MASS SPECTROSOPY

CONTENTS:

INTRODUCTION

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule. The MS principle consists of ionizing chemical compounds to generate charge molecules or molecule fragments and measurement of their mass-to-charge ratio by using the one of a variety of techniques. Mass Spectrometry is a powerful technique for identifying unknowns, studying molecular structure, and probing the fundamental principles of chemistry.

THEORY:

The relative abundance of positively charged fragments of various mass-to-charge ratios is the characteristic feature of the molecule that serve to identify the substance.

It is determined by

$1/2mv^2=eV$[∴kinetic energy= $1/2\ mv^2$] ....(1) where, m=mass of ion

v=velocity of ion

V=accelerating voltage

e=charge on ion

$$r = \left(\frac{2V}{H2}\, m/e\right) 1/_2$$

DEFINITION:

Mass spectrometry (MS) is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions.

PRINCIPLE:

A mass spectrometer generates multiple ions from the sample under investigation

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 mass-to-charge ratio

OBTAINING MASS SPECTRA CONSIST OF 2 TYPES:

Conversion of neutral molecule into a charged molecule, preferably to a positively charged molecule.

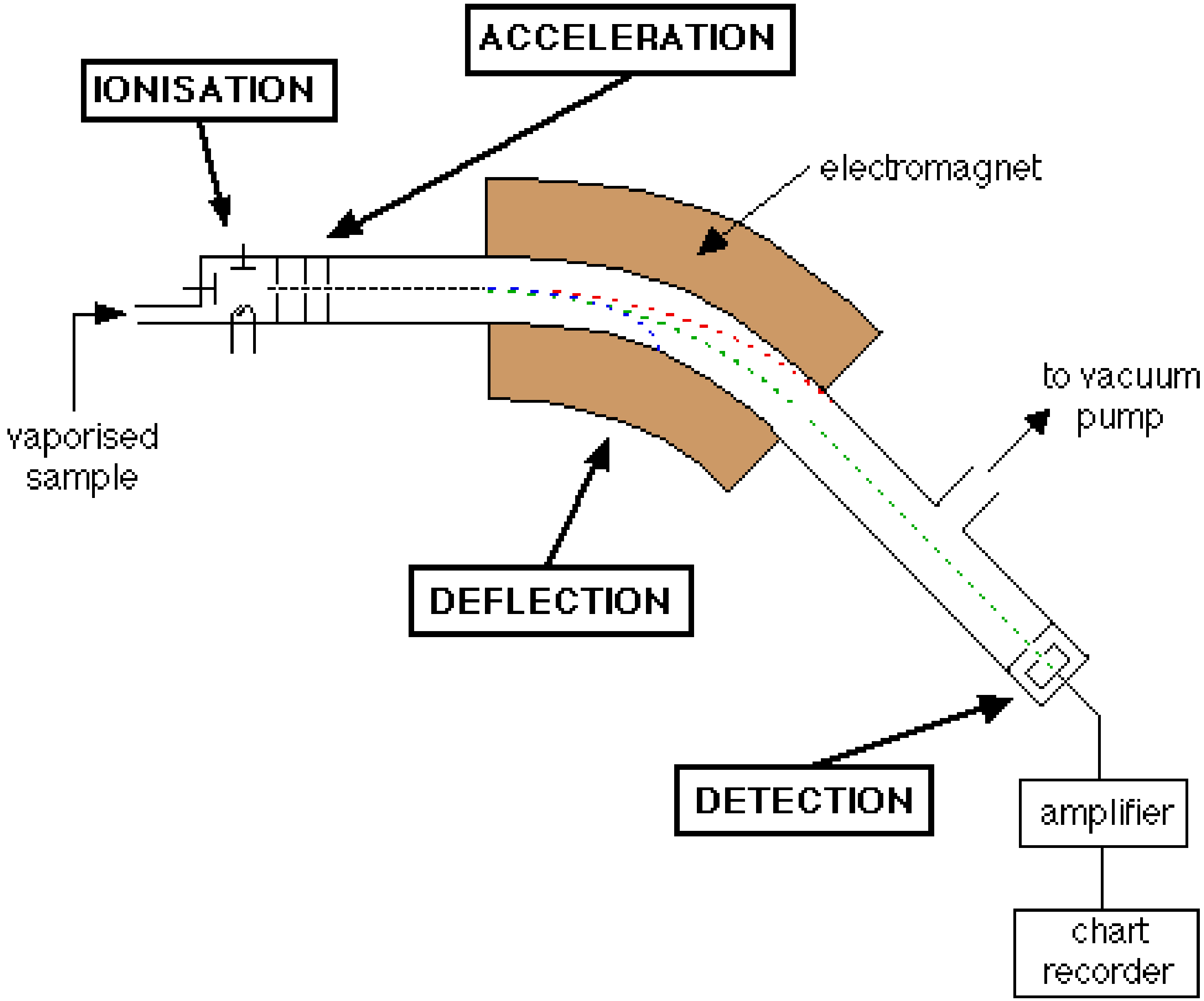
Separation of the positively charged fragments formed, based on their masses, by using electrical or magnetic field or both.

