COLUMN CHROMATOGRAPHY

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COLUMN 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 a number of different kinds of chromatography, which differ in the mobile and the stationary phase used.

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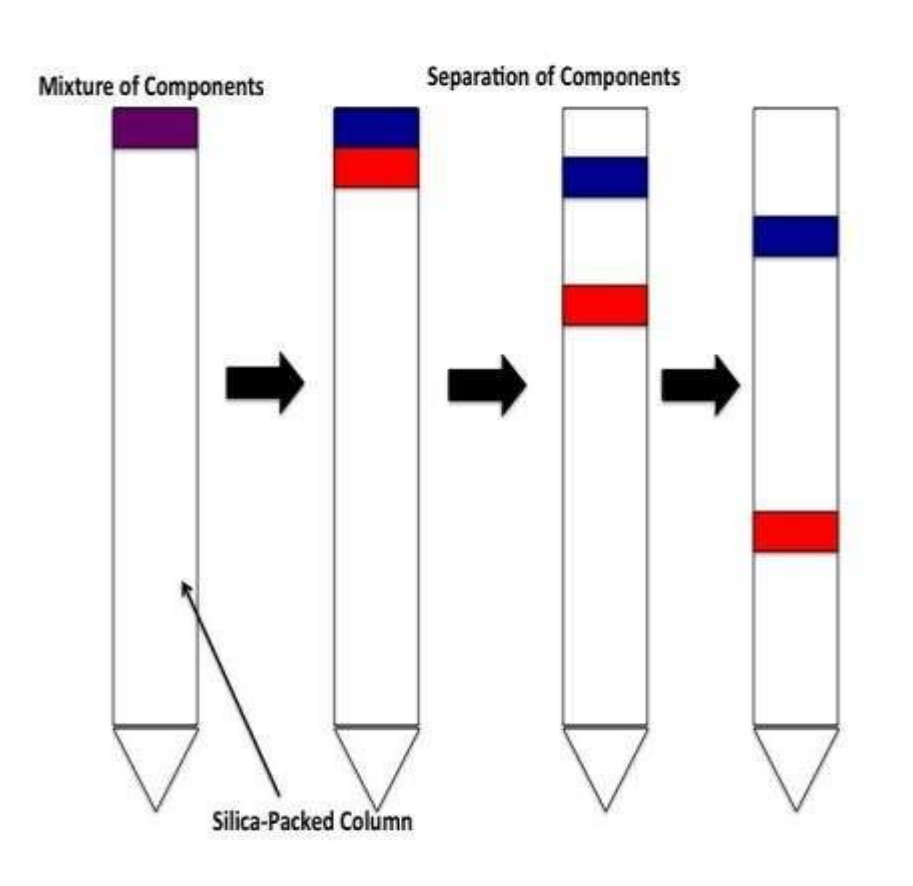
- Column Chromatography was developed by the American chemist D.T Day in 1900, M.S. Tswett,the Polish botanist, in 1906 used adsorption columns in his investigations of plant pigments.



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- Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids.
- This is a solid - liquid technique in which the stationary phase is a solid & mobile phase is a liquid.

PRINCIPLE

- **Adsorption**
- Mixture of components dissolved in the M.P is introduced in to the column. Components moves depending upon their relative affinities.

