

CHROMATOGRAPHY

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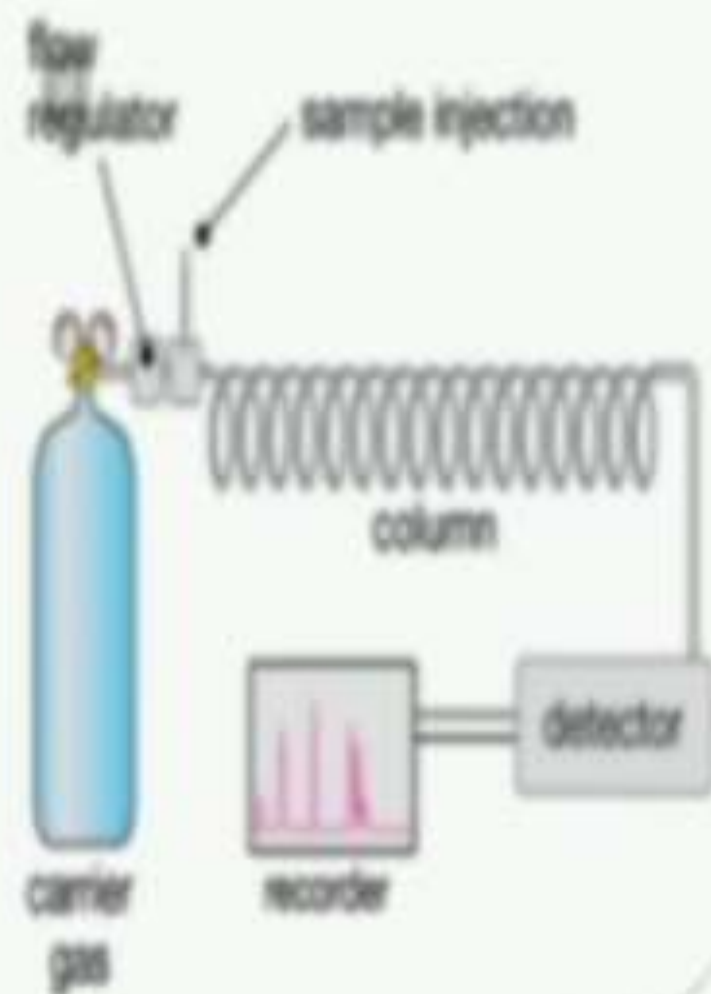
Introduction:

- ▶ **Chromatography is a combination of two Greek words chroma & Graphe.**
- ▶ **Chroma- color.**
- ▶ **Graphein-to write.**

DEFINITION:

Chromatography
-Is a technique that separating mixtures into their components in order to analyze, identify & purify the mixture or components.

Diagram of Chromatography



Principle of Chromatographic separation:

- Extracting the solutes back and forth between fresh portion of the two phases.
- The two phases are called the Mobile phase and the stationary phase.
- The principle of Chromatographic separation is very simple. The process is achieved distributing the substances to be separated between a mobile phase and a stationary phase.