INSTRUMENTATION:

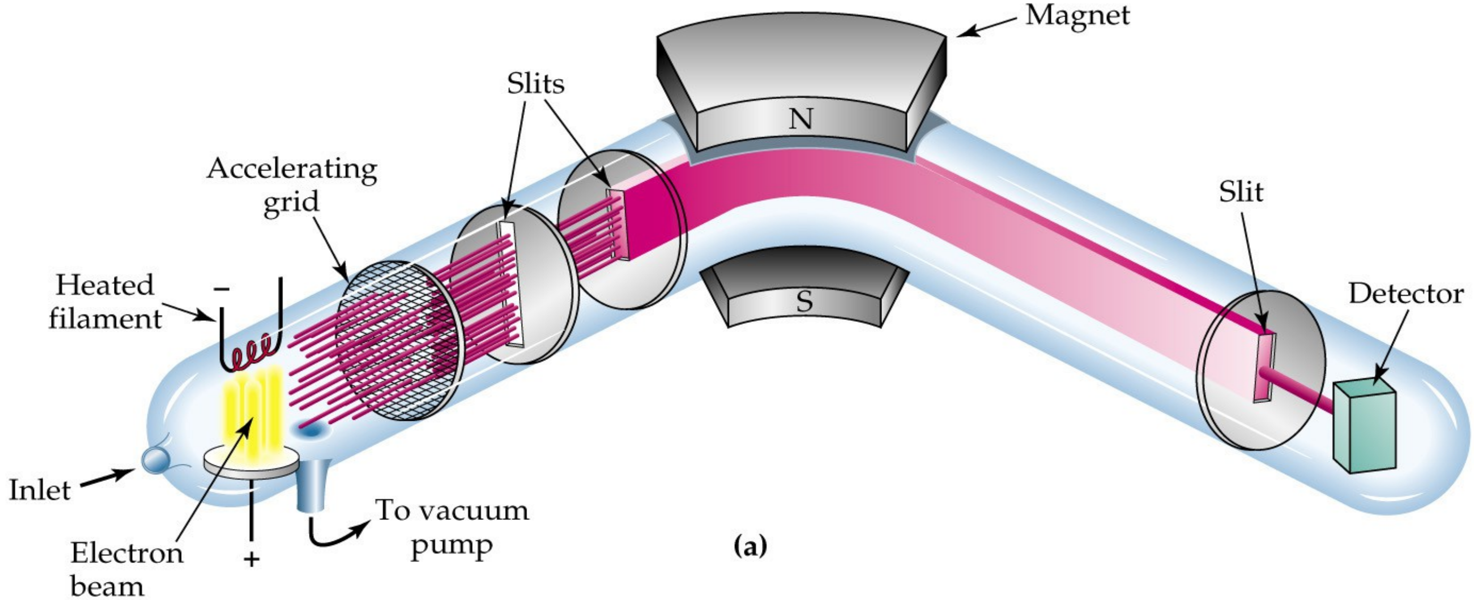
The instrument consists of three major components:

- **Ion Source:** For producing gaseous ions from the substance being studied.
- **Analyzer:** For resolving the ions into their characteristics mass components according to their mass-to-charge ratio.
- **Detector System:** For detecting the ions and recording the relative abundance of each of the resolved ionic species.

