UNIT III

MASS SPECTROMETRY [MS]

Mass spectrometry is the most accurate method for determining the molecular mass of the compound and its element composition. In this technique molecules are bombarded with a beam of energetic electron .the molecules are ionized and broken up into many fragments some of which are positive ion .each kind of ions has a particular ratio of mass to charge m/e ratio. Mass spectrometry is based on the principle that a charged particle [ion] travelling in a magnetic field will travel in a circular path, the radius of which is proportional to the particle's mass/charge ratio [m/e].

Stage 1: Ionisation

The atom is ionized by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Mass spectrometers always work with positive ions.

Stage 2: Acceleration

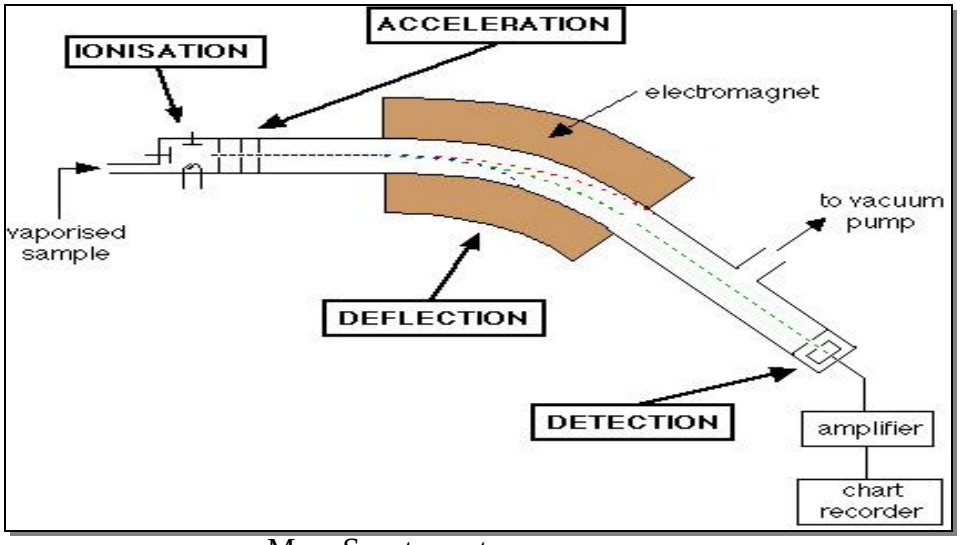
The ions are accelerated so that they all have the same kinetic energy.

Stage 3: Deflection

The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they are deflected.The amount of deflection also depends on the number of positive charges on the ion - in other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.

Stage 4: Detection

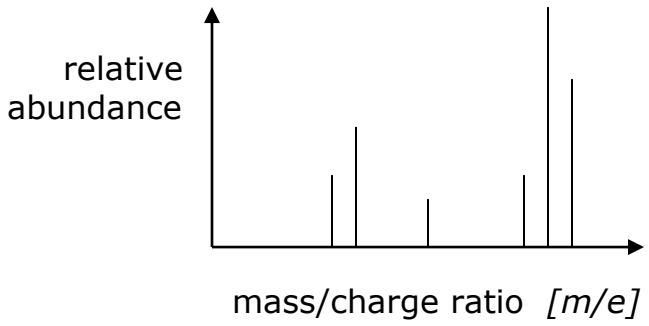
The beam of ions passing through the machine is detected electrically.



Mass Spectrometer

- The sample is heated and vaporized before entering the ionisation chamber

- In the chamber, the sample vapour is bombarded with electrons, which knock off one or more electrons to produce positively charged ions
- These (+) ions are accelerated by an electric field and enter a magnetic field perpendicular to their path
- The magnetic field sends the positively charged ions into a circular path, the radius of which is proportional to their mass/charge [*m/e*] ratio
- The ion collector will only measure ions with a particular *m/e* ratio [the magnetic and electric fields can be varied to measure ions with other *m/e* ratios]
- The *m/e* ratio allows identification of the atoms [or molecules] in the sample and the size of the ion current determines their amount …the data is displayed as a mass spectrum or "stick diagram"



- In the mass spectrum of an element, each peak represents a different isotope of that element the isotopic masses and % abundance being used to calculate the relative atomic mass of the element.
- The mass spectrum of an organic compound is quite different, each line in the spectrum representing either the molecular ion or molecular fragments of it

