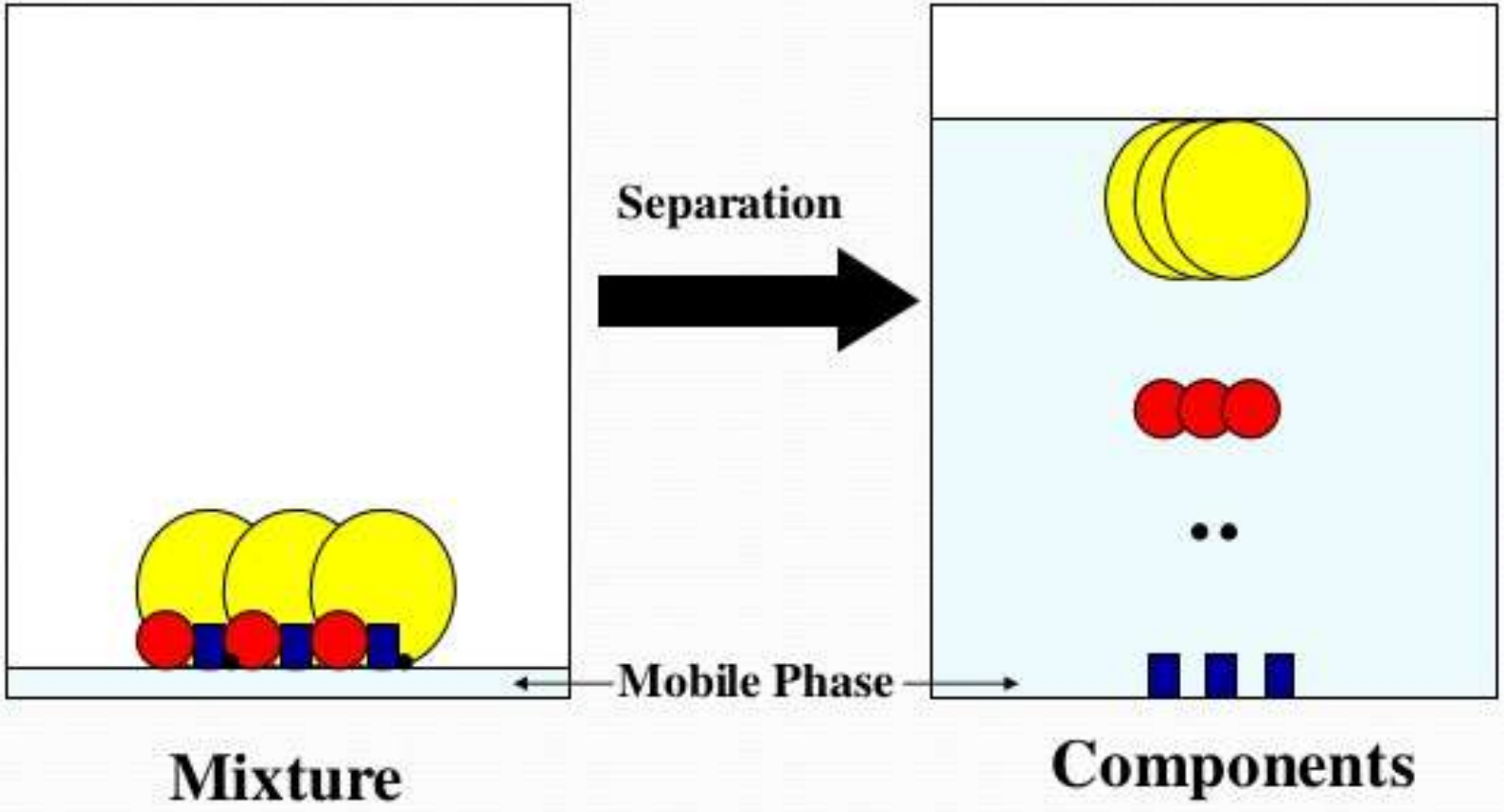
Chromatography

For Sem IV

Chromatography

- Chromatography is a method of separation in which the components to be separated are distributed between two phases, one of these is called a stationary phase and the other is a mobile phase which moves on stationary phase in a definite direction. The component of the mixture redistribute themselves between two phases by a process which may be adsorption, partition, ion exchange or size exclusion.
- The stationary phase can be solid or a liquid and the mobile phase can be liquid, gas or a supercritical fluid.

Illustration of Chromatography



Introduction

- The Term Chromatography (chroma = a colour; graphein = to write) is the collective term for a set of laboratory techniques for the separation of mixtures.
- Chromatography involves a sample (or sample extract) being dissolved in a *mobile phase* (which may be a gas, a liquid or a supercritical fluid).
- The mobile phase is then forced through an immobile, immiscible *stationary phase*.
- The phases are chosen such that components of the sample have differing solubilities in each phase.
- A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase.