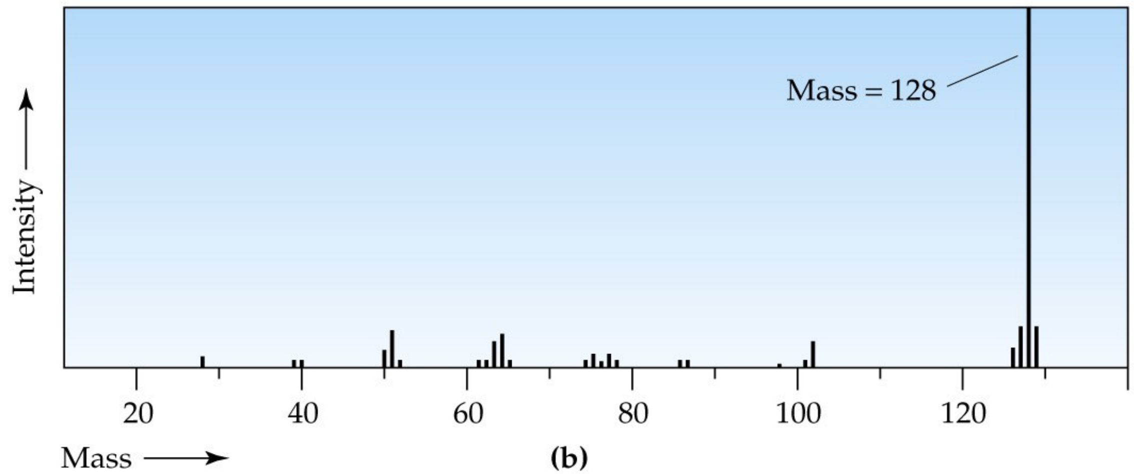
MASS SPECTROMETER



- In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum requirements (~10-6 to 10-8 mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries.


Magnet
Slits
N
Accelerating
grid
Slit
Heated
filament
−
S
Detector
Inlet
Electron
beam
+
To vacuum
pump

(a)


Mass = 128
Intensity
20
40
60
80
100
120
Mass

(b)

With all the above components, a mass spectrometer should always perform the following processes:

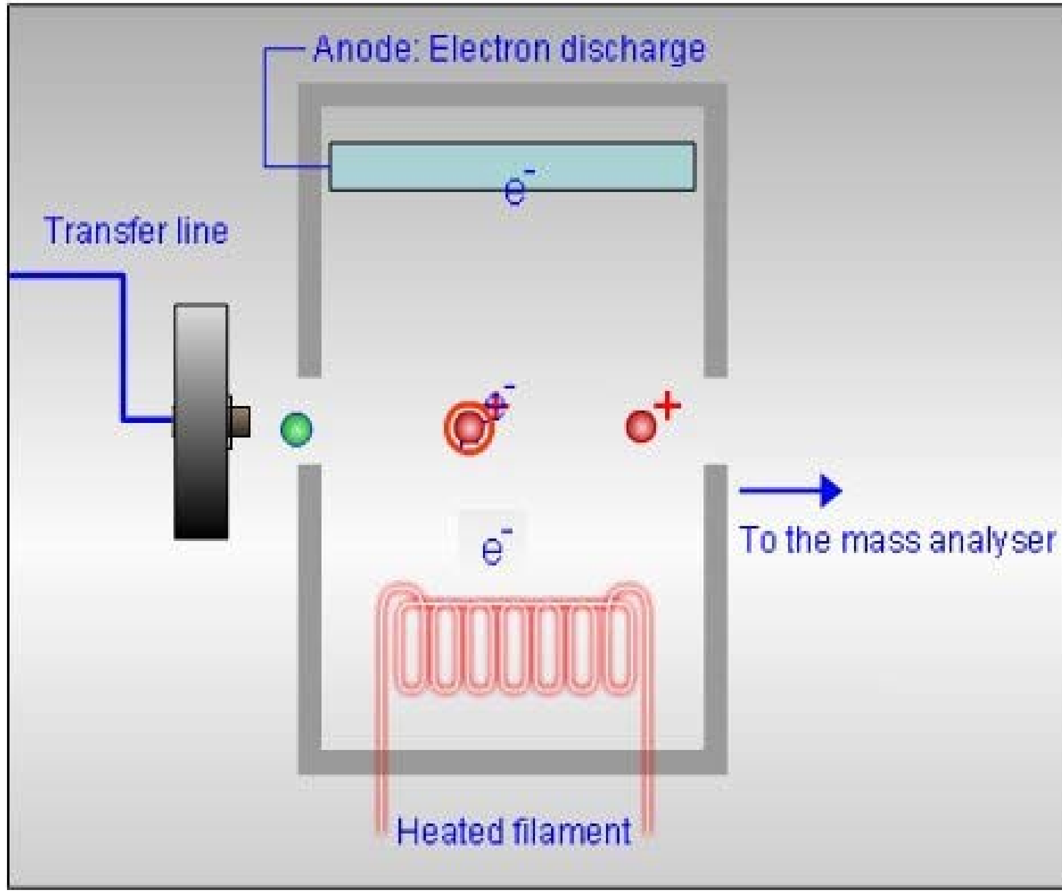
- ✓ Separate these ions according to their mass-to-charge ratio in the mass analyzer.
- ✓ Eventually, fragment the selected ions and analyze the fragments in a second analyzer.
- ✓ Detect the ions emerging from the last analyzer and measure their abundance with the detector that converts the ions into electrical signals.
- ✓ Process the signals from the detector that are transmitted to the computer and control the instrument using feedback.