Fragmentation of an Organic Molecule

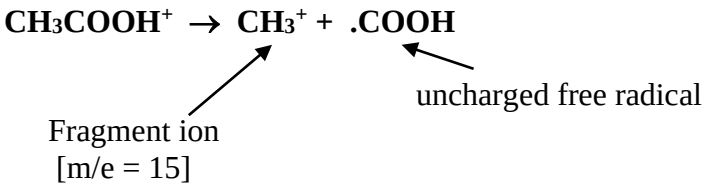
ETHANOIC ACID MASS SPECTROMETER

- a high energy electron knocks 1 electron off the molecule to produce a positive ion

$$CH_3COOH + e^- \rightarrow \underset{\text{molecular ion}}{.CH_3COOH^+} + 2e^-$$

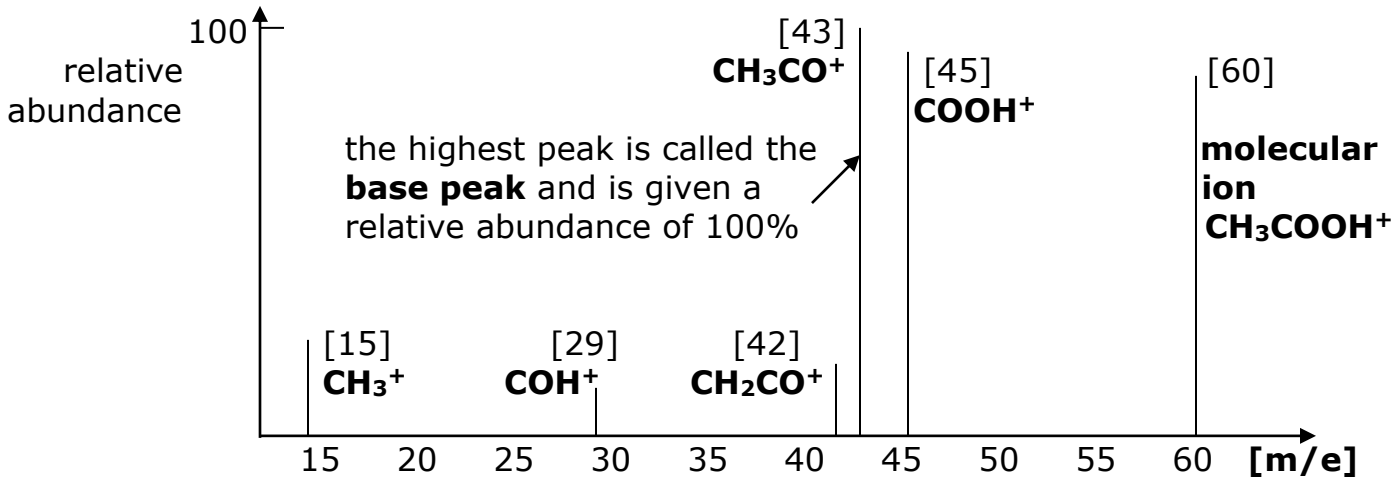
- this molecular ion, $.CH_3COOH^+$, will have the highest m/e ratio [=60] and be found at the end of the mass spectrum

- the molecular ion, being a radical with a bonding electron knocked off it, is chemically unstable and may fragment into smaller fragments.



- Fragmentation continues, producing other positively charged fragment ions and uncharged free radicals. Only the positive ions reach the detector and are included in the mass spectrum.

Simplified Mass Spectrum of Ethanoic Acid [CH_3COOH]



- The presence of and the intensity of an ion peak depends on how readily the fragment forms and how stable it is

IONIZATION TECHNIQUE

1-Chemical ionization [CI]
2-eletrospray ionization [ESI]
3-fast atom bombardment [FAB]
4-Matrix assisted lasser desorption ionization [MALDI]

Chemical ionization (CI) is an ionization technique used in mass spectrometry. Chemical ionization is a lower energy process than electron ionization (EI). The lower energy yields less or sometimes no fragmentation, and usually a simpler spectrum. The lack of fragmentation limits the amount of structural information that can be determined about the ionated species. However, a typical CI spectra has an easily identifiable protonated molecular ion peak $[M+1]^+$ which allows for determination of molecular mass. CI is thus useful in cases where the energy from the bombarding electrons in EI is so great that impact almost exclusively causes fragmentation to occur and molecular ions are not formed in large enough quantities to produce an identifiable molecular ion peak.