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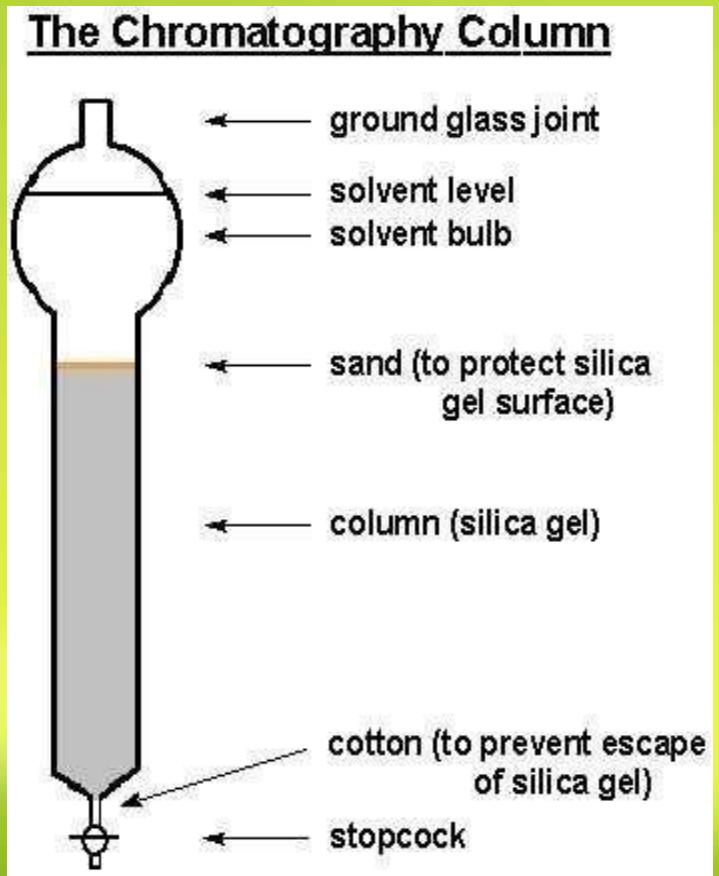
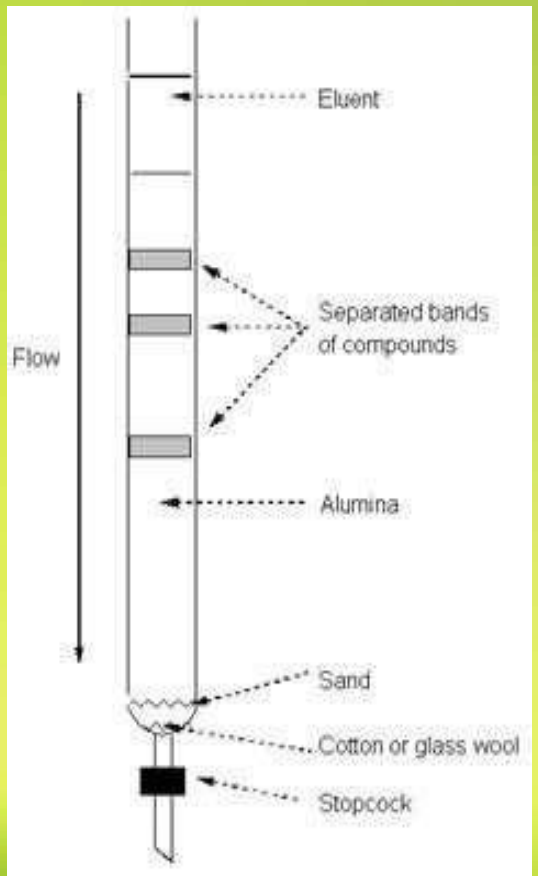
- Adsorption column chromatography, the adsorbent, packed in a glass column, and a solvent, the mobile phase, that moves slowly through the packed column. A solvent used as a mobile phase is called an eluent.





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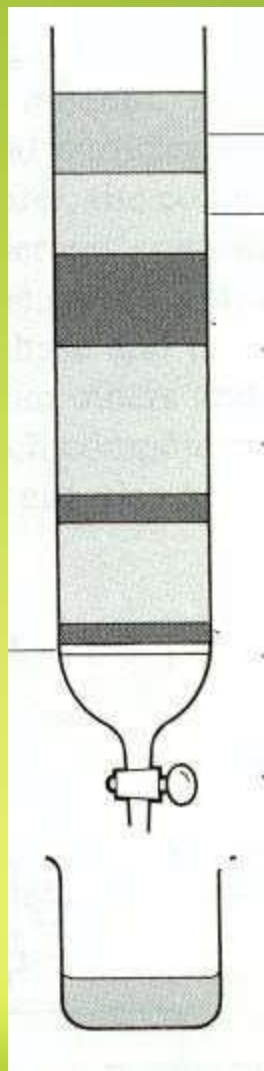
- A compound attracted more strongly by the mobile phase will move rapidly through the column, and elute from, or come off, the column dissolved in the eluent.
- In contrast, a compound more strongly attracted to the stationary phase will move slowly through the column.



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- Experimental aspects of column chromatography:
- **Adsorbents:** The usual adsorbents employed in column chromatography are silica, alumina, calcium carbonate, calcium phosphate, magnesia, starch, etc.,
- Alumina is generally suitable for chromatography of less polar compounds. Silica gel gives good results with compounds containing polar functional groups.

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- **Adsorbent in C.C should meet following criteria**

  - Particles should be spherical in shape & uniform in size
  - Mechanical stability must be high
  - They shouldn't react chemically
  - It should be useful for separating for wide variety of compounds
  - It should be freely available & inexpensive

  (The particle size of the commercially available grade is in the range 50 – 200 µm.)

