Introduction to chromatography

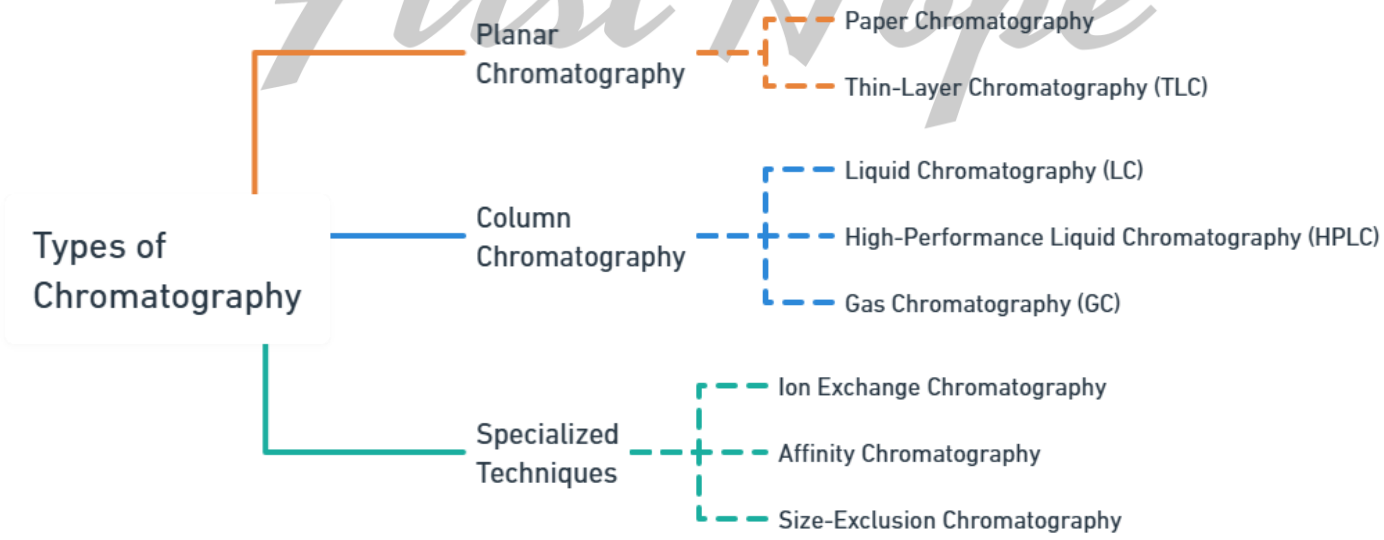
Introduction to Chromatography

- Chromatography is a versatile analytical technique used to separate, identify, and sometimes quantify components within complex mixtures.
- It is widely applied in chemistry, biochemistry, environmental science, food science, pharmaceuticals, and more, enabling the isolation and study of individual substances.

Fundamental Principle

- Chromatography operates on the differential interactions of components in a mixture with two phases:
    1. **Mobile Phase**: A liquid or gas that carries the sample through the system.
    2. **Stationary Phase**: An immobile material (solid or liquid-coated solid) that interacts differentially with the components.
- Components in the sample have varying affinities for the stationary phase and solubilities in the mobile phase, causing them to move at different rates.
- This differential movement separates the mixture into its individual components, which can then be detected and analyzed.

Types of Chromatography



1. Planar Chromatography

- **Paper Chromatography**: Uses coated paper as the stationary phase and a solvent as the mobile phase to separate components as spots.
- **Thin-Layer Chromatography (TLC)**: Employs a thin layer of adsorbent (e.g., silica gel) on a plate, providing better control and resolution than paper chromatography.

2. Column Chromatography

- **Liquid Chromatography (LC)**: A liquid mobile phase carries the sample through a column packed with a solid stationary phase, separating components into fractions.

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- **High-Performance Liquid Chromatography (HPLC)**: A refined form of LC that uses high pressure for faster, high-resolution separations of similar compounds.
- **Gas Chromatography (GC)**: Uses a gas mobile phase and a liquid-coated column. The mixture is vaporized, and separation occurs based on boiling points and stationary phase interactions.

3. Specialized Techniques

- **Ion Exchange Chromatography**: Separates ions based on their affinity for oppositely charged sites on the stationary phase.
- **Affinity Chromatography**: Exploits specific biological interactions (e.g., enzyme-substrate or antigen-antibody binding) for highly selective purification.
- **Size-Exclusion Chromatography**: Separates molecules by size, with larger molecules eluting faster than smaller ones.