In a CI experiment, ions are produced through the collision of the analyte with ions of a reagent gas that are present in the ion source. Some common reagent gases include: methane, ammonia, and isobutane. Inside the ion source, the reagent gas is present in large excess compared to the analyte. Electrons entering the source will preferentially ionize the reagent gas. The resultant collisions with other reagent gas molecules

will create an ionization plasma. Positive and negative ions of the analyte are formed by reactions with this plasma

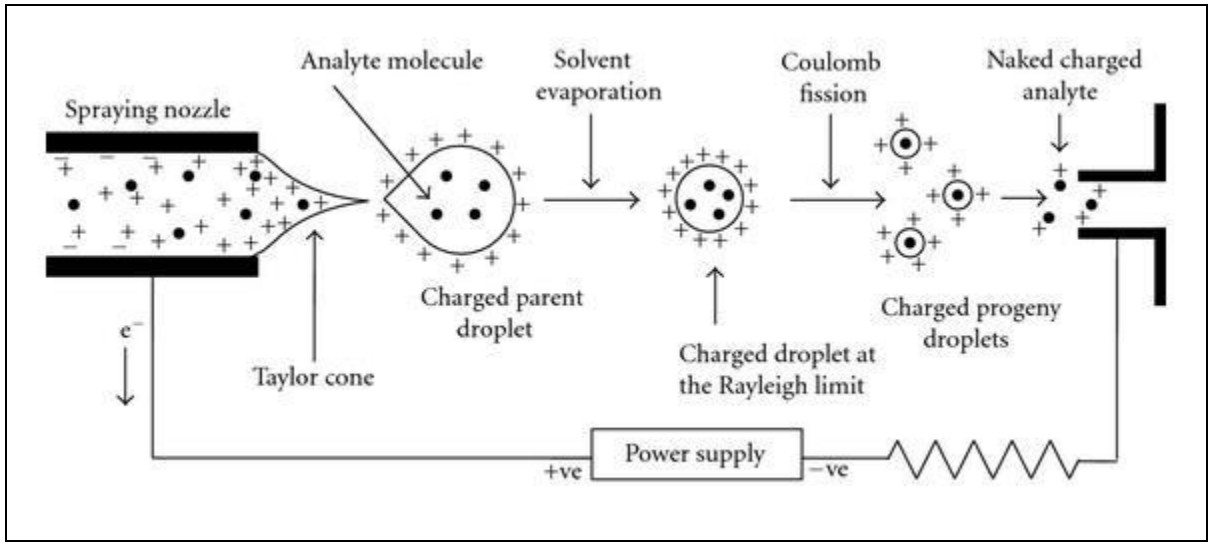
Electrospray ionization (ESI)

is a technique used in mass spectrometry to produce ions using an electrospray in which a high voltage is applied to a liquid to create an aerosol. It is especially useful in producing ions from macromolecules because it overcomes the propensity of these molecules to fragment when ionized. ESI is different from other atmospheric pressure ionization processes (e.g. MALDI) since it may produce multiple charged ions, effectively extending the mass range of the analyser to accommodate the kDa-MDa orders of magnitude observed in proteins and their associated polypeptide fragments. Mass spectrometry using ESI is called electrospray ionization mass spectrometry (ESI-MS) or, less commonly, electrospray mass spectrometry (ES-MS). ESI is a so-called 'soft ionization' technique, since there is very little fragmentation. This can be advantageous in the sense that the molecular ion (or more accurately a pseudo molecular ion) is always observed, however very little structural information can be gained from the simple mass spectrum obtained

IONIZATION MACHNISM

The liquid containing the analyte(s) of interest is dispersed by electro spray, into a fine aerosol. Because the ion formation involves extensive solvent evaporation (also termed desolvation), the typical solvents for electrospray ionization are prepared by mixing water with volatile organic compounds (e.g. methanol, acetonitrile). To decrease the initial droplet size, compounds that increase the conductivity (e.g. acetic acid) are customarily added to the solution. These species also act to provide a source of protons to facilitate the ionization process. Large-flow electrosprays can benefit from nebulization a heated inert gas such as nitrogen or carbon dioxide in addition to the high temperature of the ESI source. The aerosol is sampled into the first vacuum stage of a mass spectrometer through a capillary carrying a potential difference of approximately 3000V, which can be heated to aid further solvent evaporation from the charged droplets. The solvent evaporates from a charged droplet until it becomes unstable upon reaching its Rayleigh limit. At this point, the droplet deforms as the electrostatic repulsion of like charges, in an ever-decreasing droplet size, becomes more powerful than the surface tension holding the droplet together. At this point the droplet undergoes Coulomb fission, whereby the original droplet 'explodes' creating many smaller, more stable droplets. The new droplets undergo desolvation and subsequently further Coulomb fissions. During the fission, the droplet loses a small percentage of its mass (1.0–2.3%) along with a relatively large percentage of its charge (10–18%). There are two major theories that explain the final production of gas-phase ions: the ion evaporation model (IEM) and the charge residue model (CRM). The IEM suggests that as the droplet reaches a certain radius the field strength at the surface of the droplet becomes large enough to assist the field desorption of solvated ions. The CRM suggests that electrospray droplets undergo evaporation and fission cycles, eventually leading progeny droplets that contain on average one analyte ion or less. The gas-phase ions form after the remaining solvent molecules evaporate, leaving the analyte with the charges that the droplet carried.A large body of evidence, which is consider either direct or indirect that small ions (from small molecules) are liberated into the gas phase through the ion evaporation mechanism while larger ions (from folded proteins for instance) form by charged residue mechanism A third model invoking combined charged residue-field emission has been proposed. Another model called chain ejection model (CEM) is proposed for disordered polymers (unfolded proteins). The ions observed by mass spectrometry may be quasimolecular ions created by the addition of a hydrogen cation and denoted $[\mathbf{M} + \mathrm{H}]^+$, or of another cation such as sodiumion, $[\mathbf{M} + \mathrm{Na}]^+$, or the removal of a hydrogen nucleus, $[\mathbf{M} - \mathrm{H}]^-$. Multiply charged ions such as $[\mathbf{M} + n\mathrm{H}]^{n+}$ are often observed. For