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Selection of Stationary Phase

- Success of chromatography depends upon proper selection of S.P, it depends on the following:

1. Removal of impurities
2. No. of components to be separated
3. Length of the column used
4. Affinity differences b/w components
5. Quantity of adsorbent used

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Mobile Phase

- They act as solvent, developer & eluent. The function of a mobile phase are:
- As developing agent
- To introduce the mixture into the column – as solvent
- To developing agent
- To remove pure components out of the column – as eluent

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- The choice of the solvent is depend on the solubility characteristics of the mixture. The solvents should also have sufficiently low boiling points to permit ready recovery of eluted material.
- However, polarity as seen the most important factor in adsorption chromatography.
- ***Different mobile phases used: (in increasing order of polarity)***
- Petroleum ether, carbon tetrachloride, cyclohexane, ether, acetone, benzene, toluene, esters, water, etc
- It can b e used in either pure form or as mixture of solvents

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COLUMN CHARACTERISTICS

- The main function of all the columns is to support the stationary phase.
- The material of the column is mostly good quality neutral glass since it shouldn't be affected by solvents. An ordinary burette can also be used as column for separation.
- Column dimensions - length & diameter ratio (10:1,30:1 or 100:1)
- Various accessories are attached to the top and bottom of the column for maintenance of the elution process.

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- The length of the column depends upon:
- Number of compounds to be separated
- Type of adsorbent used
- Quantity of the sample
- Affinity of compounds towards the adsorbent used

- Better separation will be obtained with a long narrow column than short thick column because number of plates will be more.

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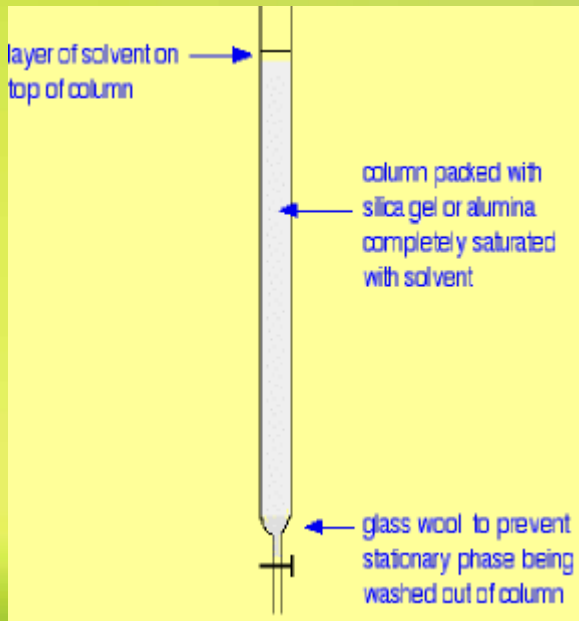
- PREPARATION OF THE COLUMN
- It consists of a glass tube with bottom portion of the column – packed with glass wool/cotton wool or may contain asbestos pad,
  - » Above which adsorbent is packed
  - » After packing a paper disc kept on the top, so that the adsorbent layer is not disturbed during the introduction of sample or mobile phase.



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Packing techniques in C.C

- There are two types of preparing the column, they are:
- **i. Dry packing / dry filling**
- **Ii. Wet packing / wet filling**

- The column should be free from impurity, before using column, it should be washed properly and dry it.
- Before filling column with stationary phase, cotton/glass wool is kept
- It should be uniformly filled

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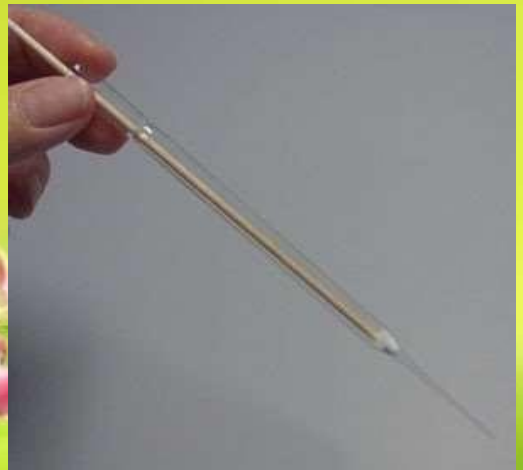
Dry Packing Technique

➢ Adsorbent is packed in the column in dry form

➢ Fill the solvent, till equilibrium is reached

DEMERIT: Air bubbles are entrapped b/w M.P & S.P→ cracks appear in the adsorbent layer.

