CHROMATOGRAPHY

Thin Layer Chromatography

Column Chromatography

Biobeads

Gel Permeation Chromatography

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Chromatography

Separation of 2 or more compounds by distribution between phases

i.e. separation is based mainly on differences between the adsorption affinities of the sample components for the surface of an active solid

Key elements:

Stationary and ***mobile*** phases

Stationary Phase

- Immobilized on support particles i.e. fixed in place for chromatographic procedure

- **Silica gel, alumina**

Mobile Phase

Consists of sample being separated -solvent that moves the sample through the stationary phase

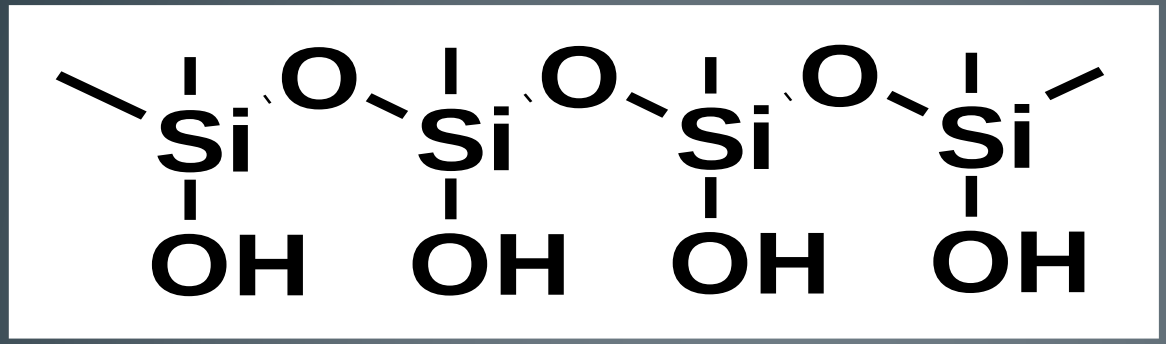
Thin Layer Chromatography (TLC)

- Stationary phase = tlc plate (sheet of glass, metal or plastic)

- Coated with solid adsorbent:-
  - **Silica** (SiO_3) = acidic

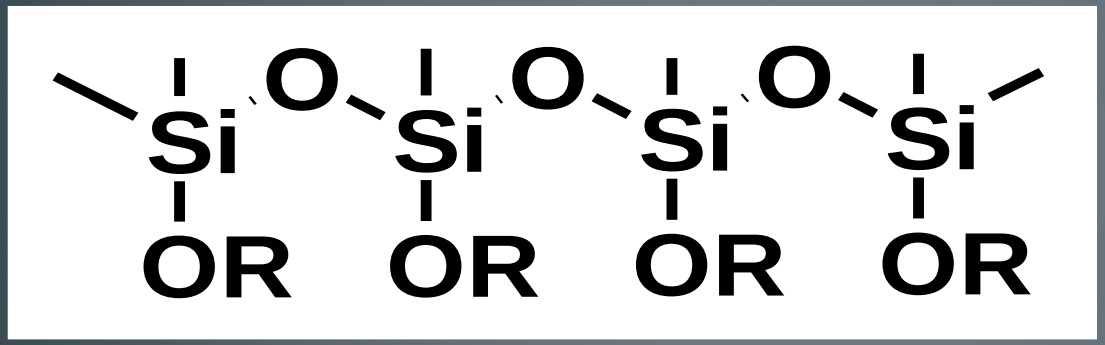
    Electropositive silicon (Si) and electronegative oxygen = very polar stationary phase
  - **Alumina** (Al_2O_3) = 3-types; acidic, basic or neutral

- Normal phase TLC
  - Polar stationary phase



  - Polar compound interacts strongly with polar stationary phase
  - Non-polar compound eluted with mobile phase

- Reverse phase TLC
  - Stationary phase = non-polar
  - Achieved by coating with mineral oil (R = long-chain alkanes)

