크로마토그래피의 원리와 분석법

Column chromatography의 원리-2

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COLUMN CHROMATOGRAPHY

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 sea sand is placed.

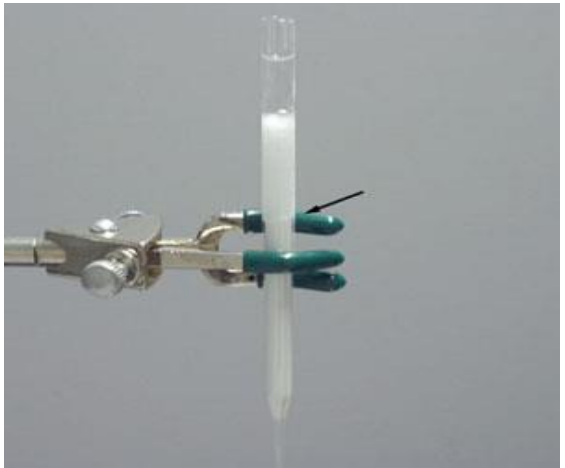
- The stationary phase settles uniformly in the column and there is no entrapment of air bubbles.
- There will not be any crack in the column of adsorbent.
- The bands eluted from the column will be uniform and ideal for separation.

동영상 참조: https://youtu.be/ltTtnVKcqDw

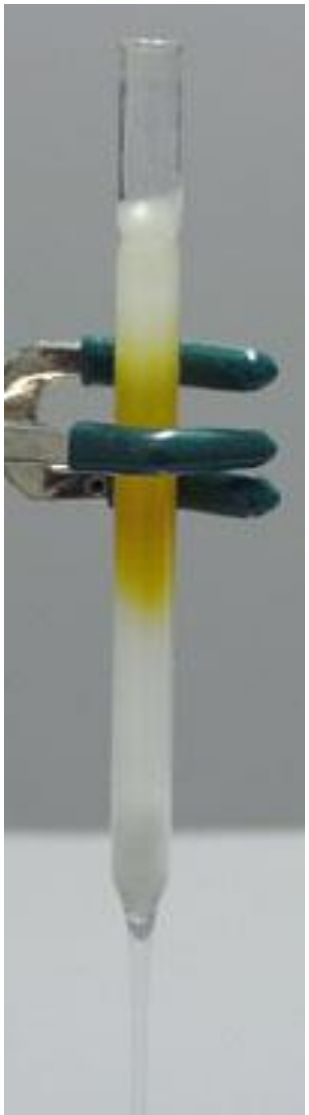
COLUMN CHROMATOGRAPHY

Introduction of the Sample

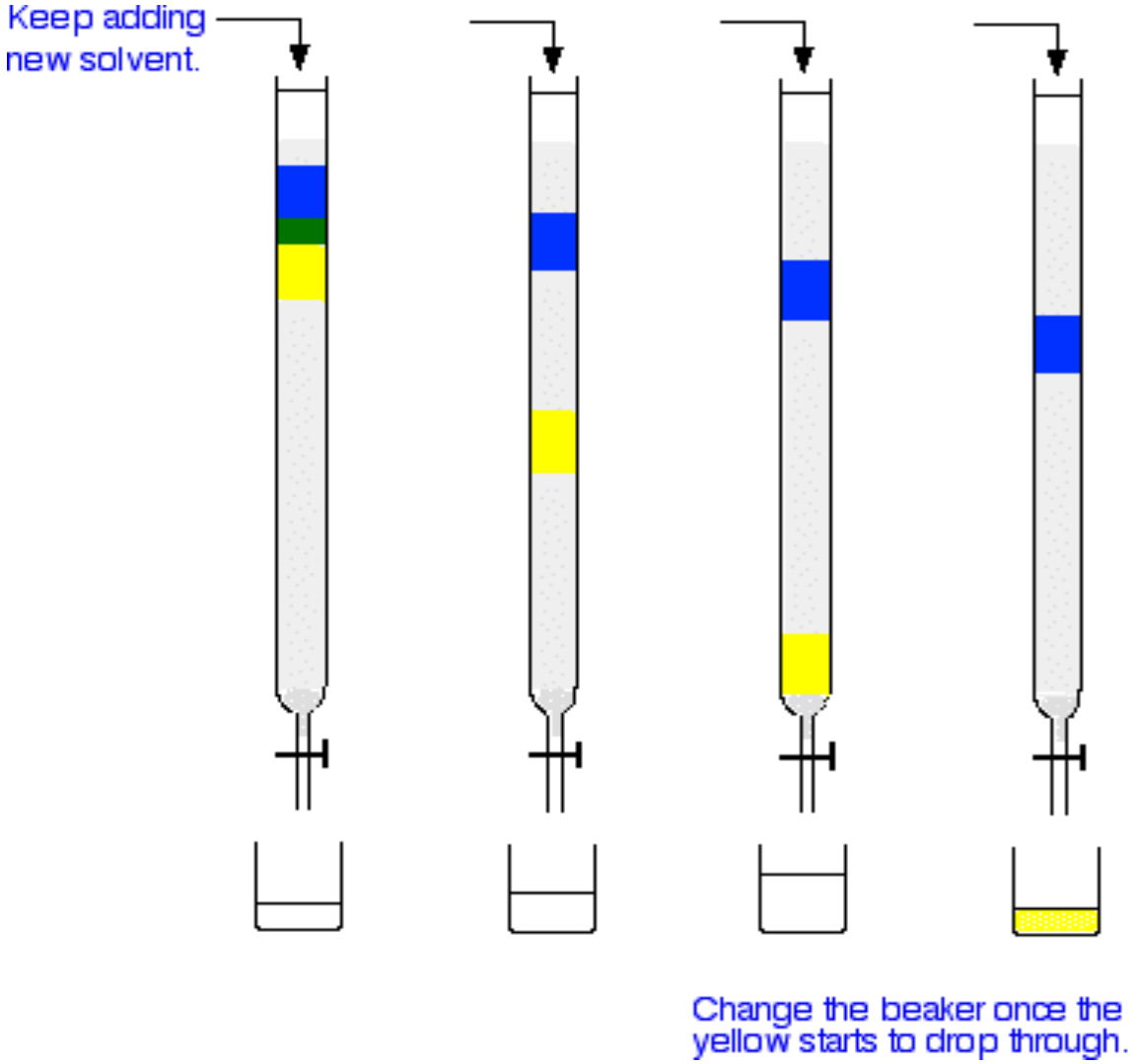
- The sample which is usually a mixture of components is dissolved in minimum quantity of the mobile phase.
- The entire sample is introduced into the column at once and get adsorbed on the top portion of the column.
- From this zone, individual sample can be separated by a process of elution.



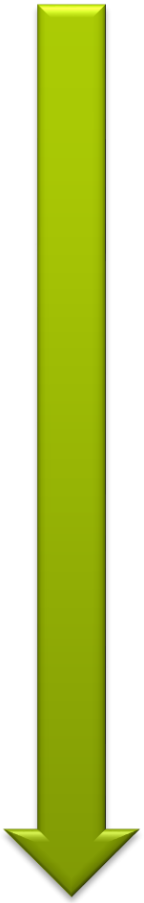
COLUMN CHROMATOGRAPHY



COLUMN CHROMATOGRAPHY



least polar



most polar

increasing polarity,
elution strength

heptane
cyclohexane, hexane
diethyl ether (ether)
benzene
toluene
dichloromethane (DCM, MC)
ethyl acetate (EA)
alcohols (MeOH, EtOH)
chloroform
acetone
water
organic acid

- Mixed eluents can be prepared by mixing low polarity and high polarity solvents and therefore creating any eluting power needed.

