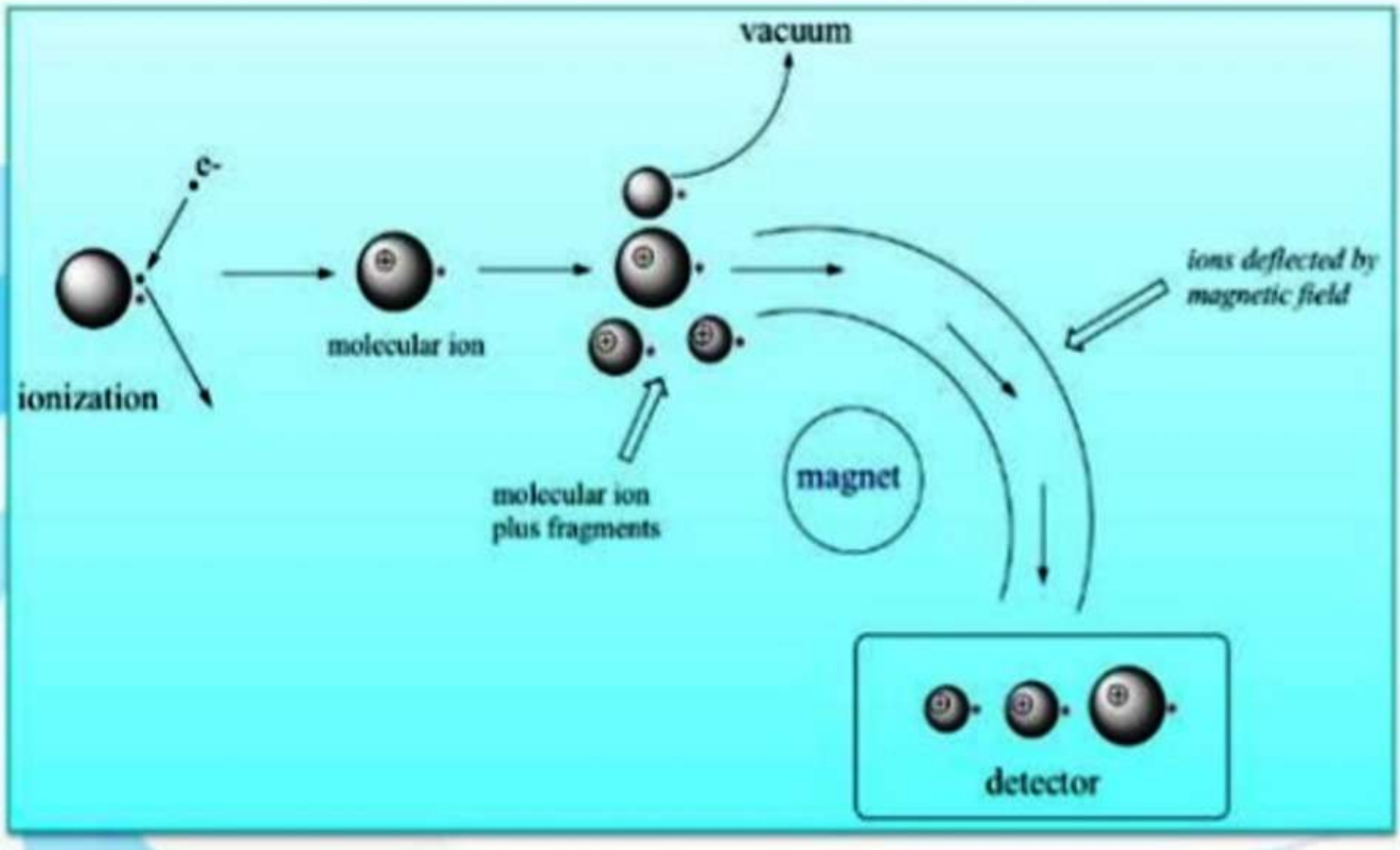
Definition

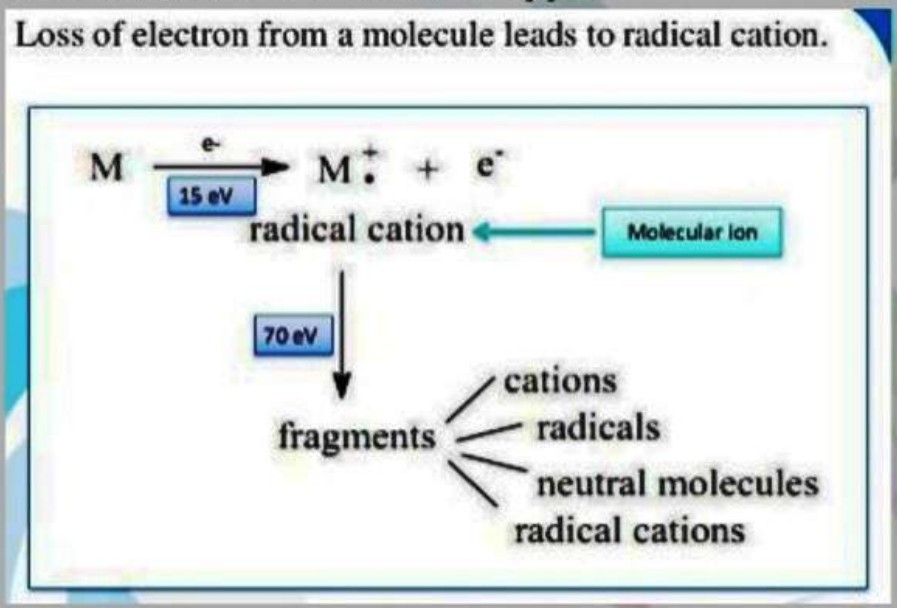
- Mass Spectrometry (MS) is a technique for the analysis of a substance in which the molecule is subjected to bombardment by high-energy electrons or atoms to cause ionization and fragmentation to give a series of ions in the gas phase that constitutes the fragmentation pattern observed by using a mass spectrometer.
- It is a process, which used to identify chemicals in a substance by their **mass** and **charge**.
- Mass spectrometers are instruments that measure mass and charge of molecules.
- A mass spectrometer also can determine how much of a compound is present in a mixture, also known as mass spectroscopy.

