# Column Chromatography

Introduction

- The separation of a mixture by distribution of its components between a mobile and stationary phase over time

- It is **a solid - liquid technique** in which the stationary phase is a solid & mobile phase is a liquid .

- The stationary phase or adsorbent in column chromatography is a solid. The most common stationary phase for column chromatography is **Silica Gel, followed by Alumina Oxide** .

- The mobile phase or eluent is a liquid. It is either a pure solvent or a mixture of different solvents .

Column Chromatography

- **<u>Column chromatography:</u>** Stationary phase is filled in a narrow tube through which the mobile phase is forced under pressure or under the effect of gravity

Solvent

Mixture of compounds

Adsorbent (stationary phase)

Glass wool

Plug
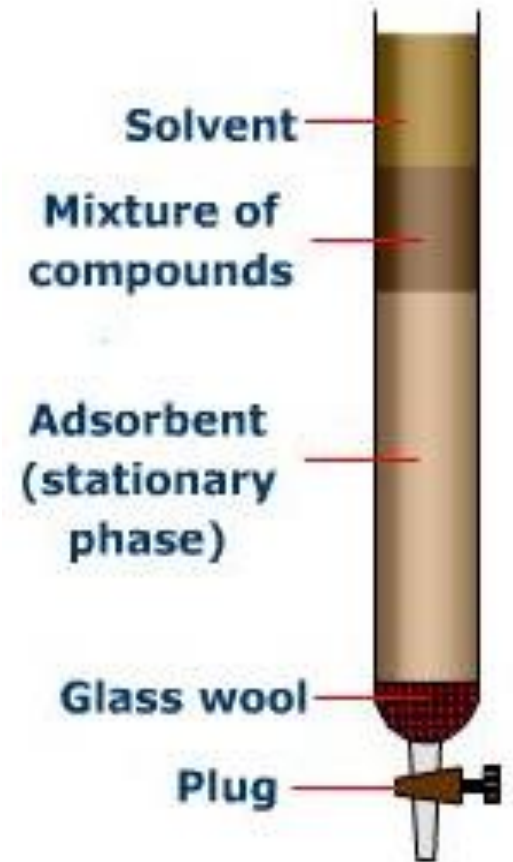
PRINCIPLE

- When a mixture of components dissolved in the mobile phase is introduced into the column , the individual components move with differentiates depending upon their relative affinities.

- The compound with lesser affinity towards stationary phase moves faster and it is eluted out of the column first.

- The one with greater affinity towards stationary phase moves slower down the column and hence it is eluted latter.

- Thus the compounds are separated .
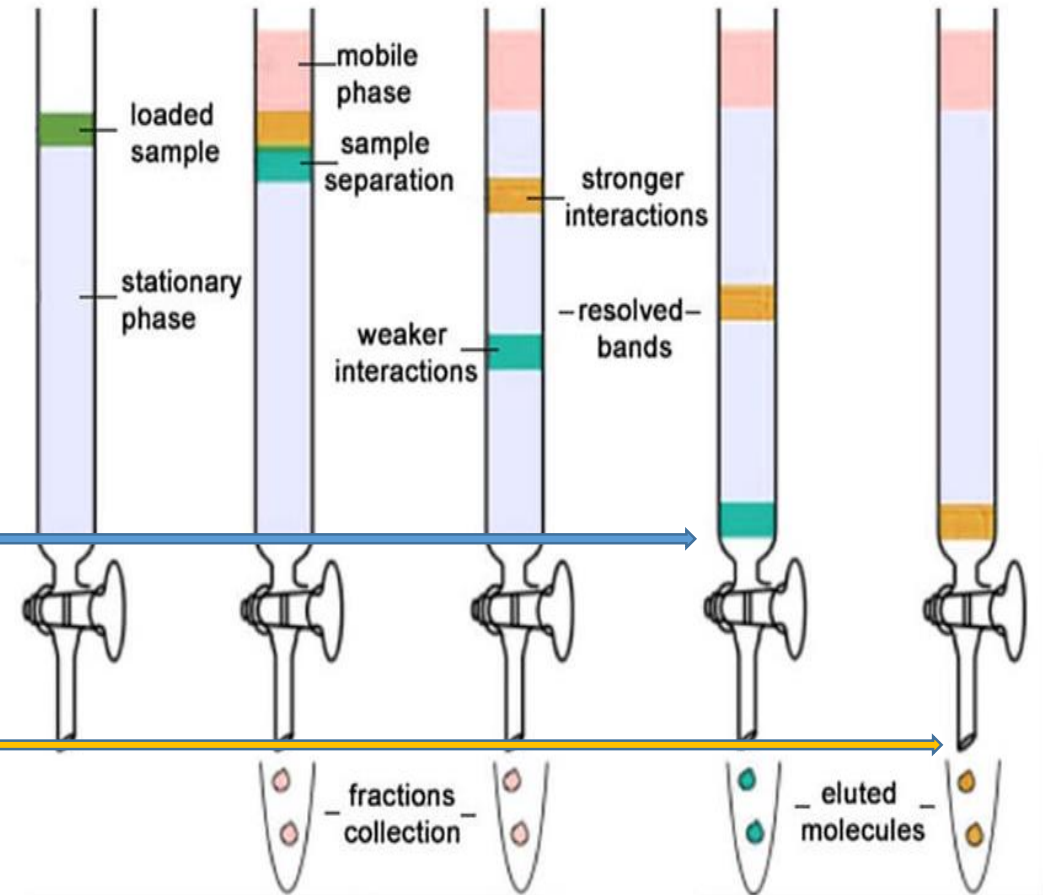
Mobile phase

- To introduce the mixture into column as solvent.

