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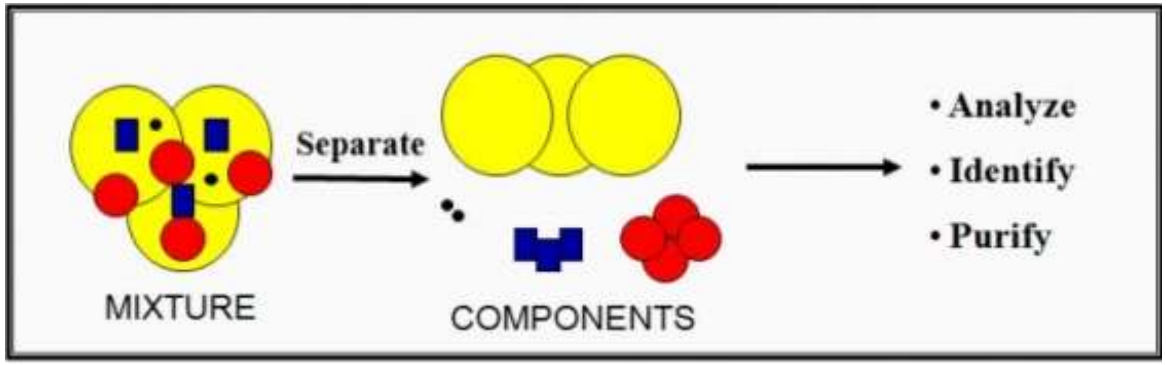
Chromatography

The most difficult operation in any phytochemical research is the isolation and purification of plant constituents. Chromatography is the most important of all purification methods now, is a comparatively simple method of separating a desired compound from its impurities, or of isolating individual components of a mixture.

- **Chromatography term represents a number of highly efficient techniques used for separation of a wide range of substances including organic & inorganic compounds.**
- **So, this technique is used for separating mixtures into their compounds in order to analyze, identify & purify the mixtures or components.**



Historic background

- **Mikhail Tswett (Tsvett), a Russian botanist, used the word 'Chromatography' to describe his separation of plant pigments, which was carried out by passing an extract of the pigments through a column packed with calcium carbonate.**

- **The result was a series of colored zones on the column and, thus, the name chromatography from the Greek words**
- ***chromatus*** **and** ***graphein*****, meaning ‘color’ and ‘to write’.**

What

Chromatograph

means

NOW????

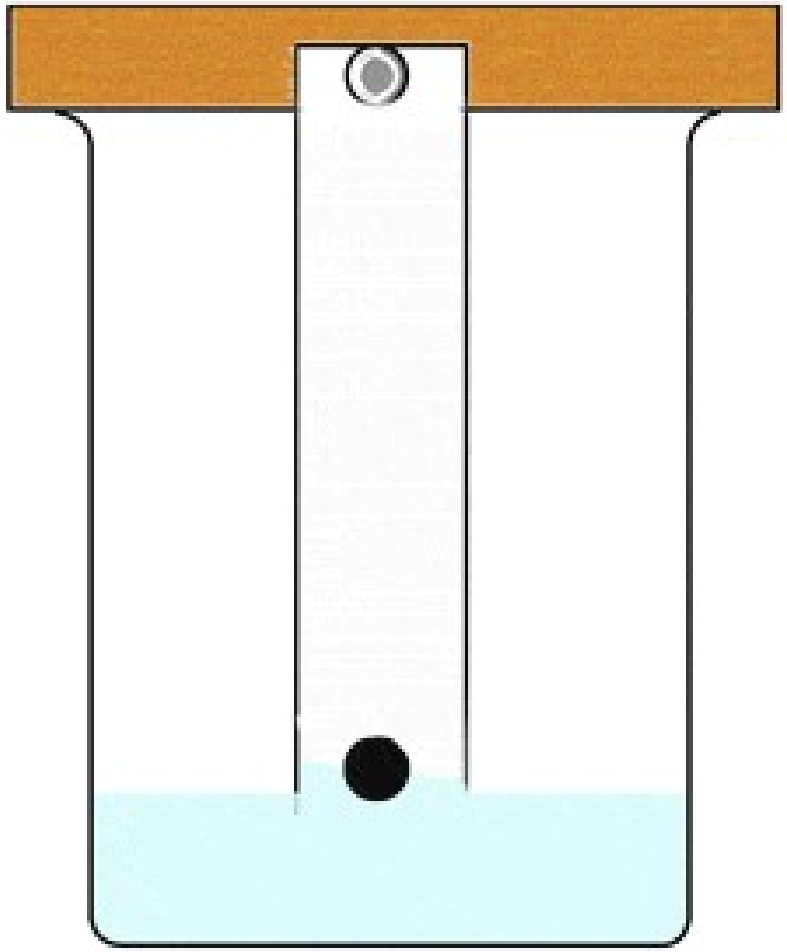
Chromatography

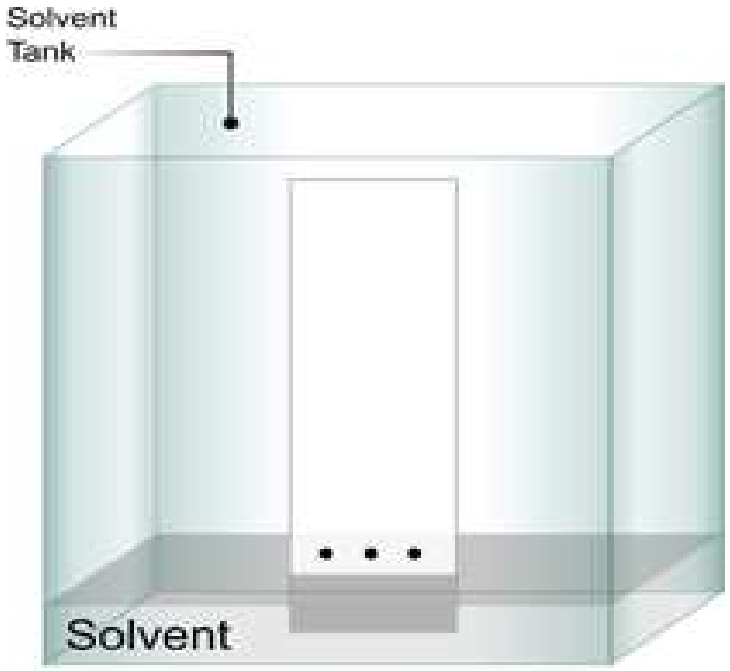
- **Chromatography is the term used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selectively absorbent stationary phase. There are number of different kinds of chromatography, which differ in the mobile and the stationary phase used.**

