Review Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2022, Volume 10, Issue 01
ISSN: 2321–3647(online)

Mass Spectroscopy and Ionization Techniques

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ABSTRACT

Mass Spectrometry- It is an analytical technique that generates charged particles in the form of ions from the substance to be analyzed to measure its mass to charge ratio. Ion Source plays an important role for generation of charged ions which further travels through analyzer and ends at detector. The production of intact molecular ions can be achieved under adequate experimental conditions, with minimal fragmentation known as soft ionization method. Neutral species either loss or gain of charge to generates ions. This paper covers an information about various type of mass ion Source (Electron impact, Chemical ionization and field ionization, Desorption-Field desorption, Electro spray ionization, matrix assisted desorption ionization, Plasma desorption) are in use for mass spectroscopy. Ions get affected by change in electrical, magnetic and radiofrequency effect as they containing either positive or negative charges this leads to better resolution of ions.

Keywords: Mass Spectroscopy, Ion source, Ionization, Desorption.

*Corresponding Author Email: ibrahim4468786@gmail.com
Received 11 December 2021, Accepted 03 January 2022

Please cite this article as: Malleswari K *et al*., Mass Spectroscopy and Ionization Techniques. American Journal of Pharmacy & Health Research 2022.

INTRODUCTION

An analytical technique which defines the chemical composition of compound or sample with reference to their mass to charge ratio is known as mass spectroscopy[1]. Ionization of sample molecule it gets converted to charged species which is permitted to enter in mass spectrometer. This charged species is in the gaseous form used for analysis, fragmentation and detection[2]. Proteins or peptides or oligosaccharide are the common compounds that get analyzed by mass spectrometer. Mass spectrometer instrument get separates into six divisions Ionization source is a mechanical device which allows ionization. Various methods of ion generation are protonation (Positive ions), Cationization (Positive ions), Deprotonation (Negative ions), Transfer of charged molecules to gas phase (which generate positive and negative ions), Electron ejection (Positive ions) and electron capture (for the generation of negative ions). The production of intact molecular ions can be achieved under adequate experimental conditions, with minimal fragmentation known as ionization method. Ion source in mass spectroscopy plays a vital role for the generation of ions.

Principle

A mass spectrometer generates multiple ions from the sample under investigation, it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type. The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation. Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on. The ions are separated in the mass spectrometer according to their mass-to-charge ratio and are detected in proportion to their abundance. A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus massto-charge ratio. Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound.

Instrumentation

Table: Division of mass spectrometer

S.NO	Division of Mass spectrometer	Type
1	1 Inlet system Solid, Liquid and Gas 1 Inlet system Solid, Liquid and Gas 1 Inlet system Solid, Liquid and Gas Inlet system	Solid, Liquid and Gas
2	Ion source	Desorption- Field desorption, Electro spray

		ionization, Matrix assisted desorption ionization, Plasma desorption, Fast atom bombardment, Secondary ion mass Spectrometry and thermospray ionization Gas phase- Electron impact, Chemical ionization and filed ionisation
3	Mass analyzer	Quadrupole , TOF, Ion Trap, FTICR
4	Ion detector	Electron multiplier, Faraday cups , Photographic plates, Scintillation counter, Channel electron multipliers, Resistive anode, Encoder image detector, High mass detection detectors Conversion dinodes – Helium leak detectors, Advance detectors Other detectors – TQD Tandem Quadrupome MS Detectors, Photonics BI Polar maldi TOF detector, flixer SQ 300 MS detectors Cryogenic detectors- Multixel photon counter
5	Vacuum system	-
6	Recorder	-

INLET SYSTEM

The inlet system can be as simple as a port through which the sample is injected or inserted into a chamber at high vacuum and heated to achieve vaporization. If the sample is a mixture of compounds, a gas chromatographic inlet system will vaporize the sample and separate the mixture into its components. Mass spectra for each of the components are recorded in succession as the components enter the mass spectrometer. Hence, mass spectra of individual components of a complex mixture can be obtained without prior separation. The mass spectrometer available to the undergraduate organic chemistry teaching laboratory has a gas chromatographic inlet system.

