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A comprehensive review on mass spectrometry

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Abstract
Mass spectrometry is an instrumental technique in which sample is converted into rapidly moving positive ions which are then separated and characterized. In mass spectrometer ions are produced from the sample, generally by electron bombardment. Mass spectroscopy is an analytical technique that identifies biomolecules or proteins present in biological samples and is also useful for studies on protein-protein interactions. The basic principle involves the fragmentation of a compound or molecule into charged species, which are accelerated, deflected, and finally focused on a detector according to their mass and charge ratio. Ion deflection is based on charge, mass, and velocity, ions separation is based on mass to charge (m/z) ratio, and detection is proportional to abundance of ions. This chapter further discusses the back ground, principle, major components, applications, and clinical significance of mass spectrometry. It is the main analytical tool measuring of molecular weight of sample. Advanced mass spectrometry methods can unambiguously identify more than 2,000 proteins in a single proteome.

Keywords: Mass spectroscopy, analytical technique, isotope ratio determination, protein identification, carbon dating

Introduction

Mass Spectroscopy (MS) is an analytical technique used to measure the mass-to-charge ratio (m/z) of ions. It provides detailed information about the molecular weight, structure, and chemical composition of a substance. Mass spectroscopy is widely used in chemistry, biology, environmental science, and pharmaceuticals for qualitative and quantitative analysis of compounds [1].

Mass spectrometry (MS) is a highly effective qualitative and quantitative analytical technique used to identify and quantify a wide range of clinically relevant analytes. Mass spectrometers expand analytical capabilities to various clinical applications when coupled with gas or liquid chromatography. In addition, mass spectrometry is an essential analytical tool in proteomics due to its ability to identify and quantify proteins [1, 2].

Most mass spectrometry data are presented in units of the mass-to-charge ratio (m/z), where m is the molecular weight of the ion (in Daltons), and z is the number of charges present on the measured molecule. For small molecules (<1000 Da), there is typically only a single charge; therefore, the m/z value is the same as the mass of the molecular ion. However, when larger molecules such as proteins or peptides are measured, they typically carry multiple ionic charges, and, therefore, the z-value is an integer greater than 1. The m/z value is a fraction of the ion's mass in these cases [2-4].

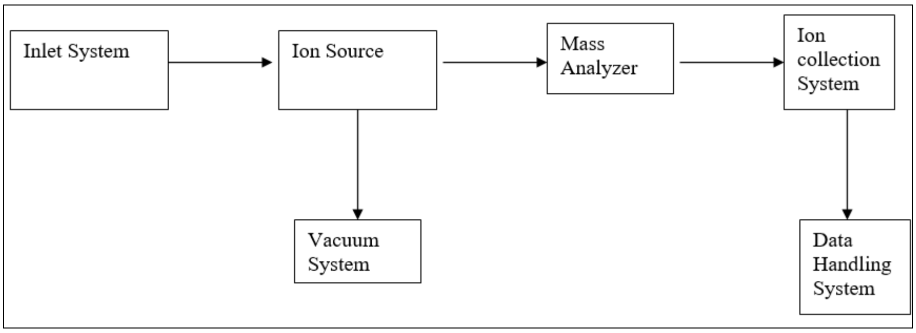

Fig 1: Block diagram of mass spectrometer
General overview

General overview

Wein (1898) showed that positive ions could be deflected by means of electric and magnetic fields. J. J. Thomson (1912) recorded the first mass spectra of simple low molecular weight molecules. Dempster (1918) and Asten (1919) designed more elaborate instruments which were used for the measurement of relative abundancies of isotopes.

Mass Spectrometry is the generation, separation, and characterization of gas phase ions according to their relative mass as a function of charge. Previously, the requirement was that the sample be able to be vaporized (similar limitation to GC), but modern ionization techniques allow the study of such non-volatile molecules as proteins and nucleotides. The technique is a powerful qualitative and quantitative tool, routine analyses are performed down to the femtogram (10^{-15} g) level and as low as the zeptomole (10^{-21} mol) level for proteins. Of all the organic spectroscopic techniques, it is used by more divergent fields

– metallurgy, molecular biology, semiconductors, geology, archaeology than any other.

Sample preparation is crucial for successful mass spectrometry, particularly when analysing complex matrices commonly encountered in clinical chemistry. This process typically involves one or more of the following steps- protein precipitations followed by centrifugation or filtration, solid-phase extraction, liquid-liquid extraction, affinity enrichment, or derivatization. Derivatization is the process of chemically modifying the target compounds to make them more suitable for analysis by mass spectrometry. This process typically involves the addition of some well-defined functional groups. The goals of derivatization vary depending on the application but typically include increased volatility, greater thermal stability, modified chromatographic properties, greater ionization efficiency, favourable fragmentation properties, or a combination of these [5-9].

