COLUMN CHROMATOGRAPHY

When a column of stationary phase is used, the technique is called as column chromatography. Based on the nature of stationary phase, i.e. whether it is solid or liquid, it is called as column adsorption chromatography or column partition chromatography.

Column adsorption chromatography:-

Principal:- A solid stationary phase and a liquid mobile phase is used and the principle of separation is adsorption. When a mixture of components dissolved in the mobile phase is introduced in to the column, the individual components move with different rates depending upon their relative affinities. The compound with lesser affinity towards the stationary phase (adsorbent) moves faster and hence it is eluted out of the column first. The one with greater affinity towards the stationary phase (adsorbent) moves slower down the column and hence it is eluted later. Thus, the compounds are separated. The type of interaction between the stationary phase (adsorbent) and the solute is reversible in nature.

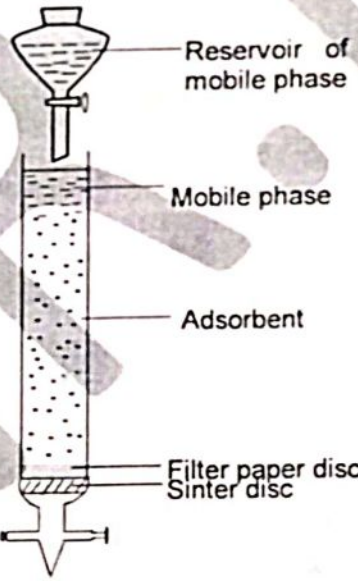
$$\text{The rate of movement of component (R)} = \frac{\text{Rate of movement of a component}}{\text{Rate of movement of mobile phase}}$$

This equation can be simplified as follows

$$\text{The rate of movement of component (R)} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

Practical requirements:-

1. Stationary phase (Adsorbent)
2. Mobile phase
3. Column characteristis
4. Preparation of column
5. Introduction of column
6. Development technique (elution)
7. Detection of components
8. Recovery of components



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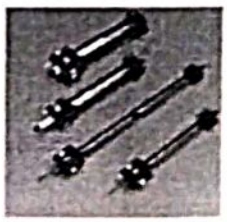


1. Stationary phase (adsorbents):- An adsorbent used in column chromatography should contain following properties

a). Particle size and geometry:- The particles should have uniform size distribution and have spherical shape. Particle size: 60-200μ.

b. Should be inert and should not react with the solute or other components.

c. Should have high mechanical stability.

d. Insoluble in the solvents or mobile phases used.

e. It should be colourless to facilitate observation of zones and recovery of components.

f. It should allow free flow of mobile phase.

g. It should be useful for separating for wide variety of compounds.

h. It should be freely available, inexpensive.

Type of adsorbents:- based upon their adsorbent activity they can be classified as weak, medium, and strong adsorbents. They are

Weak	Medium	Strong
Sucrose	$CaCO_3$	Activated Mg Silicate (silica gel)
Starch	$Ca_3(PO_4)_2$	Activated Alumina
Inulin	$MgCO_3$	Activated Charcoal
Talc	MgO	Activated Magnesia
Na_2CO_3	$Ca(OH)_2$	Fuller's earth

The most commonly used adsorbent is Silica gel which has a particle size of 60-200μ

2) Mobile Phase:-

Mobile phase serve several functions. They act as solvent, developer and as eluent. The functions of a mobile phase are

To introduce the mixture into the column – As solvent
To develop the zones for separation – As developing agent
To remove pure component out of the column – As eluent

Different mobile phases used are petroleum ether, carbontetrachloride, cyclohexane, carbondisulphide, ether, acetone, benzene, toluene, esters chloroform, alcohols (methanol, ethanol etc), Water, pyridine, organic acids (acetic acid, etc)

These solvents can be used in either pure form or as mixture of solvents of varying compositions.