Ion sources for mass spectrometry

Ionization is essential for any MS analysis, for which there are many methods suited to different sample types and applications. Broadly, these can be broken down into gas phase methods, desorption methods, and spray methods. An outline of each is given below.

GAS PHASE METHODS

Electron ionization (EI)

Analyte molecules must be in the vapor phase to allow effective interaction with the energetic electrons produced in a vacuum by a heated filament. EI can be considered a fairly harsh method of molecule fragmentation and ionization and is most commonly used when samples are relatively volatile and have low molecular weight.[3]

Chemical ionization (CI)

A gas is introduced into an EI ionization chamber at a concentration higher than the analyte. The interaction of the carrier gas with the electrons will produce several molecular ions, which will subsequently react further with the excess carrier gas and form different molecular ions. These ions will then react with the analyte molecules to form analyte molecular ions through several different mechanisms. CI is a very soft ionization technique and does not lead to extensive fragmentation.[4]

DESORPTION

Matrix assisted laser desorption ionization (MALDI)

A "matrix", dictated by the type of molecule to be detected, is added in excess to the sample to be analyzed. The sample is then irradiated by a laser, vaporizing the analyte molecules with little to no fragmentation or decomposition. Both positively and negatively charged ions can be created. MALDI is one of the major "soft" ionization methods, particularly useful for the analysis of large or labile molecules.[5]

Fast atom bombardment (FAB)

A beam of accelerated ionized atoms is focused onto the sample to be analyzed, ejecting and ionizing target analyte.[6,7] This is a soft ionization technique, able to produce positively and negatively charged ions.

Thermal ionization sources

Heated Cs, producing positive ions, is the most common primary ion source and can be focused with electrostatic ion optics for secondary ion MS.

Plasma ionization sources

Commonly used to produce beams of gaseous ions, electrons are emitted into a gas, often pure oxygen, ionizing it and creating a plasma. The ions can then be filtered by charge and accelerated into a beam.

MASS ANALYSER

Following sample ionization, the ions must be separated and this occurs in the mass analyzer. Commonly used mass analyzers include:

Time-of-flight (TOF)

Ions are separated according to their m/z ratio based on the length of time it takes them to travel through a flight tube of known length to reach a detector.

Quadrupole

Ions entering the quadrupole have their trajectory deflected by electrical potential in a manner that is proportional to their m/z value. Changing the potential allows only ions of specific m/z values to reach the chamber end and be detected.

Magnetic sector

Magnetic fields disperse ions in trajectories according to their m/z ratios in a manner that is analogous to the way a glass prism disperses light into its various wavelengths or colors.

Ion trap

Works similarly to a quadrupole but the electrodes are ring shaped and ions are separated and detected by discharging ions with unstable oscillations from the system and into the detector rather than detecting those with stable oscillations.

ION DETECTOR

Detectors

Electron multiplier (EM)

A serial connection of discrete metal plates that amplify a current of ions by a factor of ~108 into a measurable current of electrons.

Faraday cup (FC)

Ions hitting the collector cause a flow of electrons from ground through the resistor and the resulting potential drop across the resistor is amplified**.**

Photomultiplier conversion dynode

Ions initially strike a dynode, resulting in electron emission. The electrons produced then strike a phosphor screen which in turn releases photons. The photons then pass into the multiplier where amplification occurs in a cascade fashion – much like the EM.[8]

VACCUM SYSTEM

All mass spectrometers operate at very low pressure (high vacuum). This reduces the chance of ions colliding with other molecules in the mass analyzer. Any collision can cause the ions to react, neutralize, scatter, or fragment. All these processes will interfere with the mass spectrum. To minimize collisions, experiments are conducted under high vacuum conditions, typically 10-2 to 10-5 Pa (10-4 to 10-7 torr) depending upon the geometry of the instrument. This high vacuum requires two pumping stages. The first stage is a mechanical pump that provides rough vacuum down to 0.1 Pa (10-3 torr). The second stage uses diffusion pumps or turbomolecular pumps to provide high vacuum. ICR instruments have even higher vacuum requirements and often include a cryogenic pump for a third pumping stage. The pumping system is an important part of any mass spectrometer but a detailed discussion is beyond the scope of this paper.