Simple mass spectrometry



Principle of Mass Spectrometry (MS)

- 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.

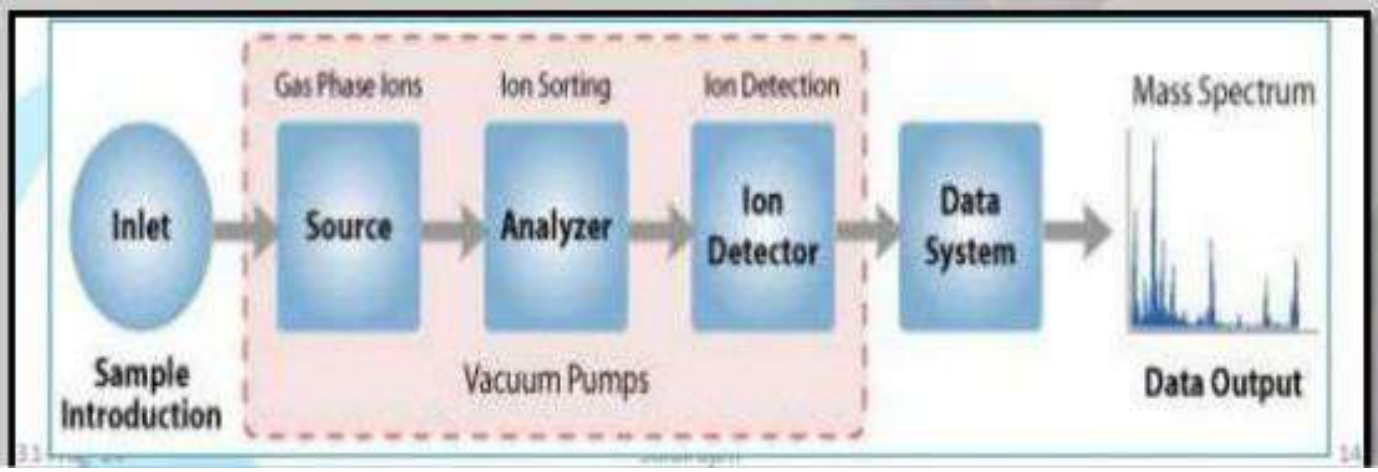


Instrumentation

- The instrument used in MS is called **mass spectrometer.**
- It produces a mass spectrum that plots the **mass-to-charge (m/z) ratio** of compounds in a mixture.
- Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.
- Mass Spectrometry (MS) is used for both qualitative and quantitative chemical analysis.
- It may be used to identify the elements and isotopes of sample, to determine the masses of molecules, and as a tool to help identify chemical structures.
- It can measure sample purity and molar mass.

Components of MS

- Inlet system
- Ion source
- Mass analyzer
- Ion detectors
- Vaccum system



Inlet system	• 1.Solid • 2.Liquid • 3.Gas
Ionization	• ESI,EI,FAB, CI,FI,FD, MALDI,PD, TI
Mass Analyser	• Quadrupole, TOF, Ion trap, FT-ICR
Detector	• Photo multiplier, Electron multiplier, Faraday cup, Micro channel plate

Ion source and Ionization Types

- Molecular ions are formed when energy of the electron beam reaches to 10-15 eV.
- Fragmentation of the ion reaches only at higher bombardment energies at 70 eV.

Ionization Types:

1.Gas Phase Ionization → • gases and vapour

2. Desorption Technique → • liquid and solid

1.Gas Phase Ionization (gases and vapour)

- Samples are ionized **outside** the ion source.
- This technique include:

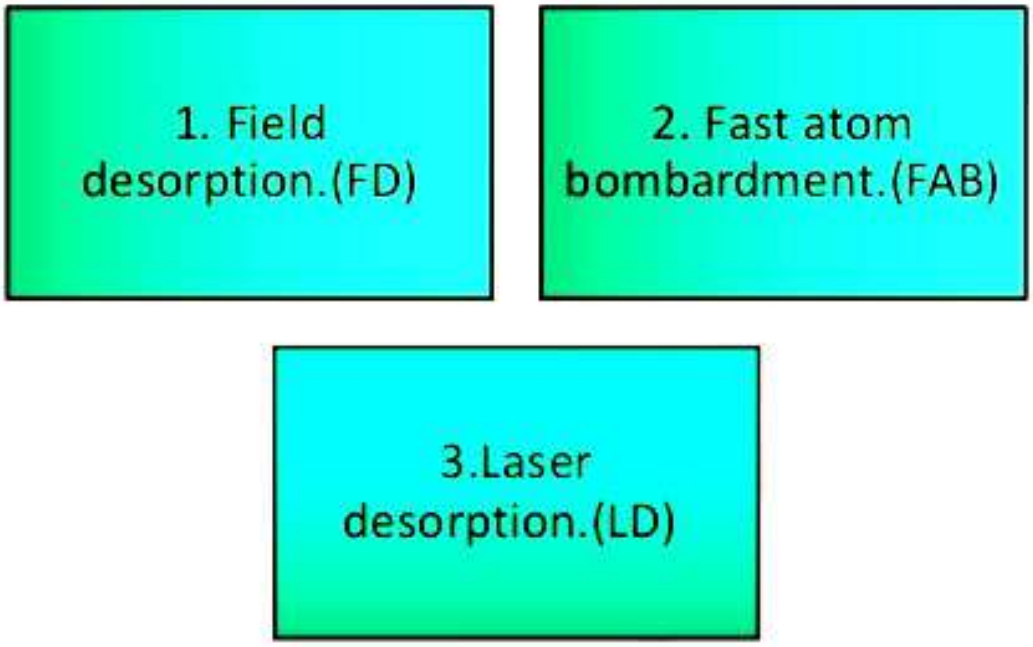
1.Electron impact ionization (EIS)