Mechanisms of Separation

- Chromatographic separation is based on different mechanisms:
  - **Adsorption**: Components adhere to a solid stationary phase.
  - **Partitioning**: Components distribute between two immiscible phases (stationary and mobile).
  - **Ion Exchange**: Charged analytes interact with oppositely charged stationary phase sites.
  - **Molecular Size**: Larger molecules pass through faster in size-exclusion chromatography.
  - **Affinity**: Highly specific binding interactions with immobilized ligands.

Detection and Analysis

- Separated components are detected using various methods:
  - **UV-Vis Detectors**: Measure light absorbance at specific wavelengths.
  - **Flame Ionization Detectors (FID)**: Common in GC, detect organic compounds by burning the sample and measuring ion production.
  - **Mass Spectrometry (MS)**: Provides detailed molecular weight and structure data, enhancing chromatographic identification.

Applications

- Chromatography plays a crucial role across industries:
  - **Pharmaceuticals**: Ensures drug purity and detects trace impurities.
  - **Environmental Science**: Detects contaminants like pesticides in water, soil, and air.
  - **Biotechnology**: Purifies biomolecules like proteins and nucleic acids for structural studies and drug development.

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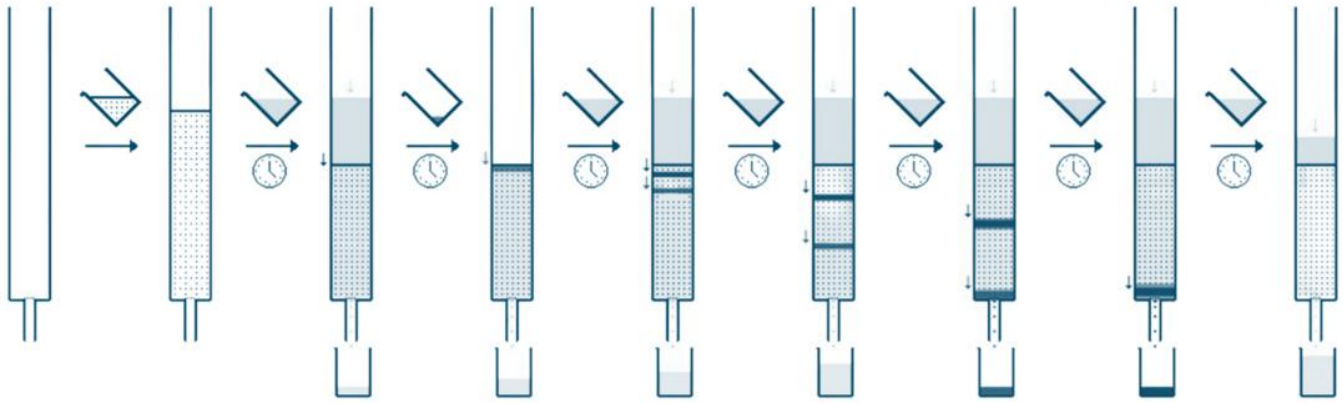
Adsorption Column Chromatography

Introduction

- Adsorption column chromatography is one of the earliest and most fundamental forms of column chromatography.
- It relies on the differential adsorption of components in a mixture onto the surface of a solid stationary phase.

Principle

- The separation is based on the varying affinities of different components for the stationary phase.
- Components that strongly adsorb to the stationary phase move more slowly through the column, while those with weaker interactions elute faster.



Methodology

1. Preparation of the Column:

- A glass column is packed with a stationary phase (e.g., silica gel or alumina).
- A cotton plug is placed at the bottom to retain the stationary phase.
- The adsorbent is slurried with a solvent and poured into the column, avoiding air bubbles.
- A layer of sand is added on top to protect the stationary phase.

2. Sample Loading:

- The mixture is dissolved in a minimal solvent and carefully added to the top of the column or applied as a dried sample pre-adsorbed on the stationary phase.

3. Elution:

- The mobile phase (solvent) flows through the column, carrying the sample.
- Components separate based on their affinity for the stationary phase; lower-affinity compounds elute first, higher-affinity compounds elute later.

4. Detection & Collection:

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