large macromolecules, there can be many charge states, resulting in a characteristic charge state envelope. All these are even-electron ion species: electrons (alone) are not added or removed, unlike in some other ionization sources. The analytes are sometimes involved in electrochemical processes, leading to shifts of the corresponding peaks in the mass spectrum. This effect is demonstrated in the direct ionization of noble metals such as copper, silver and gold using electrospray



Electro spray ionization

FAST ATOM BOMBARDMENT (FAB)

Fast atom bombardment (FAB) is an ionization technique used in mass spectrometry in which a beam of high energy atoms strikes a surface to create ions. It was developed by Michael Barber at the University of Manchester. When a beam of high energy ions is used instead of atoms (as in secondary ion mass spectrometry), the method is known as liquid secondary ion mass spectrometry (LSIMS). In FAB and LSIMS, the material to be analyzed is mixed with a non-volatile chemical protection environment, called amatrix, and is bombarded under vacuum with a high energy (4000 to 10,000 electron volts) beam of atoms. The atoms are typically from an inert gas such as argon or xenon. Common matrices include glycerol,thioglycerol, 3-nitrobenzyl alcohol (3-NBA), 18-crown-6 ether, 2-nitrophenyloctyl ether, sulfolane,diethanolamine, and triethanolamine. This technique is similar to secondary ion mass spectrometry and plasma.

IONIZATION MACHNISM;

FAB is a relatively low fragmentation (soft) ionization technique and produces primarily intact protonated molecules denoted as $[M + H]^+$ and deprotonated molecules such as $[M - H]^-$. The nature of its ionization mechanism is similar to matrix-assisted laser desorption/ionization (MALDI) and chemical ionization

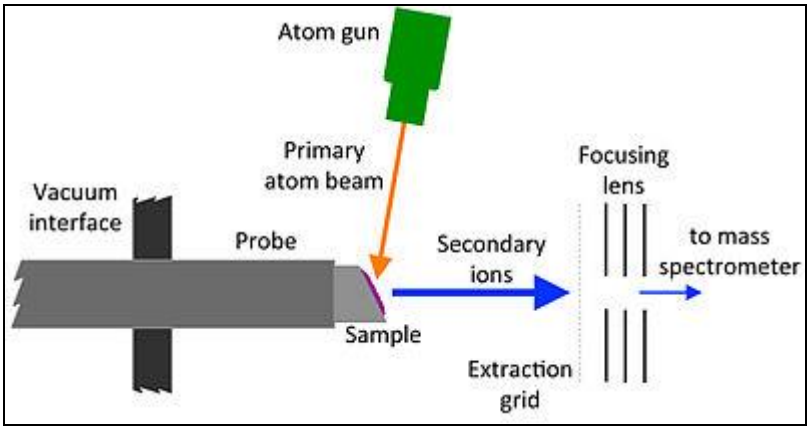
IN CONTINUOUS FLOW FAST ATOM BOMBARDMENT (CF-FAB)

In this method the sample is introduced into the mass spectrometer insertion probe through a small diameter capillary. When a metal frit is used to disperse the liquid on the probe, the technique is known as frit