2.Chemical ionization.(CI)

3.Field ionization.(FI)

2.Desorption Technique(liquid and solid)

- Samples are ionised **inside** the ion source.
- This technique include:



Ionizing Agents

Basic Type	Name and Acronym	Ionizing Agent
Gas phase	Electron impact (EI)	Energetic electrons
	Chemical ionization (CI)	Reagent gaseous ions
	Field ionization (FI)	High-potential electrode
Desorption	Field desorption (FD)	High-potential electrode
	Electrospray ionization (ESI)	High electrical field
	Matrix-assisted desorption/ionization (MALDI)	Laser beam
	Plasma desorption (PD)	Fission fragments from ^{252}Cf
	Fast atom bombardment (FAB)	Energetic atomic beam
	Secondary ion mass spectrometry (SIMS)	Energetic beam of ions
	Thermospray ionization (TS)	High temperature

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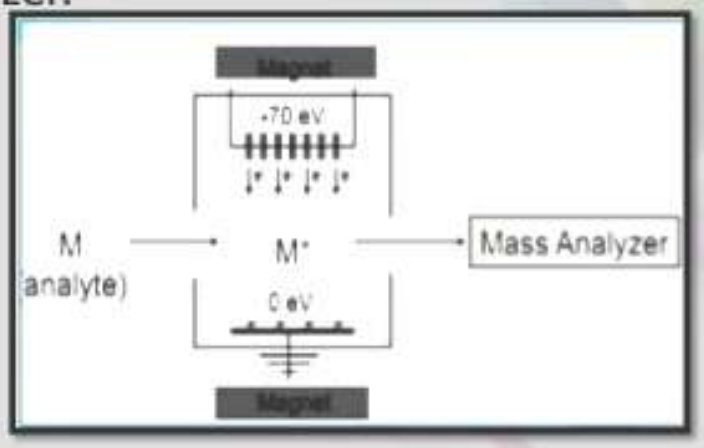
Ionization Method

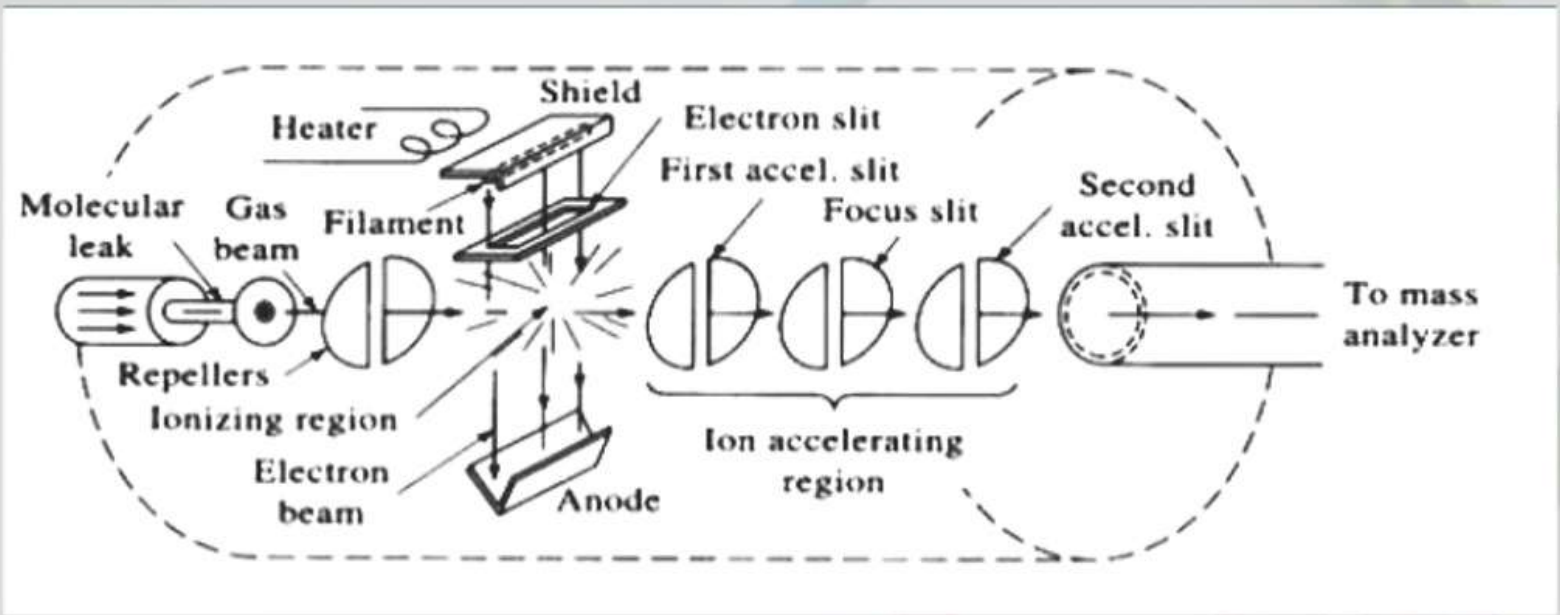
IONISATION METHOD	COMPOUNDS	MASS RANGE
Electron impact ionisation	Thermally volatile and stable	500 Da
Chemical ionisation	Thermally volatile and Stable	500 Da
Electro spray ionisation	Polar and Basic	70000 Da
Fast atom bombardment	Peptides	7000 Da
Field ionisation	Thermally volatile	1000 Da
MALDI	Large Biomolecules	3,00,000 Da
Plasma desorption	Neutral compounds	500 Da
APCI	Thermally liable	1000 Da
SIMS	Same as FAB	300-13000 Da
Laser desorption	Elemental analysis	500 Da

Electron impact ionization

- A beam of electrons passes through a gas-phase sample and collides with neutral analyte molecules (M) to produce a positively charged ion or a fragment ion.
- Generally electrons with energies of 70 eV are used to form a fragment ions.
- The positive ions are collected in focusing plates and passed to mass analyzer.