Mass spectrometers convert molecules into ions, which are then manipulated using electric and magnetic fields. This process requires 3 main components as follows:

- **Ion source:** A sample is placed into the mass spectrometer, which is then ionized by the apparatus.
- **Mass analyzer:** Ions are sorted in the device based on their mass-to-charge ratio (m/z).
- **Detector:** Ions are measured and displayed on the mass spectrum chart.

Principle

- Mass spectroscopy is most accurate method for determining the molecular mass of the compound and its elemental composition.
- In this technique, molecules are bombarded with a beam of energetic electrons.
- The molecules are ionized and broken up into fragments, some of which of positive ions.
- Each kind of ion has ratio of mass charges that is m/e ratio (value) [2,10].

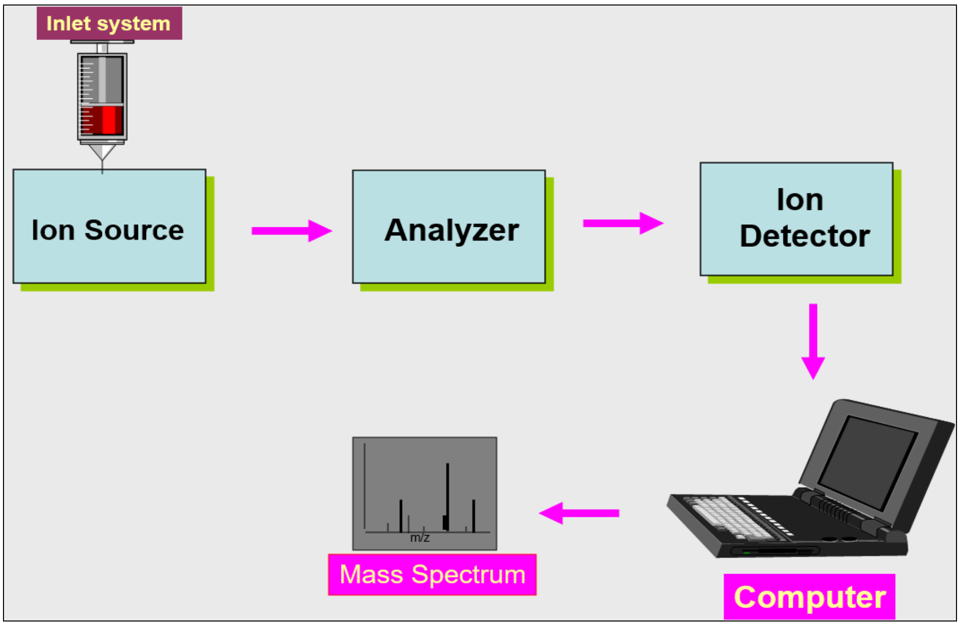

Fig 2: Components of a mass spectrometer

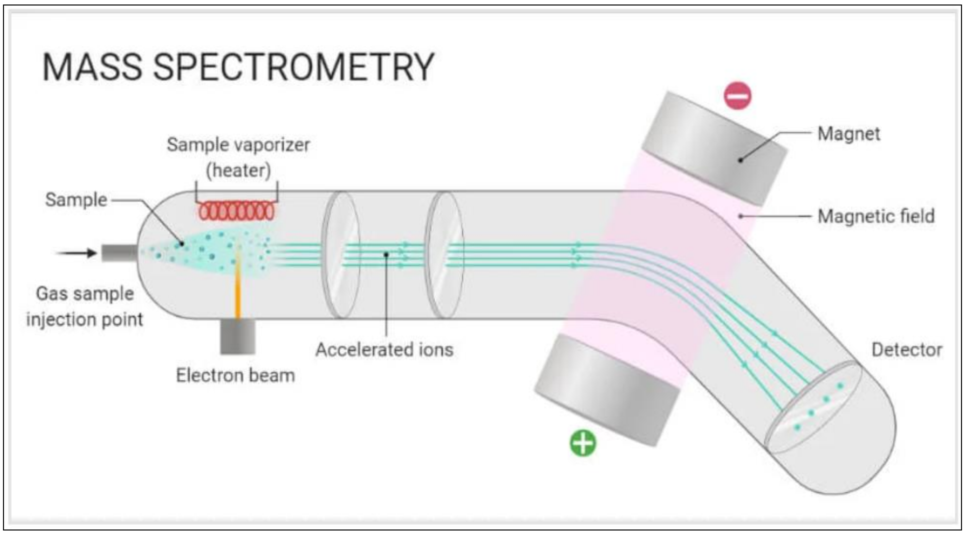

Fig 3: Principle of mass spectrometry

Applications of mass spectroscopy

Mass Spectroscopy (MS) is a powerful and versatile analytical tool used in many scientific fields. Here are some of its major applications:

1. Identification of Unknown Compounds

- Determining molecular weight and structural features of unknown substances.
- Useful in organic chemistry to identify newly synthesized molecules.