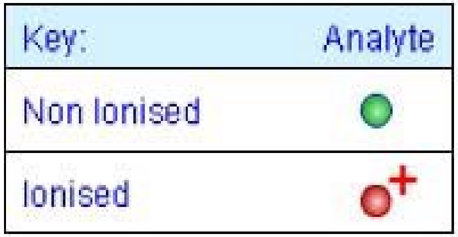
IONIZATION TECHNIQUES:

1. Electron impact (EI)
2. Chemical Ionisation (CI)
3. Fast atom bombardment (FAB)
4. Field ionization
5. Plasma desorption

1.ELECTRON IMPACT (EI):

In the Electron Impact (EI) process, electrons are emitted from a heated filament (usually made of tungsten or rhenium) and are accelerated across the source by using an appropriate potential (5-100V) to achieve the required electron energy (sufficient to ionize the molecule).



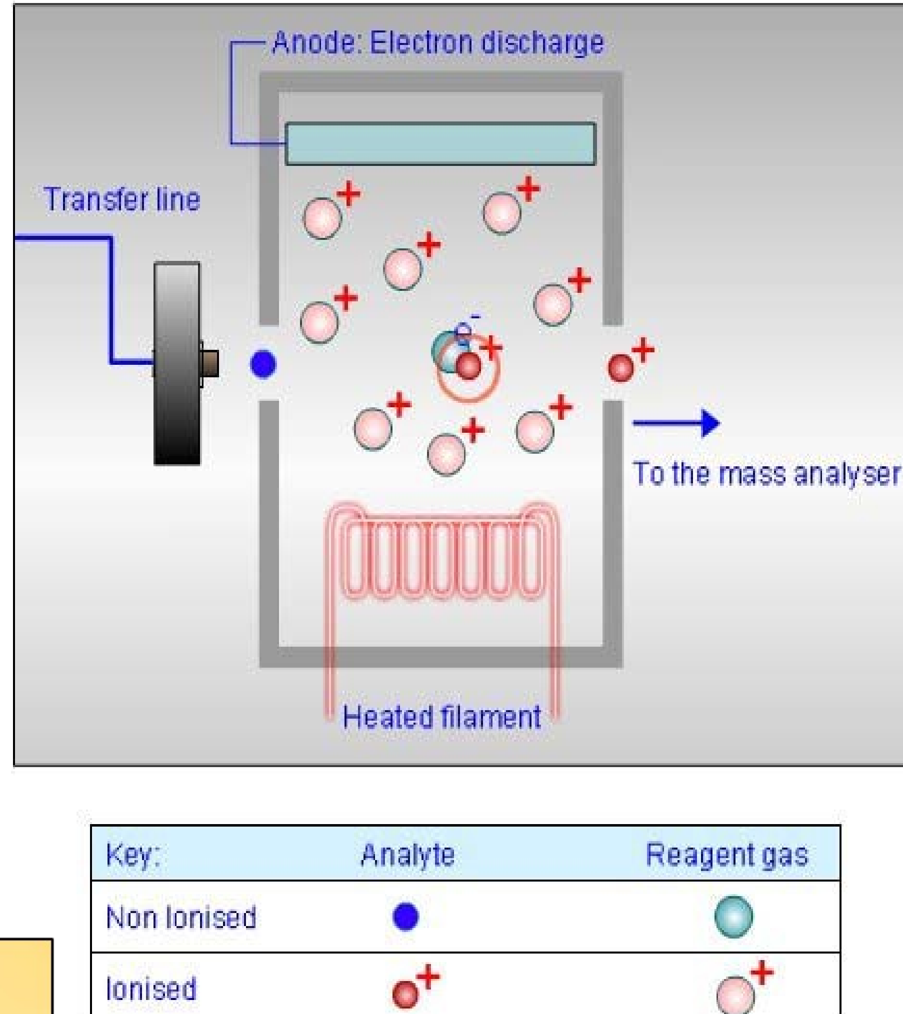


$$M_{(g)} + e^- \qquad M^+ + 2e^-$$

2.CHEMICAL IONISATION (CI)