$$M + e^{-}(\sim 70\,eV) \rightarrow M^{+} + 2e^{-}$$



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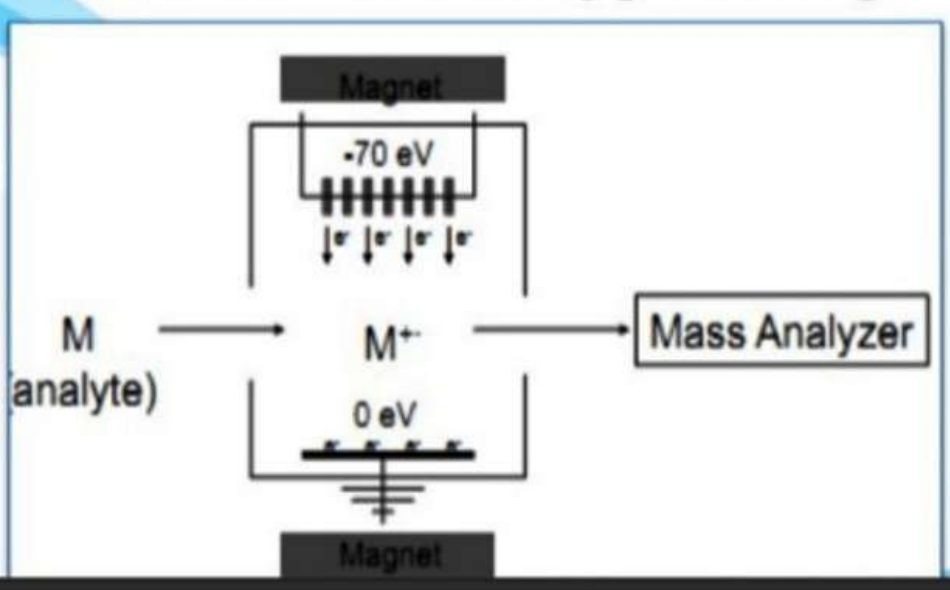

Chemical Impact Ionization

- Chemical Impact Ionization between interactions of sample with large amount of reagent gas.
- Commonly used reagent gases include methane, ammonia, isobutane.
- Oxygen and hydrogen are used in Negative ion chemical ionization in MS.

- The vaporised sample is introduced into the mass spectrometer with an excess of a reagent gas (methane) at pressure of about 1 torr.
- The excess carrier gas is ionized by electron impact to the primary ions $CH_4^{\cdot+}$ and CH_3^+.
- These may react with the excess methane to give secondary ions

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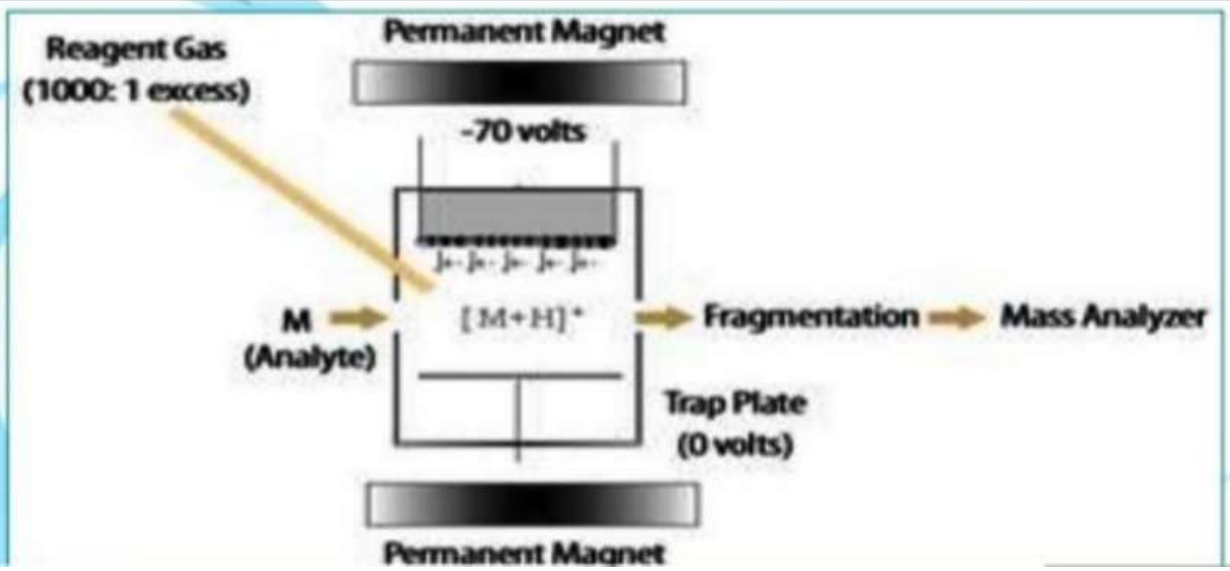


Chemical ionization

- In chemical ionization, the ionization of the analyte is achieved by **interaction of it's molecules with ions of a reagent gas** in the chamber or source.
- Chemical ionization is carried out in an **instrument similar to electron impact** ion source with some modifications such as:-
- Addition of a vacuum pump.
- Narrowing of exit slit to mass analyzer to maintain reagent gas pressure of about 1 torr in the ionization chamber.
- Providing a gas inlet.

* **TYPES OF CI:**
* Depending upon the type of ions formed CI is categorized as:-
* 1. Positive Chemical Ionization
* 2. Negative Chemical Ionization
* **1. Positive Chemical Ionization**
* In this technique **positive ions** of the sample are produced.
* In positive chemical ionization, gases such as Methane, Ammonia, Isobutane etc are used.

- For example,
- Ammonia is used as reagent gas.
- First ammonia radical cations are generated by electron impact and this react with neutral ammonia to form ammonium cation (reactive species of ammonia CI).

- NH^{4+} reacts with the sample molecules by proton transfer to produce sample ions

- **Negative Chemical Ionization**
- Negative chemical ionization is **counterpart** of Positive chemical ionization.
- In this technique, **negative ions** of the sample are formed.
- Oxygen and Hydrogen are used as reagent gasses.
- This method is used for ionization of **highly electronegative samples**.