Difference between partition & adsorption chromatography

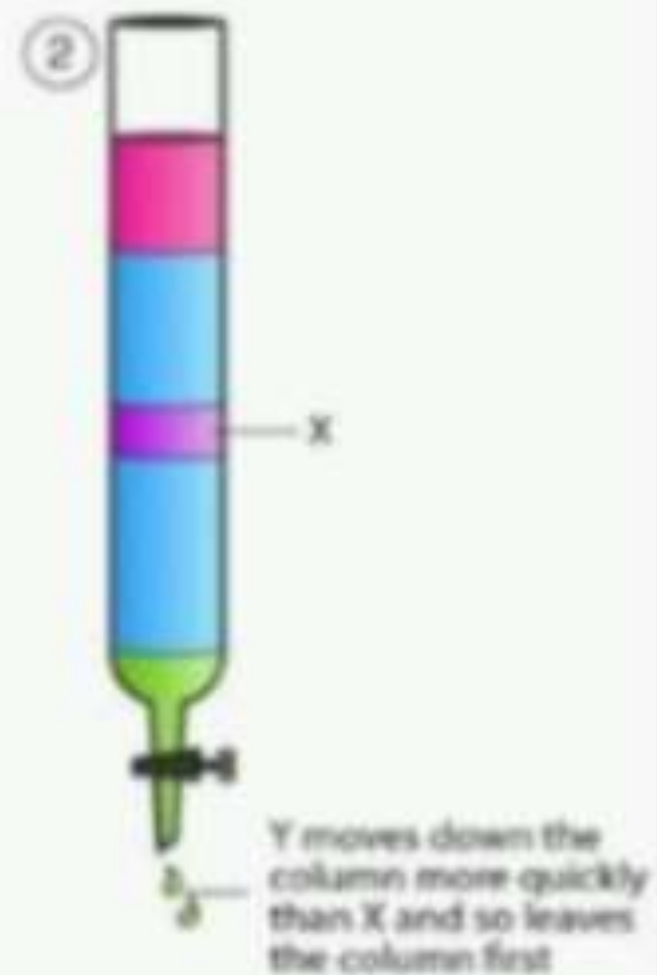
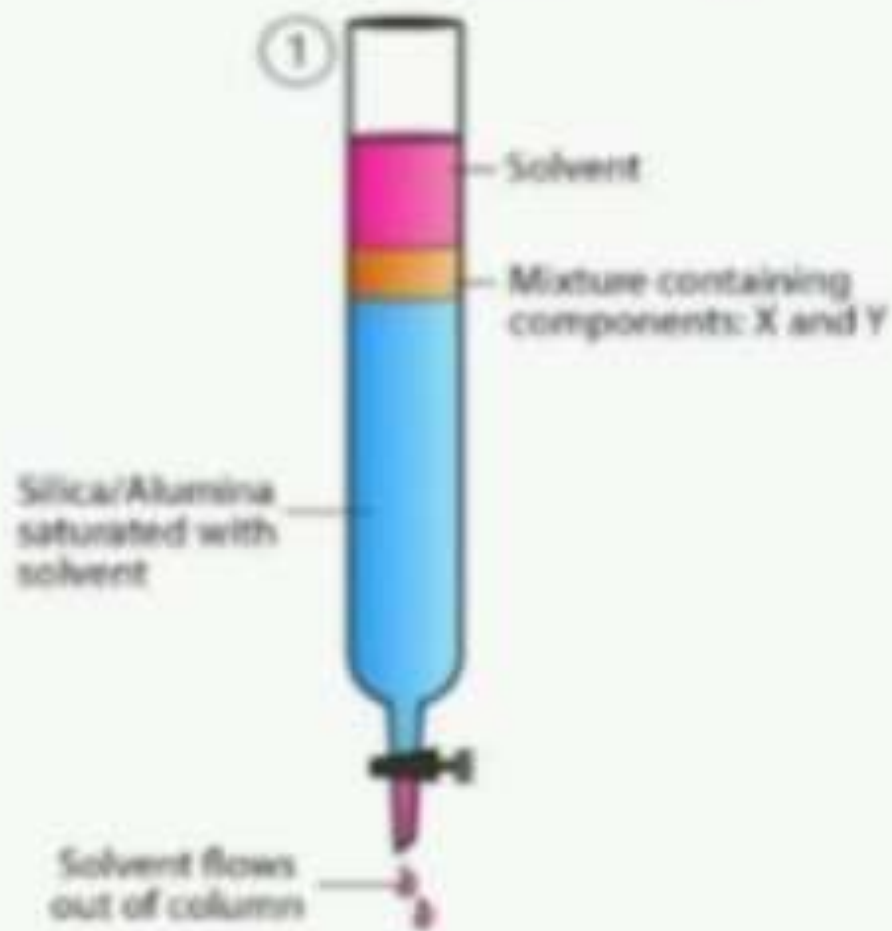
Partition chromatography