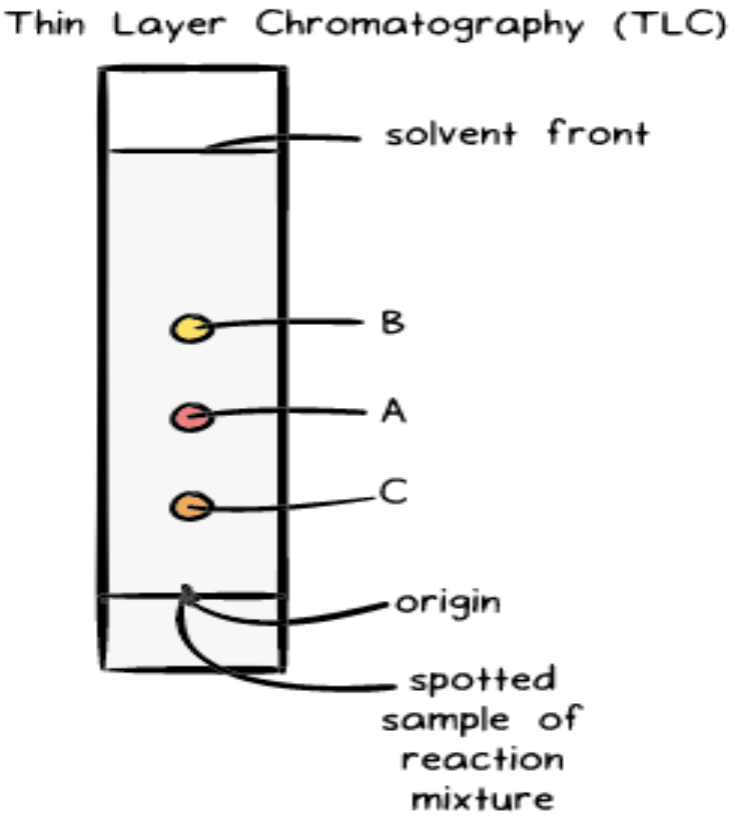


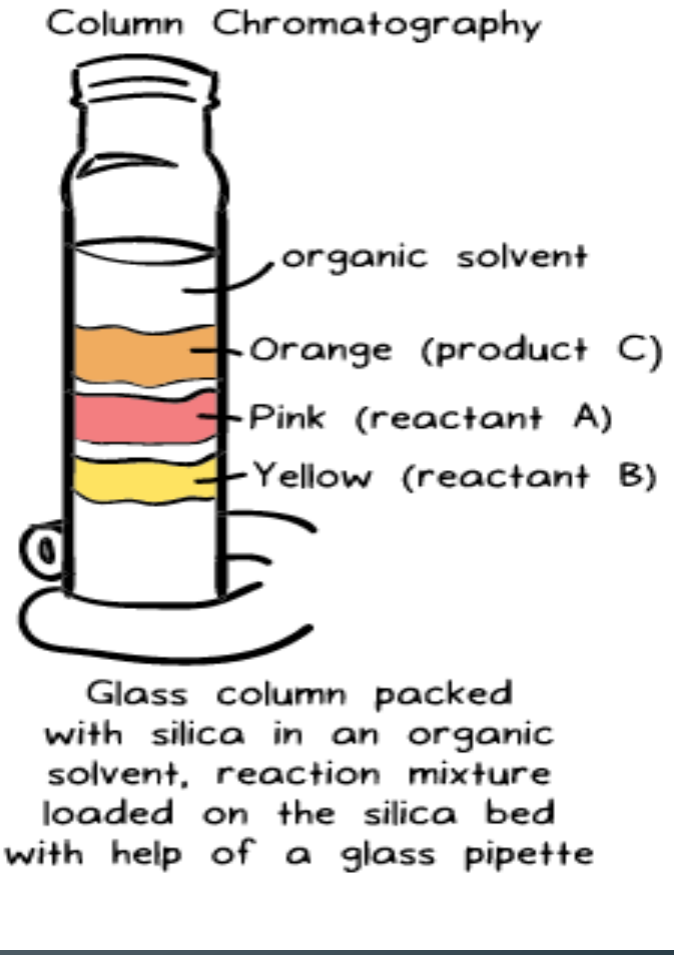
  - Non-polar compound interacts strongly with non-polar stationary phase
  - Polar compound eluted with solvent

Thin Layer Chromatography (TLC)

- Used to :
  - Determine no. of components in a mixture
  - Monitor reaction progress
  - Verify substance identity (Retention factor)

Determine order of elution i.e. determine appropriate conditions for column chromatography