Chemical ionisation involves the ionisation of a reagent gas, such as methane at relatively high pressure (~1 mbar) in a simple electron impact source. Once produced, the reagent gas ions collide with the analyte molecules producing ions through gas phase reaction processes such as proton

$$nNH_3 + e^- \quad NH_4^+ + 2e^-$$
$$NH_4^+ + M \quad NH_3 + [M+H]^+$$
$$NH_4^+ + M \quad [M+NH_4]^+$$

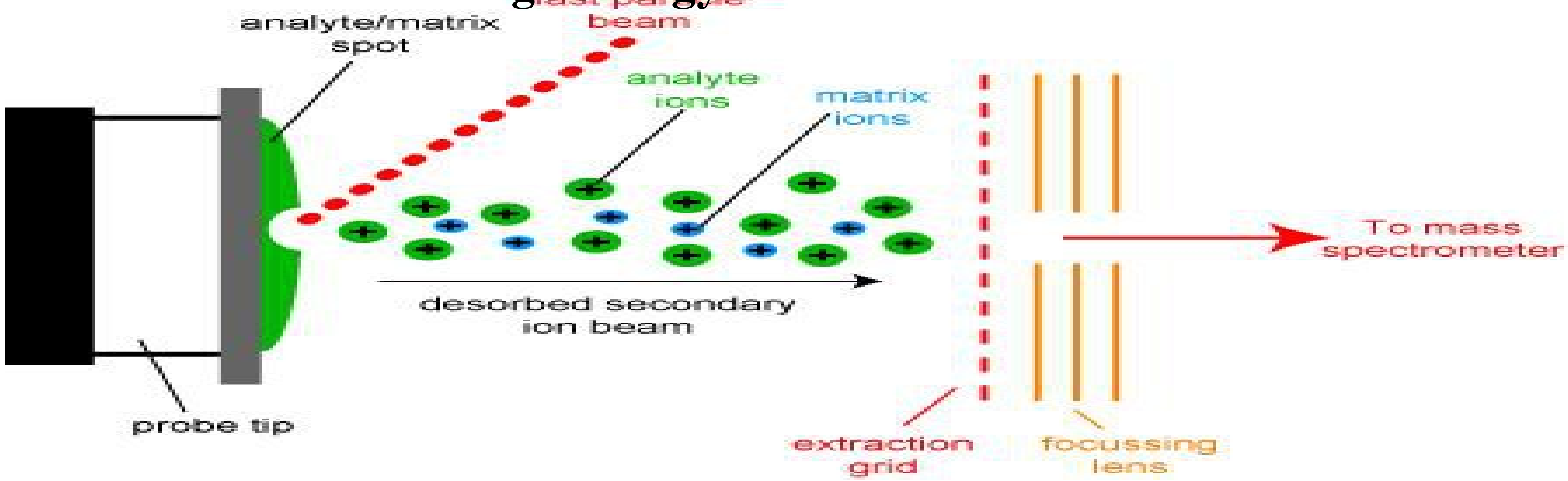


FAST ATOM BOMBARDMENT (FAB)

A high-energy beam of netural atoms, typically Xe or Ar, strikes a solid sample causing desorption and ionization. It is used for large biological molecules that are difficult to get into the gas phase. FAB causes little fragmentation and usually gives a large molecular ion

peak, making it useful for molecular weight determination.