- ▶ **Partition chromatography :** separation on the stationary phase occurs by partition due to differences in partition coefficients.
- ▶ **Used for liquid -liquid gas chromatography.**

Adsorption chromatography

- ▶ **Adsorption chromatography:** relative differences in adsorption of constituents of given sample .
- ▶ **Used only for solid-liquid (or) solid-gas Chromatography.**

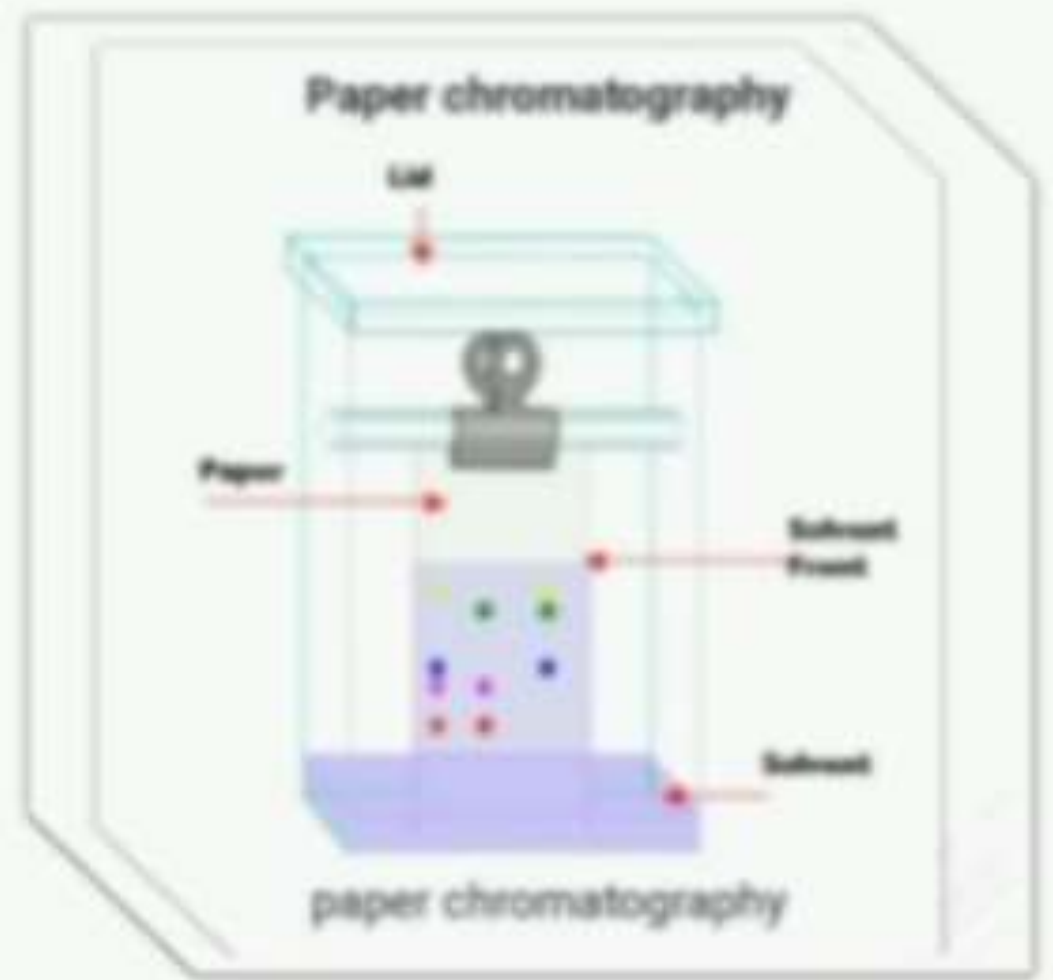
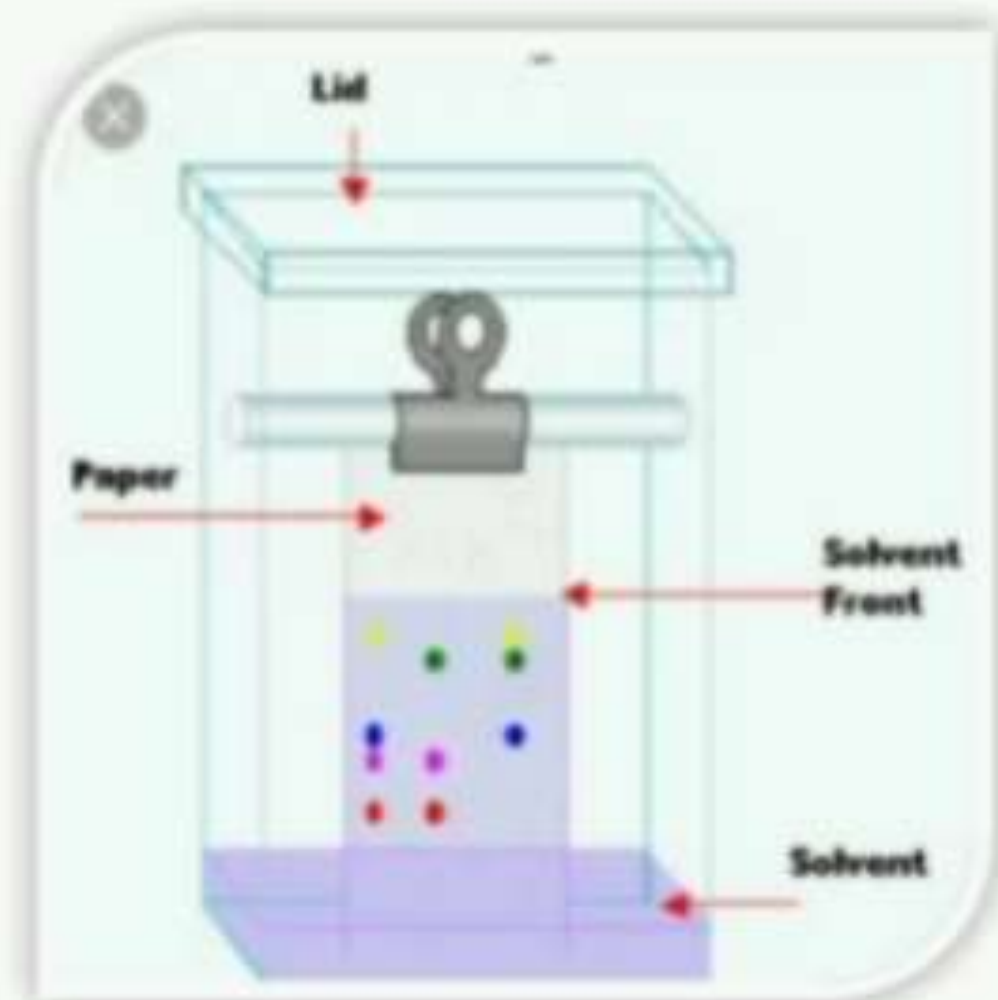
ADSORPTION CHROMATOGRAPHY