3) Column characteristics:- The material of the column is mostly good quality neutral glass since it should not be affected by solvents, acids or alkalies. An ordinary burette can also be used as column for separation. The column dimensions are important for effective column dimensions. The length: diameter ratio ranges from 10:1 to 30:1. For more efficiency, the length: diameter ratio can be 100:1. The length of the column depends on

* Affinity of compounds towards the adsorbent used.
* Number of compounds to be separated.
* Type of adsorbent used.
* Quality of the sample.

4) Preparation of the column:-

The bottom portion of the column is packed with cotton wool or glass wool or may contain a asbestos pad, above which the column of adsorbent is packed. A whatman filter paper disc can also be used. After packing the column with adsorbent, a similar paper disc is kept on the top, so that the adsorbent layer is not disturbed during the introduction of sample or mobile phase. Disturbance in the layer of adsorbent will lead to irregular bands in separation.

They are two types of preparing the column, which are called as packing techniques. They are

i) Dry packing technique:- In this technique, the required quantity of adsorbent is packed in the column in dry form and the solvent allowed to flow through the column till equilibrium is reached. The air bubbles are entrapped between the solvent and the stationary phase and the column may not be uniformly packed. Cracks appear in the adsorbent present in the column. Hence clear band of the separated component may not be obtained.

ii) Wet packing technique:- This is the ideal technique. The required quantity of the adsorbent is mixed with the mobile phase solvent in a beaker and poured into the column. The stationary phase settles uniformly in the column and there is no entrapment of air bubbles. There will not be any crack in the column of adsorbent. The bands eluted from the column will be uniform and ideal for separation.

5) Introduction of sample:-

The sample which is usually a mixture of components is dissolved in minimum quantity of the mobile phase used for preparing the column or a solvent of minimum polarity. The entire sample is introduced into the column at once and gets adsorbed on to the top portion of the column. From this zone, the individual samples can be separated by a process of elution.

6) Development technique:- (elution)

After the introduction of the sample the individual components are separated out from the column by the elution techniques, the two techniques are

Isocratic elution technique: In this elution technique, the same solvent compositions or solvent of same polarity is used throughout the process of separation.

Eg: Chloroform only, pet.ehter: Benzene = 1:1 only etc.

Gradient elution technique: (gradient – gradually) In this elution technique, solvents of gradually increasing polarity or increasing elution strength are used during the process of separation. Initially low polar solvent is used followed by gradually increasing the polarity to a more polar solvent.

eg: initially Benzene, then chloroform, then Ethyl acetate, then to methanol, etc.

Detection of components:-

The detection of coloured components can be done visually. Different coloured bands are seen moving down the column which can be collected separately. But for colourless compounds, the technique depends upon the properties of the components. Different techniques which can be used are

i) Absorption of light (UV/Vis) – using UV/Vis detector

ii) Fluorescence or light emission characteristics –using fluorescence detector

iii) By using flame ionisation detector

iv) Evaporation of the solvent and weighing the residue

Recovery of components:- The best technique is to recover the components by a process called as elute, the solvent called as eluent and the process of removing the components from the column is called as elution. The different elution techniques are discussed already. Recovery is done by collecting as different fractions of mobile phase of equal volume like 10m, 20ml, etc or unequal volume. They can also be collected time wise. i.e. a fraction every 10 or 20 minutes etc. The recovered fractions are detected by using above techniques. Similar fractions are mixed so that the bulk of the compound of each type is obtained in a pure form. If a fraction, still contains several components, it can be resolved by using another column.

Advantages of column chromatography:-

i) Any type of mixture can be separated by column chromatography.

ii) Any quantity of the mixture can be separated (µg to mg of substance)

iii) Wider choice of mobile phase.

iv) In preparative type, sample can be separated and reused.

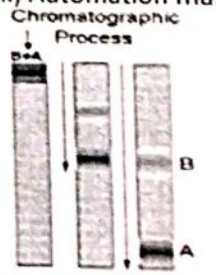
V) Automation is possible.

Disadvantages of column chromatography:-

i) Time consuming method.

ii) More amounts of solvents are required which are expensive.

iii) Automation makes the technique more complicated and expensive.



Elution through column