Review Article

Mass spectrometry: An analytic tool in biological research

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Abstract
Mass spectrometry has become an essential analytical tool in biological research, enabling the characterization of a wide range of biomolecules, including proteins, sugars, and oligonucleotides. The technique involves the ionization of molecules, followed by their separation based on the mass-to-charge ratio (m/z), and subsequent detection. The integration of MS with advanced techniques such as liquid chromatography allows for high-resolution analysis of complex biological samples. This paper discusses the components, processes and applications of mass spectrometry in biological studies.

Keywords: Mass spectrometry, mass/charge ratio, liquid chromatography, ionization, MALDI

Introduction

The interaction between electromagnetic radiation and matter forms the basis for spectrometry. Mass Spectrometry (MS) is a potent tool for identifying unknown compounds and studying molecular structure based on the fundamental principles of chemistry. In it, the compound to be characterised is ionised and the ionic molecules are then separated based on their mass/charge ratio (m/z) and the number of ions representing each mass/charge unit is recorded as a spectrum. [1] It has become an essential analytical tool in biological research and can be used to characterise a wide variety of biomolecules such as proteins, sugars and oligonucleotides. The ions formed are very reactive and short-lived. [2,3] So, their formation and manipulation must be conducted in a vacuum to minimise ion-molecule reactions, scattering, and neutralisation of the ions.

Components

A mass spectrometer consists of the following components: the inlet system, ion source, mass analyser and detector (Figure 1).

Inlet system

The function of an inlet system is to introduce a small amount of sample into the ion source with minimal loss of vacuum. The samples should be in the vapour phase prior to ionisation. Gases and samples with high vapour pressure are introduced directly into the source region of the mass spectrometer through a needle valve. Liquids and solids are usually heated to increase the vapour pressure for analysis. Modern mass spectrometers are equipped with different kinds of inlet systems like batch inlets, direct probe inlets, chromatographic and capillary electrophoretic inlet systems. The batch inlet system is the conventional and simplest one where the sample is volatilised externally and then allowed to leak into the evacuated

ionisation region. Direct probe inlets are used for the injection of solid and non-volatile liquid into the ionisation region by means of a sample probe. Chromatographic inlet systems in Gas Chromatographic Mass Spectrometry (GC-MS) and Liquid Chromatographic Mass Spectrometry (LC-MS), capillary electrophoretic units in Capillary Electrophoresis Mass Spectrometry (CE-MS) are employed for the separation and identification of the components in the sample mixture by giving independent mass spectra. Gas chromatography is the commonly used technique for introducing volatile samples into a mass spectrometer while liquid chromatography is used for thermally labile compounds which are not easily separated by gas chromatography.

Ion source

A sample molecule for analysis by mass spectrometer must be converted to gas phase-charged particles by ionisation process. In the ion source region, neutral sample molecules are ionised and then accelerated into the mass analyser. The ions are generated by inducing either the loss or gain of charge. The various methods of ionisation include electron ionisation, chemical ionisation, desorption ionisation and electrospray ionisation.

In electron ionisation, a beam of high energy electrons strikes the molecules. The electron molecule collision releases an electron from the molecule creating a cation or electron gain to produce anions as follows.

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

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

The product in both instances is a radical, and also an ion. Hence it is represented as $M^{\bullet +}$ or $M^{\bullet -}$, + and – signs indicate ionic state and dot (.) represents a radical. It may be called a radical ion or molecular ion or parent ion. In chemical ionisation, the sample molecules are combined with an ionised reagent gas. On collision with ionised reagent gas (methane, ammonia, isobutene), sample molecules are ionised by proton transfer, electron transfer, and adduct formation. In desorption ionisation, the sample to be analysed is dissolved in a matrix and placed on a high energy beam of ions or high intensity photons. When high intensity photons are used it is termed MALDI (Matrix-assisted laser desorption/ionisation). [4] The analyte is placed in a light absorbing solid matrix which protects the analyte from being destroyed by direct laser beam. The matrix is composed of energy absorbing molecules such as 2,5-dihydroxybenzoic acid or cyano-4-hydroxycinnamic acid. With a short pulse of laser light, the analytes are ionised into gas phase and desorbed from the matrix into the vacuum system. In electrospray ionisation (ESI), the samples are dissolved in a polar, volatile solvent and pumped through a narrow, stainless-steel capillary. A high voltage of 3 or 4 kV is applied to the tip of the capillary. The sample emerging from the tip is dispersed into an aerosol of highly charged droplets. The solvent evaporates and ions are released from the droplets. It is one of the most important techniques for analysing biomolecules such as polypeptides, proteins, and oligonucleotides having molecular weights of 100,000 Da or more**.** It produces multiply charged ions based on ion evaporation process. [5,6]