Paper vs Thin Layer Chromatography

Paper Chromatography	Thin-Layer Chromatography
Cheap	Faster
Little Preparation	Detects smaller amounts
More efficient for polar and water-soluble compounds	Better separation of less polar compounds
Easy to handle and store	Corrosive materials can be used
A wide range of stationary phases available	

Thin layer chromatography/paper chromatography



Difference between HPLC & HPTLC

HPLC

- ▶ **A form of liquid chromatography to separate compounds dissolved in a solution.**
- ▶ **High pressure liquid chromatography or high performance liquid chromatography.**
- ▶ **A type of column chromatography.**
- ▶ **Takes 2-60 min per sample.**

HPTLC

- ▶ **A most advanced form of planar Chromatography.**
- ▶ **High performance thin layer chromatography.**
- ▶ **A type of planar Chromatography.**
- ▶ **Takes 1-30 minutes per sample.**

HPTLC:

Instrumentation of HPTLC.

Applicator



Automatic developing chamber



Digital camera for photo documentation

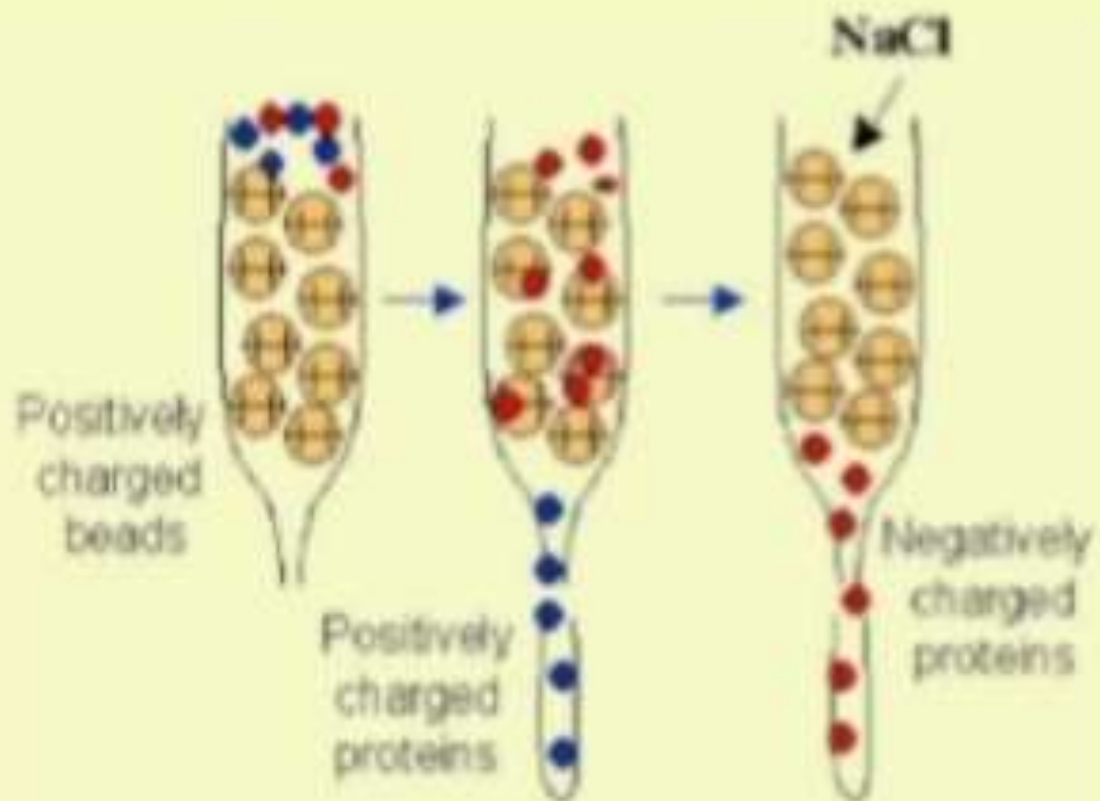


Scanner

Ion exchange chromatography

Definition: Ion exchange chromatography Usually referred as ion Chromatography uses an ion exchange mechanism to separate molecules on basis of their electrical chargers.