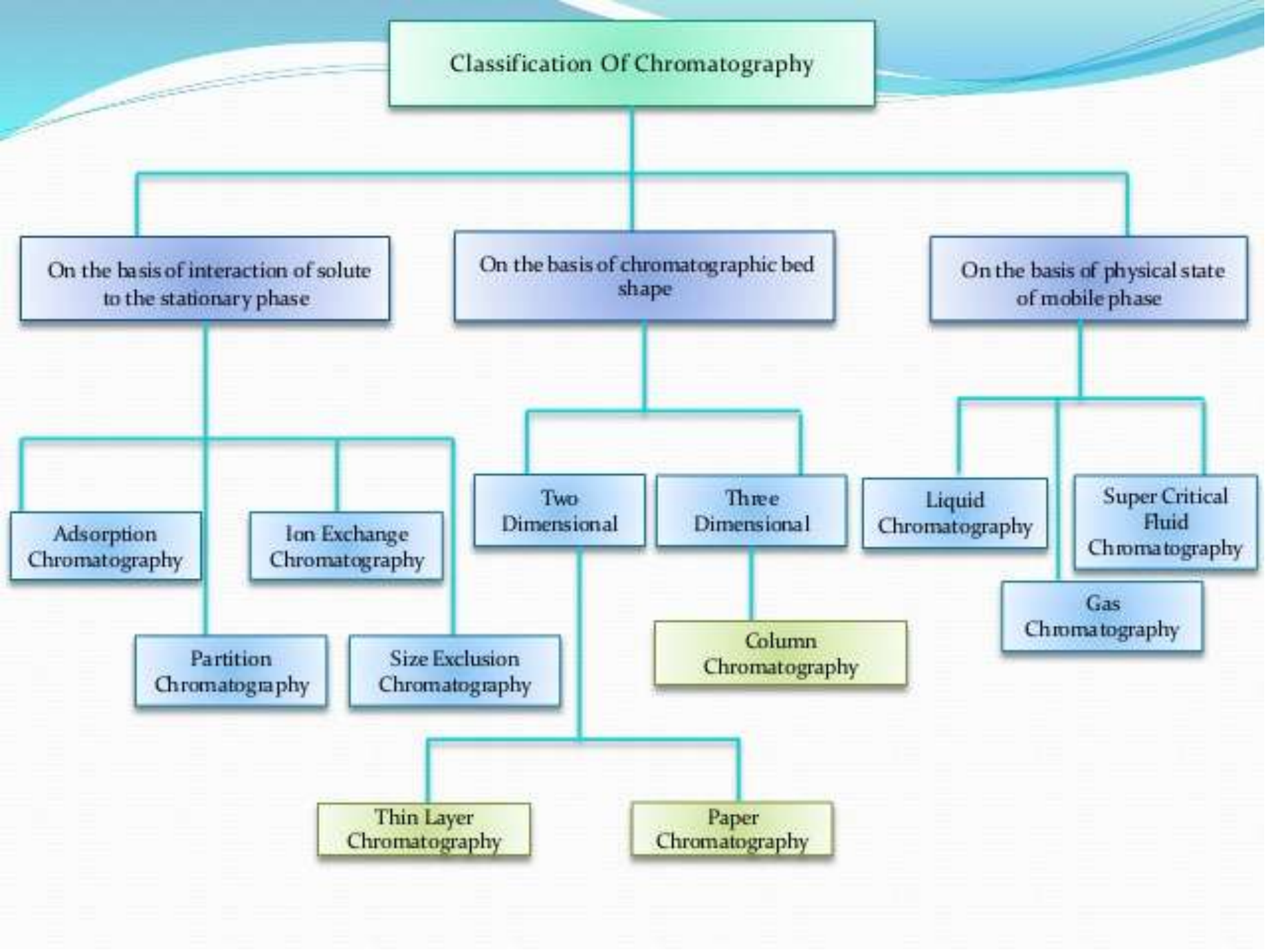
- As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.
- Techniques such as H.P.L.C. (High Performance Liquid Chromatography) and G.C. (Gas Chromatography) use *columns* - narrow tubes packed with stationary phase, through which the mobile phase is forced.
- The sample is transported through the column by continuous addition of mobile phase. This process is called *elution*.

History

- The subject of Chromatography was introduced into scientific world in a very modest way by M. Tswett in 1906.
- He employed a technique to separate various pigments such as chlorophylls and xanthophylls by passing the solution of these compounds into the glass column which was packed with finely divided calcium carbonate.
- After the later, Thompson and Way had realized the Ion Exchange properties of soils.
- Almost after three decades, in 1935 Adams and Holmes observed the Ion Exchange characteristics in crushed phonograph. This observation opened the field for preparation of Ion Exchanged resins.
- The concept of Gas-Liquid Chromatography was first introduced by Martin and Synge in 1941.

- They were also responsible for the development in Liquid-Liquid chromatography.
- In 1944, from Martin laboratory, the separation of amino acid by paper chromatography was reported.
- In 1952, the importance of the chromatography was observed when both Synge and Martin were awarded with Nobel Prize.
- In 1959, a technique known as Gel Filtration chromatography was observed which is used to separate low molecular weight substances from high molecular substances.
- In 1960, further improvement in liquid chromatography led to the development of High Performance Liquid Chromatography.
- The following decade of 1970's saw an improvement in the field of adsorption chromatography in the form of Affinity chromatography which was mainly based on biological interactions.

- A new field was originated which was supercritical fluid chromatography.
- Supercritical fluid chromatography is a hybrid of gas and liquid chromatography and combine advantageous feature of the both gas and liquid chromatography.
- It will not be wrong to say that the entire twentieth century can be named as the century of chromatography.


Classification Of Chromatography
On the basis of interaction of solute to the stationary phase
On the basis of chromatographic bed shape
On the basis of physical state of mobile phase
Adsorption Chromatography
Ion Exchange Chromatography
Partition Chromatography
Size Exclusion Chromatography
Two Dimensional
Three Dimensional
Column Chromatography
Thin Layer Chromatography
Paper Chromatography
Liquid Chromatography
Super Critical Fluid Chromatography
Gas Chromatography

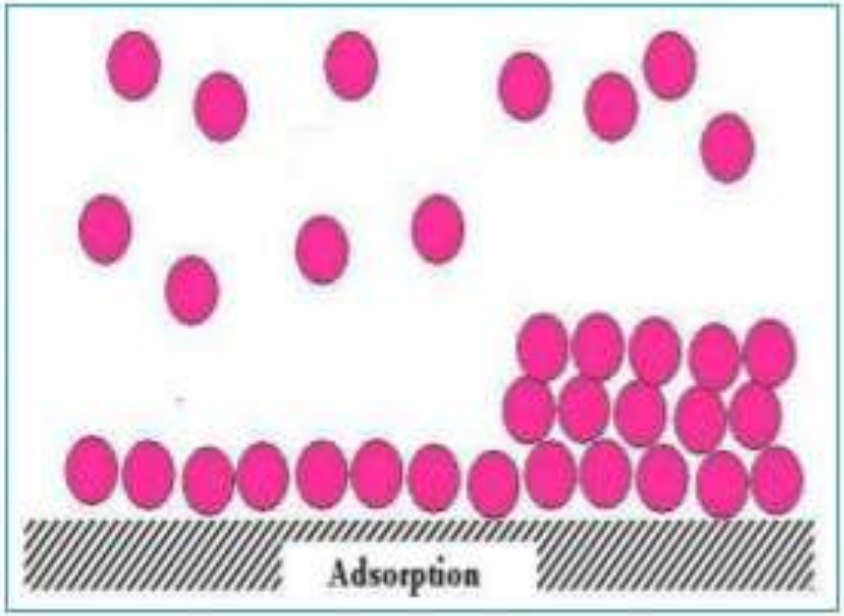
On the basis of interaction of solute to the stationary phase

