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Chromatography

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Introduction

Chromatography is a technique used to separate mixtures into their individual components. The process involves two phases:

1. **Stationary Phase:** This is the phase that remains fixed in place. It can be a solid or a liquid coated onto a solid support.
2. **Mobile Phase:** This phase moves through or over the stationary phase. It can be a liquid or a gas.

The separation of components occurs because they interact differently with the stationary and mobile phases. As the mobile phase moves, components in the mixture travel at different rates, allowing them to be separated and analyzed.

Chromatography is widely used in chemistry and biology for purifying substances, analyzing mixtures, and identifying compounds. Common types include Thin-Layer Chromatography (TLC), Column Chromatography, Gas Chromatography (GC), and Liquid Chromatography (LC).

Chromatography principle

The principle of chromatography is based on the differential partitioning of components between two phases: the stationary phase and the mobile phase. Components in a mixture move at different rates depending on their varying affinities for each phase, leading to their separation.

Chromatography Instrumentation

Chromatography instrumentation refers to the equipment used for performing and analyzing chromatographic separations. Key components include:

1. **Thin-Layer Chromatography (TLC)**
 - **TLC Plate:** Coated with stationary phase material.
 - **Development Chamber:** For solvent-based separation.
 - **Spotting Device:** Applies sample to the plate.
 - **Visualization System:** For detecting separated components, e.g., UV lamps or stains.

2. Column Chromatography

- **Chromatography Column:** Contains stationary phase material.
- **Elution System:** Delivers the mobile phase through the column.
- **Fraction Collector:** Collects separated components.

3. Gas Chromatography (GC)

- **Injector:** Introduces the sample into the system.
- **Chromatography Column:** Coated with stationary phase, housed in a temperature-controlled oven.
- **Carrier Gas Supply:** Provides the mobile phase.
- **Detector:** Identifies and quantifies separated components (e.g., FID, MS).
- **Data System:** Records and analyzes data.

4. Liquid Chromatography (LC)

- **Pump:** Delivers mobile phase through the column.
- **Chromatography Column:** Contains stationary phase material.
- **Injector:** Introduces the sample.
- **Detector:** Monitors eluted components (e.g., UV-Vis, Fluorescence).
- **Fraction Collector:** Optionally collects separated fractions.
- **Data System:** Analyzes chromatographic data.

5. High-Performance Liquid Chromatography (HPLC)

- **High-Pressure Pump:** Delivers mobile phase under high pressure.
- **Chromatography Column:** Packed with high-resolution stationary phase.
- **Sample Injector:** For precise sample introduction.
- **Detector:** Measures eluted components (e.g., UV-Vis, Mass Spectrometry).
- **Data System:** Analyzes and interprets results.

6. Supercritical Fluid Chromatography (SFC)

- **Supercritical Fluid Pump:** Delivers supercritical fluid as mobile phase.
- **Chromatography Column:** Contains stationary phase suitable for supercritical fluids.
- **Injector:** Introduces the sample.
- **Detector:** Detects separated components (e.g., UV-Vis, Mass Spectrometry).
- **Data System:** For data acquisition and analysis.

Chromatography Combination chart

Chromatography Technique	Stationary Phase	Mobile Phase	Key Instrumentation	Applications
Thin-Layer Chromatography (TLC)	Coated plate (e.g., silica gel, alumina)	Solvent system	TLC plate, Development chamber, Spotting device, Visualization system (e.g., UV lamp)	Qualitative analysis, Purity testing, Preliminary screening
Column Chromatography	Packed column (e.g., silica gel, alumina)	Solvent or liquid phase	Chromatography column, Elution system (e.g., gravity-fed or pump), Fraction collector	Purification of compounds, Isolation of active ingredients
Gas Chromatography (GC)	Coated column (e.g., capillary or packed column)	Carrier gas (e.g., helium, nitrogen)	Injector, Chromatography column, Carrier gas supply, Detector (e.g., FID, MS), Data system	Analysis of volatile compounds, Environmental monitoring, Forensic analysis
Liquid Chromatography (LC)	Packed column (e.g., silica)	Liquid phase	Pump, Chromatography column, Injector, Detector (e.g., UV-Vis)	Analysis of non-volatile compounds, Pharmaceutical analysis, Biochemical analysis

Chromatography Technique	Stationary Phase	Mobile Phase	Key Instrumentation	Applications
High-Performance Liquid Chromatography (HPLC)	polymer-based)		Fluorescence), Fraction collector, Data system	
	High-resolution column (e.g., C18, reverse-phase)	Liquid phase	High-pressure pump, Chromatography column, Injector, Detector (e.g., UV-Vis, Mass Spectrometry), Data system	Detailed analysis of complex mixtures, Quality control, Research
Supercritical Fluid Chromatography (SFC)	Packed column (e.g., silica, polymer-based)	Supercritical fluid (e.g., CO ₂)	Supercritical fluid pump, Chromatography column, Injector, Detector (e.g., UV-Vis, Mass Spectrometry), Data system	Separation of thermally labile compounds, Specialty chemical analysis

Key Points

- **Stationary Phase:** The material that interacts with the components of the mixture to facilitate separation.
- **Mobile Phase:** The phase that moves through or over the stationary phase and carries the components of the mixture.

Applications

- **Thin-Layer Chromatography (TLC):** Qualitative analysis, purity testing, reaction monitoring, forensic analysis.
- **Column Chromatography:** Compound purification, isolation of active ingredients, separation of complex mixtures.
- **Gas Chromatography (GC):** Analysis of volatile compounds, environmental monitoring, quality control, forensic analysis.
- **Liquid Chromatography (LC):** Analysis of non-volatile compounds, pharmaceutical quality control, biochemical research, environmental testing.
- **High-Performance Liquid Chromatography (HPLC):** Detailed mixture analysis, drug development, quality control, research.

References

- **"Principles and Practice of Chromatography"** by W. W. (William W.) Christie
 - This book covers the fundamental principles and techniques used in chromatography, including both theoretical and practical aspects.
- **"Introduction to Modern Liquid Chromatography"** by Lloyd R. Snyder, Joseph J. Kirkland, and John W. Dolan
 - A comprehensive guide to liquid chromatography, discussing both theoretical foundations and practical applications.
- **"Chromatography: Concepts and Contrasts"** by James M. Miller
 - Offers a broad overview of chromatographic techniques and their various applications, emphasizing both theory and practical considerations.

- **"Modern Chromatographic Analysis of Vitamins"** by John K. D. Sutherland
 - Focuses on the application of chromatography in the analysis of vitamins, providing both theoretical and practical insights.
- **"Chromatography: Basic Principles, Sample Preparations, and Related Methods"** by R. K. B. R. F. K. J. W. I. T. D. (Authors and Editors)
 - This book provides a detailed overview of chromatography principles, sample preparation, and related methods.
- **"Handbook of Chromatography"** edited by Jack Cazes
 - A multi-volume reference work that provides extensive information on various chromatography techniques and their applications.