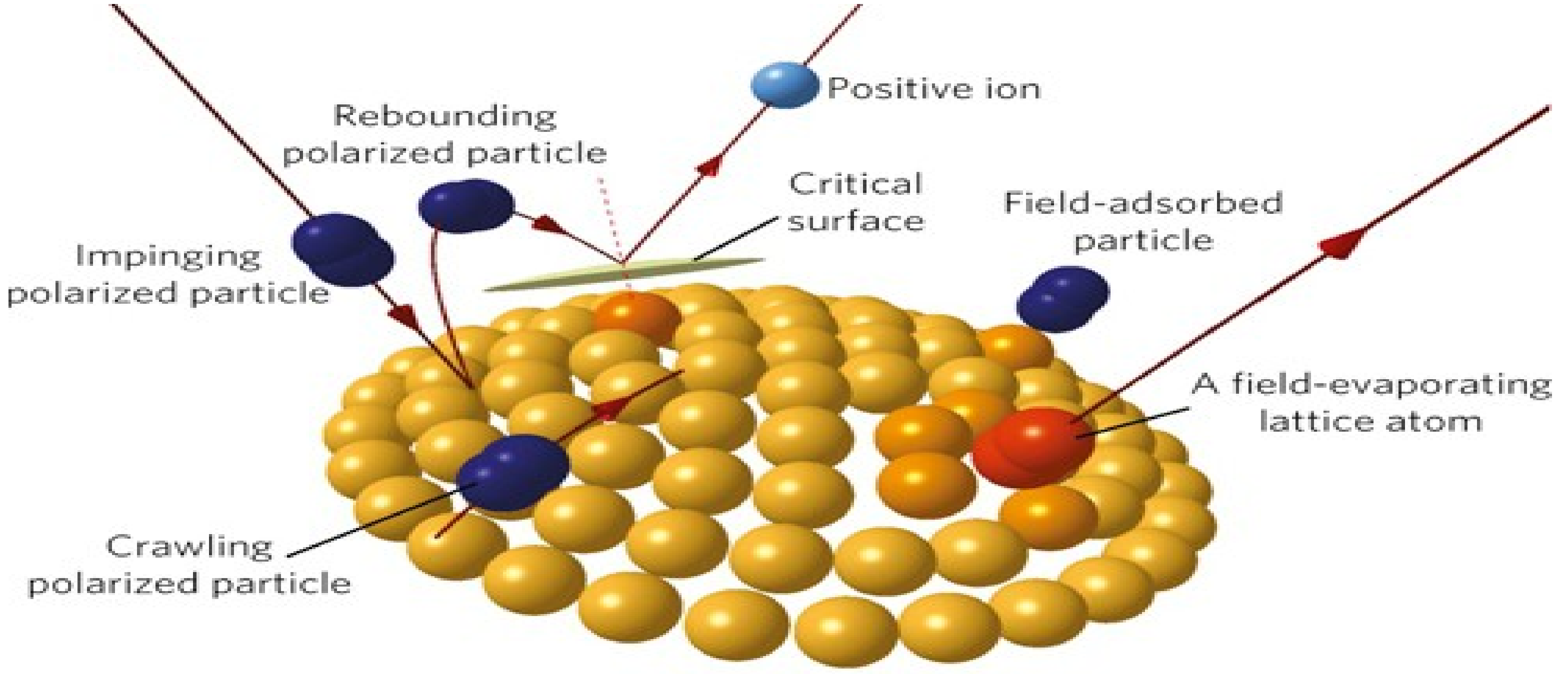
The atomic beam is produced by accelerating ions from an ion source though a charge-exchange cell. The ions pick up an electron in collisions with netural atoms to form a beam of high energy atoms.



4.FIELD IONIZATION:

