

Chromatography

Introduction to Chromatography

Definition:

- Chromatography is a physicochemical separation technique used to separate, identify, and quantify components in a mixture.
- The separation is based on differential partitioning between a mobile phase and a stationary phase.

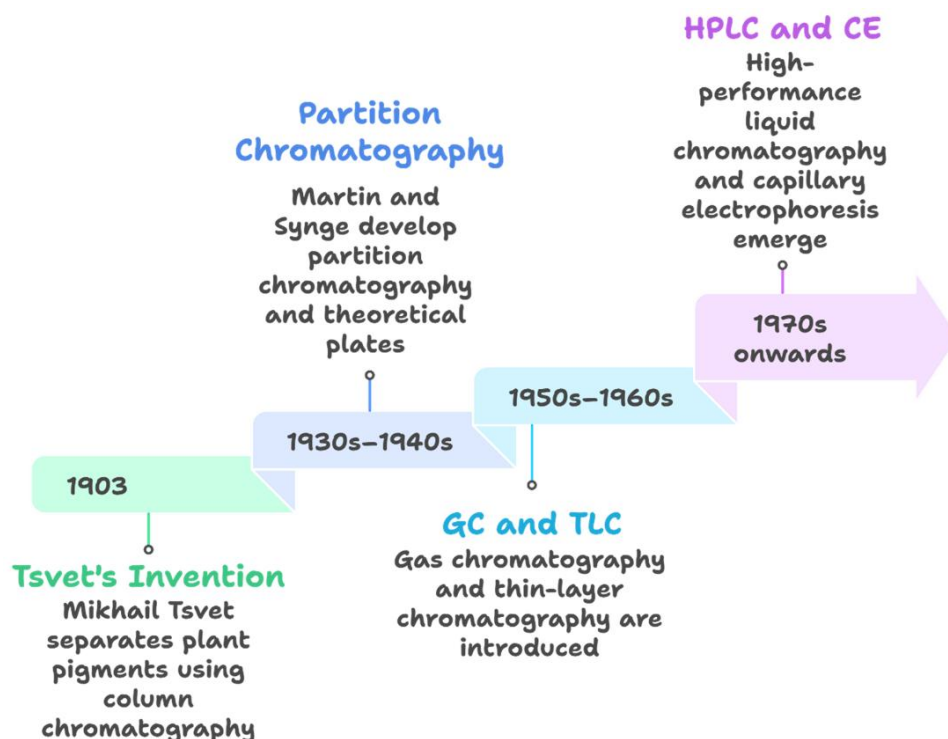
Relevance in Pharmaceuticals:

- Chromatography is indispensable in pharmaceutical analysis for:**
 - Drug purity testing
 - Quantification of active pharmaceutical ingredients (APIs)
 - Detection of impurities and degradation products
 - Pharmacokinetic and bioavailability studies
 - Quality control and assurance

Principle:

- Chromatography operates on the principle that different compounds distribute themselves to varying extents between two phases – the stationary phase (solid or liquid supported on a solid) and the mobile phase (liquid or gas) – leading to differential migration and hence separation.