- After filling tapping can be done to remove void spaces.



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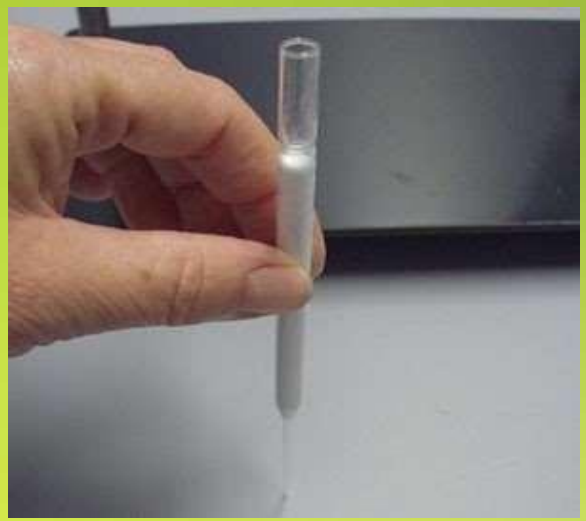

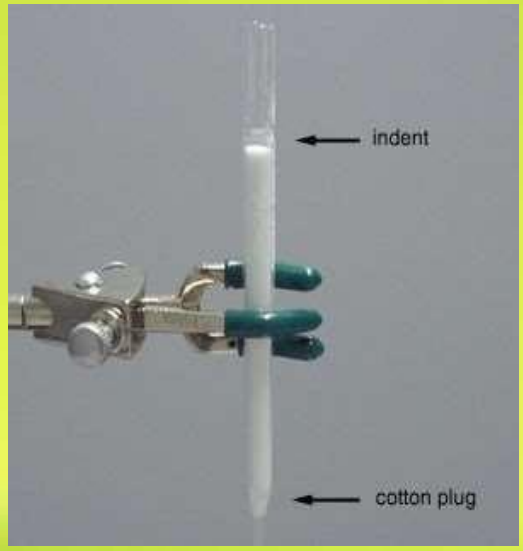

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Wet Packing Technique

» ideal & common technique

The material is slurried with solvent and generally added to the column in portions.

◊ S.P settles uniformly & no crack in the column of adsorbent.

» solid settle down while the solvent remain upward.

» this solvent is removed then again cotton plug is placed.

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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 at once and get adsorbed on the top portion of the column.
- From this zone, individual sample can be separated by a process of elution.

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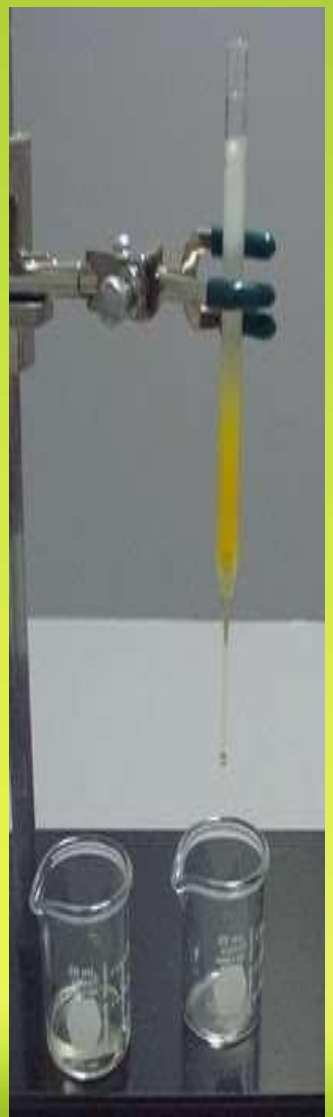
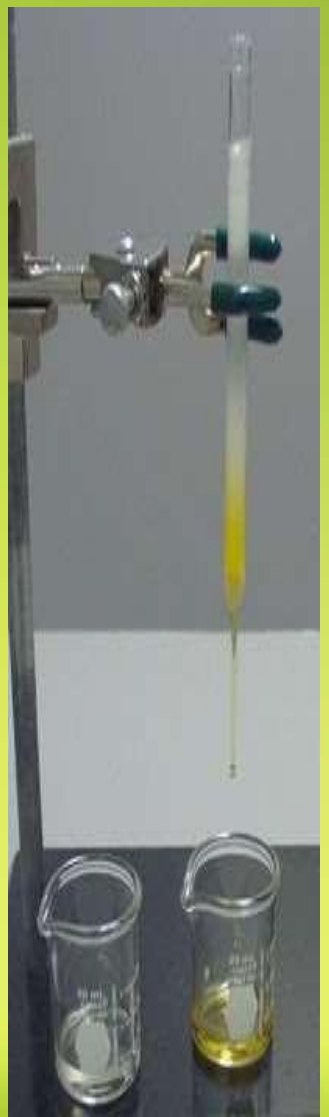