2. Structural Elucidation of Molecules

- Helps in determining the structure of complex molecules by analysing the fragmentation patterns.
- Commonly used in studying natural products, polymers, and complex organic molecules.

3. Quantitative and Qualitative Analysis

- Quantifies the amount of substance in a mixture with high sensitivity and accuracy.
- Determines presence/absence of specific components in complex mixtures.

4. Proteomics and Genomics

- Identifies proteins, peptides, and nucleic acids.
- Used in protein sequencing and post-translational modification analysis.
- Plays a key role in biological and medical research.

5. Pharmaceutical Industry

- Drug discovery and development - helps identify drug candidates and their metabolites.
- Pharmacokinetics and pharmacodynamics studies.
- Quality control and purity analysis of pharmaceutical products.

6. Environmental Analysis

- Detects pollutants, pesticides, and toxins in air, water, and soil.
- Used in trace analysis for monitoring environmental safety.

7. Food Industry

- Detection of contaminants, such as pesticides, toxins, and additives.
- Authentication of food products (e.g., verifying origin, species of meat or fish).
- Quality control and flavour compound analysis.

8. Clinical and Biomedical Research

- Disease biomarker identification (e.g., for cancer, metabolic disorders).
- Therapeutic drug monitoring.
- Analysing metabolomics and lipidomics (study of metabolites and lipids in biological systems).

9. Forensic Science

- Drug testing, identification of illicit substances.
- Toxicology analysis - identifying poisons or drugs in biological samples.
- Explosives and chemical warfare agent detection.

10. Petrochemical Industry

- Analysis of hydrocarbons, fuels, and lubricants.
- Characterization of crude oil components and additives [11-15].

Clinical significance

Mass spectrometry is applicable across diverse fields, including forensic toxicology, metabolomics, proteomics, pharma/biopharma, and clinical research. Specific applications of mass spectrometry include drug testing and discovery, food contamination detection, pesticide residue analysis, isotope ratio determination, protein identification, and carbon dating.

- **Applications of mass spectrometry in the diagnosis of disease**

Mass spectrometers are primarily used in clinical settings to diagnose diseases due to biomarkers. Biomarkers are used in diagnoses, prognoses, and treatment. For example, the enzyme amylase can be used as a biomarker for pancreatitis in disease diagnosis. Similarly, natriuretic peptides are monitored in patients with cardiovascular diseases to predict patient outcomes. Mass spectrometry can be used to examine the metabolic profile of pharmacologic agents to monitor the efficacy of certain therapies.

Mass spectrometers can measure biomarkers, ranging in size from small molecules to large macromolecules. The principle of mass spectrometers also applies to human body samples such as plasma and serum blood, urine, saliva, sweat, and skin secretions. Using liquid or gas chromatography allows for biomarkers to be separated and analyzed in smaller pieces, which optimizes the sensitivity and specificity of the spectrometer. Thus, mass spectrometers thereby improve clinical decision-making. Mass spectrometers analyze a sample and its biomarker profile to diagnose disease. Of note, 2 critical biomarkers are proteins and lipids. In earlier examples, it was stated that enzymes, which are proteins, can be used to detect diseases. Similarly, a lipid panel has often been used to diagnose diseases such as metabolic syndrome.

If the biomarker is a protein, the principles of proteomics can be applied to mass spectrometry. Mass spectrometry has been used to quantify differences between 2 biological states of proteomes; a set of proteins produced in a specific organism. A protein sample is placed into the spectrometer, and the mass of the individual components of the protein is tagged based on the isotopes generated through spectral analyses. The mass spectrometer is useful in this setting because it generates spectral data for proteomes, allowing the human body to analyze proteins in urine and serum.

If the biomarker is a lipid, the principles of lipidomics can be applied to generate a clinical profile for the patient using mass spectrometry. Lipidomics consists of the lipid profile and set of reactions generated within biology. Lipids are used for energy storage and assist in the endocrine regulation of the body system. Mass spectrometry has been used to characterize masses of important ingredients in lipid oxidation reactions, helping quantify the various molecules of lipid reactions and, thus, their biological properties within the human body.

- **Applications of mass spectrometry in COVID-19**

During the peak of the coronavirus pandemic, many countries were hindered by inadequate testing due to supply chain shortages and inconsistencies in mass-produced testing kits. Clinical laboratories thus created a method to use mass spectrometry. Nasal swab samples of patients were analyzed using mass spectrometry, and the pattern generated by the spectrum was used to categorize patients if they were COVID-19 positive or negative.