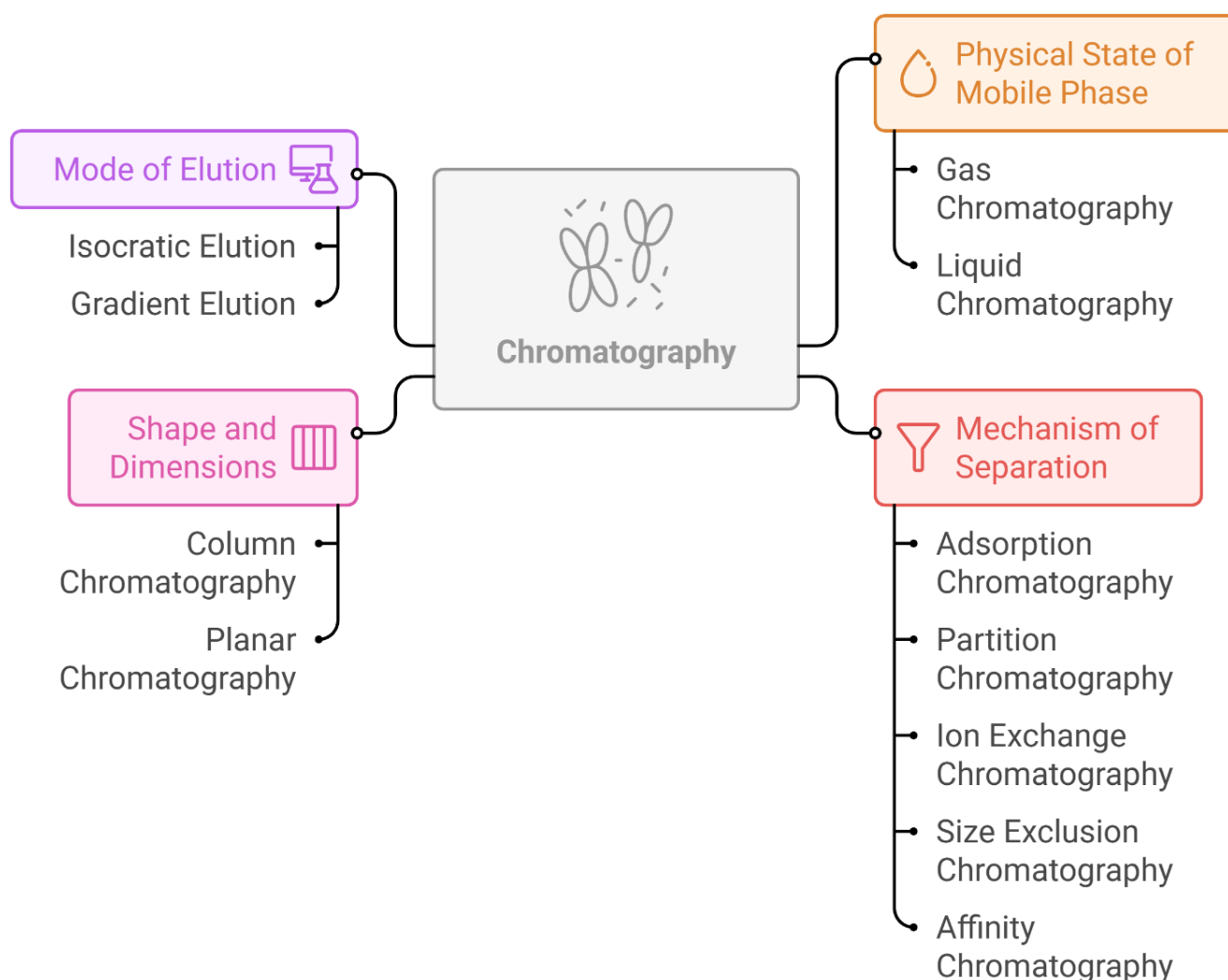
History of Chromatography



- **1903:** The Russian botanist Mikhail Tsvet is credited with the invention of chromatography.
 - He separated plant pigments (chlorophylls and carotenoids) using a column packed with calcium carbonate and an organic solvent as the mobile phase.
- **1930s–1940s:** The development of partition chromatography by Martin and Synge revolutionized the technique.
 - They also introduced the concept of theoretical plates, a key idea in understanding chromatographic performance.
- **1950s–1960s:** Introduction of gas chromatography (GC) and thin-layer chromatography (TLC) expanded the utility of chromatographic techniques.
- **1970s onwards:** The development of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) brought about improved sensitivity, resolution, and reproducibility.

Classification of Chromatography

Chromatography can be classified based on:



1. Physical State of Mobile Phase

- **Gas Chromatography (GC):** Mobile phase is a gas.
- **Liquid Chromatography (LC):** Mobile phase is a liquid.

2. Mechanism of Separation

- **Adsorption Chromatography:** Separation based on adsorption of analytes on solid stationary phase (e.g., TLC, column chromatography).
- **Partition Chromatography:** Based on partitioning between two immiscible liquids (e.g., paper chromatography).
- **Ion Exchange Chromatography:** Based on ionic interactions between analytes and charged groups on the stationary phase (e.g., analysis of amino acids).
- **Size Exclusion Chromatography:** Separation based on molecular size (e.g., analysis of proteins and polymers).
- **Affinity Chromatography:** Highly selective method based on specific binding interactions (e.g., antibody-antigen, enzyme-substrate).

3. Shape and Dimensions

- **Column Chromatography:** Stationary phase is packed into a column.
- **Planar Chromatography:** Stationary phase is spread on a flat surface (e.g., paper, glass plate).

4. Mode of Elution

- **Isocratic Elution:** Single solvent system used throughout.
- **Gradient Elution:** Composition of mobile phase changes over time (common in HPLC).

Separation Techniques in Chromatography

Separation is influenced by the **interaction** of analytes with stationary and mobile phases:

A. Adsorption-Based Techniques

- Molecules adhere to the surface of the stationary phase.
- More strongly adsorbed substances move slowly (e.g., TLC, column chromatography).

B. Partition-Based Techniques

- Molecules distribute themselves between stationary and mobile phases.
- The ratio of this distribution is called the partition coefficient.

C. Ion-Exchange Techniques

- Analytes are separated based on their charge interactions with oppositely charged sites on the stationary phase.
- Useful in separating charged drug molecules, nucleotides, peptides.

D. Size Exclusion (Gel Filtration)

- Molecules are separated according to size.
- Larger molecules elute first as they are excluded from the pores of the stationary phase.

E. Affinity-Based Techniques

- Most specific form of chromatography.
- Binding is highly selective – for example, enzymes can be isolated using substrate-bound columns.

F. Chiral Chromatography

- Specialized form to resolve enantiomers.
- Uses chiral stationary phases or chiral mobile phase additives.
- Critical in pharmaceutical analysis since enantiomers can have different pharmacological or toxicological profiles.

Choice of Chromatographic Methods in Pharmaceutical Analysis

The selection of a chromatographic technique depends on several **critical factors**:

1. Nature of the Analyte

- **Volatile compounds**: Gas Chromatography
- **Non-volatile, polar compounds**: HPLC
- **Macromolecules like proteins**: Size exclusion or affinity chromatography

2. Analytical Objective

- **Identification**: TLC, HPLC, GC-MS
- **Quantification**: HPLC, GC
- **Purity Testing**: HPLC with UV or MS detection
- **Chiral Separation**: Chiral HPLC or GC

3. Sensitivity Required

- For **trace analysis**, GC-MS or LC-MS/MS is preferred.

4. Sample Matrix

- Biological samples like blood/plasma: Need sample clean-up using SPE (solid phase extraction) followed by HPLC or GC.
- Formulated products: May require dissolution and filtration before analysis.

5. Regulatory Compliance

- According to ICH Q2 guidelines, methods should be validated for:
 - Accuracy
 - Precision
 - Specificity
 - Linearity



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