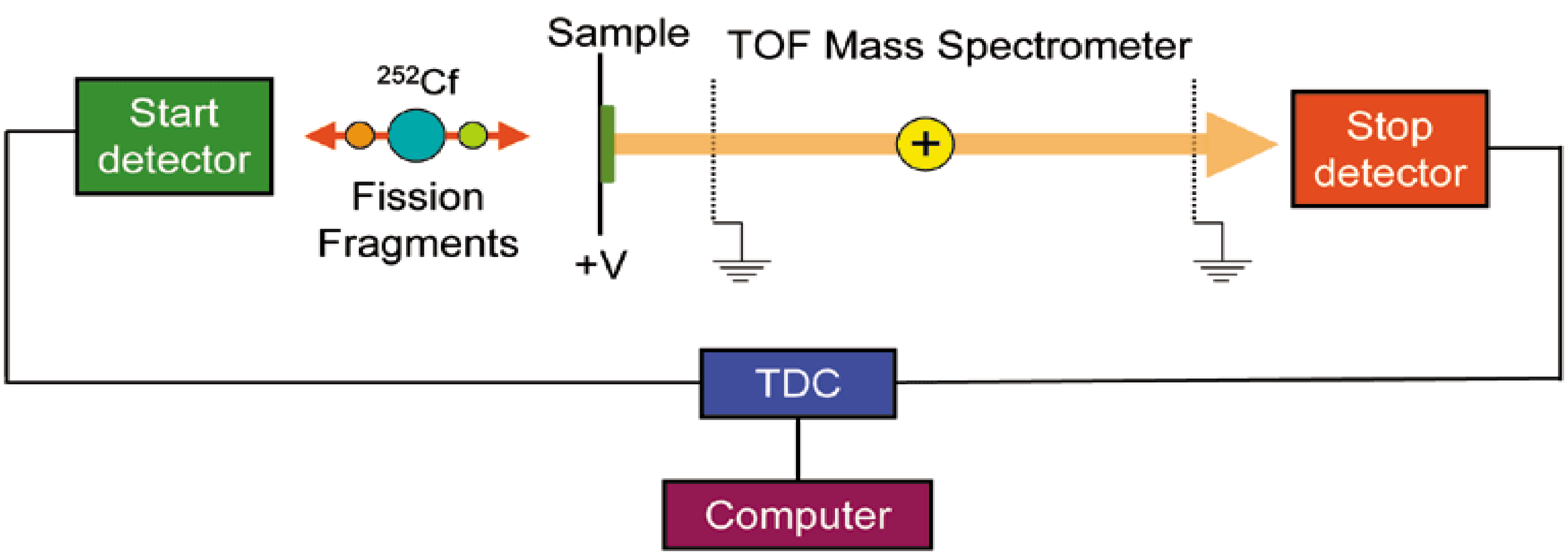
Field ionization (FI) is a method that uses very strong electric fields to produce ions from gas-phase molecules. It is perfectly suited for the analysis of synthetic polymers or man made polymer .

Examples: poly siloxane, poly phosphazene backelite, nylon etc.,



5.Plasma desorption:

Plasma desorption ionization mass spectrometry (PDMS), also called fission fragment ionization, is a mass spectrometry technique in which ionization of material in a solid sample by bombarding it with ionic or neutral atoms formed as a result of the nuclear fission of a suitable nuclide, typically the californium isotope ^{252}Cf.



CHROMATOGRAPHIC 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 separation techniques.
- GCMS
- LCMS

GAS CHROMATOGRAPHY :

- 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.

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 electro spray ionization source is used in LC/MS. Other popular and commercially available LC/MS ion sources are atmospheric pressure chemical ionization and atmospheric pressure photo ionization.

- There are also some newly developed ionization techniques like laser spray.

APPLICATIONS:

Mass spectrometry has both qualitative and quantitative uses.

1.Structure elucidation

2.Detection of impurities

3.Quantitative analysis

4.Drug metabolism studies

5.Clinical, toxicological and forensic applications

6.GC MS

MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

QUALITATIVE APPLICATIONS

1.Determination of molecular weight:

Mass spectrometry serves as the best possible technique for the determination or confirmation of molecular weight of compounds that can be easily volatilized.

2.Determination of molecular formula :

For the determination of molecular formula b mass spectrometry, it is essential to identify the molecular ion peak as well as its exact mass.

3. Determination of partial molecular formula:

Generally, atoms are polyisotopic. In mass spectrometer, the ions are selected according to their actual mass. Exact information about the atomic composition ao the selected ions is furnished by the mass distribution of molecular ions

4.Determination of structure of compounds:

Bombardment of vaporized sample molecules with a high beam of electrons results in their fragmentation producing a large number of ions with varying masses.

QUANTITATIVE APPLICATIONS

1.Determination of isotope abundance:

Although differences in the masses of isotopes of an element are very small, the isotope abundance i.e., the isotopic composition of molecules within an easily vaporizable sample can be determined with mass spectrometry.

The information so obtained may be useful for:

(a) tracer studies with isotopes

(b) determination of atomic weights of compounds

(c) determination of age rocks and minerals

(d) study of origin as well as nature of solar system

2 Determination of isotope ratio :

Mass spectroscopy is used to determine isotope ratio which in turn helps to determine the concentration of individual components present in complex mixture from which it cannot be separated quantitatively.