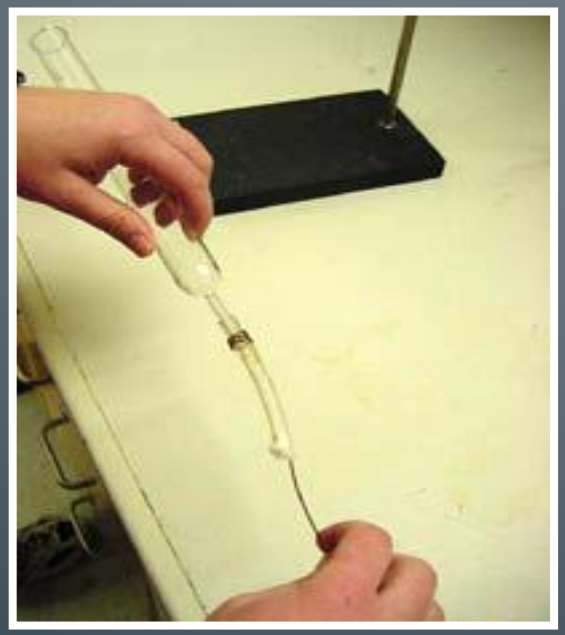


Column Chromatography

- Stationary phase mounted in vertical glass column
- Mobile phase added to top of column
- Mobile phase flows under influence of gravity (gravity column chromatography)
- Components of sample separated

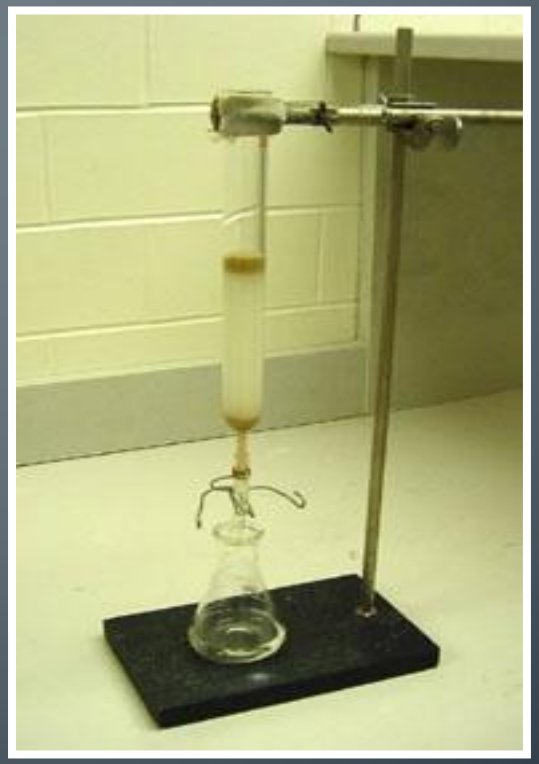
- ***Packing a (silica gel) column:-***

1. Add plug of cotton to bottom of column
2. Add enough sand to fill curved portion of column
3. Fill column to 1/4 to 1/3 full with ***initial*** eluent



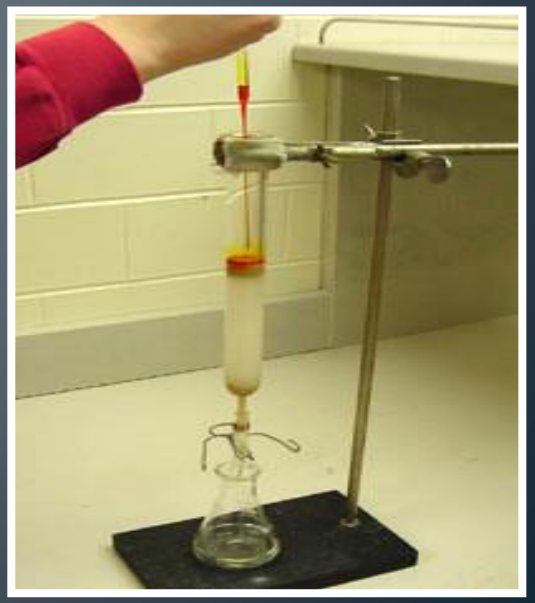
4. Prepare slurry of silica in ***initial eluent*** (~ 20 mL silica gel to double volume of eluent, 40 mL)
5. (With tap open) Quickly but carefully pour slurry into column

6. To help silica settle uniformly tap on side of column with rubber stopper or tubing
7. Rinse excess silica on column sides with eluent
8. Once silica has settled, carefully add sand to top of column



Loading the sample:-

9. Drain eluent from column
10. Carefully add sample (dissolved in min. amount of initial eluent) to column with pasteur pipette
11. Drain eluent from column until no sample remains above surface of sand