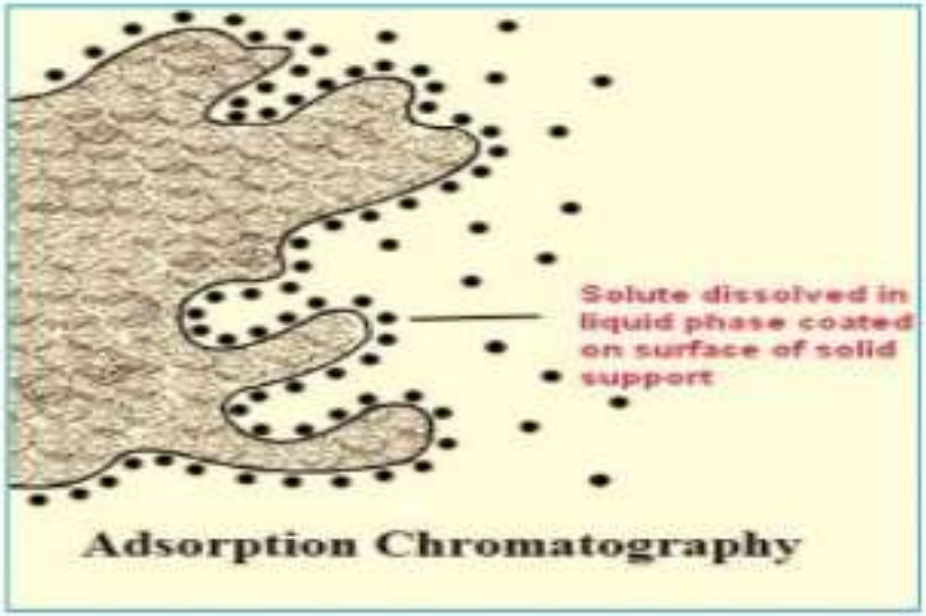
- Adsorption Chromatography
- Partition Chromatography
- Ion Exchange Chromatography
- Size Exclusion Chromatography

Adsorption Chromatography

- **Definition:**

Adsorption chromatography is probably one of the oldest types of chromatography around. It utilizes a mobile liquid or gaseous phase that is adsorbed onto the surface of a stationary solid phase. The equilibration between the mobile and stationary phase accounts for the separation of different solutes.





- **Principle:**

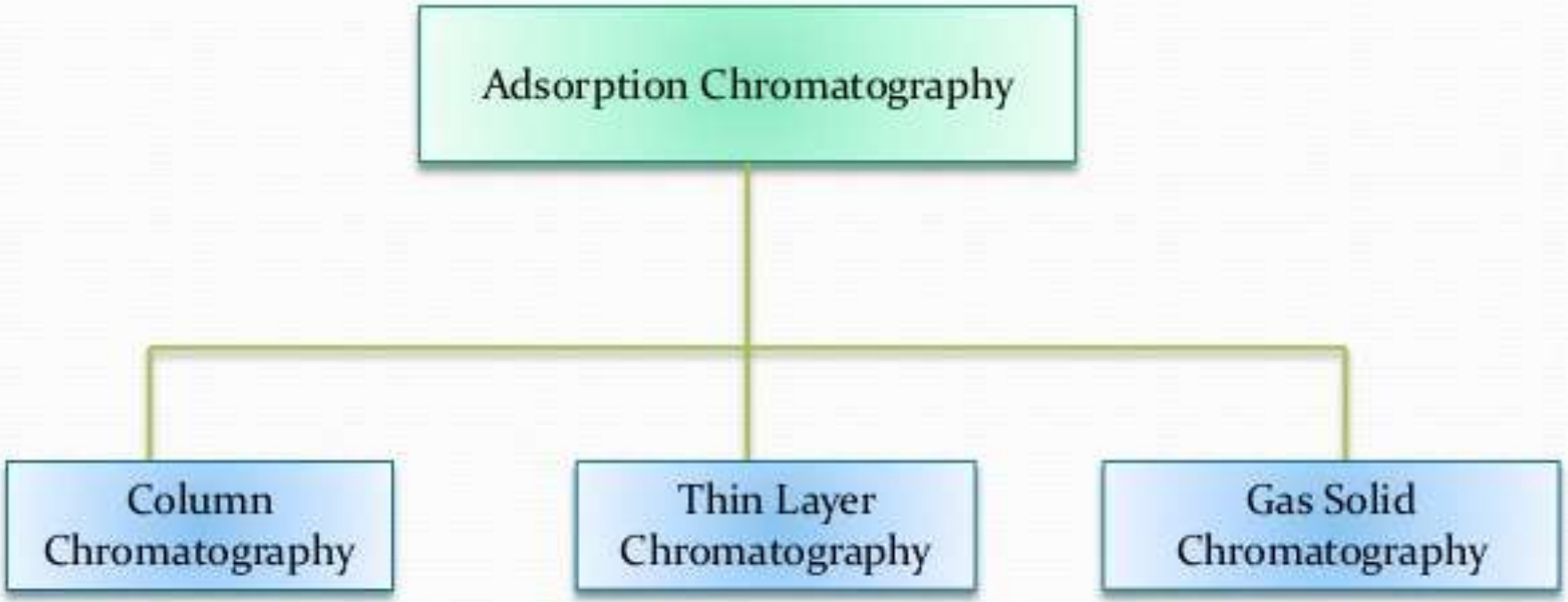
  Principle of Adsorption Chromatography involves competition of components of sample mixture for active site on adsorbent. These active sites are formed in molecule due to

- 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.