- **ADVANTAGES**
- Used for high molecular weight compounds.
- Used for samples which undergo rapid fragmentation in EI.
- **LIMITATIONS**
- Not suitable for thermally unstable and non-volatile samples.
- Relative less sensitive then EI ionization.
- Samples must be diluted with large excess of reagent gas to prevent primary interaction between the electrons and sample molecules.

Field Ionization

- FI is used to produce **ions from volatile compounds** that do not give molecular ions by EI.
- It produces molecular ions with **little or no fragmentation**.
- Application of very **strong electric field induces emission** of electrons.
- FI utilizes 10-micron diameter tungsten emitter wires on which carbon whiskers, or dendrites, have been grown.
- A high electric field gradient (10^{10} V/cm) at the tips of the whiskers produces ionization

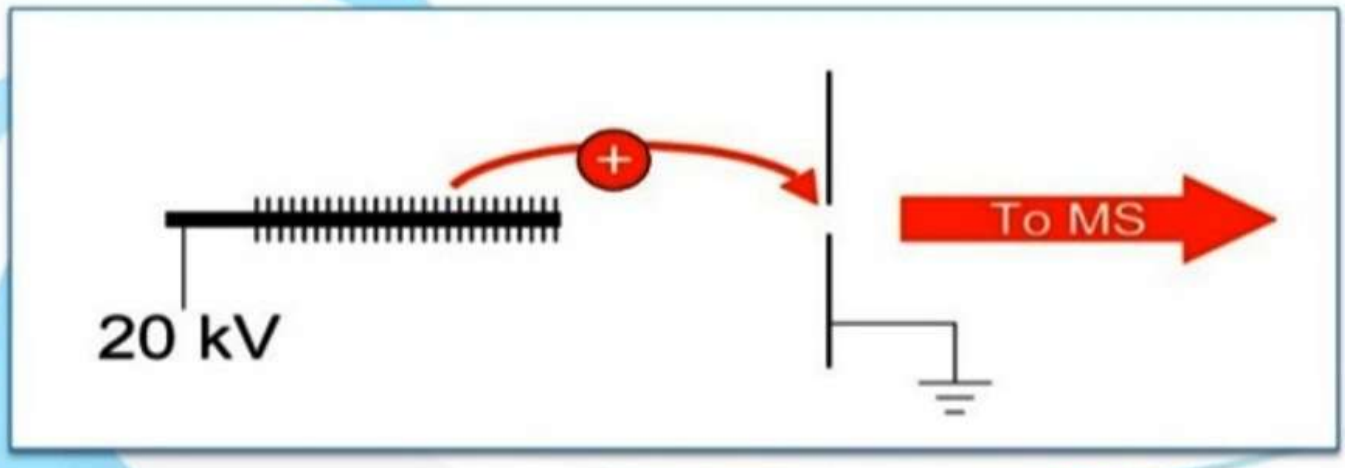
Field ionisation:-

In this method the molecule pass through sharp metal anode carrying an electric field of 10^{10} v m^{-1}

Electrons are analysed in primary focusing cathode slit.

ADV :- ↑ abundance of molecular ions.

DISADV :- lower resolution.



Field Desorption

- Also known as offspring of field ionization.
- In field desorption method, a **multitipped emitter** (made up of tungsten wire with carbon or silicon whiskers grown on its surface) similar to that used in FI is used.
- The sample solution is deposited on the **tip of the emitter whiskers** either by
- dipping the emitter into analyte solution or
- using a microsyringe.
- Then the sample is ionized by applying a **high voltage to the emitter**.

Electrospray ionization

- Electrospray ionization is a technique used in mass spectrometry to **produce ions from macromolecules** such as proteins, polypeptides and oligonucleotides **having molecular weights of 10,000 Da or more**.
- The method generates ions from solution of a sample by **creating fine spray of charged droplets**.
- • A solution of sample is **pumped through** a fine, **charged stainless steel capillary needle** at a rate of few microlitres/minute.
- The needle is maintained at **a high electric field** (several kilovolts) with respect to cylindrical electrode.
- • The liquid pushes itself out of the capillary **as a mist or aerosol of fine charged droplets**.

Field Ionization

- In this method the molecule pass through sharp metal anode carrying an electric field of 10^{10} vm^{-1}.
- Electrons are analysed in primary focusing cathode slit.
- ADV :- ↑ abundance of molecular ions.
- DISADV :- lower resolution.

- These charged droplets are then passed through **desolvating capillary** where the solvent is evaporated in the vacuum and attachment of charge to the analyte molecules takes place.
- Desolvating capillary uses **warm nitrogen** as nebulising gas.
- The desolvating capillary is maintained under high pressure.
- • As the droplets evaporate the analyte molecules comes closer together.

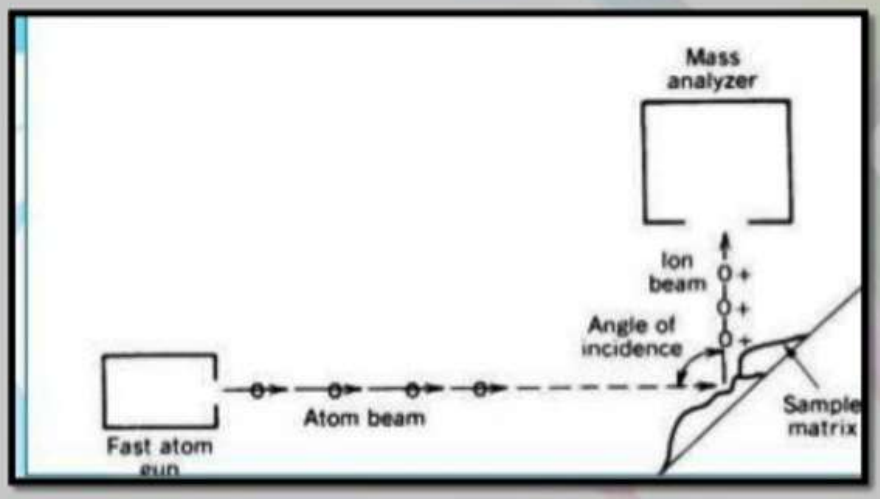
- These molecules become unstable as the similarly charged molecules comes closer together and the droplets explode once again. This is referred as ***Coulombic fission***.
- • The process **repeats itself** until the analyte is **free from solvent and is alone ion**.
- • The ion then moves to the mass analyzer.