3.Differentiation between Cis and Trans isomers:

Mass spectrometry may be used to differentiate between cis and trans isomers. Both the isomers yield similar spectra but are differentiated from the intensity of the molecular ion peaks. The molecular ion peak of trans isomer is more intense than that of cis isomer.

4.Mass spectrometry in thermodynamics:

(a) determination of heat of vapourization

To determine the heat of vapourization of high temperature compounds, data from the spectrum is collected and a graph is plotted by taking ion intensities on Y-axis and temperature on X-axis.

(b) Determination of heat of sublimation

To determine the heat of sublimation of a compound, vapours of the sublimed solids are passed into the ionization chamber of the mass spectrometer. The spectrum is recorded in which the obtained peak intensities are directly proportional to the vapour pressure (VP) of the sample in the ionization chamber.

5.Measurement of ionization potential :

Ionization potential is the minimum energy required by the bombarding electrons to produce the molecular ions from a molecule of an atom.

6.Detrmination of ion-molecule reactions:

Mass spectrometry finds its use in the study of ion molecule reaction i.e., the reactions in between the fragment ion and the unionized molecules. The rate of these reactions directly depend on the operating pressure.

7.Detction of impurity:

The impurities present sample even in low concentration (parts per million) can be detected by spectrometry, provide the molecular weights of the impurities differ considerably from the major components.

8.Identification of unknown compounds:

Mass spectrometry can be used to identify the unknown compounds. this can be achieved by recording the spectrum of the unknown compounds and comparing it with the spectrum of the standard compound recorded under identical conditions.

9.Identification of proteins:

Mass spectrometry serves as valuable tool in the study of structure and functions of proteins (proteomics). Electro spray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are the widely used ionization methods for this purpose. Mass spectrometry in the proteomics particularly deals with the analysis of protein digested by protease like trypsin.

ADVANTAGES :

•**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.

DISADVANTAGES:

1. Doesn't directly give structural information (although we can often figure it out).
2. Needs pure compounds .
3. Difficult with non-volatile compounds.

CONCLUSIONS AND FUTURE PROSPECTS:

The inherent analytical advantages of mass spectrometry, including sensitivity and speed, combined with recent advances promise to make MS a mainstay of drug design and discovery. The development of ionization techniques such as ESI and MALDI now allows almost any compound to be studied by MS. In addition, MS/MS and MS^n add the capability for structural analysis of compounds that are present at low levels and/or are present in complex mixtures.

REFERENCES:

1.e, M. S. & Kerns, E. H. LC/MS applications in drug development. Mass Spectrom. Rev. 18, 187–279 (1999).

2.Cheng, X. & Hochlowski, J. Current application of mass spectrometry to combinatorial chemistry. Anal. Chem. 74, 2679–2690 (2002).

3.Triolo, A., Altamura, M., Cardinali, F., Sisto, A. & Maggi, C. A. Mass spectrometry and combinatorial chemistry: a short outline. J. Mass Spectrom. 36, 1249–1259 (2001).

4.Shin, Y. G. & Breemen, R. B. v. Analysis and screening of combinatorial libraries using mass spectrometry. Biopharm. Drug Dispos. 22, 353–372 (2001).

5.Kassel, D. B. Combinatorial chemistry and mass spectrometry in the 21st century drug discovery laboratory. Chem. Rev. 101, 255–267 (2001).

6.Yamashita, M. & Fenn, J. B. Electrospray ion source. another variation of the free-jet theme. J. Phys. Chem. 88, 4451–4459 (1984).

The initial publication in the development of electrospray as a useful ionization technique for mass spectrometry.

7.Karas, M., Bachmann, D., Bahr, U. & Hillenkamp, F. Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int. J. Mass Spectrom. Ion Processes 78, 53–68 (1987).

8.Seminal publication describing the use of a matrix to enhance the ionization efficiency of non-volatile compounds — this method of laser/desorption ionization is the most commonly used today.

9.Horning, E. C., Horning, M. G., Carroll, D. I., Dzidic, I. & Stillwell, R. N. New Picogram detection system based on a mass spectrometer with an external ionization source at atmospheric pressure. Anal. Chem. 45, 936–943 (1973