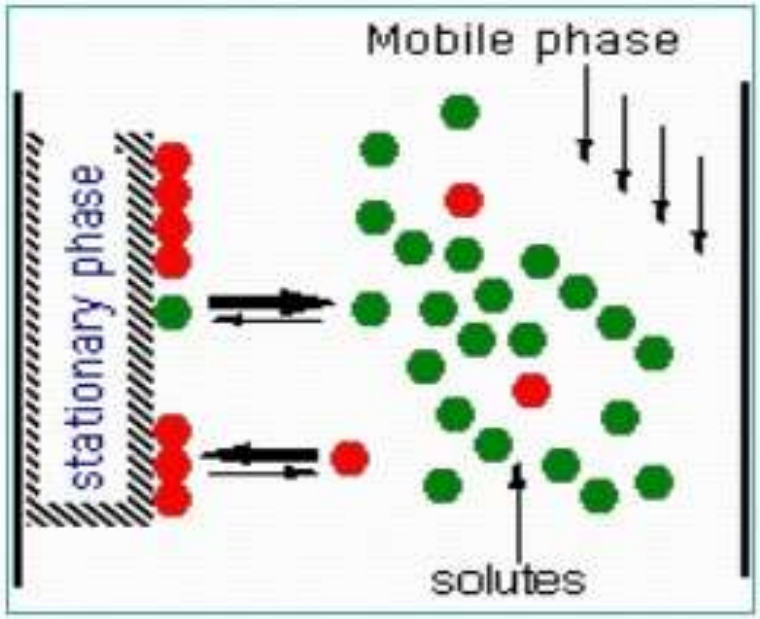
Types:

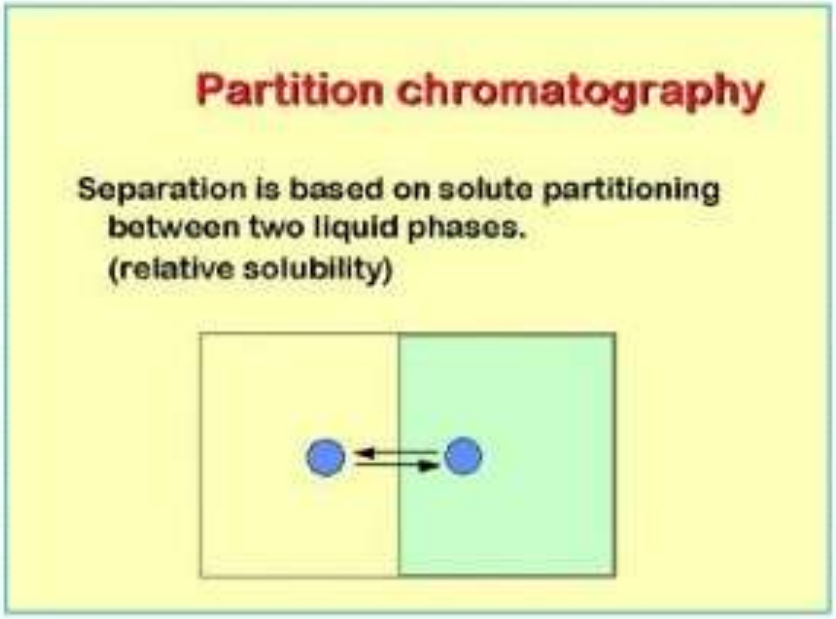

Adsorption Chromatography
Column Chromatography
Thin Layer Chromatography
Gas Solid Chromatography

Partition Chromatography

- **Definition:**

This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase. Solute equilibrates between the mobile phase and the stationary liquid.