- **Applications of mass spectrometry in pharmaceuticals**

Mass spectrometry plays a crucial role in the analysis of pharmaceutical drugs. The ionization process within the apparatus helps differentiate the molecules that create the drugs. This capability is essential for conducting faster and more accurate screenings during clinical analysis of patient samples, leading to improved drug monitoring and safety.

- **Applications of mass spectrometry in the analysis of glycans**

Oligosaccharides are molecules formed by associating several monosaccharides linked through glycosidic bonds. Determining the complete structure of oligosaccharides is more complex compared to that of proteins or oligonucleotides. This process involves the determination of additional components because of the isomeric nature of monosaccharides and their capacity to form linear or branched oligosaccharides. Knowing the structure of an oligosaccharide requires not only the determination of its monosaccharide sequence and its branching pattern but also the isomer position and the anomeric configuration of each of its glycosidic bonds. Advances in glycobiology involve a comprehensive study of the structure, biosynthesis, and biology of sugars and saccharides. Mass spectrometry is emerging as an enabling technology in the field of glycomics and glycobiology.

- **Applications of mass spectrometry in the analysis of oligonucleotides**

Oligonucleotides, DNA or RNA, are linear polymers of nucleotides. These nucleotides are composed of a nitrogenous base, a ribose sugar, and a phosphate group. Oligonucleotides may undergo several natural covalent modifications, which are commonly present in transfer RNA and ribosomal RNA, or unnatural ones resulting from reactions with exogenous compounds. Mass spectrometry plays a vital role in identifying these modifications and determining their structure and position in the oligonucleotide. This technique allows the determination of the molecular weight of oligonucleotides and, directly or indirectly, their sequences.

- **Applications of mass spectrometry in environmental analysis**

Drinking water testing, pesticide screening and quantitation, soil contamination assessment, monitoring carbon dioxide levels and pollution and conducting trace elemental analysis of heavy metals leaching.

- **Applications of mass spectrometry in forensic analysis**

In forensic science, mass spectrometry is used to analyze trace evidence, such as fibers from carpets and polymers found in paints. Mass spectrometry is also essential in arson investigations to detect fire accelerants, confirm drug abuse, and identify explosive residues in bombing investigations.

- **Quality control and lab safety**

When working in the laboratory, it is crucial to exercise caution while preparing samples before introducing the compound into the mass spectrometer for analysis. For example, using the proper measuring tools for accuracy during chromatography is essential. If quality control is not used, resultant peaks may form in the mass spectrum, which does not correspond to ions within the sample.

Operating the mass spectrometer requires safety to be taken into consideration. The mass spectrometer apparatus can have high temperatures, resulting in burns. Thus, it is important not to touch any part of the apparatus while the spectral data is generated. In addition, ionization occurs within the device, so it is essential to be cautious when running a mass spectrometer. Lastly, the samples placed in the spectrometer could be hazardous upon exposure to the skin or inhalation. Thus, wearing the appropriate personal protective equipment, such as safety glasses, long pants, closed-toed shoes, a long lab coat, and gloves, is highly recommended.

- **Enhancing healthcare team outcomes**

Interprofessional communication is essential when using mass spectrometers. For example, if a patient presents to the emergency department after ingesting an unknown compound, lab technicians and pathologists can analyze the patient's sample to identify the components present in the substance. This collaboration enables laboratory professionals to support healthcare workers, including nurses and physicians, in hospital or clinic settings. In the emergency room, clinicians play a crucial role in fostering this interprofessional environment by sharing the patient's medical history and physical examination findings. This information can help narrow the possible compounds in the patient's sample, facilitating a more accurate and timely diagnosis and treatment plan [15-20].

Conclusion

Mass spectrometry is an analytical tool used for measuring the molecular mass of a sample. For large samples such as Biomolecules, molecular masses can be measured to within an accuracy of 0.01% of the total molecular mass of the sample. i.e. within 4 Daltons (Da) or atomic mass units (amu) error for a sample of 40,000 Da. Structural information can be generated using certain types of Mass spectrometer. Usually those with Multiple Analysers which are known as Tandem Mass Spectrometers. This is achieved by fragmenting the sample inside the instrument and analysing the products generated This procedure is useful for the structural elucidation of organic compounds and for peptide or oligonucleotide sequencing. Mass spectrometers are used in industry and academia for both routine and research purposes. Mass spectrometer helps to determine the ionization potential value. (i.e. The minimum energy required to convert the neutral molecule to an ion). By employing ionization efficiency curves (from plots of peak height against beam energy).

Conflicts of interest

None.

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