

# 크로마토그래피의 원리와 분석법

## Column chromatography의 원리-1

Soonchunhyang University

Department of Chemical Engineering

Prof. Jungkyun Im

순천향대

나노화학공학과

임정균 교수

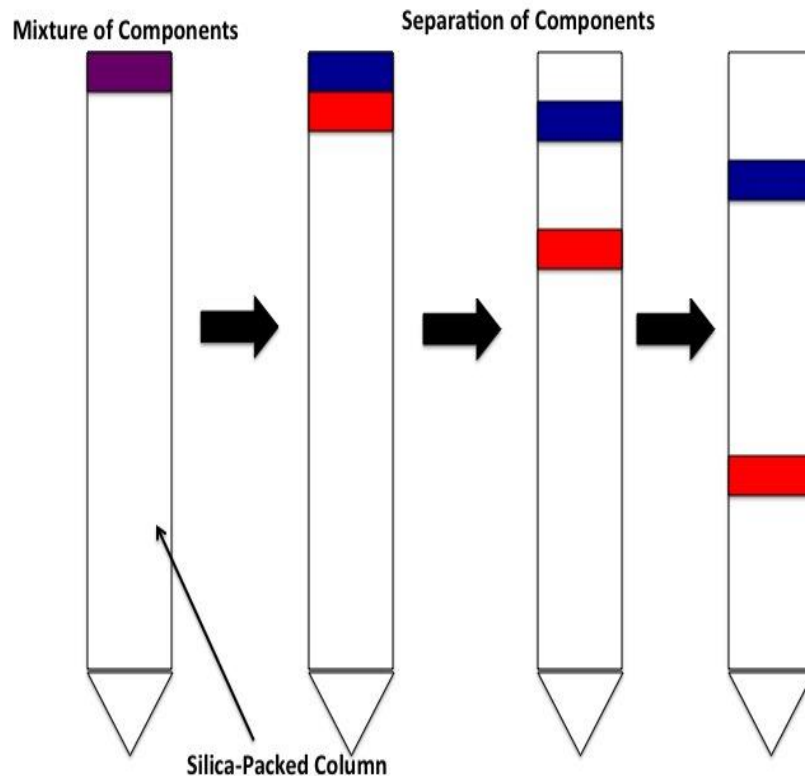


# COLUMN CHROMATOGRAPHY



Column Chromatography was developed by M.S. Tswett, the Russian botanist, in 1906 used adsorption columns in his investigations of plant pigments.

희랍어어원 color + write = chromatography

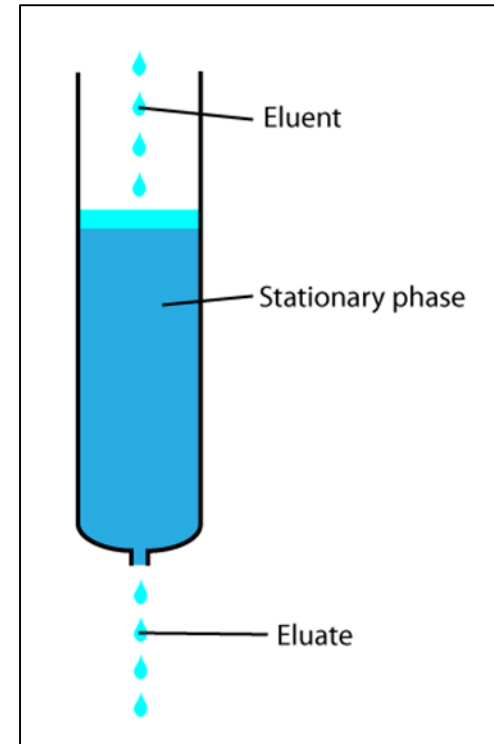


# Terms in column chromatography

- Stationary phase, adsorbent
- Mobile phase, solvent, eluent, developing agent(TLC)
- Sample, adsorbate
- Elution (process)
- Eluate

이동상 : column을 통해 이동하는 용매는 액체 혹은 기체

고정상 : column 안에 머물러있는 상은 보통 모세관 내벽에 혹은 column에 충전된 고체입자 표면에 덮여진 점도가 있는 액체 혹은 고체입자



# COLUMN CHROMATOGRAPHY

- 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.