ANALOGY…

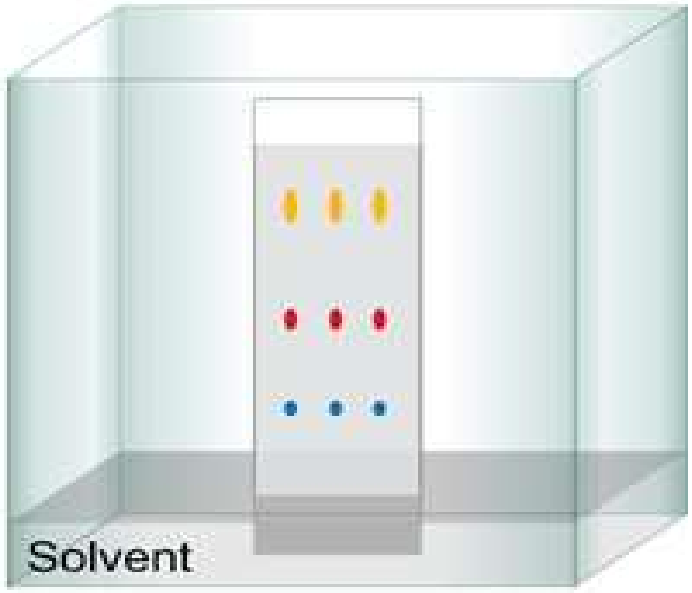
Mobile phase

Stationary phase

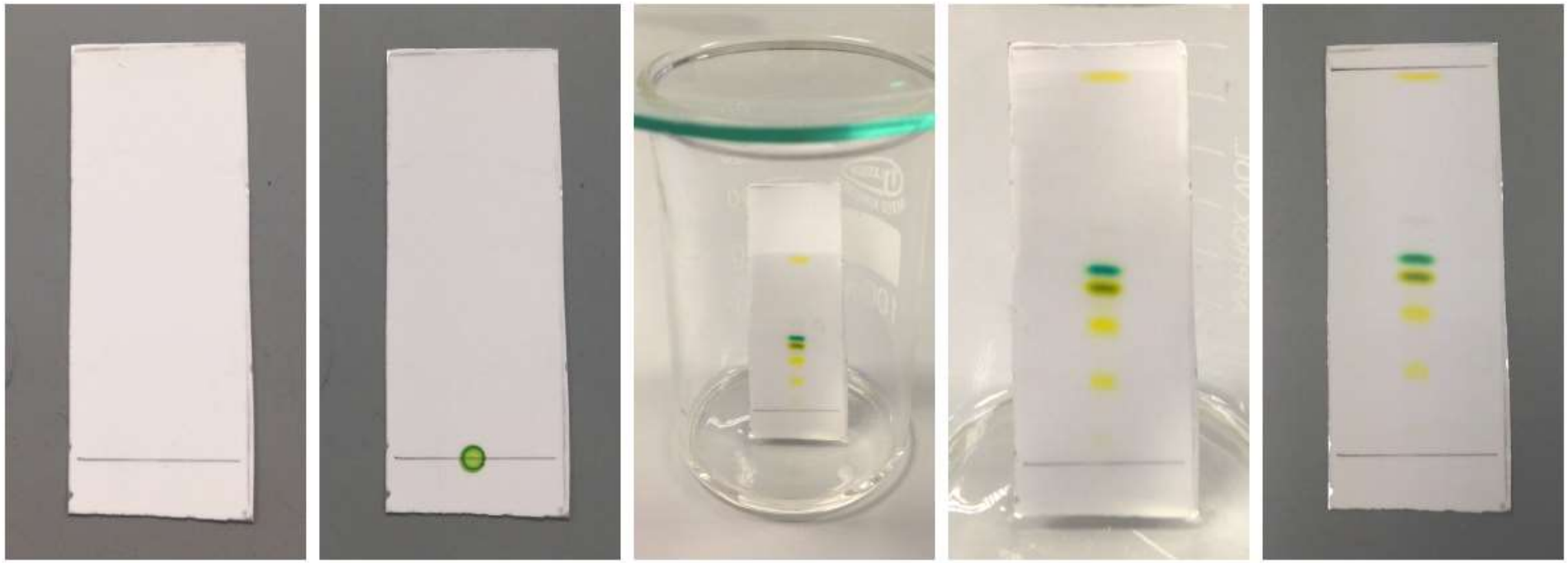




Time Zero



After Ten Minutes



In Chromatography we have 2 phases:

1) The first is called the Stationary phase ((called also **adsorbent** or sorbent phase))

- It is the phase in which the sample component to be separated is applied & it could be **solid** or **liquid**.

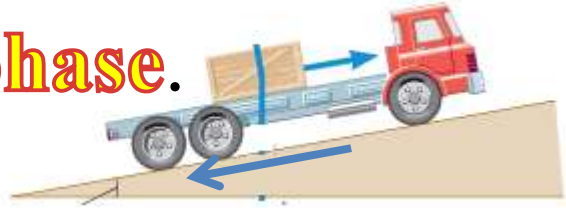
2) The second phase is called the mobile phase ((also called the **solvent system** or **eluent**))

- It is the phase which is going to move along the stationary phase resulting in separation of the sample component. This phase may be **liquid** or **gas**.

Chromatographic separation techniques are based on the principle of an unequal distribution or differential distribution of the mixture components between these two phases(stationary & mobile phase) by the action of two forces:

1) Driving force of the mobile phase.

2) Retarding force of **stationary phase**.

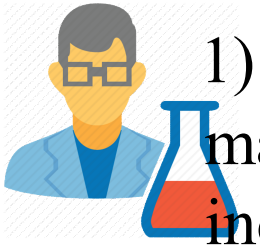


Generally, Chromatography can be considered as both **qualitative & quantitative** method of analysis.

By qualitative we mean that we can know the **quality of each component present in the mixture**. Such as determining the type of amino acid (a.a.) constituting the protein under study

By quantitative we mean that we can determine the **percent or the quantity of each component** present in the sample under study.

Aim of using chromatography:



1) Scientific research: chromatography is widely used in many scientific studies as in pharmaceutical industry, oil industry, geology……& all most all sciences.

2) Analytical procedures: Chromatography is used to determine the quantity & the percent of each component in the mixture or the sample to be separated & it is mainly used in medical field or chemistry.

