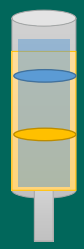
Column Chromatography
(Adsorption & Partition)



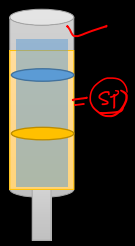
- ✓Basic Introduction
- ✓Types
- ✓Principle
- ✓Procedure
- ✓Application
- ✓Advantages

Chromatography
Instrumental Analysis

COLUMN CHROMATOGRAPHY

Introduction:

- When stationary phase (solid or liquid) is used in the form of column (usually made up of glass), the techniques called column chromatography
- It is separation techniques based on the affinity of analyte towards the stationary phase and mobile phase.
- It is widely used in the pharmacy in the form or simple column or HPLC, GC column.
- **Principle:** On the basis of nature of stationary phase and mechanism of seperration it can be divided into two:
  - 1. Adsorption column chromatography
  - 2. Partition column chromatography



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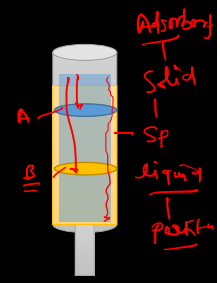
Principle:

- **Adsorption Chromatography**
- Adsorption Chromatography involves the separation of a chemical mixture based on the interaction of the adsorbate with the adsorbent
- Separation based on Affinity of analyte to Stationary Phase (Solid) and Mobile Phase (Liquid or Gas)
- **Partition Chromatography**
- The separation of components between two liquid phases viz original solvent (Mobile phase-Liquid/Gas) and the film of solvent/Liquid used in the column (Stationary phase)
- Rate of movement of component

  R = Rate of movement of component/ Rate of movement of mobile phase

  R = Distance moved by solute/ Distance moved by Solvent



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Principle:

- Rate of movement of component

$$R = \frac{\text{Rate of movement of component}}{\text{Rate of movement of mobile phase}}$$

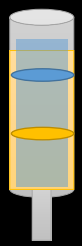
R = Distance moved by solute/ Distance moved by Solvent

$$R = Am / Am + \alpha As$$

Am- Avg cross section of mobile phase

As- Avg cross section of Stationary phase

α – Partition Cofficient



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Procedure:

- **Mobile phase** – This phase is made up of solvents and it performs the following functions:
  - It acts as a solvent- to make mixture
  - It acts as a developing agent – to separate the components
  - It acts as an eluting agent – to elute the compenent
  - Some examples of solvents used as mobile phases based on their polarity are – ethanol, acetone, water, acetic acid, pyridine, etc.
- **Stationary phase** – It is a solid/liquid material which should have good adsorption properties and meet the conditions given below:
  - Shape and size of particle: Particles should have a uniform shape and size in the range of 60 – 200µ in diameter.

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Procedure:

- **Stationary phase** –
  - Stability and inertness of particles: high mechanical stability and chemically inert. Also, no reaction with acids or bases or any other solvents was used during the experiment.
  - It should be colourless, inexpensive and readily available.
  - Should allow free flow of mobile phase
  - It should be suitable for the separation of mixtures of various compounds.
  - E.g., silica, alumina, calcium phosphate, calcium carbonate, starch, and magnesia,

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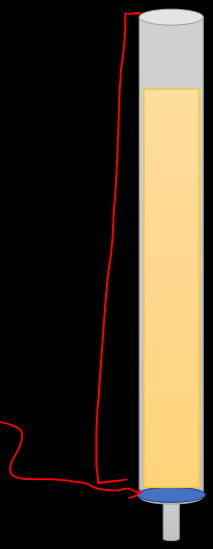
Procedure:

- **Column-**
  - made by glass,
  - Length:diameter ratio- 10:1 to 100:1
  - Commonly available size- WxL(mm)- 26x460, 36x460, 49x460, 70x460
  - Bottom- packed with cotton wool, glass wool, and Whatman filter paper
- **Stationary Phase Preparation-**
  - dry packing technique
  - Wet packing technique- use 25-50% slurry of adsorbent



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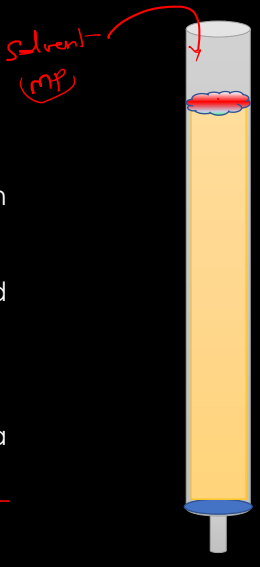
Procedure:

- **Sample Introduction-**
  - made by dissoving in minimum quantity of solvent sample,
  - At one instant, the sample is introduced into the column and on the top portion of the column, it is absorbed.
  - Through the elution process, the individual sample can be isolated from this zone.
- **Elution Techniques- Separation Steps**
  - 1. Isocratic elution technique – Throughout the procedure, a solvent of the same polarity or same solvent composition is utilized.
  - E.g., Chloroform Alone



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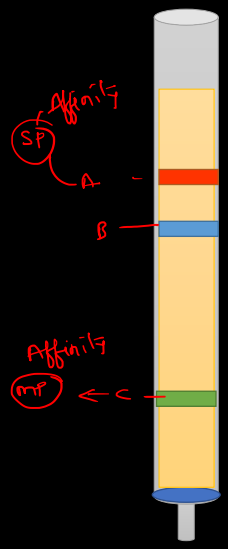
Procedure:

- **Elution Techniques- Separation Steps**
  - 2. Gradient elution technique – Throughout the separation procedure, solvents of gradually increased polarity or increased elution strength are utilized.
  - Example: Benzene → Chloroform → Ethyl acetate → Chloroform
- **Detection**
  - In case the mixture separated in a column chromatography procedure are colored compounds, then monitoring the separation progress is simple.
  - In case the compounds undergoing separation are colorless, then small fractions of the eluent are sequentially collected in tubes that are labeled. Thorugh TLC, the composition of each fraction is determined.



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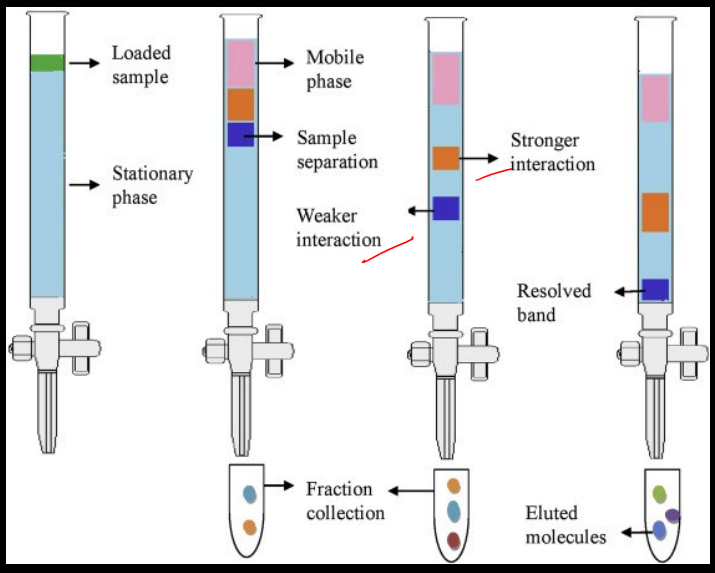
Procedure:



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Application:

1.To isolate active constituents

2.To separate compound mixtures

3.To remove impurities or carry purification process

4.To isolate metabolites from biological fluids

5.To estimate drugs in drug formulations or crude extracts

6.Advantages

- Any type and any quantity of mixture can be separated
- Wider choice of mobile phase
- Automation is possible
- In preparative type, the sample can be separated and reused

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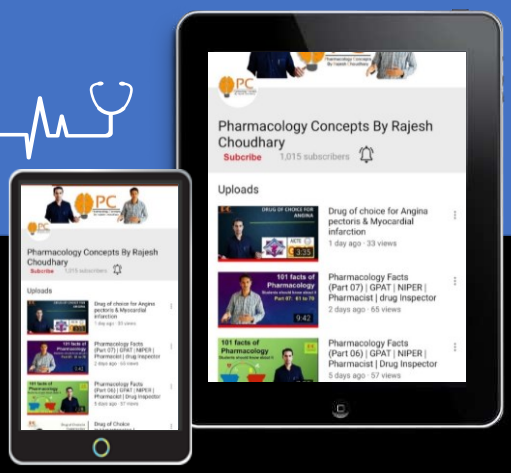
Thanks for Watching



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