Separation techniques combined with mass spectrometry

An important enhancement to the mass resolving and mass determining capabilities of mass spectrometry is using it in tandem with chromatographic and other separation techniques.

Gas chromatography

A gas chromatograph (right) directly coupled to a mass spectrometer (left)

Main article: Gas chromatography–mass spectrometry

A common combination is gas chromatography-mass spectrometry (GC/MS or GC-MS). In this technique, a gas chromatograph is used to separate different compounds. This stream of separated compounds is fed online into the ion source, a metallic filament to which voltage is applied. This filament emits electrons which ionize the compounds. The ions can then further fragment, yielding predictable patterns. Intact ions and fragments pass into the mass spectrometer's analyzer and are eventually detected.[9] However, the high temperatures (300°C) used in the GC-MS injection port (and oven) can result in thermal degradation of injected molecules, thus resulting in the measurement of degradation products instead of the actual molecule(s) of interest.[10]

Liquid chromatography

Indianapolis Museum of Art conservation scientist performing liquid chromatography–mass spectrometry Similar to gas chromatography MS (GC-MS), liquid chromatography-mass spectrometry (LC/MS or LC-MS) separates compounds chromatographically before they are introduced to the ion source and mass spectrometer. It differs from GC-MS in that the mobile phase is liquid, usually a mixture of water and organic solvents, instead of gas. Most commonly, an electrospray ionization source is used in LC-MS. Other popular and commercially available LC-MS ion sources are atmospheric pressure chemical ionization and atmospheric pressure photoionization. There are also some newly developed ionization techniques like laser spray.

Capillary electrophoresis–mass spectrometry

Capillary electrophoresis–mass spectrometry (CE-MS) is a technique that combines the liquid separation process of capillary electrophoresis with mass spectrometry.[11] CE-MS is typically coupled to electrospray ionization.[12]

Ion mobility

Ion mobility spectrometry-mass spectrometry (IMS/MS or IMMS) is a technique where ions are first separated by drift time through some neutral gas under an applied electrical potential gradient before being introduced into a mass spectrometer.40 Drift time is a measure of the radius relative to the charge of the ion. The duty cycle of IMS (the time over which the

experiment takes place) is longer than most mass spectrometric techniques, such that the mass spectrometer can sample along the course of the IMS separation. This produces data about the IMS separation and the mass-to-charge ratio of the ions in a manner similar to LC-MS.[13] The duty cycle of IMS is short relative to liquid chromatography or gas chromatography separations and can thus be coupled to such techniques, producing triple modalities such as LC/IMS/MS.[14]

APPLICATIONS

Mass spectrometry has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now commonly used in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds. As an analytical technique it possesses distinct advantages such as: Increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter, reduces background interference, Excellent specificity from characteristic fragmentation patterns to identify unknowns or confirm the presence of suspected compounds, Information about molecular weight, Information about the isotopic abundance of elements, Temporally resolved chemical data. A few of the disadvantages of the method is that it often fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and p-positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions.