Sample elution:-

Eluting solvent added to top of column as necessary

Polarity of eluent affects order of elution

Eluent composition can be changed as column progresses

NB ALWAYS start with least polar solvent/mixture

Individual (coloured) fractions collected as solvent drips from bottom of column

TLC used to analyze fractions

- Variation in mobile phase polarity:-

Petroleum Ether
Hexanes
Cyclohexane
Toluene
Chloroform
Ethyl acetate
Tetrahydrofuran
Dichloromethane
t-Butyl methyl ester
Diethyl ether
n-Propanol
Acetone
Pyridine
Ethanol
Methanol
Water

Increasing solvent polarity (towards polar functional groups)

Fastest - elute with non-polar mobile phase

Alkene hydrocarbons
Alkyl halides
Alkenes
Dienes
Aromatic hydrocarbons
Aromatic halides
Ethers
Esters
Aldehydes
Amines
Alcohols
Phenols
Carboxylic acids
Sulfonic acids

Increasing functional group polarity

Slowest - eluted with polar mobile phase

- Important pointers:-
  - **Uniform column packing and loading**

    i.e. level sand and silica
  - **Drying out**

    cracks in silica affect separation
  - **Sand at bottom of column**

    to create uniform silica gel line
  - **Sand at top of column**

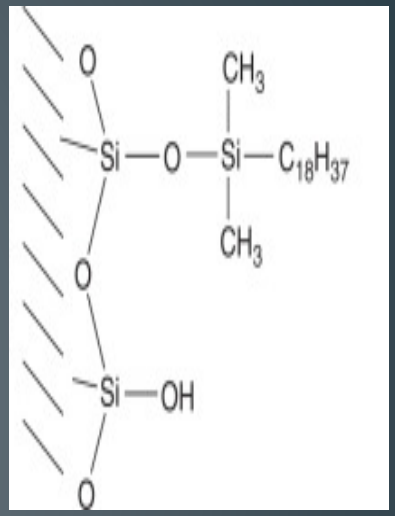
    to aid even loading of sample

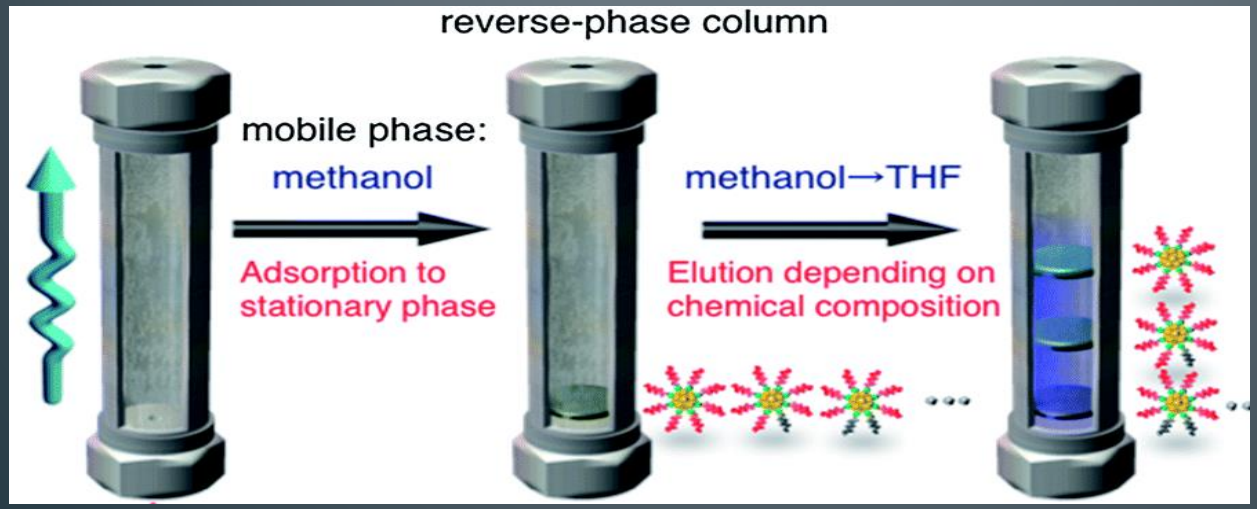
Flash Column Chromatography

- Air pressure applied
- Rapid chromatographic separation
- Fast flow rate
- Increased resolution and decreased band dispersion