Ion – exchange Chromatography



Gel filtration chromatography

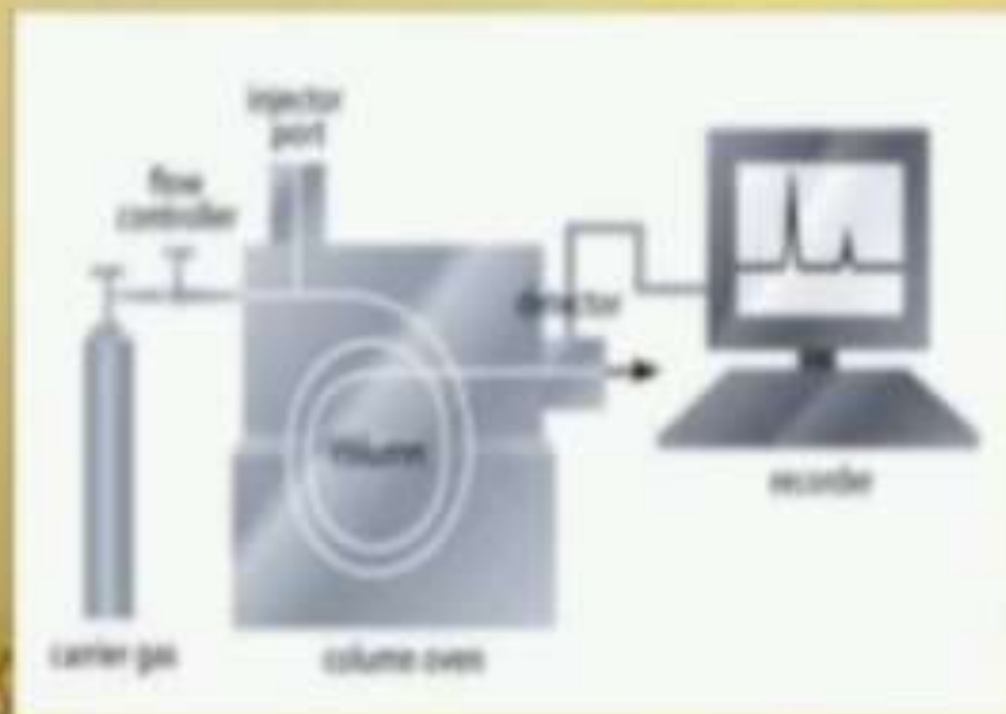
- It is also known as gel permeation chromatography (GPC) or size exclusion chromatography (SEC).
- It separates molecules according to their size, shape and molecular weight.

Difference b/w affinity & ion exchange chromatography

	Affinity Chromatography	Ion Exchange Chromatography
DEFINITION	A biochemical technique used to separate components in a mixture depending on interactions between these components.	A form of liquid chromatography in which we can analyze ionic substances.
COMPONENTS	Can separate both charged and uncharged particles.	Can separate only charged particles.
PRINCIPLE	Target molecules have a high affinity for the stationary phase.	Target molecules have an opposite charge to that of the stationary phase surface.
EXAMPLES	Antigens, antibodies, proteins, DNA, enzymes, substrates, etc.	Inorganic anions and cations, organic ions, proteins, etc.



Gas Chromatography



- **Gas chromatography is a technique by which a mixture is separated into its constituents by a moving gas phase over a stationary phase.**

- **GLC(gas liquid chromatography):**

It is a technique where be the components of a mixture in the gaseous state are separated as the sample passes over a stationary phase & a gaseous mobile phase.

Based on stationary phase GC is classified into two types:

Gas-solid Chromatography (GSC).

Gas-liquid chromatography (GLC)