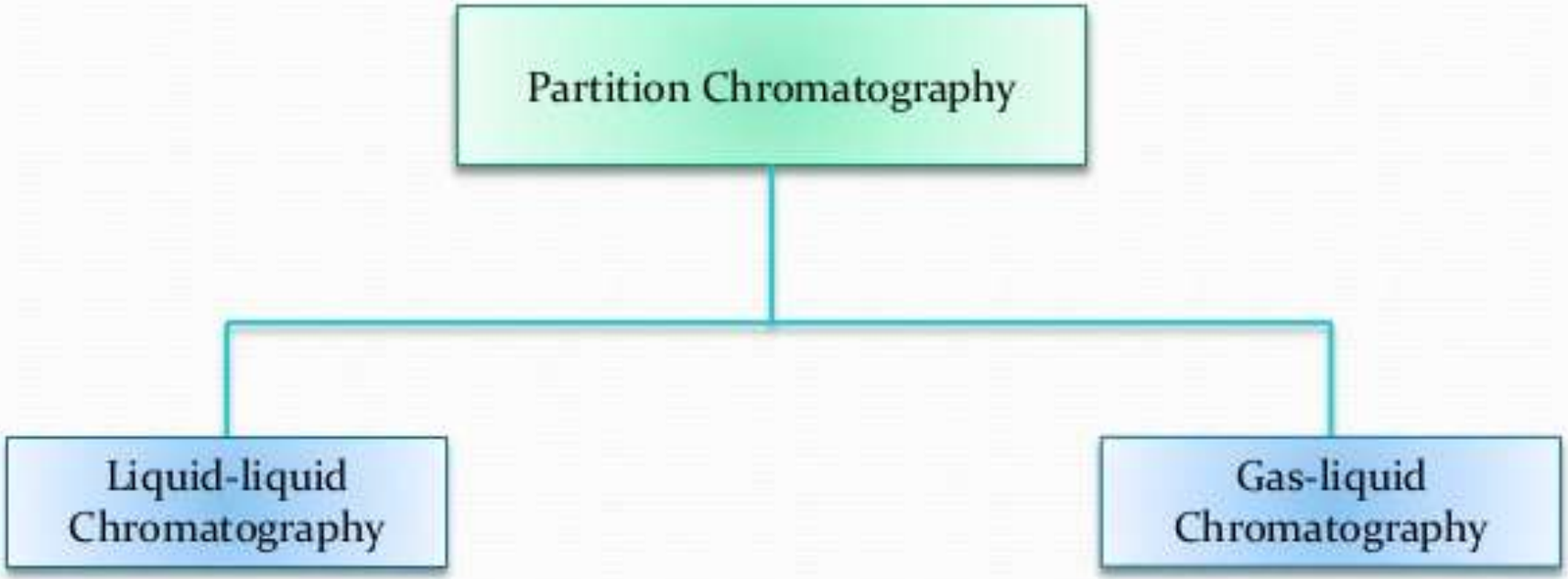
- **Principle:**

Separation of components of a sample mixture occurs because of partition. Stationary phase is coated with a liquid which is immiscible in mobile phase.

Partition of component of sample between sample and liquid/ gas stationary phase retard some components of sample more as compared to others. This gives basis for separation.

The stationary phase immobilizes the liquid surface layer, which becomes stationary phase. Mobile phase passes over the coated adsorbent and depending upon relative solubility in the coated liquid, separation occurs. The component of sample mixture appear separated because of differences in their partition coefficient.

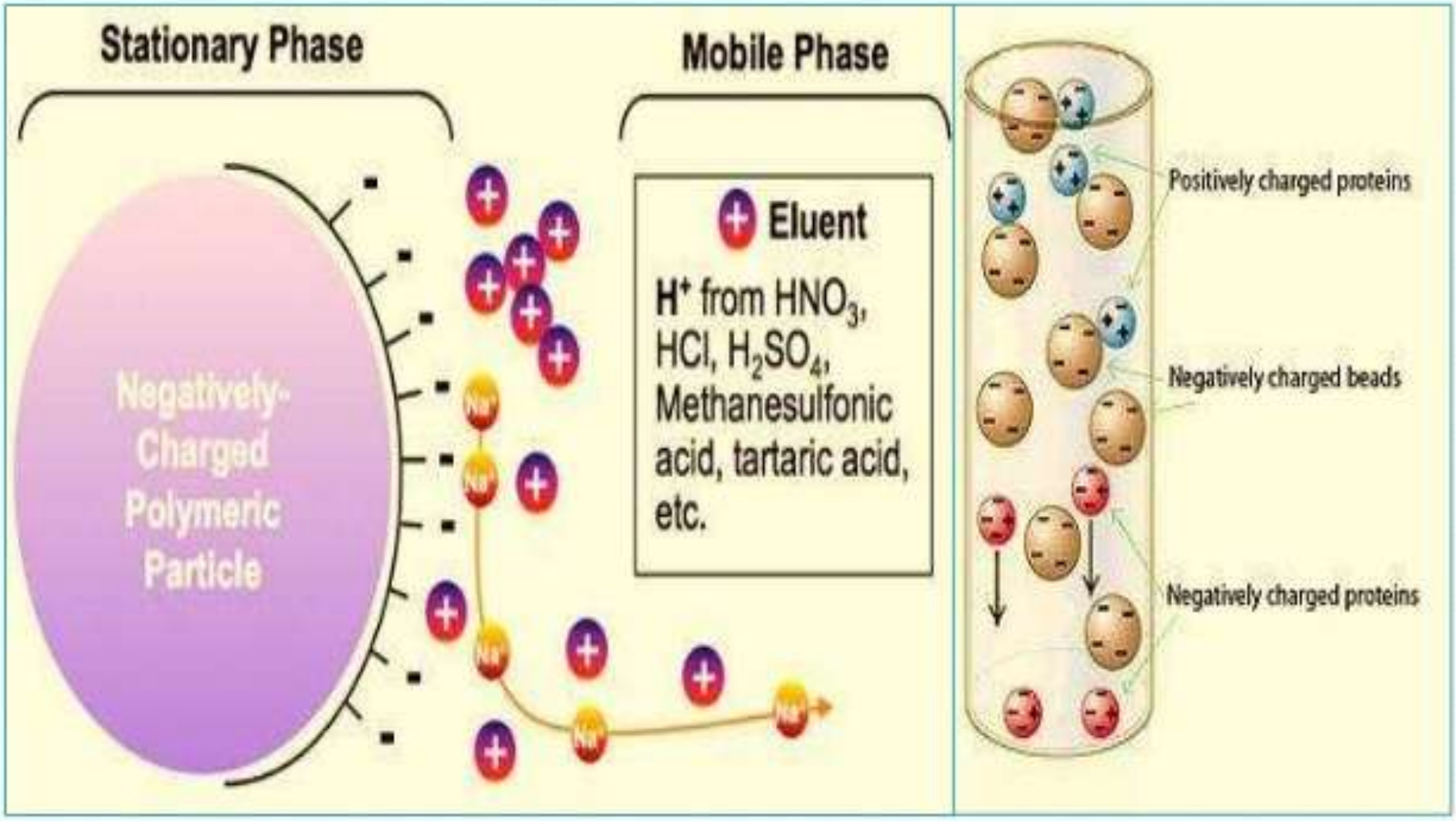
Types:


Partition Chromatography
Liquid-liquid Chromatography
Gas-liquid Chromatography

Ion Exchange Chromatography

- **Definition:**

Ion Exchange Chromatography (Ion Chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecules including large protein, small nucleotide and amino acids. The solution to be injected is called Sample and individually separated components are called analytes. It is often used in protein purification, water analysis, and quality control.