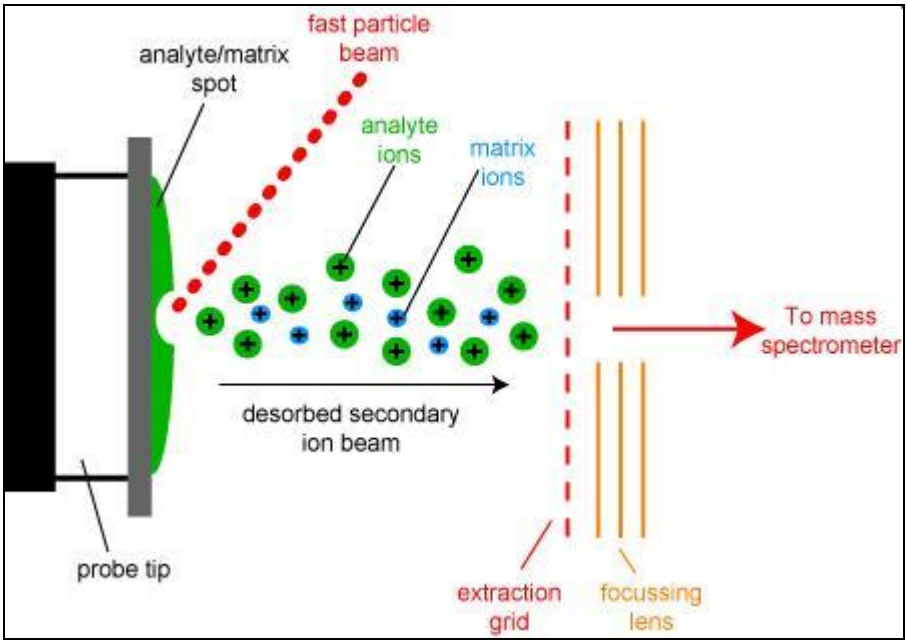
FAB. Samples can be introduced by flow injection, microdialysis, or by coupling with liquid chromatography. Flow rates are typically between 1 and 20 µL/min. CF-FAB has a higher sensitivity compared to static FAB.

APPLICATION OF THE FAB

Elucidation of the amino acid sequence of the oligopeptide efrapeptin D. This contained a variety of very unusual amino acid residues. The sequence was shown to be: N-acetyl-L-pip-AIB-L-pip-AIB-AIB-L-leu-beta-ala-gly-AIB-AIB-L-pip-AIB-gly-L-leu-L-iva-AIB-X. PIP = pipecolic acid, AIB = alpha-amino-isobutyric acid, leu = leucine, iva = isovaline, gly = glycine. This is a potent inhibitor of mitochodrial ATPase activity.



FAB



IONIZATION MACHNISM

MATRIX-ASSISTED LASER DESORPTION/IONIZATION (MALDI)

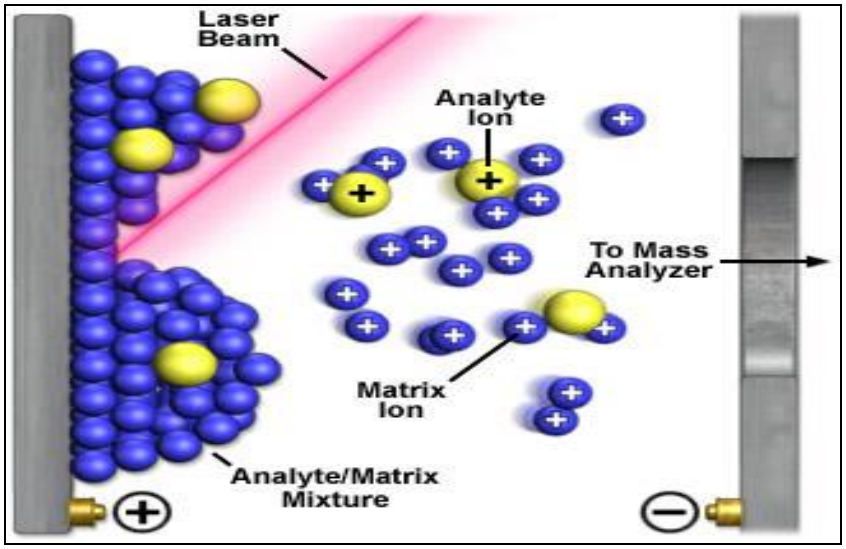
Matrix-assisted laser desorption/ionization (**MALDI**) is a soft ionization technique used in mass spectrometry, allowing the analysis ofbiomolecules (biopolymers such as DNA, proteins, peptides and sugars) and large organic molecules (such as polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods. It is similar in character to electrospray ionization (ESI) in that both techniques are relatively soft ways of obtaining ions of large molecules in the gas phase, though MALDI produces far fewer multiply charged ions.