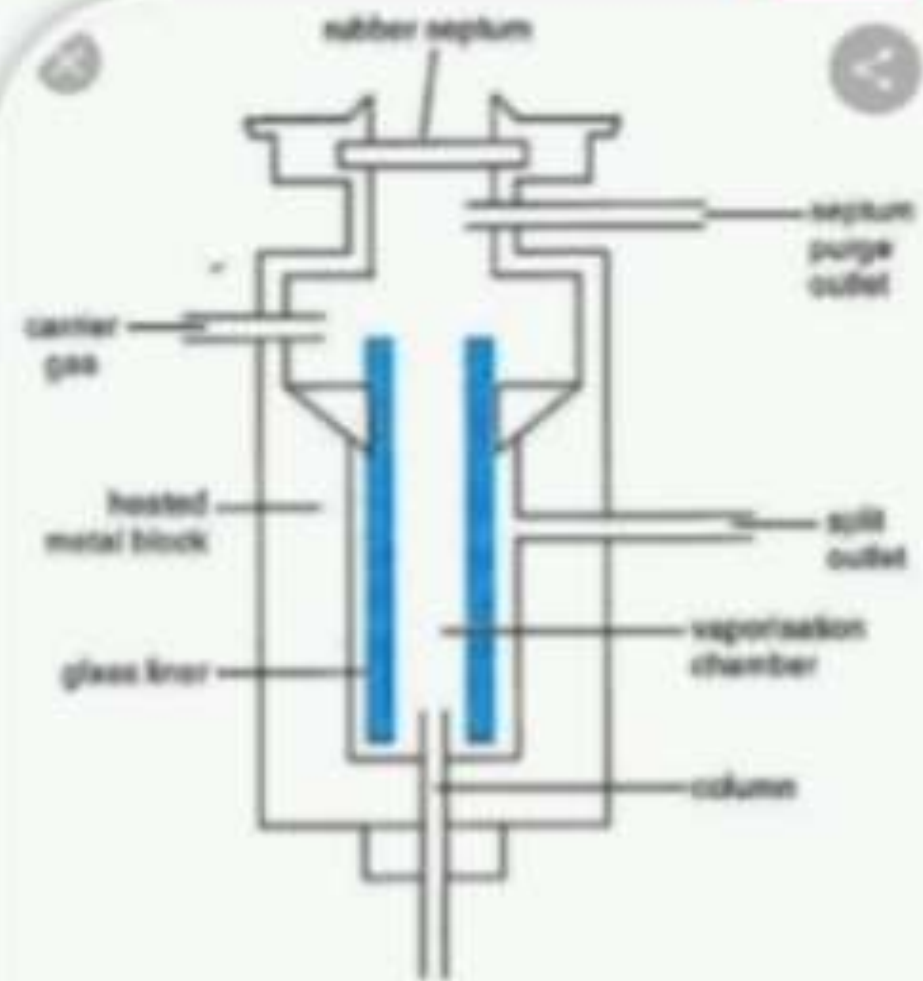
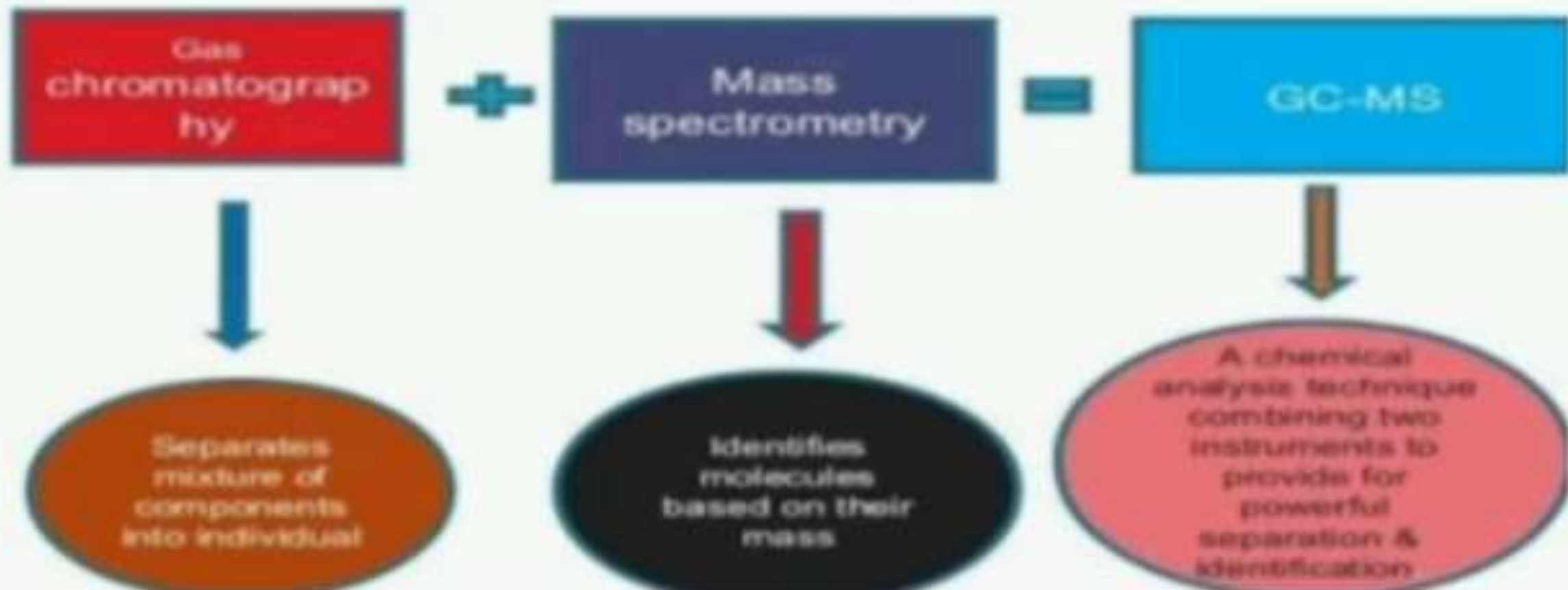