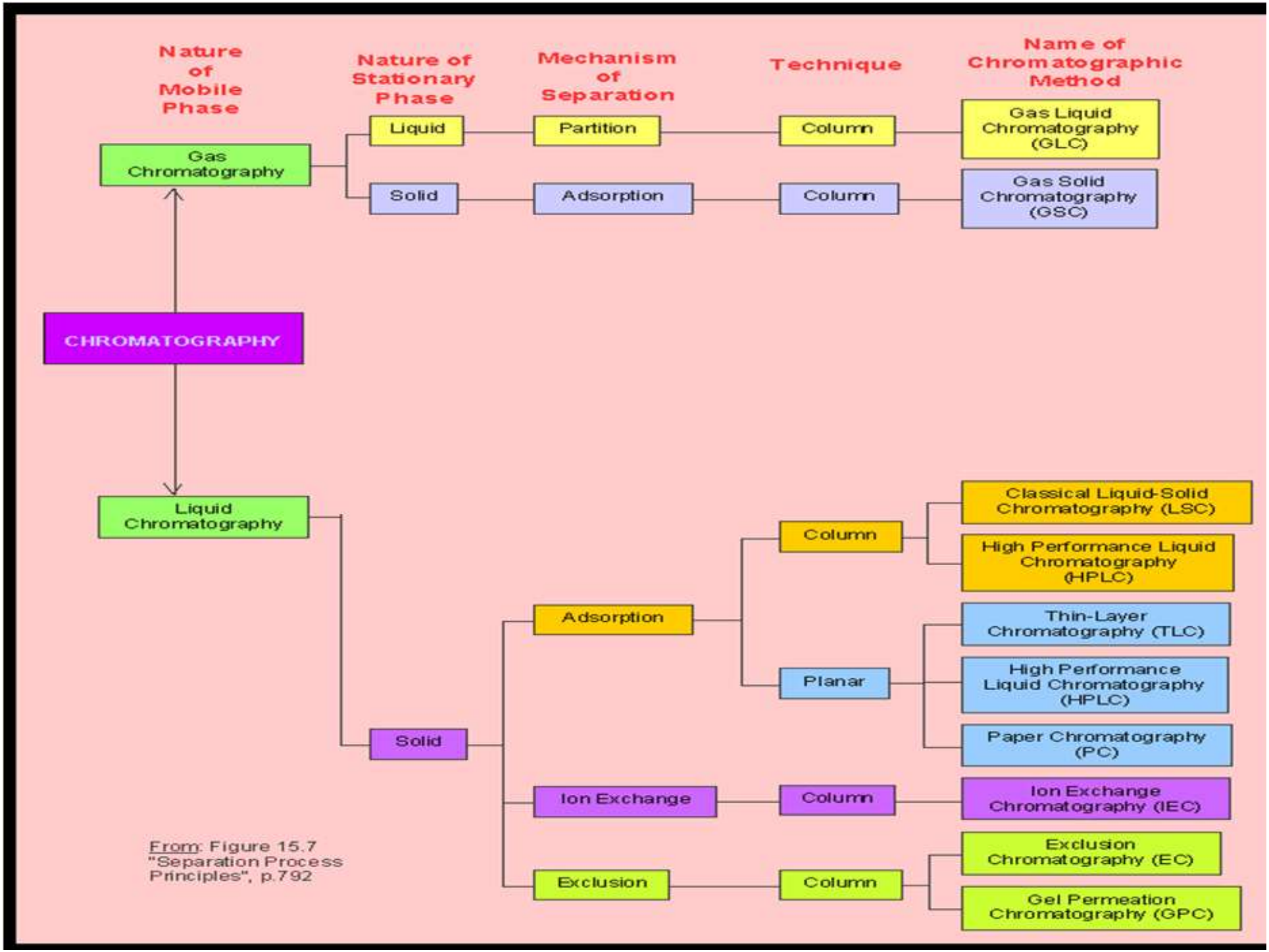
3) Purification: chromatography is used to purify large quantity of the desired compound by preparative techniques

Advantages:

1) The high selectivity in separation shown by the technique.

2) Another important advantage of chromatography is that its use involves mild experimental conditions as, for example, it can be generally carried out at room temperature.

Classification of Chromatography


Nature of Mobile Phase
Nature of Stationary Phase
Mechanism of Separation
Technique
Name of Chromatographic Method
CHROMATOGRAPHY
Gas Chromatography
Liquid
Partition
Column
Gas Liquid Chromatography (GLC)
Solid
Adsorption
Column
Gas Solid Chromatography (GSC)
Liquid Chromatography
Solid
Adsorption
Column
Classical Liquid-Solid Chromatography (LSC)
High Performance Liquid Chromatography (HPLC)
Planar
Thin-Layer Chromatography (TLC)
High Performance Liquid Chromatography (HPLC)
Paper Chromatography (PC)
Ion Exchange
Column
Ion Exchange Chromatography (IEC)
Exclusion
Column
Exclusion Chromatography (EC)
Gel Permeation Chromatography (GPC)
From: Figure 15.7 "Separation Process Principles", p.792

Classification of chromatography according to mechanism of separation

1) Adsorption

2) Partition

3) Ion-exchange

4) Gel filtration (molecular sieves, exclusion)