MALDI methodology is a three-step process. First, the sample is mixed with a suitable matrix material and applied to a metal plate. Second, a pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material. Finally, the analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases, and can then be accelerated into whichever mass spectrometer is used to analyse them

MATRIX AND SAMPLE PREPARATION:

The matrix consists of crystallized molecules, of which the three most commonly used are 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), α-cyano-4-hydroxycinnamic acid (CHCA, alpha-cyano or alpha-matrix) and 2,5-dihydroxybenzoic acid (DHB). A solution of one of these molecules is made, often in a mixture of highly purified water and an organic solvent such as acetonitrile (ACN) or ethanol. A counter ion source such as Trifluoroacetic acid (TFA) is usually added to generate the [M+H] ions. A good example of a matrix-solution would be 20 mg/mL sinapinic acid in ACN: water: TFA (50:50:0.1).

The matrix solution is mixed with the analyte (e.g. protein-sample). A mixture of water and organic solvent allows both hydrophobic and water-soluble (hydrophilic) molecules to dissolve into the solution. This solution is spotted onto a MALDI plate (usually a metal plate designed for this purpose). The solvents vaporize, leaving only the recrystallized matrix, but now with analyte molecules embedded into MALDI crystals. The matrix and the analyte are said to be co-crystallized. Co-crystallization is a key issue in selecting a proper matrix to obtain a good quality mass spectrum of the analyte of interest.



Compound	Solvent	Wavelength (nm)	Applications
2,5-dihydroxy benzoic acid[9]	acetonitrile,water,methanol,acetone,chloroform	337, 355, 266	peptides,nucleotides,oligonucleotides,oligosaccharides
3,5-dimethoxy-4-hydroxycinnamic acid[7][10]	acetonitrile, water, acetone, chloroform	337, 355, 266	peptides, proteins, lipids
4-hydroxy-3-methoxycinnamic acid[7][10]	acetonitrile, water,propanol	337, 355, 266	proteins
α-Cyano-4-hydroxycinnamic acid[11]	acetonitrile, water, ethanol, acetone	337, 355	peptides, lipids, nucleotides
Picolinic acid[12]	Ethanol	266	oligonucleotides
3-hydroxy picolinic acid[13]	Ethanol	337, 355	oligonucleotides

APPLICATION

Biochemistry

In proteomics, MALDI is used for the rapid identification of proteins isolated by using gel electrophoresis: SDS-PAGE, size exclusion chromatography, affinity chromatography, strong/weak ion exchange, isotope coded protein labelling (ICPL),and two-dimensional gel electrophoresis.

Organic chemistry

Some synthetic macromolecules, such as catenanes and rotaxanes, dendrimers and hyper branched, and other assemblies, have molecular weights extending into the thousands or

tens of thousands, where most ionization techniques have difficulty producing molecular ions. MALDI is a simple and fast analytical method that can allow chemists to rapidly analyze the results of such syntheses and verify their results.

Polymer chemistry

In polymer chemistry MALDI can be used to determine the molar mass distribution. Polymers with polydispersity greater than 1.2 are difficult to characterize with MALDI due to the signal intensity discrimination against higher mass oligomers.

A good matrix for polymers is dithranolor AgTFA. The sample must first be mixed with dithranol and the AgTFA added afterwards; otherwise the sample would precipitate out of solution.