- To develop the zones for separation as developing agent.

- To remove pure component out of the column as eluent .

Keep adding new solvent.

Change the beaker once the yellow starts to drop through.
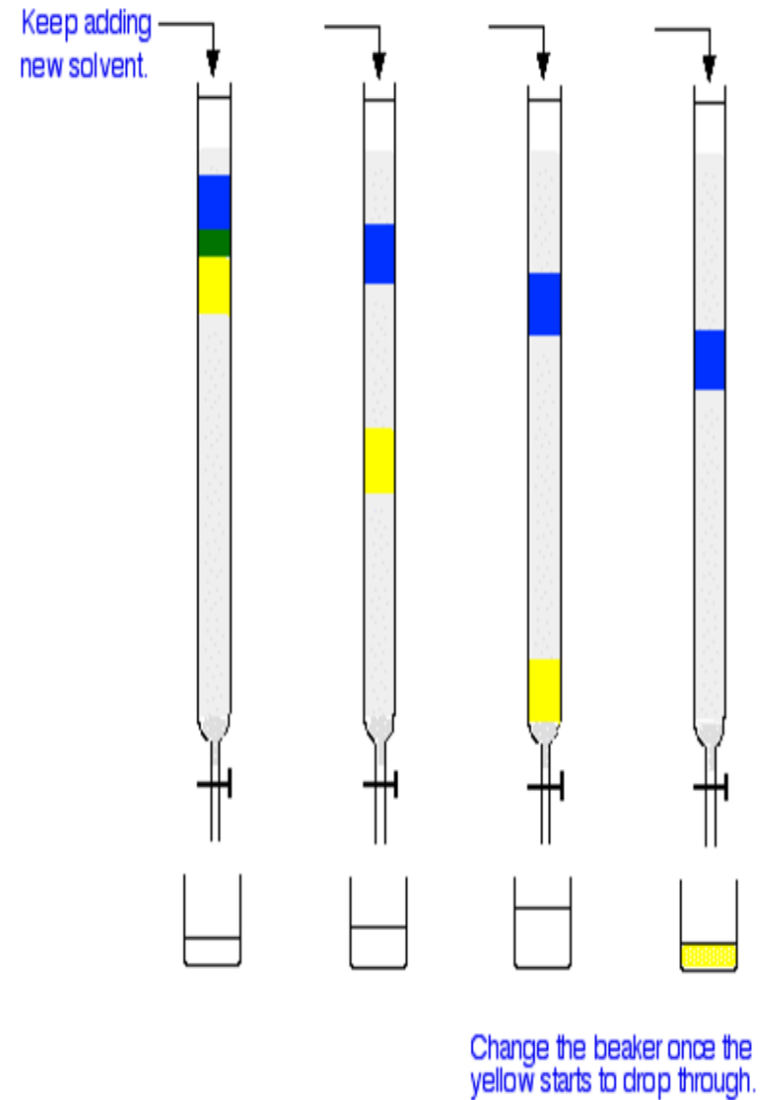
Introduction of the Sample

• The sample which is usually a mixture of components is dissolved in minimum quantity of the mobile phase.

• The entire sample is introduced into the column <u>at once and get adsorbed on the top portion of the column.</u>

• From this zone, individual sample can be separated by a process of elution.

Factors affecting Separation

Factors due to Stationary Phase :

- <u>Particle size of the stationary phase:</u> Reducing the particle size increases the surface area and improve separation. However , reduction of the particle size will decrease the flow rate of the mobile phase .

- <u>Adsorbent activity:</u> The choice of the suitable adsorbent is very important.

- <u>Uniformity of packing of the column:</u> If the stationary phase is not packed uniformly then the bands will be irregular and less uniform resulting in poor separation.

- <u>Concentration of the mixture:</u> the proper ratio between sample to be separated and the amount of stationary phase is very important too much samples resulted in bad separation.

Factors due to Mobile Phase :

- Selection of the proper mobile phase: Very polar mobile phase will wash out all components without any separation. On the other hand very non polar mobile phase will result in broad band and poor separation.

- Rate of flow: Slower flow rate usually resulted in a better separation and narrower bands.

- Consistency of flow: The continuous flow of the mobile phase during the whole experiment gives better separation than interrupting the flow then continue it later

Factors due to Columns :

- Column dimensions: Increasing the length of the column improve separation. However, that usually leads to slower flow rate. Also increasing the column length some times is impractical.

- Column temperature: Increasing the temperature usually reduces the adsorption power of the stationary phase and increase elution speed. This may leads to decrease in the efficiency separation.

Normal and Reverse Phase

Normal Phase

- It uses a polar stationary phase and a non polar (low polarity solvents) mobile phase

- Non polar compounds elute faster than polar compounds

- When polarity of mobile phase is increased, elution time will increase

- Mobile phase is nonpolar, e.g. hexane, dichloromethane, chloroform, ethyl ether, and isopropyl alcohol (IPA)

Reverse Phase

- It uses a non plar stationary phase and polar mobile phase

- Polar compounds elute faster than non polar compounds

- When polarity of mobile phase is increased, elution time will decrease

- Mobile phase is polar, e.g. water, acetonitrile, methanol