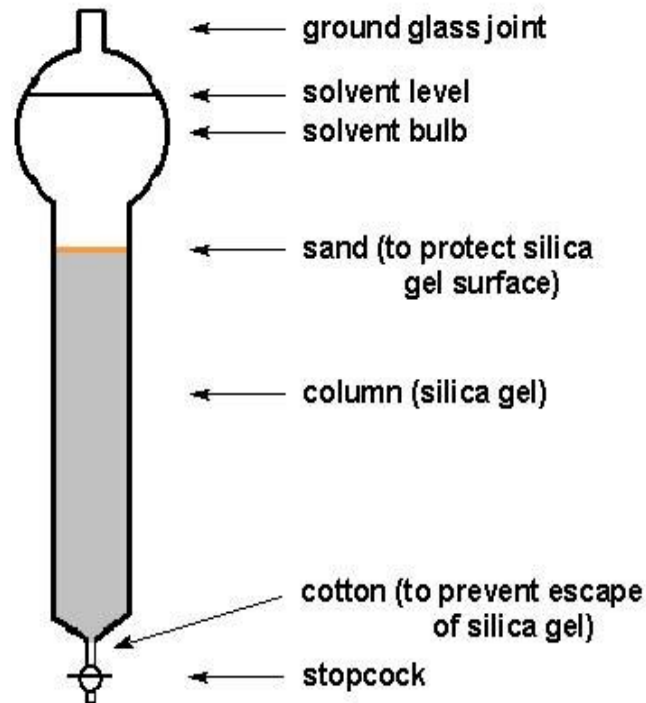
## PRINCIPLE

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

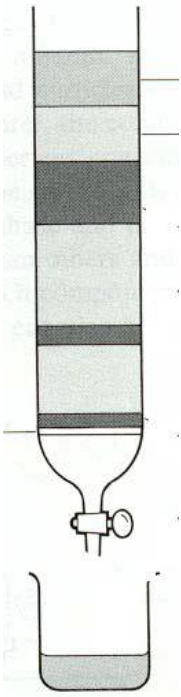
- Adsorption column chromatography, the adsorbent, packed in a glass column, and a solvent, the mobile phase, that moves slowly through the packed column. A solvent used as a mobile phase is called an eluent.

### The Chromatography Column

(also known as gravity chromatography)



- 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.



When a mixture of components dissolved in the mobile phase is introduced into the column, the individual components move with different rates 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.

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





- **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  $\mu\text{m}$ .)

## **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

## 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

- 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 be used in either pure form or as mixture of solvents

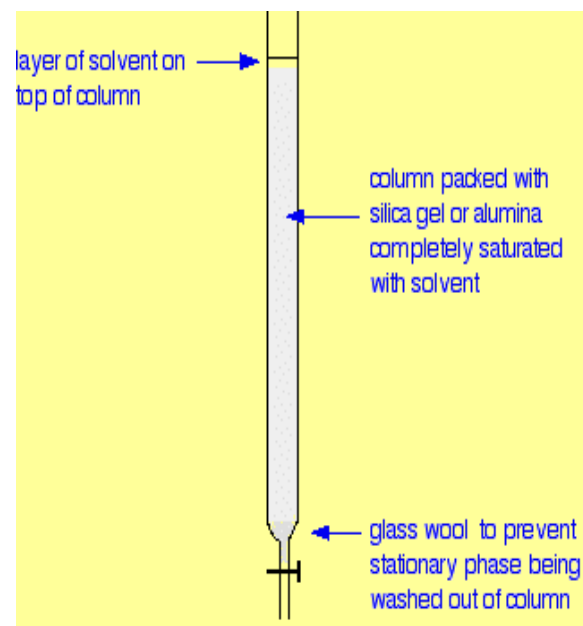
## COLUMN CHARACTERISTICS

- 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.

- 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.