Mass analysis

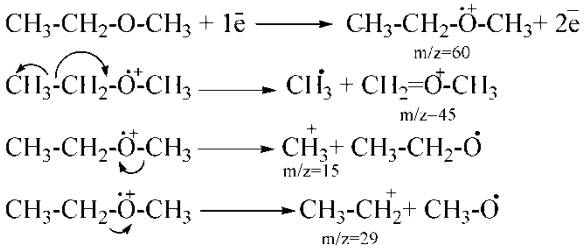
The mass analyser is the main component of the mass spectrometer. Once the sample has been ionised, the ions are accelerated by an electric field into mass analyser where the ions are separated based on their m/z ratio and finally detected. General types of mass analysers are quadrupole mass analyser, time of flight mass analyser (TOF), magnetic sector mass analyser, electrostatic sector mass analyser, quadrupole ion trap mass analysers and ion cyclotron resonance. Quadrupole mass analyser and time of flight mass analyser are the common mass analysers. In quadrupole mass analyser, ions are transmitted through an electric field created by an array of four parallel metal rods, the quadrupole. TOF mass analyser measures ion flight time. The arrival time to the detector is dependent upon mass, charge and kinetic energy of the ions. The velocity of two ions with the same kinetic energy will vary depending on their masses. The lighter ion will have higher velocity and reach the detector first. Ion trap mass analysers function to trap molecular ions in a 3-D electric field which increases sensitivity.

Detector

The ion collection system measures the relative abundance of ion fragments of each mass. Several types of detectors are available for mass spectrometers. Photographic plates are used in older instruments. Electron multiplier tube, Faraday cup and Array detectors are commonly used.

Interpretation

The m/z ratio is used to describe ions observed in mass spectrometry (MS). A mass spectrum is represented as a vertical bar graph, in which each bar represents an ion having a specific m/z ratio and the length of the bar indicates the relative abundance of the ion (Figure 2). "m" is the numerical value for the mass of the ion and "z" is the numerical value for the charge of the ion which is equal to the number of electrons lost or gained. Mass of the ion is expressed in Dalton (Da) for polymers, peptides and other large molecules. Generally, one electron is lost during ionisation, so z is 1 and the m/z value is equivalent to the relative molecular mass of the ion. The mass fragmentation of ether is shown below



Molecular ions are the intact ionised analyte molecule. The molecular ion provides the molecular mass of the analyte, and it will be the highest mass in a spectrum (parent peak) and it is the first clue to interpret a mass spectrum (Figure 2). Fragment ions are formed by subsequent fragmentation of molecular ions. The base peak is the tallest peak because it represents the commonest, stable fragment ion to be formed. This is usually given an arbitrary height of 100, and the height of other ions is relative to this. The nitrogen rule, illogical peaks, isotope effects, and their relative abundance are also to be considered for mass interpretation.

The structural information is obtained from the fragmentation patterns of the mass spectrum. Functional groups and overall structure determine how some portions of molecules will resist fragmenting, while other portions will fragment easily. The mass spectra of many compounds have been published and may be used to identify unknowns. Mass spectral libraries are also used for their identification.

Applications in Biology

Mass spectrometry has become one of the most widely used analytical techniques in life sciences. It is widely used to measure the molecular mass of biomolecules such as polypeptides and in nucleic acid sequencing and elucidation of protein structure. It is also used in the analysis of complex biological systems, drug metabolism, lipid analysis, metabolomics, quantitative proteomics, and clinical microbiology. [7,8]

Tandem MS is the combination of two mass analysers in one mass spectrometer instrument (Figure 3). Tandem MS is used to identify compounds in a mixture. The mass analysis of the mixture by the first analyser would give a mixed spectrum. In such instances, a particular peak is selected and then subjected to further degradation and analysis using a second analyser. [9] For example, in protein identification, a protein is treated with protease and hydrolysed into a mixture of peptides. The mixture is then injected into the mass spectrometer. The first mass analyser sorts different peptides so that only one peptide is selected for further analysis. The selected peptide is further fragmented in the collision cell with a collision gas. CO-NH, CH-CO, NH-CH bonds are broken to produce neutral and charged species. The m/z of product ions is measured in the second mass analyser. This is usually done in a triple quadrupole MS or a Q-TOF. MALDI-TOF is commonly used for peptide mass fingerprinting, protein identification and in large scale proteomics work because of its speed and

sensitivity. It also helps in qualitative and quantitative analysis of amino and organic acids.