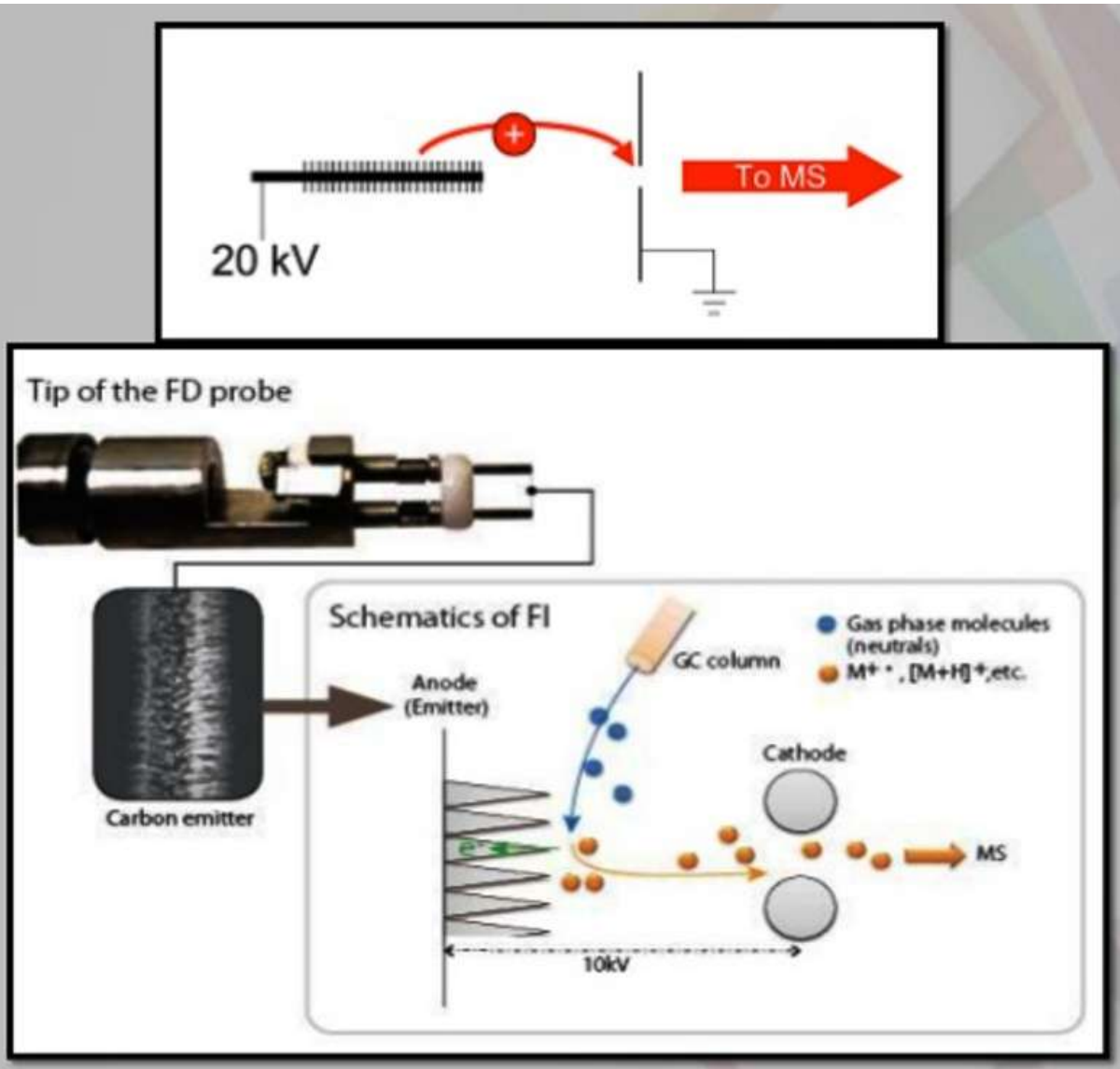
- **ADVANTAGES**
- Most important techniques for analysis of **high molecular weight biomolecules** such as polypeptides, proteins, oligonucleotides and synthetic polymers.
- Can be used along with **LC and capillary electrophoresis**.

- **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.
- 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)**

Fast Atom Bombardment (FAB)

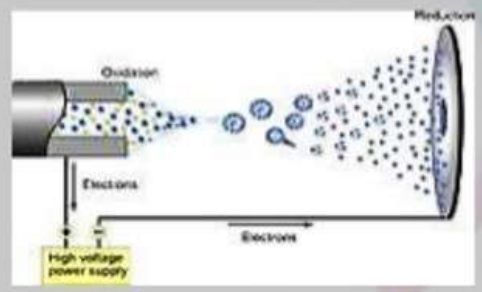
- Argon gas ionised by hot filament and focused beam that bombards the sample.
- Beam impinges the sample, a series of molecular reactions occur and analyse in MS analyser.
- Ex:-Insulin, Amino glycosides, Phospholipids.