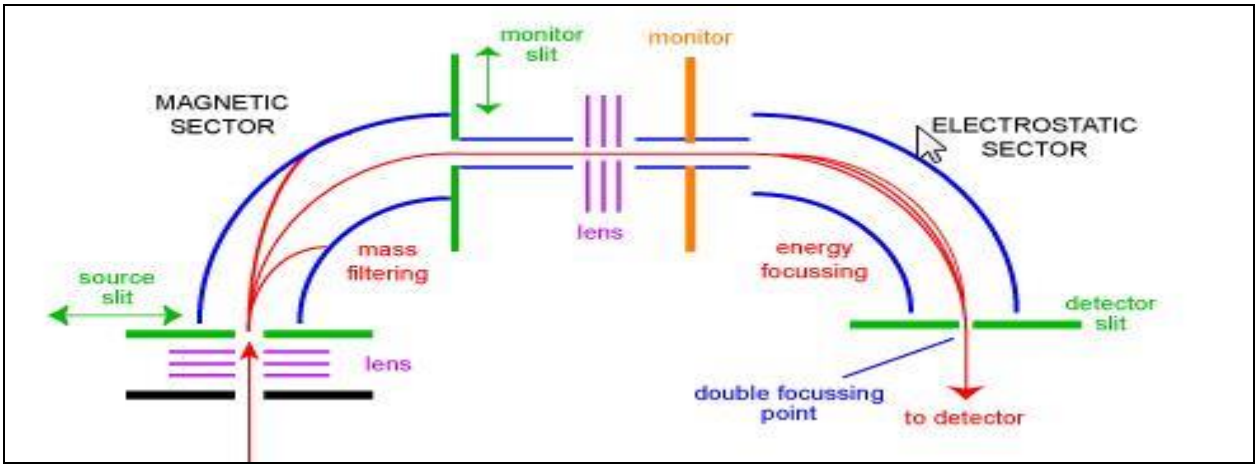
Microbiology

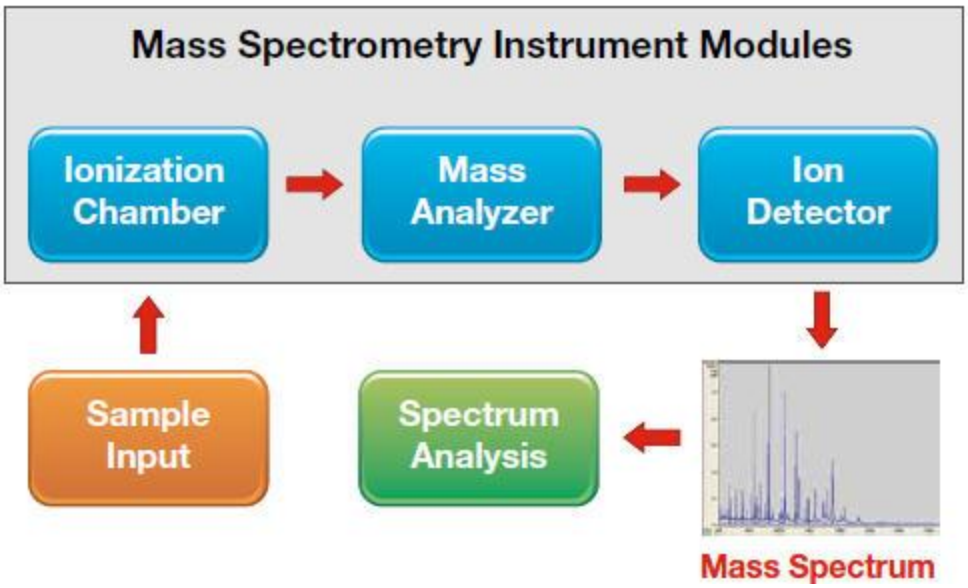
MALDI/TOF spectra are used for the identification of micro-organisms such as bacteria or fungi. A portion of a colony of the microbe in question placed onto the sample target and overlaid with matrix.

Medicine

MALDI spectra are often utilized in tandem with other analysis and spectroscopy techniques in the diagnosis of diseases. MALDI is a diagnostic tool with much potential because it allows for the rapid identification of proteins and changes to proteins without the cost or computing power of sequencing nor the skill or time needed to solve a crystal structure in X-ray crystallography.

INSTRUMENTION





IONIZATION CHAMBER-
1-Chemical ionization [CI]
2-eletrospray ionization [ESI]
3-fast atom bombardment [FAB]
4-Matrix assisted lasser desorption ionization [MALDI]