Fig. 9. Injection port.

GC-MS



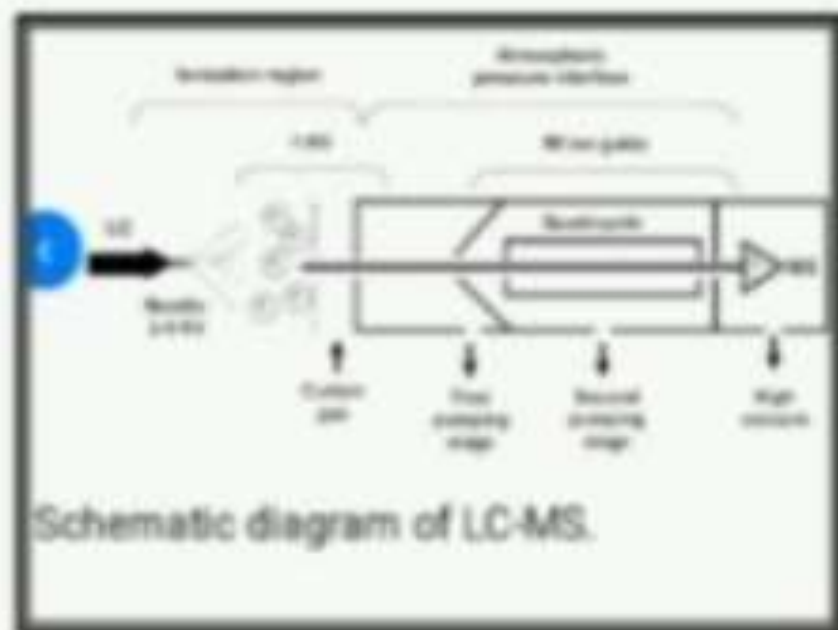
LC-MS

- LC-MS (liquid chromatography & Mass spectrometry).
- LC-MS was introduced by russian botanist in 1900s

Application:

1. High polar doping agents- Screening analysis.
2. Analysis Impurities-pharma.

Diagrammatic representation of LC-MS



Chromatography technique is very rapidly increasing technology in present day.

Conclusion: I here by conclude my presentation by saying few words.

It has application in majority of fields.

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THANK YOU