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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. Iso means same/ similar.
- Example: chloroform only, petroleum: ether=1:1

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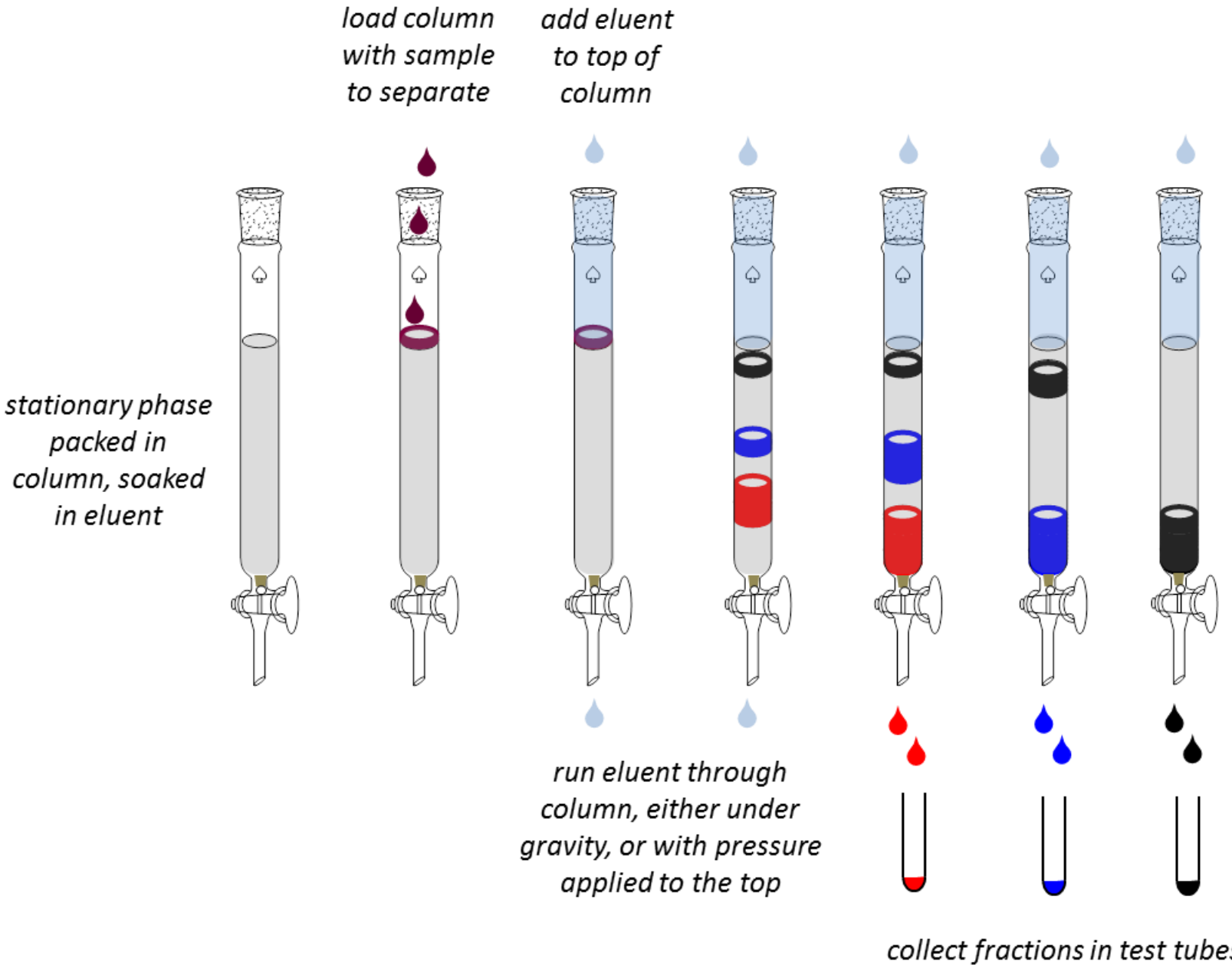
(ii) Gradient elution techniques:

- Solvents of gradually ↑ polarity or ↑ elution strength are used during the process of separation.
- Initially low polar solvent is used followed by gradually increasing the polarity.
- E.g. initially benzene, then chloroform, then ethyl acetate then chloroform


load column with sample to separate
add eluent to top of column
stationary phase packed in column, soaked in eluent
run eluent through column, either under gravity, or with pressure applied to the top
collect fractions in test tubes

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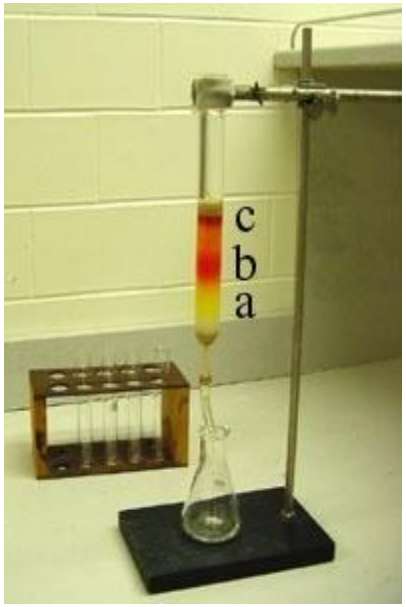
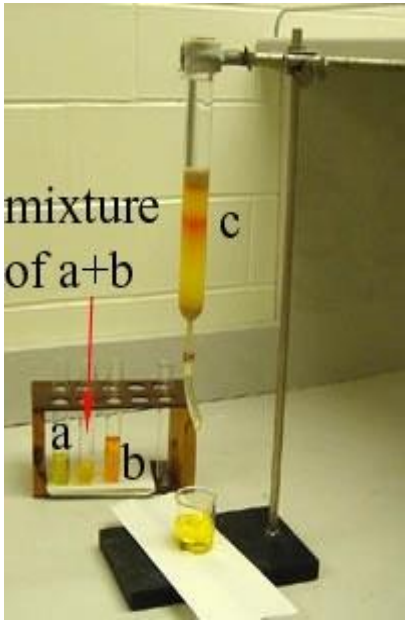
- **Eluting the sample:** Components a, b, and c separate as column progresses.





- Fractions can be collected in test tubes, vials, beakers, or Erlenmeyer flasks.
- Recovery is done by collecting different fractions of mobile phase of equal volume like 10ml, 20ml etc or unequal volume.
  They can also be collected timewise i.e. a fraction for every 10min or 20min etc.The recovered fractions are detected by using different techniques.

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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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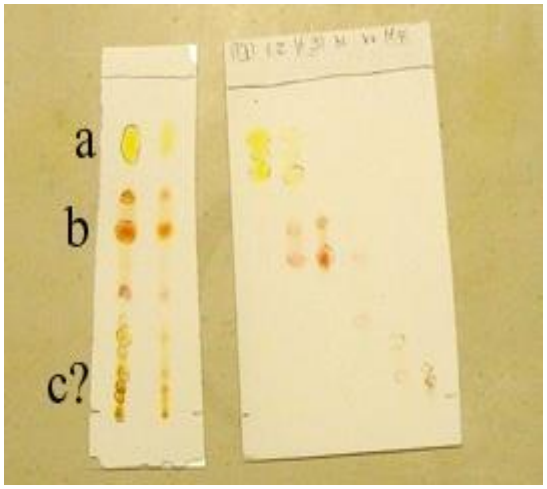
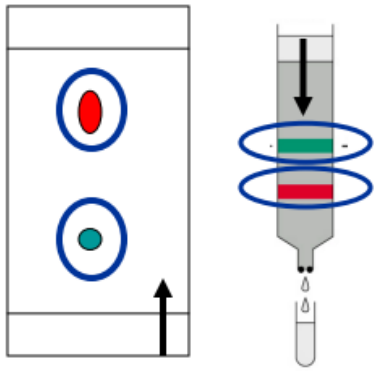
Analyzing the fractions:

- Analyze the fractions by thin-layer chromatography

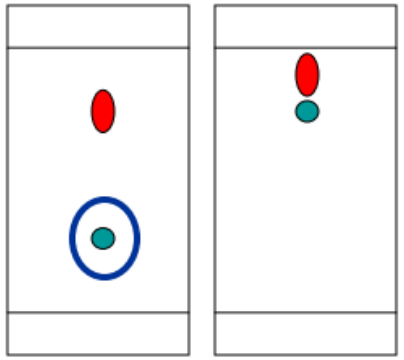


TLC vs. Column Chromatography

- Why?
  - **Compounds** with less attraction for the adsorbent move rapidly with the eluent.
  - **Compounds** with more attraction for the adsorbent move slowly with the eluent.



- **The more polar the eluent, the more rapidly a compound moves.**



- **Calculate R_f** for each spot on the plate with the best separation (measure from center of spot)

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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.
7. Pressure

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APPLICATIONS

- ►Separation of mixture of compounds
- ►Purification process (removal of impurities)
- ►Isolation of active constituents
- ►Estimation of drugs in formulation
- ►Isolation of active constituents
- ►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

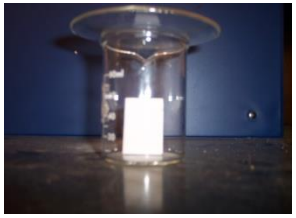
- Disadvantages of C.C

» Time consuming

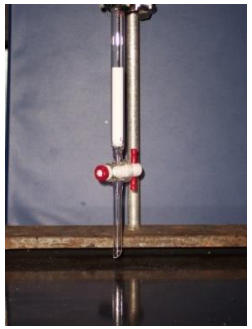
» More amount of Mobile Phase are required

» Automation makes the techniques more complicated & expensive

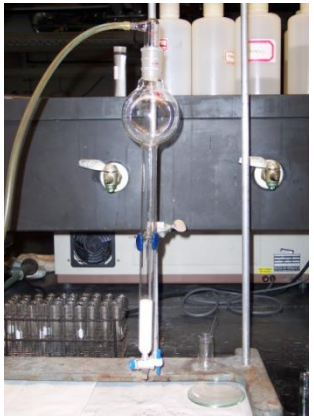
Types of Liquid Chromatography



(TLC) Paper Chrom.



Gravity Chrom. Tsvett, 1903



Flash Chrom. 1978

HPLC 1952

UPLC 2004

Chromatography - Classification

- **Basis of shape**
  - Column Chromatography – Open column, flash, vacuum
  - Planar Chromatography – TLC, HPTLC, OPLC, Centrifugal TLC
- **Mode of Separation**
  - Adsorption (NPC, LSC)– separates molecules based on polarity, least polar eluting first
  - Partition - (RPC, LLC) – Separates molecules based on combination of solubility parameters, partition coefficients, and polarity, most polar eluting first
  - Ion exchange – Separates molecules on basis of molecular charge
  - Size exclusion (GPC, GFC) – separation based on molecular size, largest eluting first
  - Affinity – based on affinity with ligand
- **Basis of Mobile Phase**
  - Liquid Chromatography – LLC, LSC
  - Gas chromatography – GLC, GSC

Various forms of Chromatography

- **Column Chromatography**
  - Prep Column Chromatography
    - Flash Chromatography (FC)
    - Vacuum liquid Chromatography (VLC)
  - Ion Exchange Chromatography
  - Gel Chromatography
    - Gel Filtration (GFC)
    - Gel Permeation (GPC)
- **Pressure Liquid Chromatography**
  - Low-Pressure LC
  - Medium Pressure LC (MPLC)
  - High Pressure LC (HPLC)
- **Normal Phase and Reversed Phase Chromatography**

The End.