# COLUMN CHROMATOGRAPHY

- 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.
- The column must be packed as uniformly as possible to minimize the distortion of the chromatographic boundaries



# COLUMN CHROMATOGRAPHY

## Packing techniques in C.C

- There are two types of preparing the column, they are:
  - i. Dry packing / dry filling
  - li. 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

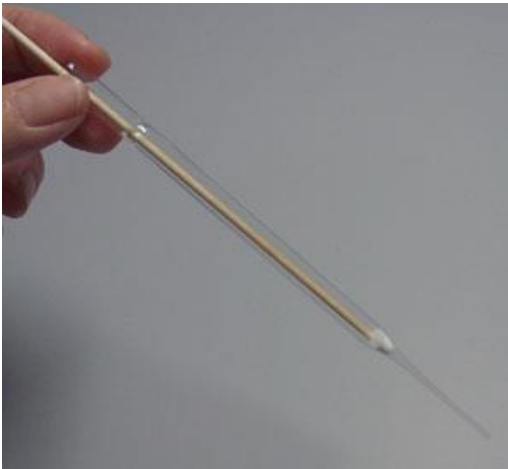


# COLUMN CHROMATOGRAPHY

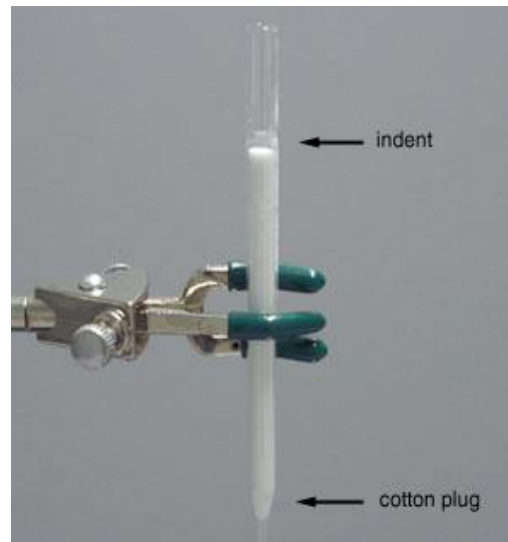
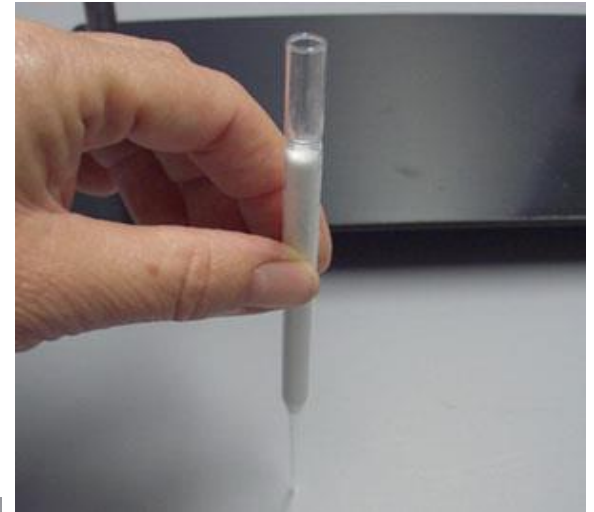
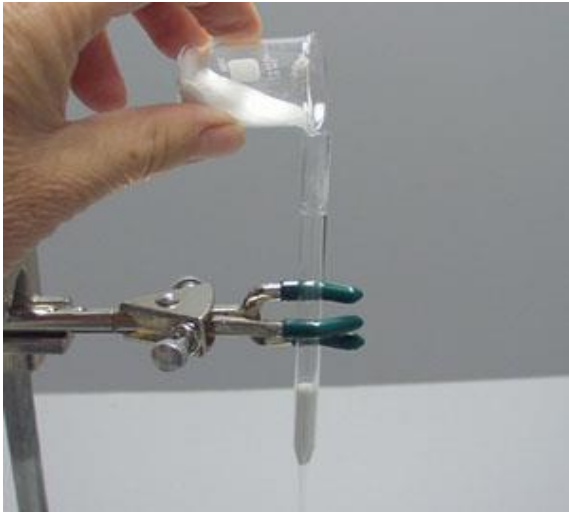
## Dry Packing Technique

- Adsorbent is packed in the column in dry form
- Fill the solvent, till equilibrium is

DEMERIT: Air bubbles are entrapped b/w M.P & S.P → cracks appear in the adsorbent layer. Hence flow characteristics and clear band of the separated component may not be obtained.



# COLUMN CHROMATOGRAPHY



동영상 참조:  
<https://youtu.be/uh3yyIFUEfl>