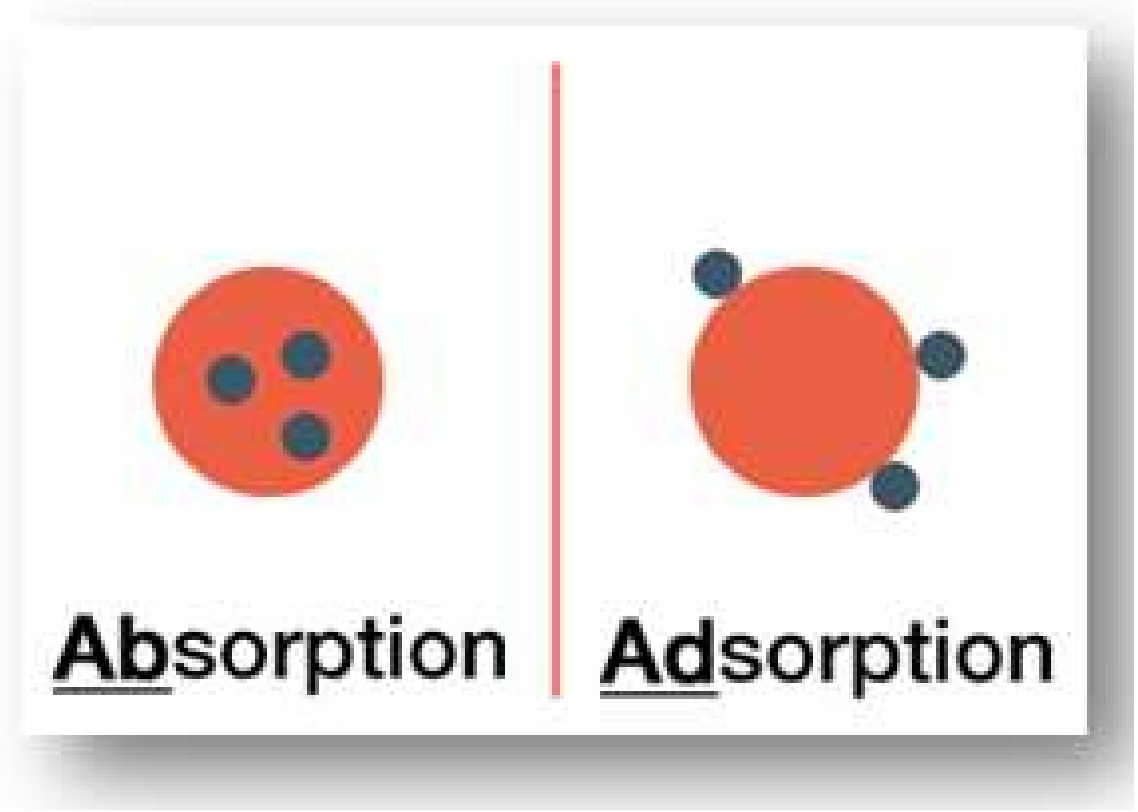
1) Adsorption

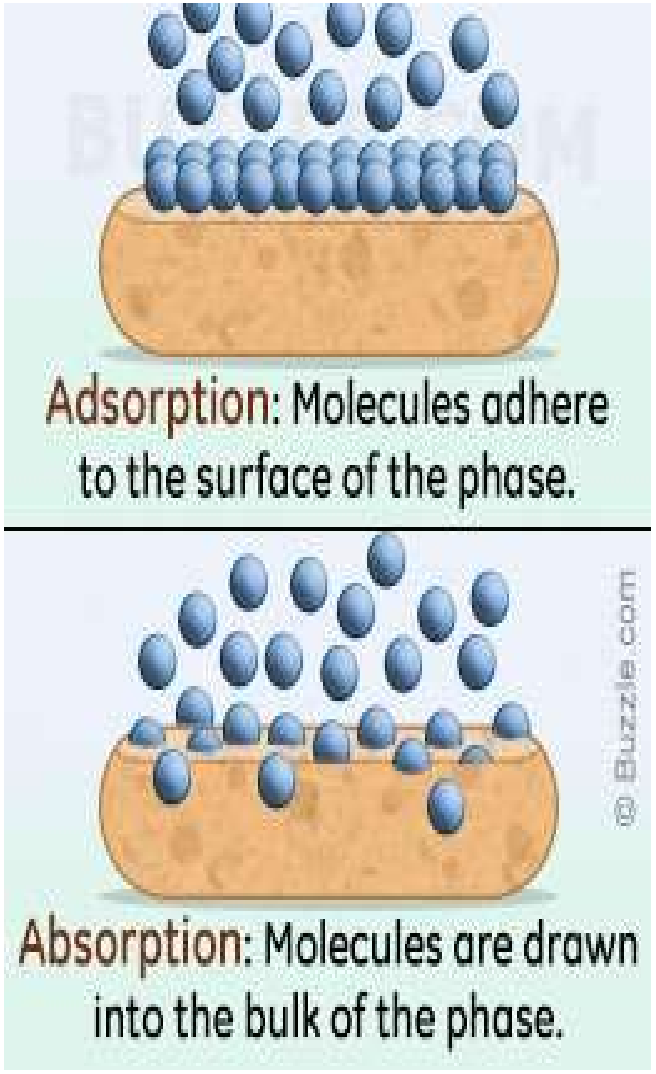
If the mechanism of separation is ((adsorption)) then the chromatography is said to be ((adsorption chromatography)).

The stationary phase is solid

The mobile phase is liquid or gas.

Adsorption versus Absorption


Absorption
Adsorption


Adsorption: Molecules adhere to the surface of the phase.
Absorption: Molecules are drawn into the bulk of the phase.
© Buzzle.com

Principal of adsorption mechanism of separation

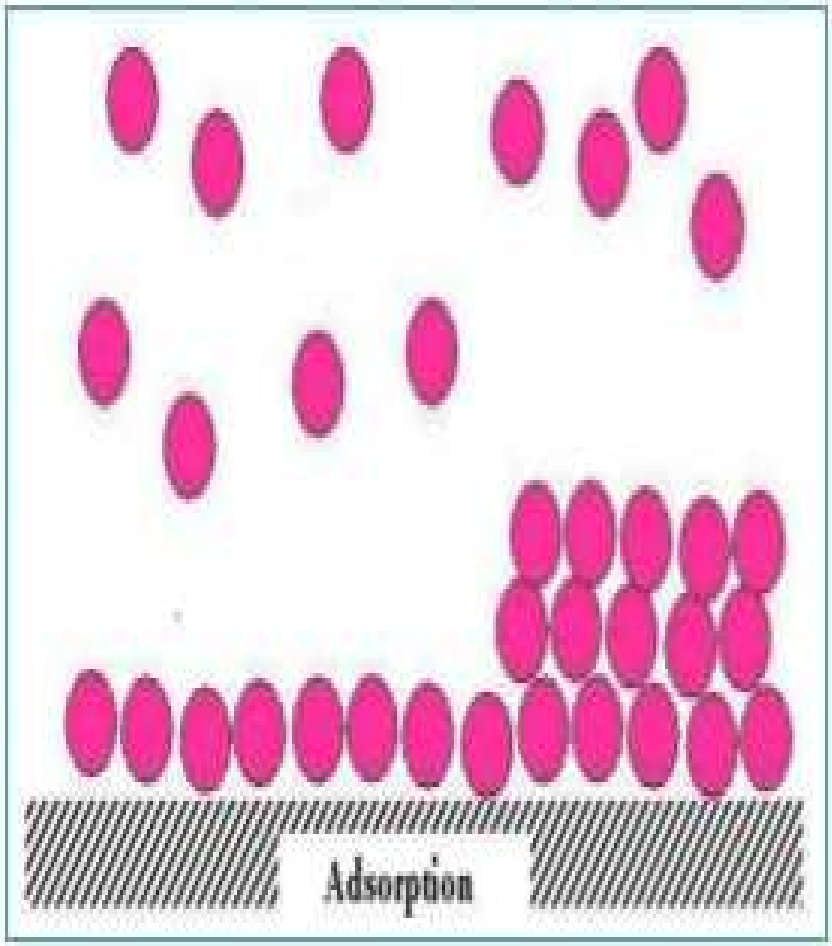
Adsorption chromatography is a process of separation of components in a mixture introduced into chromatography system **based on** the **relative differences** in adsorption of components to the stationary phase present in the chromatography column.

