**Study Material: Chromatographic Techniques**

**I. Core Principle of Chromatography**

**Definition:** Chromatography is a laboratory technique for the separation of a mixture into its individual components. The separation is based on the differential partitioning of the components between a **stationary phase** and a **mobile phase**.

* **Stationary Phase:** A fixed substance that does not move (e.g., silica gel on a plate, coating inside a column).
* **Mobile Phase:** A fluid (gas or liquid) that moves through or over the stationary phase, carrying the sample mixture with it.

**The Key Concept: Partitioning**
Components in the mixture have different affinities (attractions) for the stationary and mobile phases.

* A component with a **stronger affinity for the stationary phase** will move more slowly.
* A component with a **stronger affinity for the mobile phase** will move more quickly.

This difference in migration rates causes the components to separate into distinct bands or spots.

**II. Key Terminology**

* **Elution:** The process of passing the mobile phase through the chromatographic system to move the sample components.
* **Eluent:** The mobile phase itself.
* **Eluate:** The mobile phase exiting the column, containing the separated components.
* **Retention Time (tᵣ):** (In Column Chromatography) The time taken for a particular compound to travel from the injector to the detector. This is a key identifying characteristic.
* **Retardation Factor (Rf):** (In Planar Chromatography) A unitless value calculated as:
**Rf = Distance traveled by solute / Distance traveled by solvent front**
Used to identify compounds.
* **Chromatogram:** The visual output of the chromatograph (usually a graph with peaks), showing the detector response as a function of time or eluent volume.
* **Resolution (Rs):** A measure of how well two peaks are separated from each other. Higher resolution means better separation.

**III. Classification of Chromatographic Methods**

Chromatography can be classified based on two main criteria:

**1. Based on the Physical State of the Mobile Phase:**

* **Gas Chromatography (GC):** Mobile phase is a gas (e.g., Helium, Nitrogen).
* **Liquid Chromatography (LC):** Mobile phase is a liquid (e.g., water, methanol, acetonitrile).

**2. Based on the Mechanism of Separation (Interaction with Stationary Phase):**

* **Adsorption Chromatography:** Separation based on adsorption to a solid surface (e.g., silica, alumina). Common in TLC.
* **Partition Chromatography:** Separation based on differential solubility in a liquid stationary phase coated on a solid support. (Most common mechanism in HPLC and GC).
* **Ion Exchange Chromatography:** Separation based on attraction between analyte ions and charged sites on the stationary phase.
* **Size Exclusion Chromatography (Gel Filtration/Permeation):** Separation based on the size of molecules. Small molecules enter pores and are delayed; large molecules flow through quickly.
* **Affinity Chromatography:** Highly specific separation based on a lock-and-key interaction (e.g., antibody-antigen, enzyme-substrate).

**IV. Major Chromatographic Techniques (Deep Dive)**

**1. Thin-Layer Chromatography (TLC)**

* **Principle:** Adsorption/Partition.
* **Setup:** Stationary phase is a thin layer of adsorbent (e.g., silica gel) coated on a glass, plastic, or aluminum plate. The mobile phase (solvent) moves up the plate by capillary action.
* **How to Analyze:** Components appear as spots. Their Rf values are compared to standards.
* **Applications:** Quick check of reaction completion, purity testing, herbal analysis, and a pilot technique for column chromatography.
* **Pros:** Cheap, fast, simple, can run multiple samples simultaneously.
* **Cons:** Less quantitative, lower resolution.

**2. Column Chromatography**

* **Principle:** Adsorption/Partition.
* **Setup:** Stationary phase (e.g., silica gel) is packed into a glass column. The mobile phase is gravity-fed or pushed through the column. Components elute at different times and are collected in fractions.
* **Applications:** Purification and isolation of compounds, especially in organic synthesis.
* **Pros:** Excellent for preparative-scale separation.
* **Cons:** Time-consuming, can use large volumes of solvent.

**3. High-Performance Liquid Chromatography (HPLC)**

* **Principle:** Partition (Reversed-Phase is most common), but also Ion-Exchange, Size-Exclusion.
* **Setup:** A high-pressure pump forces the liquid mobile phase through a tightly packed column containing very small particles. A detector (e.g., UV-Vis) analyzes the eluting compounds.
* **Key Feature:** **High Pressure** allows for faster flow rates and better separation efficiency.
* **Types:**
	+ **Reversed-Phase HPLC:** Non-polar stationary phase (e.g., C18) and polar mobile phase. Most common type.
	+ **Normal-Phase HPLC:** Polar stationary phase and non-polar mobile phase.
* **Applications:** Quantitative and qualitative analysis in pharmaceuticals (drug testing), environmental monitoring, food and beverage (e.g., caffeine content), forensics.
* **Pros:** Highly accurate, quantitative, excellent resolution, versatile, automatable.
* **Cons:** Expensive instrumentation and maintenance, requires technical skill.

**4. Gas Chromatography (GC)**

* **Principle:** Partition.
* **Setup:** The mobile phase is an inert gas (carrier gas). The sample is vaporized and injected onto the column, which is housed in an oven. Separation occurs based on volatility and polarity. A detector (e.g., Flame Ionization Detector - FID, Mass Spectrometer - MS) analyzes the eluting gases.
* **Key Feature:** Requires samples to be **volatile** and **thermally stable**.
* **Applications:** Analysis of fuels, essential oils, environmental pollutants, blood alcohol content, volatile organic compounds (VOCs).
* **Pros:** Very high resolution, excellent for complex volatile mixtures, highly sensitive detectors (like GC-MS).
* **Cons:** Limited to volatile/thermostable compounds, not suitable for large biomolecules like proteins.

**5. Comparison Table: HPLC vs. GC**

| **Feature** | **HPLC** | **GC** |
| --- | --- | --- |
| **Mobile Phase** | Liquid | Gas (e.g., He, N₂) |
| **Sample** | Non-volatile, thermally unstable, large molecules (e.g., drugs, proteins, sugars) | Volatile, thermally stable, small molecules (e.g., solvents, fuels, essential oils) |
| **Temperature** | Usually ambient | High temperatures (controlled oven) |
| **Principle** | Partitioning between liquid and solid | Partitioning between gas and liquid |
| **Detection** | UV-Vis, Fluorescence, Refractive Index | FID, TCD, Mass Spectrometry (M |