mass spectrometer.
- Thus, a mass spectrometer helps in analysing the aesthetic content in a patient's breath during the surgery.

Analysis Of Aerosol Particles:

- Aerosols are particles that may be solid, or liquids suspended in gas that measure in the range of 3 nm to 100 micrometres.
- They are produced either naturally or by human influence and may contain wind-blown suspension or combustion of biomass (organic material) or fossil fuels (oil, natural gas, coal, etc.,).
- Analysing aerosols helps in predicting global climate change, the extent of regional air pollution, and its potential consequences on human health.

- Analysis of aerosols is complex because it contains different chemicals in a single particle. Therefore, it can be done either by electron ionization mass spectrometer or by using TOF aerosol mass spectrometers.

DISCUSSION

Mass spectroscopy (MS) has become a key tool in many areas of science because it offers highly sensitive, precise measurements, and flexible use. Its works ionizing molecules, separating the based on their mass -to-charge ratio, and detecting the ions. It explains not only these basic steps but also describes the instruments and types of analyzer and detectors used. How a molecule breaks apart during ionization (fragmentation) is very important for understanding its structure. The pattern in the resulting spectra often follow known principle such as Stevensons rule and the Even-Electron rule. Mass spectroscopy is used in a wide range of scientific areas such as proteomics, geonomics, forensic investigations, herbal analysis, and environmental studies. It plays a major role in identifying proteins, studying changes that occur after proteins are made (post translational modifications), and even in hospitals to monitor gases used in anesthesia in real time. Mass spectroscopy Also supports advanced application like analyzing tiny particles in the air and identifying genetic variation such as SNPs. Combining MS with techniques like High Performance Liquid Chromatography (HPLC) and gas chromatography (GC) its ability to detect very small amounts of substances. Fragmentation reactions like the rearrangements and retro-diels-alder reaction are especially used for interpreting the spectra of organic and biological compound when conforming their structure.



CONCLUSION

Mass spectrometry is a highly effective and essential method in modern scientific research. Its ability to break down molecules, measure their mass, and detect small fragments helps identify structures, check compound purity, and determination of fragmentation and instrumentation. The technique operates by ionizing chemical compounds and analyzing the resulting ions based on their mass to charge ratios. With advanced fragmentation patterns and specialized detectors, mass spectrometry provides detailed molecular information that supports both research and industrial applications. Its broad use from identifying biological molecules to analyzing particles in the environments shows how important MS is in both research and industry. With ongoing technological improvements, mass spectrometers are becoming more sensitive and accurate, ensuring that MS continue to be one of the top tools for chemical, biological, and forensic analysis.

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