Reverse phase Column Chromatography

- Non-polar stationary phase
- Start with polar mobile phase in elution





BIOBEADS

- Bio-Beads SM are neutral, macroporous adsorbent of non-polar polystyrene
- Adsorption of organics from aqueous solutions
- Adsorption of non-polar compounds or surface active agents

- Prepare slurry of Bio-Beads and allow it to flow down the sides of the column……prevent bubble formation
- Allow adsorbent to settle and elute excess solvent from the column
- Equilibrate adsorbent with buffer (phosphate buffer)
- Apply sample and elute with buffer
- Regenerate beads by washing with 100% methanol

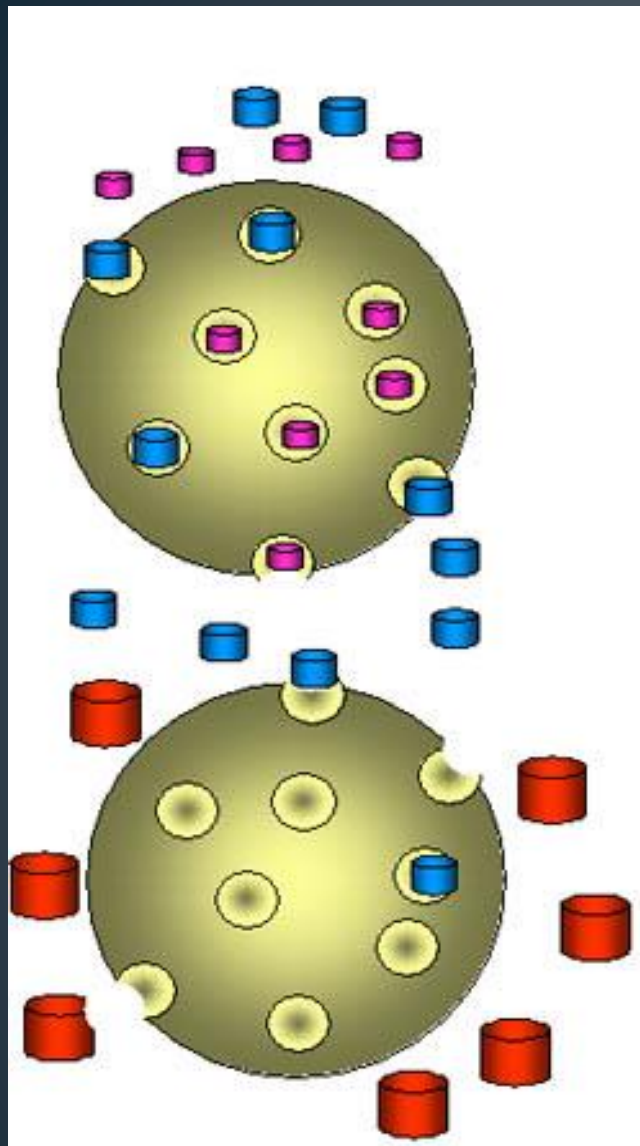
Gel Permeation Chromatography

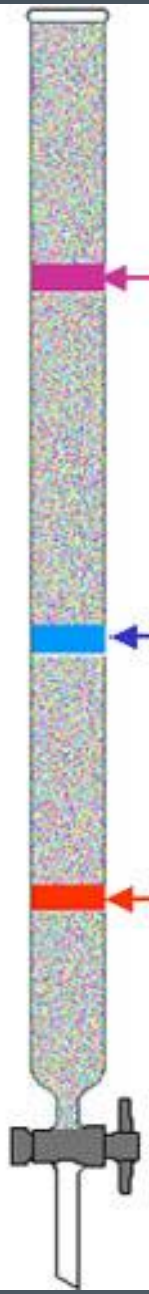
- Size Exclusion Chromatography
- A high performance liquid chromatography technique
- Employs a swollen gel as stationary phase
- For separation of molecules based on their ***molecular size*** in solution

- Mobile phase flows through millions of highly porous, rigid particles (stat. phase)
- Pore sizes of these particles controlled and available in range of sizes

Molecules diffuse through gel at rates depending on molecular size

Low molecular weight molecules retained in column longer than high molecular weight material





very small molecules enter many pores in the gel, equilibrating between the gel and the moving buffer, and so travel slowly and are eluted later

medium sized molecules enter some pores in the gel, equilibrating between the gel and the moving buffer

large molecules enter few pores in the gel, and so travel rapidly and are eluted sooner

Thank you