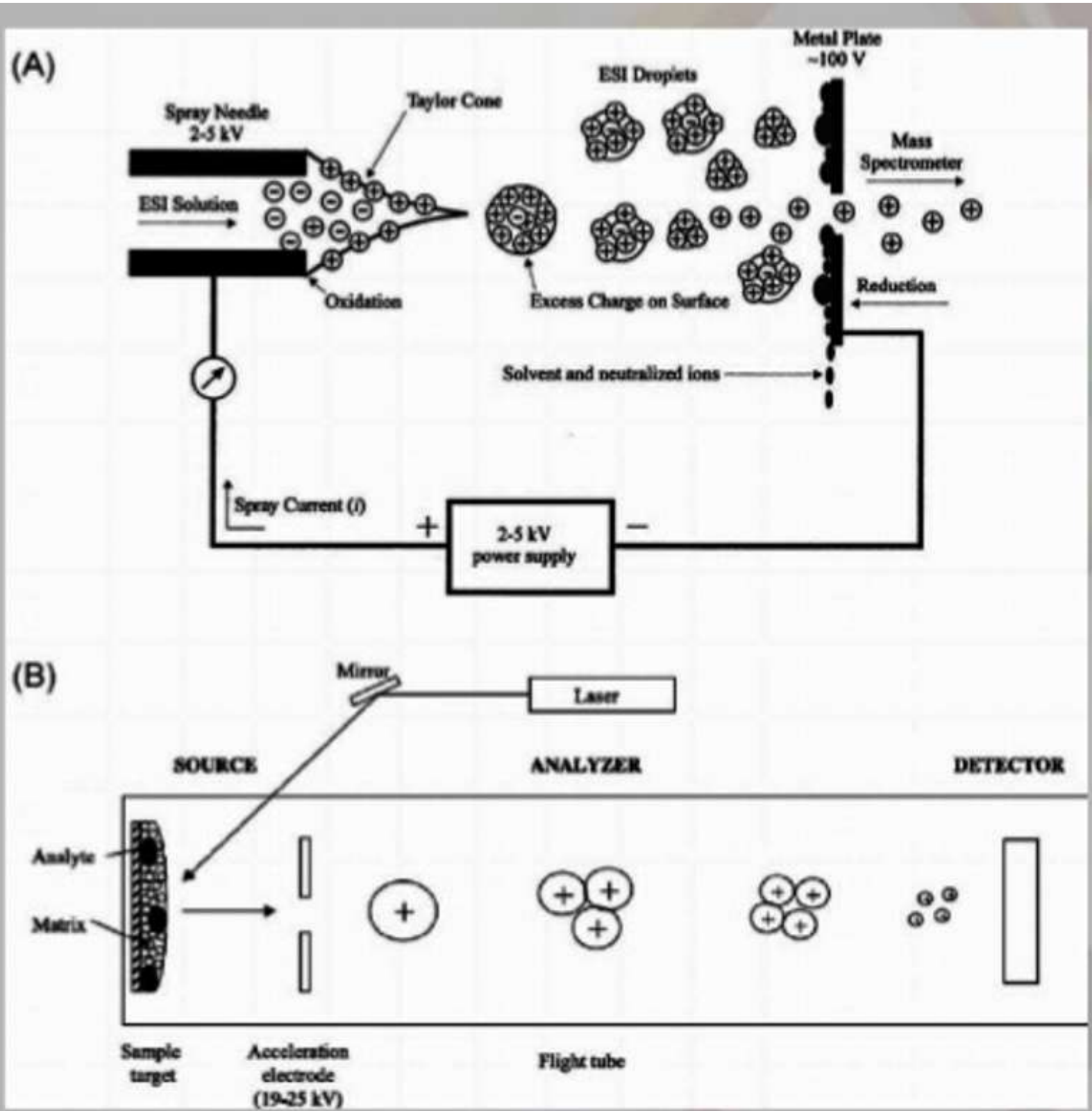



To MS
20 kV
Tip of the FD probe
Carbon emitter
Schematics of FI
Anode
(Emitter)
GC column
Gas phase molecules
(neutrals)
M+ · , [M+H] +,etc.
Cathode
MS
10kV

Electro Spray Ionization (ESI)

- The ESI source consists of a very fine needle and a series of skimmers.
- A sample solution is sprayed into the source chamber to form droplets.
- When droplets carry charge exit the capillary end, as the solvent evaporates, the droplets disappear leaving highly charged analyte molecules.

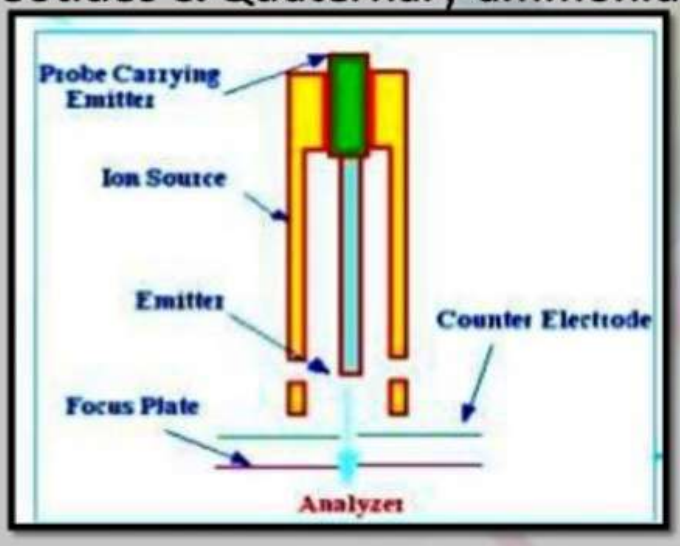



(A)
Metal Plate
~100 V
ESI Droplets
Taylor Cone
Spray Needle
2-5 kV
Mass
Spectrometer
ESI Solution
Oxidation
Excess Charge on Surface
Reduction
Solvent and neutralized ions
Spray Current (i)
+
2-5 kV
power supply
−
(B)
Mirror
Laser
SOURCE
ANALYZER
DETECTOR
Analyte
Matrix
Sample
target
Acceleration
electrode
(19-25 kV)
Flight tube

Other Ionization Methods

Field Desorption

- Useful for nonvolatile and thermo-labile compounds.
- Sample is applied to field ion emitter and the solvent allowed to evaporate.
- Evaporated sample that leads to chemical ionization or EIS. •
- Example:- Nucleotides & Quaternary ammonium compounds.