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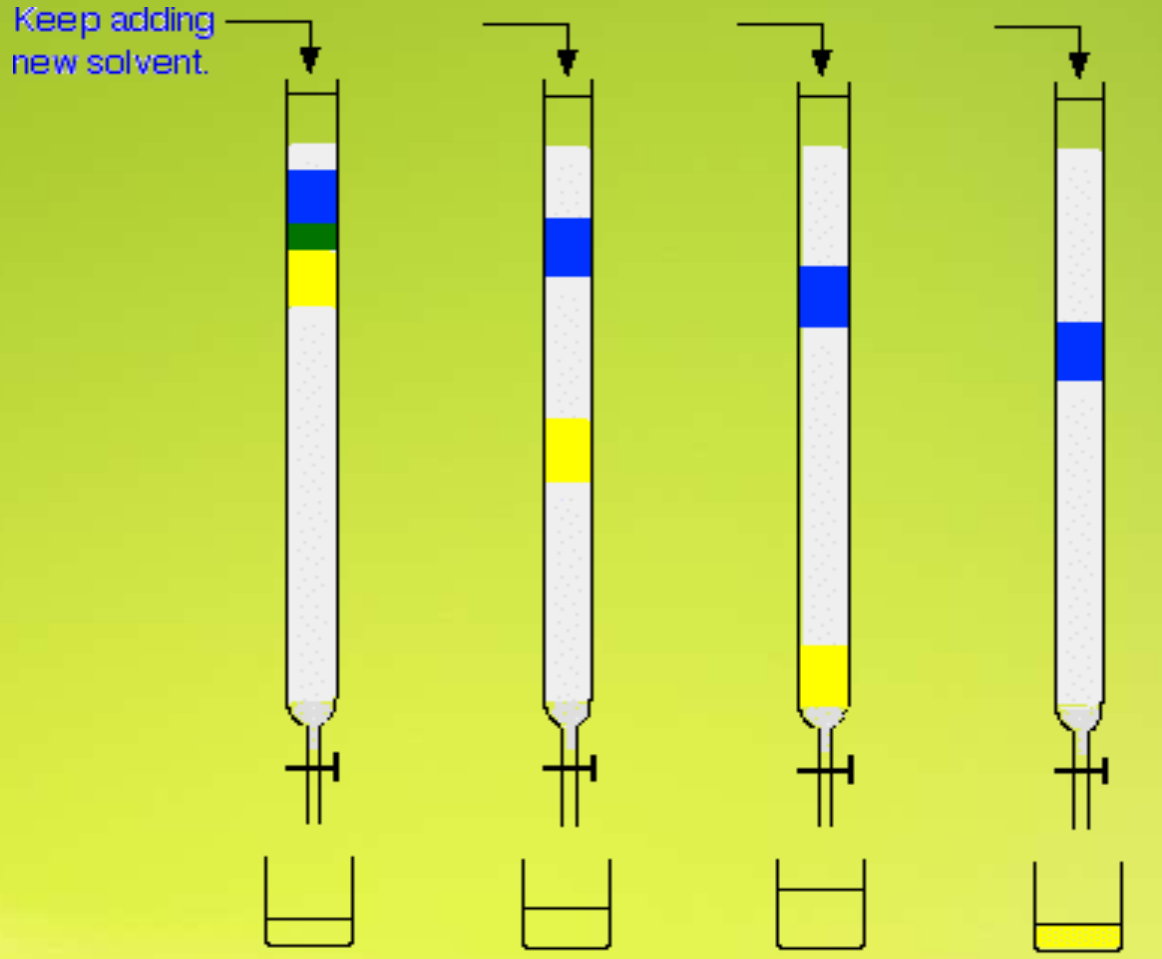

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- Development technique (Elution)
- By elution technique, the individual components are separated out from the column. The two techniques are:
- (i)Isocratic elution technique : in this elution technique , same solvent composition or solvent of same polarity is used throughout the process of separation.
- Example: chloroform only

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(ii) Gradient elution techniques:
(gradient – gradually)

- Solvents of gradually ↑ polarity or ↑ elution strength are used during the process of separation.
- E.g. initially benzene, then chloroform, then ethyl acetate then chloroform

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- DETECTION OF COMPONENTS
- If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually.