Stationary Phase
Mobile Phase
Negatively-Charged Polymeric Particle
Eluent
H+ from HNO3, HCl, H2SO4, Methanesulfonic acid, tartaric acid, etc.
Positively charged proteins
Negatively charged beads
Negatively charged proteins

- **Principle:**

Ion Exchange Chromatography is based on the relative retention of the ions during their progress through an ion exchange column which has functional group of opposite charge attached to its surface. The stronger the charge on the ion, the greater is the retention time in the column.

Ion chromatography is used to separate organic or inorganic charged substances. The stationary phases used are based on typical ion exchange resins.

Types:

Ion Exchange Chromatography

Cation Exchange Chromatography

Solute cations are attached to the negatively charged sites covalently bond to the stationary phase

Anion Exchange Chromatography

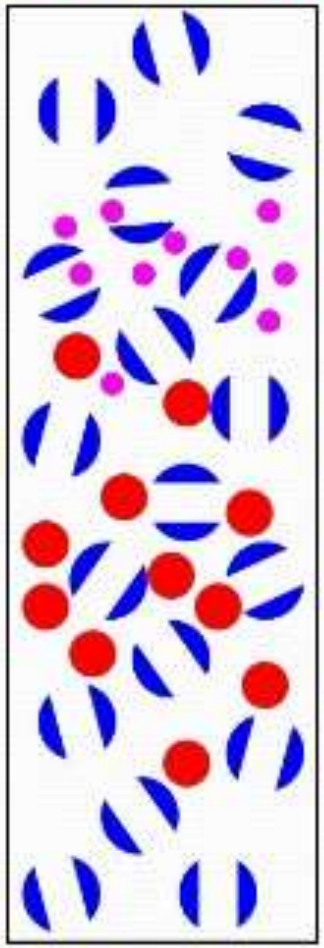
Solute anions are attached to the positively charged sites covalently bond to the stationary phase

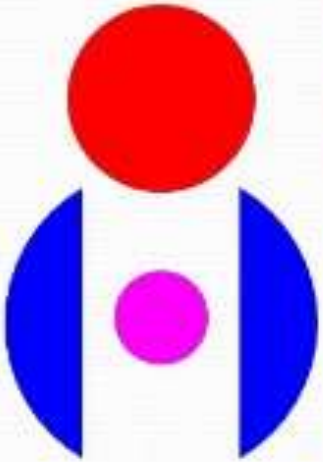
Size Exclusion Chromatography

- **Definition:**

Size-Exclusion Chromatography (SEC) is a chromatographic method in which molecules in a solution are separated by their size, and in some cases molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers. Typically, when an aqueous solution is used to transport the sample through the column, the technique is known as **gel-filtration chromatography**, versus the name **gel-permeation chromatography,** , which is used when an organic solvent is used as a mobile phase.

Size Exclusion Chromatography

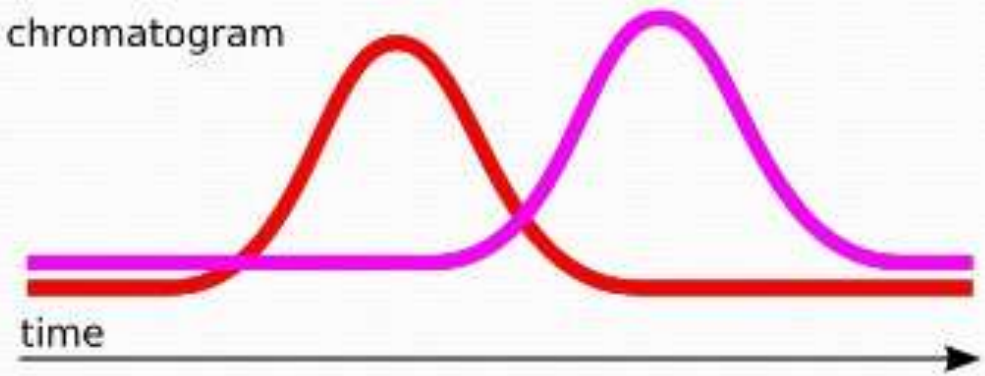




Large particles cannot enter gel and are excluded. They have less volume to traverse and elute sooner.

Small particles can enter gel and have more volume to traverse. They elute later.

flow



- **Principle:**
- A mixture of molecules dissolved in liquid (the mobile phase) is applied to a chromatography column which contains a solid support in the form of microscopic spheres, or "beads" (the stationary phase).
- The mass of beads within the column is often referred to as the column bed.
- The beads act as "traps" or "sieves" and function to filter small molecules which become temporarily trapped within the pores.
- Larger molecules are "excluded" from the beads .
- Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access most or all pores.
- Thus, large molecules elute first, smaller molecules elute later, while molecules that can access all the pores elute last from the column.
- Particles of different sizes will elute(filter) through a stationary phase at different rates.