PLASMA DESORPTION

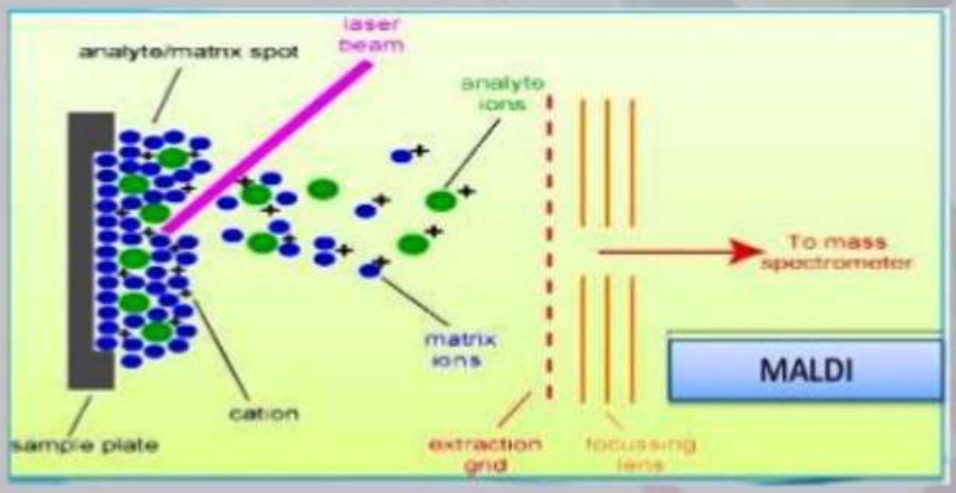
- Sample is coated with a high energetic fragment Californium 252.
- This fission fragment desorbs positive, negative, and neutral molecules.
- ^{252}cf generates 10^{12} power at 10,000k, this may ionize the target molecule.

LASER DESORPTION

- This method involves the interaction of laser beam with sample to produce both vaporization and ionization.
- The vaporized sample passed to mass spectrometers for analysis.
- Appl:-used for elemental analysis.

Matrix Assisted Laser Desorption /Ionization (MALDI)

- MALDI is a LIMS method of vaporizing and ionizing and sample molecules are dispersed in a solid matrix such as nicotinic acid.
- A UV laser pulse ablates the matrix which carries some of the large molecules into the gas phase in an ionized form so they can be extracted into a mass spectrometer.



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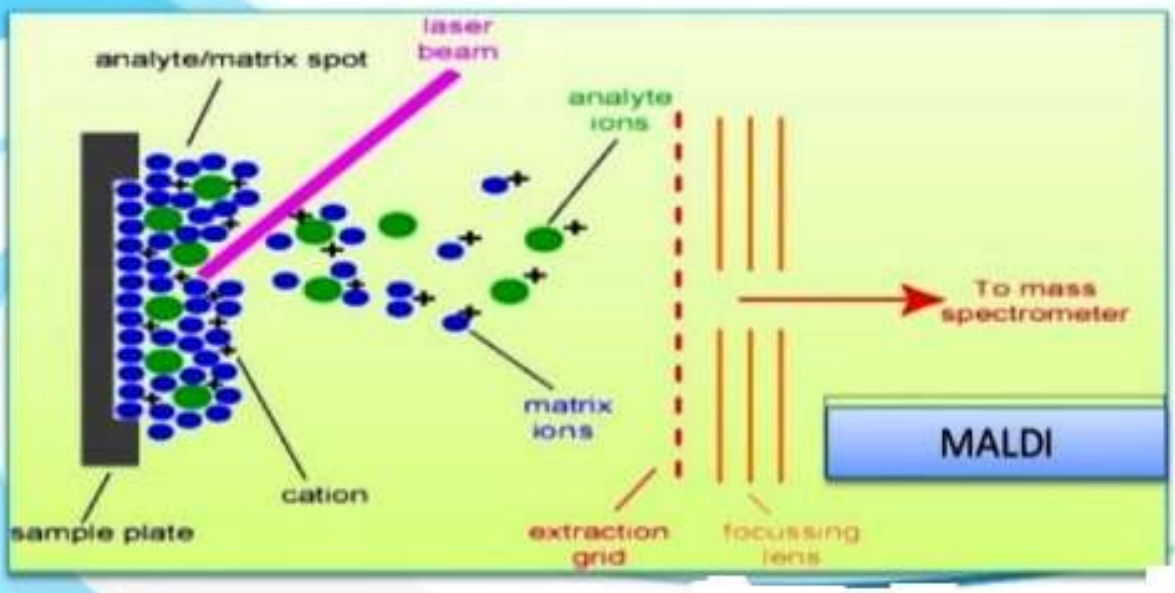

Matrix assisted laser desorption (MALDI)

- Matrix assisted laser desorption is a technique in mass spectrometry for ionization of biomolecules (polymers such as proteins, polypeptides and sugars) and synthetic polymers that are more fragile and form fragments when ionized by conventional methods.
- It consist of two components
- **1 Matrix :** Matrix is used in MALDI to
- Absorb the laser energy.
- Prevent analyte agglomeration.
- Protect analyte from being destroyed by direct laser beam

- Matrix consists of a crystallized molecules of which the most commonly used are
- Sinapinic acid)
- α – cyano cinnamic acid (α –cyano or α – matrix)
- Dihydroxy benzoic acid (DHB)
- Nicotinic acid
- Matrix solution is then mixed with the analyte to be investigated.
- The solution is then **spotted in a air tight chamber** on the **tip of the sample probe**.

- With a vacuum pump the air is removed and vacuum is created which leads to evaporation of the solvent leaving behind a layer of recrystalized matrix containing analyte molecules.
- **2 Laser**
- The **solid mixture** is then exposed **to pulsed laser beam**.
- The matrix absorbs the laser energy and transfers some of this energy to the analyte molecules which results in the **sublimation of sample molecules** as ions or the matrix after
- Absorbing the laser energy gets ionized and **transfer part of this charge** to the sample molecules and ionize it.