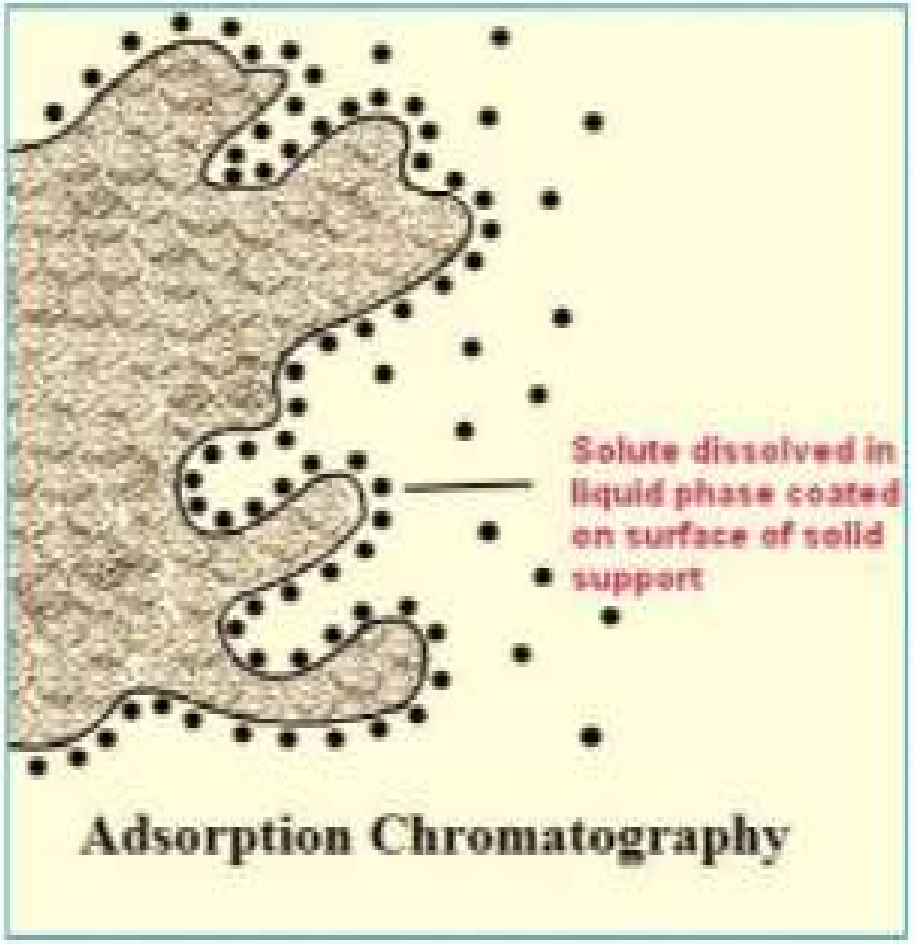
Here the molecules or components of the mixture travel with different rates due to differences in their affinity towards stationary phase.

Adsorption means
a physical attachment between the compound and the particles of stationary phase. Based on the nature, polar compounds adsorb with stronger or greater intensity to the polar stationary phase while non-polar compounds adsorb better to the non-polar stationary phase than polar components.

Hence during separation of components, when we use a polar stationary phase, polar components elute out late due to greater adsorption and non-polar components get out of the column or elute out first. This is exactly reverse on using a non-polar stationary phase.

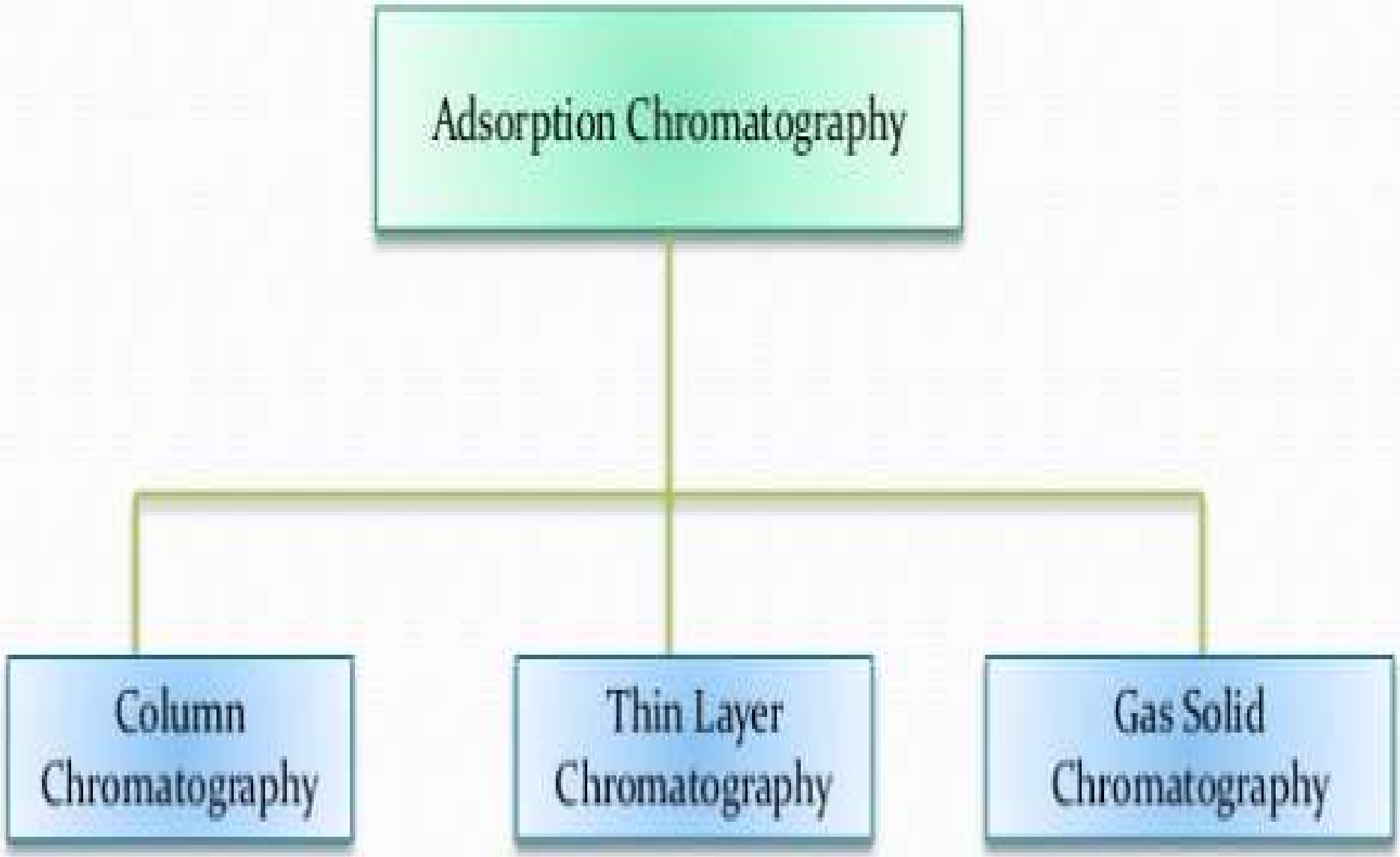
This adsorption chromatography applies to only **solid-liquid** or **solid-gas** chromatography. **Because the adsorption phenomenon is inherent property of solids** and hence it is used with only solid stationary phase chromatography.