On the basis of chromatographic bed shape

- Two dimensional

i. Thin Layer Chromatography

ii. Paper Chromatography

- Three dimensional

i. Column Chromatography

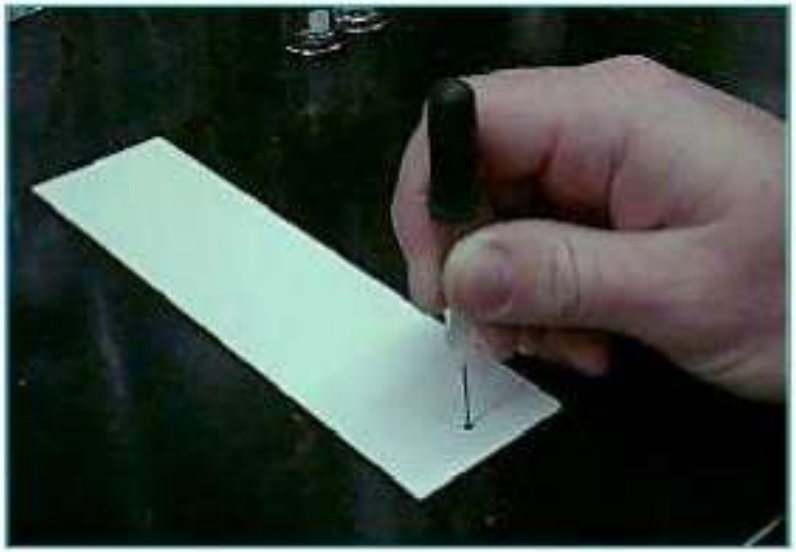
Thin Layer Chromatography

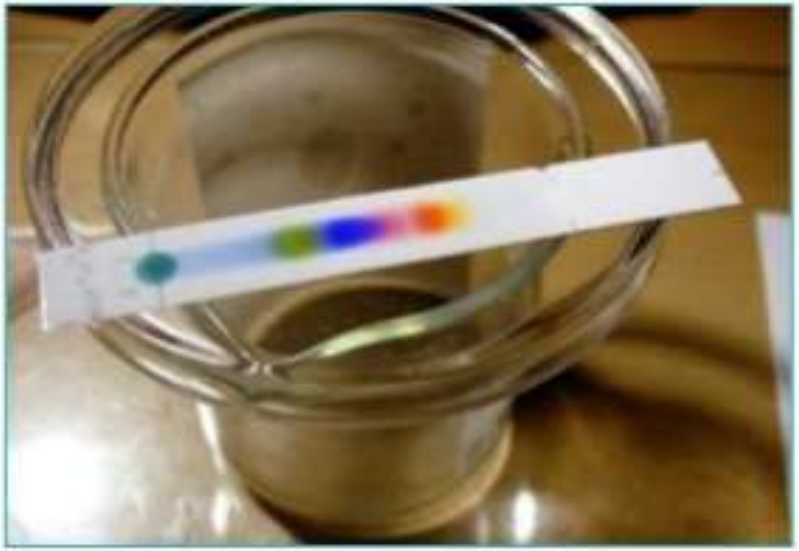
- **TLC is a method for identifying substances and testing the purity of compounds.**
- **TLC is a useful technique because it is relatively quick and requires small quantities of material.**
- **Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.**
- **The stationary phase: is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.**
- **The mobile phase: is a developing liquid which travels up the stationary phase, carrying the samples with it.**
- **Components of the samples will separate on the stationary phase according to how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.**

Thin Layer Chromatography

- **Definition:**

  Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products.



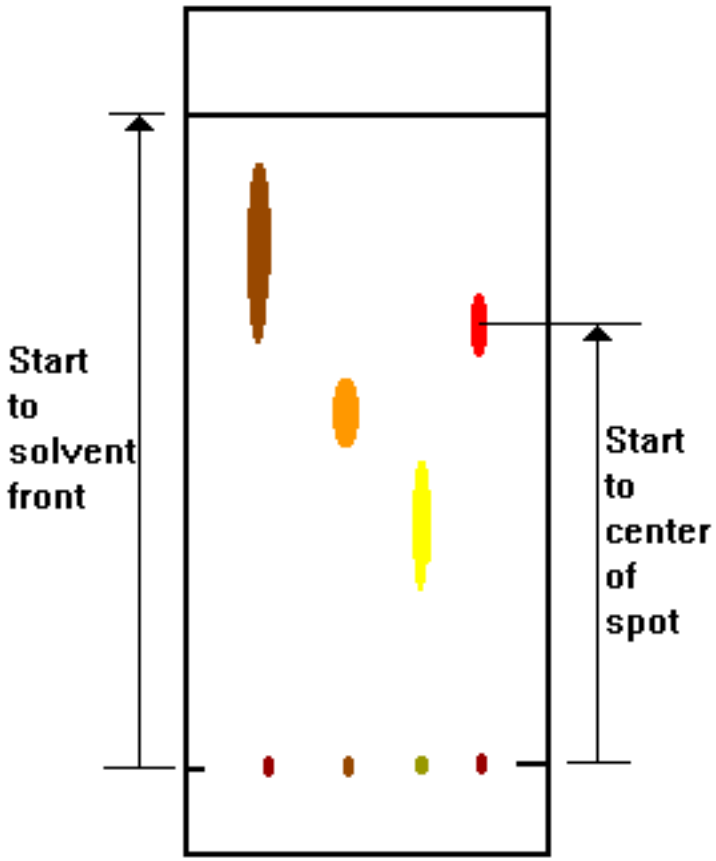


- **Principle:**

Similar to other chromatographic methods TLC is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved.

Once separation occurs individual components are visualized as spots at respective level of travel on the plate. Their nature or character are identified by means of suitable detection techniques.