Ion Separation

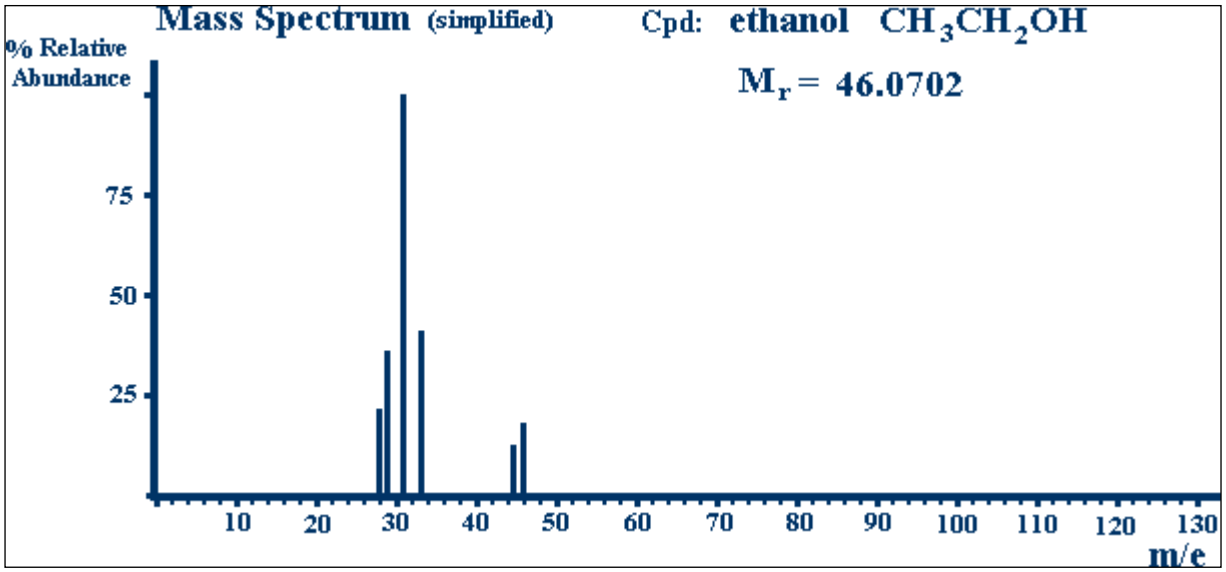
Mass Analyser

Quadrupole
Magnetic Sector Field
Electric Sector Field
Time-Of-Flight (TOF)
Ion Trap

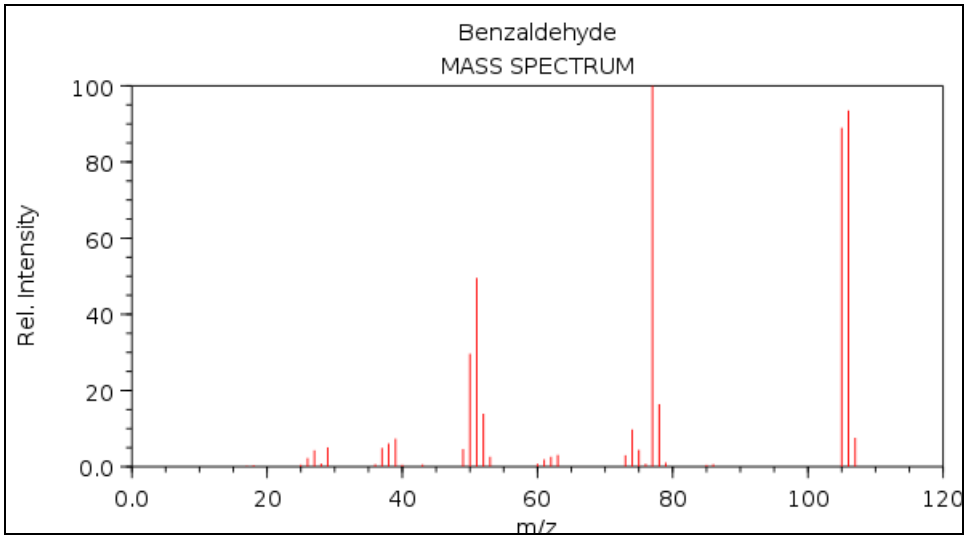
Ion Detection

Detector

Electron Multiplier
Multichannel plate
Faraday Cup



Mass spectrum of ethanol



Mass spectrum of benzaldehyde

MASS APPLICATION

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 in very common use 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 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.

2- 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 require

3-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.

4-Atom probe

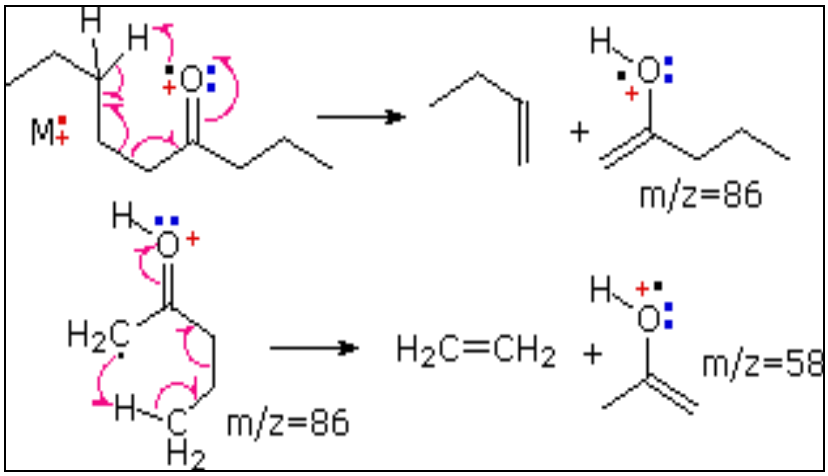
An atom probe is an instrument that combines time-of-flight mass spectrometry and field-evaporation microscopy to map the location of individual atoms.

5-Protein characterization

6- Space exploration

McLafferty rearrangement

The **McLafferty rearrangement** is a reaction observed in mass spectrometry. It is sometimes found that a molecule containing a keto-group undergoes β-cleavage, with the gain of the γ-hydrogen atom. This rearrangement may take place by a radical or ionic mechanism


M+
m/z=86
H2C=CH2
m/z=58
m/z=86