- If the compounds to be isolated from column chromatography are colorless. In this case, small fractions of the eluent are collected sequentially in labelled tubes and the composition of each fraction is analyzed by TLC.

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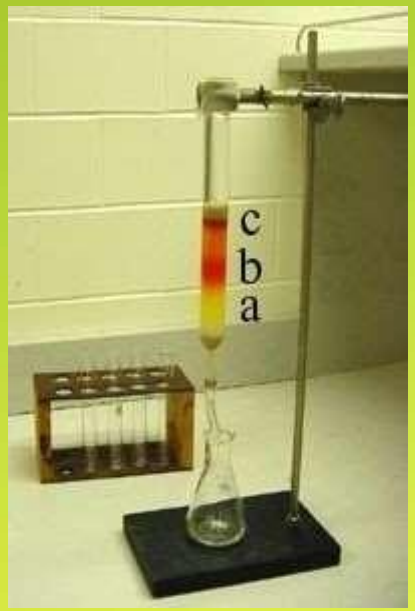
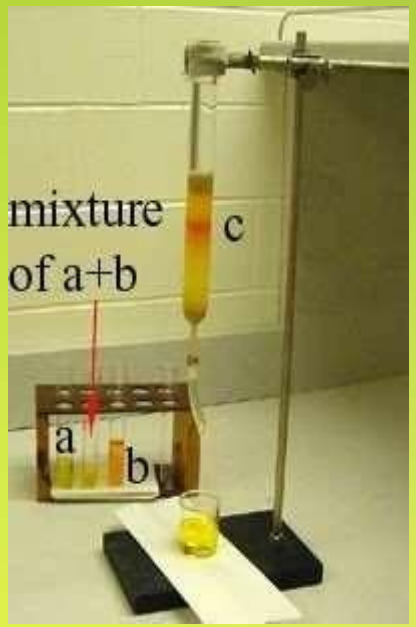
- **Eluting the sample:** Components a, b, and c separate as column progresses.





- Fractions can be collected in test tubes, vials, beakers, or Erlenmeyer flasks.

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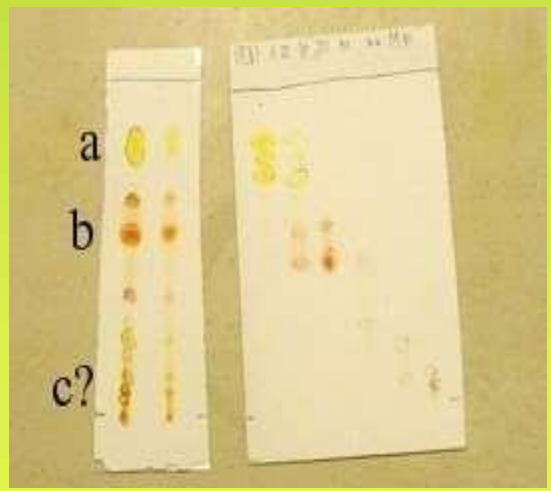
Analyzing the fractions:

- Analyze the fractions by thin-layer chromatography



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- FACTORS AFFECTING COLUMN EFFICIENCY

1. Dimension of the column: column efficiency has been improved by increasing length/width ratio of the column.
2. Particle size of column packing: separation to be improved by decreasing the particle size of the adsorbent.
3. Activity of the adsorbent
4. Temperature of the column: The speed of the elution increases at higher temperatures.
5. Packing of the column
6. Quality of solvents: solvents having low viscosities is giving better results.

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APPLICATIONS

- Separation of mixture of compounds
- Purification process
- Isolation of active constituents
- Estimation of drugs in formulation
- Isolation of active constituents
- Determination of primary and secondary glycosides in digitalis leaf.
- separation of diastereomers

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- Advantages of C.C

» Any type of mixture can be separated

» Any quantity of mixture can be separated

» Wider choice of Mobile Phase

» Automation is possible

- Disadvantages of C.C

» Time consuming

» more amount of Mobile Phase are required

» Automation makes the techniques more complicated & expensive

thank you . . .