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$

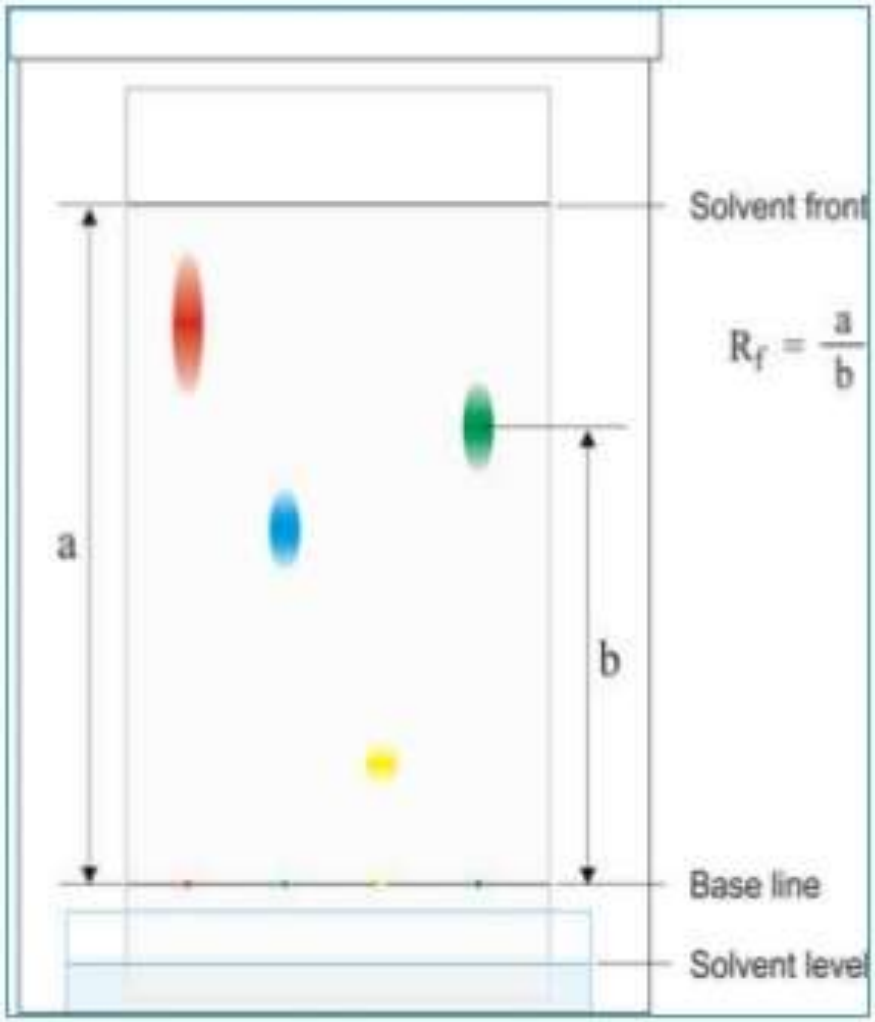


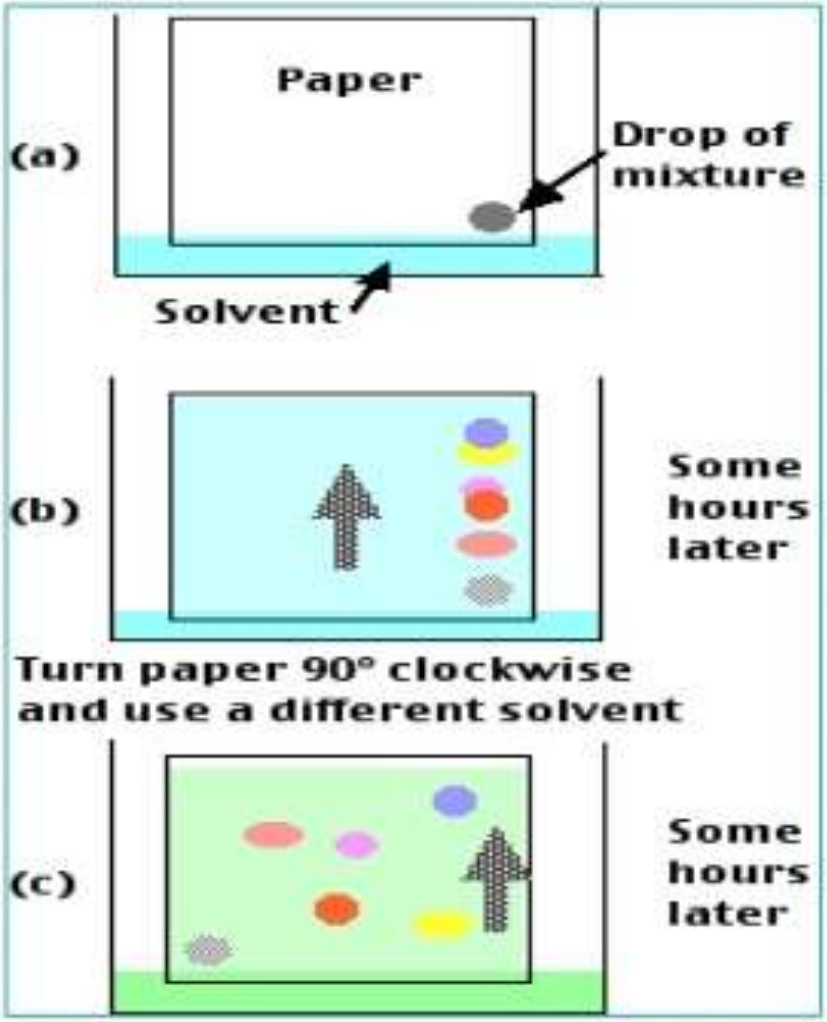
The Rf (retention factor) value for each spot should be calculated.
It is characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates.
Hence, known Rf values can be compared to those of unknown substances to aid in their identifications.

Paper Chromatography

- **Definition:**

Paper chromatography is an analytical method that is used to separate coloured chemicals or substances, especially pigments. This can also be used in secondary or primary colours in ink experiments. This method has been largely replaced by thin layer chromatography, but is still a powerful teaching tool. **Double-way paper chromatography**, also called two-dimensional chromatography, involves using two solvents and rotating the paper 90° in between. This is useful for separating complex mixtures of compounds having similar polarity, for example, amino acids. If a filter paper is used, it should be of a high quality paper. The mobile phase is developing solutions that can travel up to the stationary phase carrying the sample along with it.


Solvent front
$R_f = \frac{a}{b}$
a
b
Base line
Solvent level


(a)
Paper
Drop of mixture
Solvent
(b)
Some hours later
Turn paper 90° clockwise and use a different solvent
(c)
Some hours later

- **Principle:**

  The principle involved is partition chromatography where in the substances are distributed or partitioned between to liquid phases. One phase is the water which is held in pores of filter paper used and other phase is that of mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water(in stationary phase) and mobile phase solvents during the movement of mobile phase under the capillary action of pores in the paper.

  The principle can also be adsorption chromatography between solid and liquid phases, where in the stationary phase is the solid surface of paper and the liquid phase is of mobile phase. But most of the applications of paper chromatography work on the principle of partition chromatography i.e. partitioned between two liquid phases.