Adsorption


Solute dissolved in liquid phase coated on surface of solid support
Adsorption Chromatography

Principle of Adsorption Chromatography

- Involves competition of components of sample mixture for active site on adsorbent. These active sites are formed in molecule due to
  - **Cracks**
  - **Edges**
- Separation occurs because of the fact that an equilibrium is established between molecules adsorbed on stationary phase and those which are flowing freely in mobile phase. The more the affinity of the molecule of particular component, less will be its movement.


Adsorption Chromatography
Column Chromatography
Thin Layer Chromatography
Gas Solid Chromatography

The most common solid adsorbants used in chromatography are:

Silica gel,

Alumina,

Activated charcoal

Adsorption chromatography is valuable in the **isolation** and **purification** of


Vitamins,

Hormones,


Many alkaloids.


Cardiac glycosides,

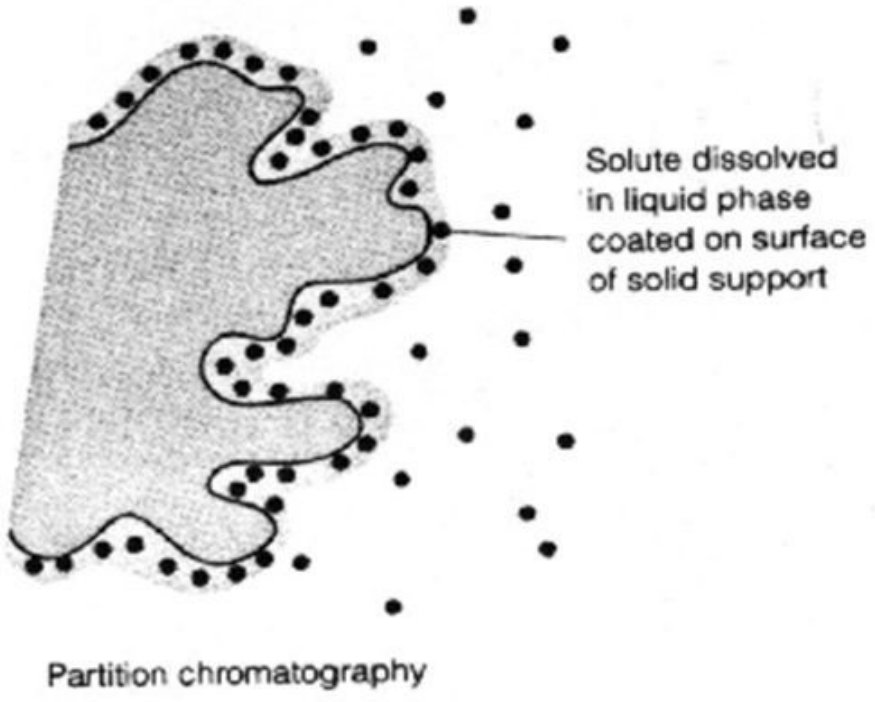

Anthraquinones, etc.

2) Partition:

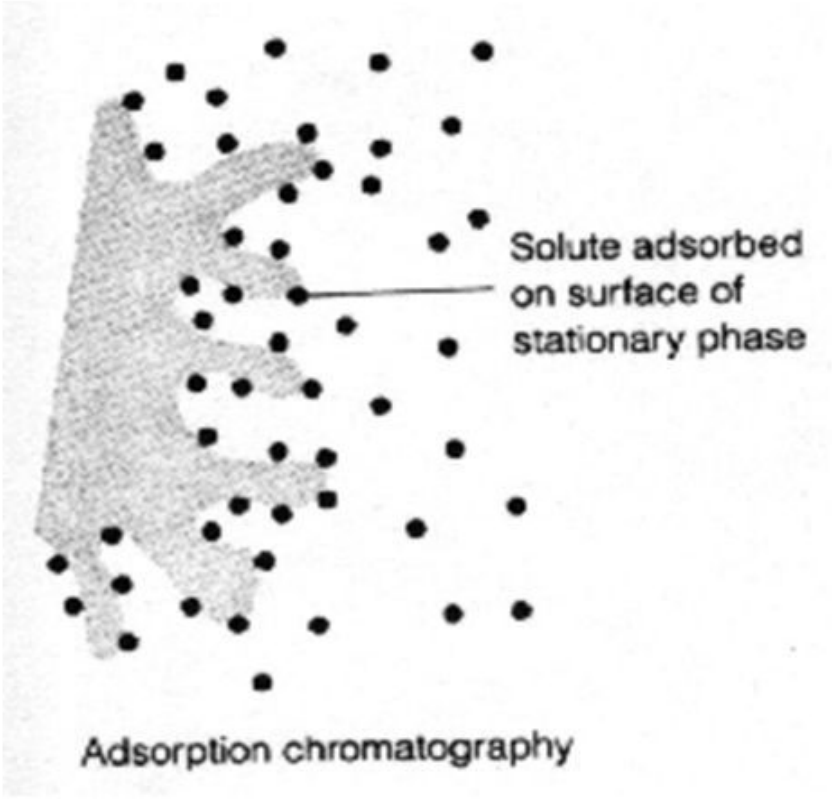
It **depends** on the **partition coefficient** or the **relative solubility of the sample** in the two phases

- **The stationary phase is liquid, while the mobile phase is liquid or gas.**
- **The difference between adsorption & partition mechanisms is the type of forces responsible for distribution of the sample between these two phases.**