Since MS enables accurate determination of molecular mass of a protein, it is the most efficient way to identify proteins and evaluate their purity based on comparison of the data obtained from the MS with those predicted for all the proteins contained in a database. This could be extended for rapid verification of the fidelity and homogeneity of proteins produced by genetic engineering. Soft ionisation methods like ESI can be used for the study of the non-covalent interactions of proteins. MALDI-TOF is also used for the precise identification of genus and species of bacteria and analysis of antibiotic resistance based on their protein profile, carbapenem hydrolysis product or antibiotic biomarkers.

The protein produced by the ribosome may undergo several post-translational modifications such as glycosylation, disulfide bridge formation, phosphorylation, sulfation, hydroxylation, carboxylation, acetylation etc. MS enables identification and localization of such modifications based on their mass differences. MS allows precise determination of the molecular weight of oligonucleotides and their sequencing. They can be analysed in both positive and negative ion mode, the latter having better sensitivity and resolution.

GC-MS has been used for the identification of monosaccharides and very small oligosaccharides, determination of their structure and quantification. Analysis of fatty acids, acylglycerols, bile acids, phospholipids, steroids, prostaglandins, sphingolipids and leukotrienes can be done successfully by MS. MS and Nuclear magnetic resonance (NMR) spectroscopy are the most widely used techniques for metabolome analysis. In comparison with NMR, MS is more sensitive and, thus, can also be used for compounds of lower concentration.

GC-MS and LC-MS are widely used to separate and identify a broader range of compounds with minimal sample preparation of pure compounds or compound mixtures of plant or animal origin. Mass fragmentation patterns produced by these compounds are specific and used for the elucidation of compound structure. They are also used in quantifying pesticides in water samples and identifying steroids in athletes.

Along with various separation techniques, MS can be used for the isolation and structure elucidation of various bioactive compounds.

Conclusion

Several modifications and improvisations are being added in the field of spectroscopy. Hybrid mass spectrometers like Electromagnetic analysers coupled

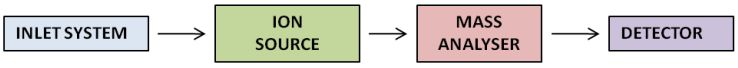


Fig.1. Components of a mass spectrometer.

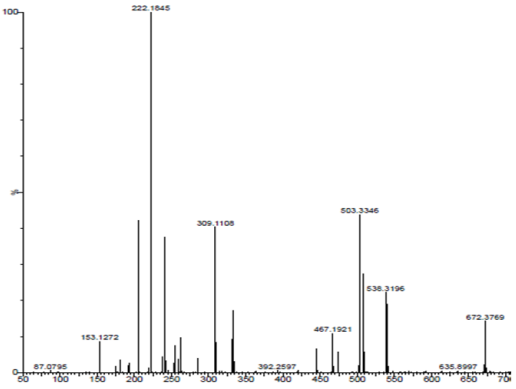


Fig.2. Mass spectrum showing molecular ion at m/z 672.37 and base peak at m/z 222.18 and other fragment ions

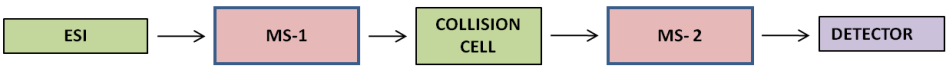


Fig.3. Schematic representation of MS/MS.

to quadrupoles or ion trap, Ion trap analyser combined with time-of-flight or ion cyclotron resonance are developed by using several types of analysers. It gives more sensitivity and accuracy in mass analysis of biomolecules. Knowledge of mass spectroscopy, its application and interpretation of the data have greatly improved the quality of research dealing with biomolecules, particularly phytocompounds.

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Conflict of interest
There are no conflicts of interest.

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