Isotope ratio MS: isotope dating and tracing

Mass spectrometry is also used to determine the isotopic composition of elements within a sample. Differences in mass among isotopes of an element are very small, and the less abundant isotopes of an element are typically very rare, so a very sensitive instrument is required. These instruments, sometimes referred to as isotope ratio mass spectrometers (IR-MS), usually use a single magnet to bend a beam of ionized particles towards a series of Faraday cups which convert particle impacts to electric current. A fast on-line analysis of deuterium content of water can be done using flowing afterglow mass spectrometry, FA-MS. Probably the most sensitive and accurate mass spectrometer for this purpose is the accelerator mass spectrometer (AMS). This is because it provides ultimate sensitivity, capable of measuring individual atoms and measuring nuclides with a dynamic range of ~1015 relative to the major stable isotope.[15] Isotope ratios are important markers of a variety of processes. Some isotope ratios are used to determine

the age of materials for example as in carbon dating. Labeling with stable isotopes is also used for protein quantification. (see protein characterization below)

Membrane-introduction mass spectrometry: measuring gases in solution

Membrane-introduction mass spectrometry combines the isotope ratio MS with a reaction chamber/cell separated by a gas-permeable membrane. This method allows the study of gases as they evolve in solution. This method has been extensively used for the study of the production of oxygen by Photosystem II.[16]

Trace gas analysis

Several techniques use ions created in a dedicated ion source injected into a flow tube or a drift tube: selected ion flow tube (SIFT-MS), and proton transfer reaction (PTR-MS), are variants of chemical ionization dedicated for trace gas analysis of air, breath or liquid headspace using well defined reaction time allowing calculations of analyte concentrations from the known reaction kinetics without the need for internal standard or calibration. Another technique with applications in trace gas analysis field is secondary electrospray ionization (SESI-MS), which is a variant of electrospray ionization. SESI consist of an electrospray plume of pure acidified solvent that interacts with neutral vapors. Vapor molecules get ionized at atmospheric pressure when charge is transferred from the ions formed in the electrospray to the molecules. One advantage of this approach is that it is compatible with most ESI-MS systems.[17,18]

Pharmacokinetics

Pharmacokinetics is often studied using mass spectrometry because of the complex nature of the matrix (often blood or urine) and the need for high sensitivity to observe low dose and long time point data. The most common instrumentation used in this application is LC-MS with a triple quadrupole mass spectrometer. Tandem mass spectrometry is usually employed for added specificity. Standard curves and internal standards are used for quantitation of usually a single pharmaceutical in the samples. The samples represent different time points as a pharmaceutical is administered and then metabolized or cleared from the body. Blank or t=0 samples taken before administration are important in determining background and ensuring data integrity with such complex sample matrices. Much attention is paid to the linearity of the standard curve; however it is not uncommon to use curve fitting with more complex functions such as quadratics since the response of most mass spectrometers is less than linear across large concentration ranges.[19,20,21]. There is currently considerable interest in the use of very high sensitivity mass spectrometry for micro dosing studies, which are seen as a promising alternative to animal experimentation.

Protein characterization

Mass spectrometry is an important method for the characterization and sequencing of proteins. The two primary methods for ionization of whole proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). In keeping with the performance and mass range of available mass spectrometers, two approaches are used for characterizing proteins. In the first, intact proteins are ionized by either of the two techniques described above, and then introduced to a mass analyzer. This approach is referred to as "top-down" strategy of protein analysis. The top-down approach however is largely limited to low-throughput single-protein studies. In the second, proteins are enzymatically digested into smaller peptides using proteases such as trypsin or pepsin, either in solution or in gel after electrophoretic separation. Other proteolytic agents are also used. The collection of peptide products are often separated by chromatography prior to introduction to the mass analyzer. When the characteristic pattern of peptides is used for the identification of the protein the method is called peptide mass fingerprinting (PMF), if the identification is performed using the sequence data determined in tandem MS analysis it is called de novo peptide sequencing. These procedures of protein analysis are also referred to as the "bottom-up" approach, and have also been used to analyse the distribution and position of post-translational modifications such as phosphorylation on proteins.[22] A third approach is also beginning to be used, this intermediate "middle-down" approach involves analyzing proteolytic peptides that are larger than the typical tryptic peptide.[23]

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