Partition chromatography



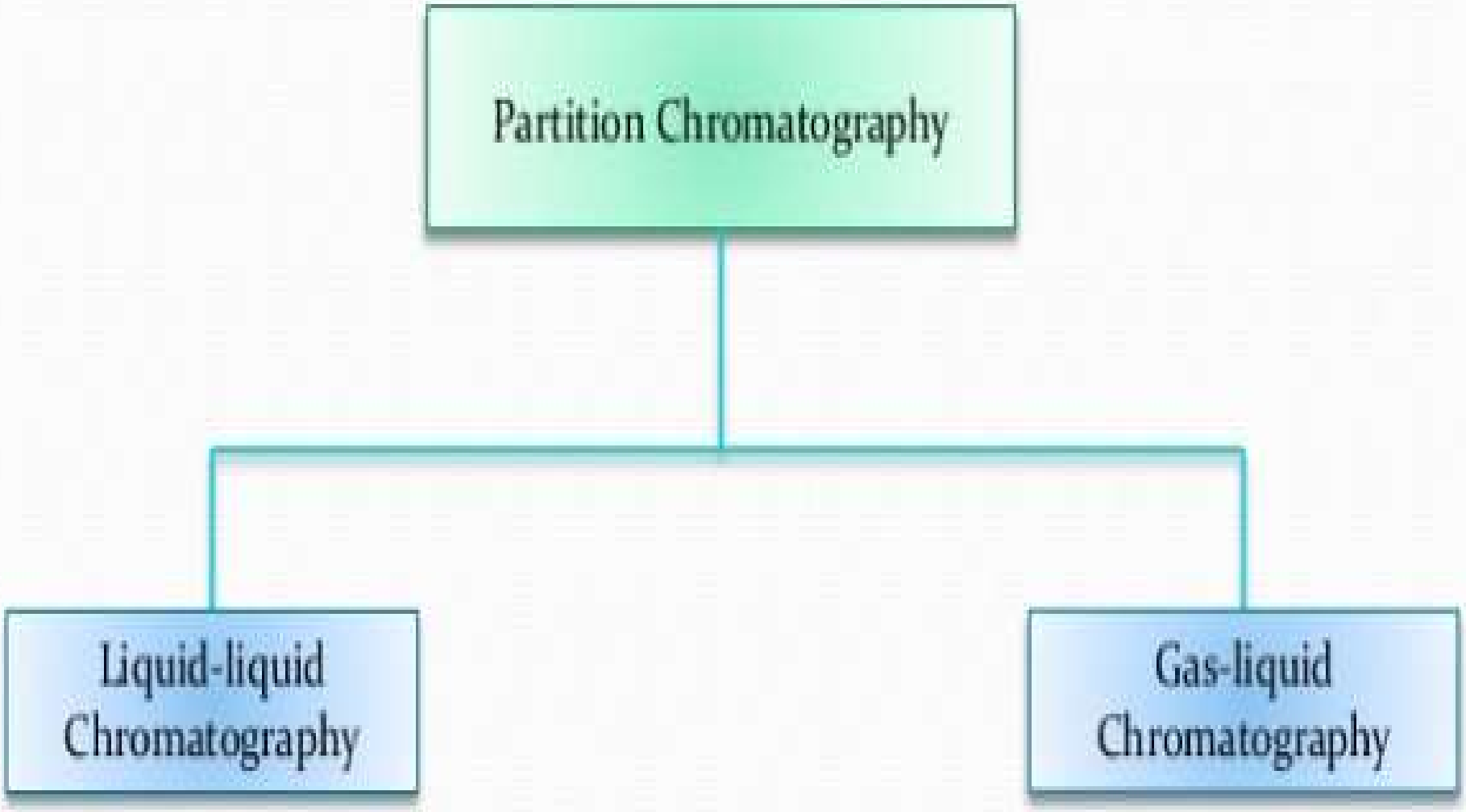
Partition chromatography



Adsorption chromatography

เช่น paper chromatography

In adsorption mechanism the adsorption coefficient is the main factor responsible for chromatography(separation) , while partition mechanism , the partition coefficient is the main factor responsible for chromatography.

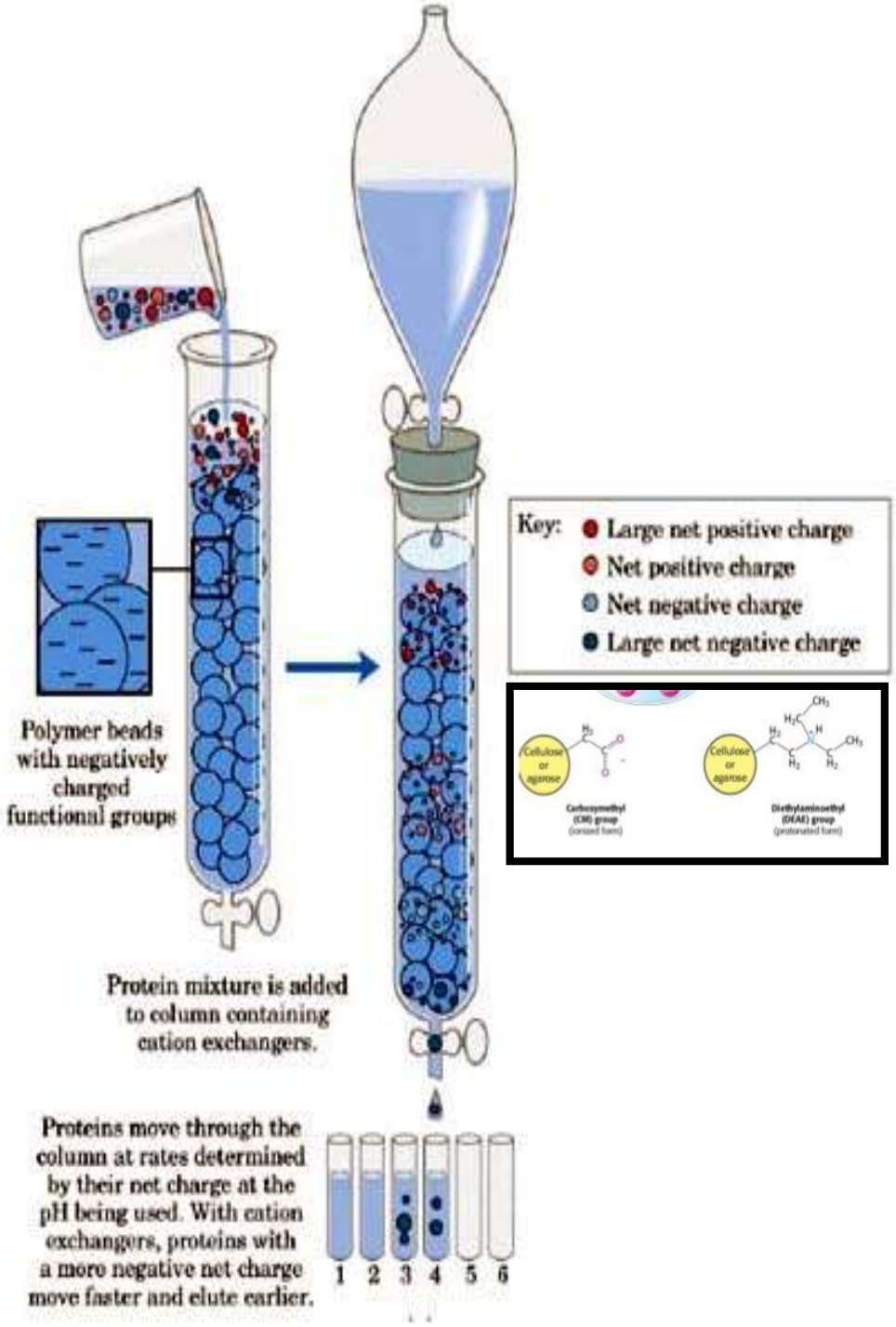

Partition Chromatography
Liquid-liquid Chromatography
Gas-liquid Chromatography

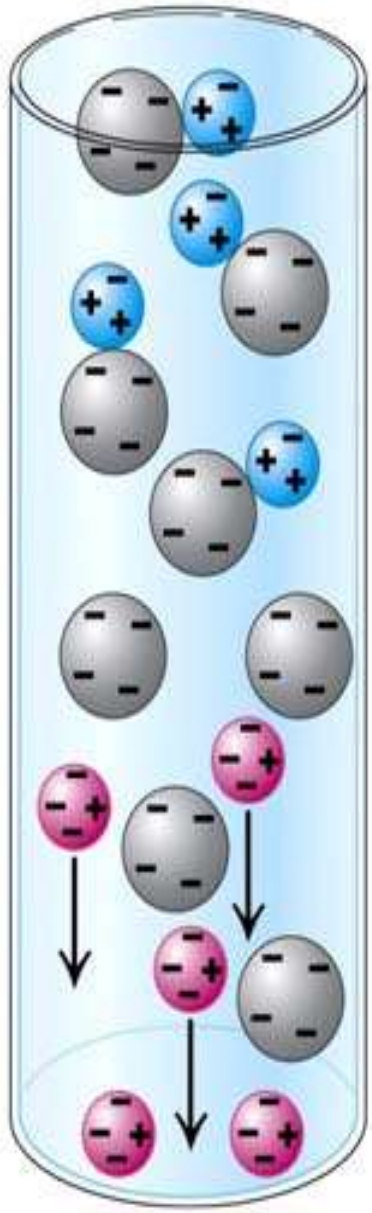
3)Ion-exchange:

- Ion-exchange chromatography (IEC) is part of ion chromatography which is an **important analytical technique for the separation and determination of ionic compounds**.

- This chromatography is one of the most important techniques **used in the separation** of peptides, **proteins**, nucleic acids and related **biopolymers** which are **charged molecules** in different molecular sizes and molecular nature

- Its suitable for separating ionic compounds or substances form ionic species in aqueous medium
- Using ionic solution (mobile phase) such as buffer & solid stationary phase such as synthetic resin prepared in the form of cross linked chain & may be in a form of beds.





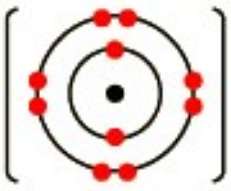
Positively charged protein binds to negatively charged bead

Negatively charged protein flows through

Ion exchange mechanism used for:

Softening the water

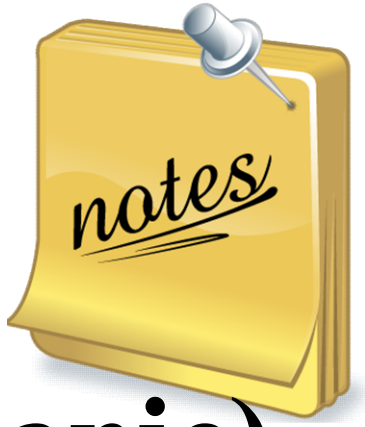
Refining sugars



Separation of metal ions preparation of deionized water

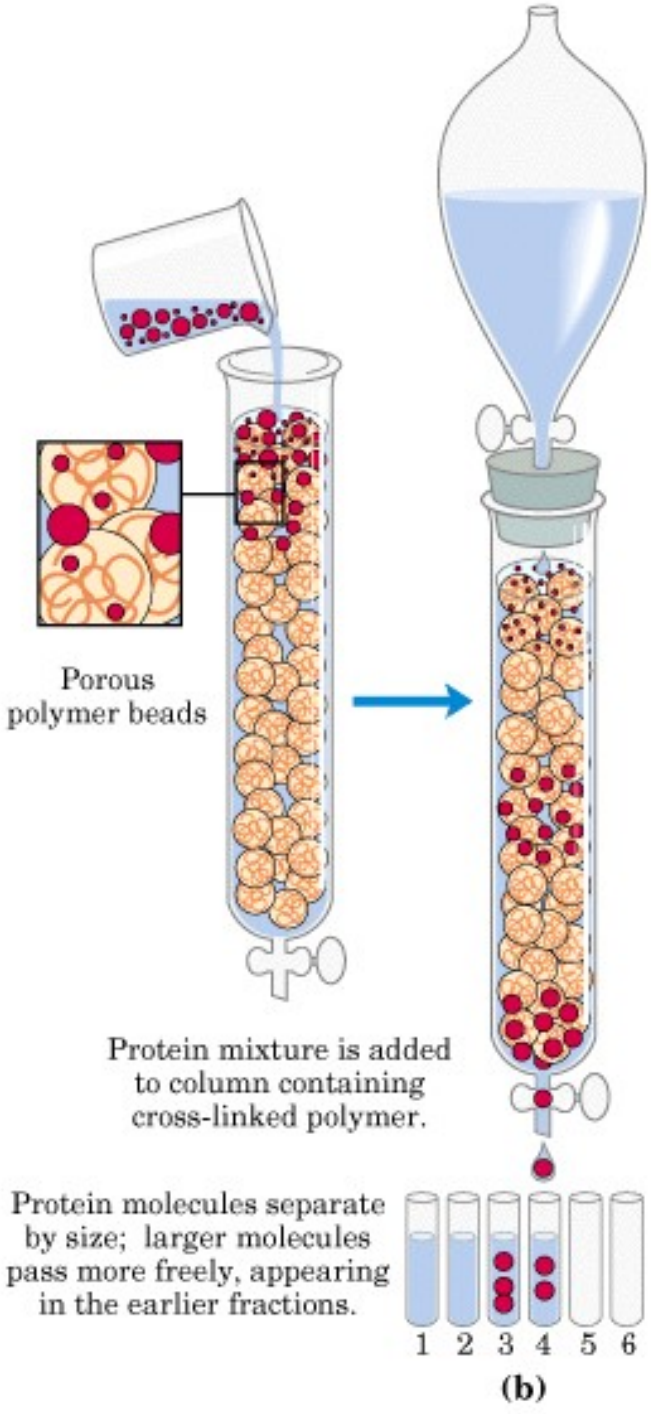
4) Gel filtration (molecular sieves)

in this mechanism the separation is made according to the molecular size & shape.



- **The mobile phase should be liquid (organic or inorganic) such as water, ethanol, methanol…..**
- **This method is used for separation of enzymes, protein & nucleic acids.**

- **Result in gel filtration chromatography, in this mechanism the separation is made according to the molecular size & shape.**
- **The gel or the stationary phase which is solid prepared by cross linking of long chain dextran & made in a form of granules or beads, the particles of the gel has pores, the pores size is determine by the degree of cross linking**


Porous
polymer beads
Protein mixture is added
to column containing
cross-linked polymer.
Protein molecules separate
by size; larger molecules
pass more freely, appearing
in the earlier fractions.
1 2 3 4 5 6
(b)


The end is
very near
Thanks!