**Study Material: Chromatographic Techniques (Separation & Purity Analysis)**

**🔷 Introduction to Chromatography**

Chromatography is a powerful analytical technique used to separate components of a mixture based on their differential affinities towards a stationary phase and a mobile phase. It is widely used for **purity testing**, **component separation**, and **qualitative and quantitative analysis**.

**🔬 Principle of Chromatography**

Chromatography works on the principle of **differential partitioning** or **adsorption** of components between two phases:

* **Stationary Phase:** A phase that remains fixed in place (e.g., paper, silica gel).
* **Mobile Phase:** A solvent or gas that moves through or over the stationary phase carrying the analyte.

As components of the mixture interact differently with the two phases, they travel at different rates, leading to their **separation**.

**🧪 Paper Chromatography**

**✅ Definition:**

Paper chromatography is a type of planar chromatography where **filter paper** acts as the stationary phase, and a **liquid solvent** acts as the mobile phase. It is especially useful for **separating pigments, amino acids, sugars**, and drugs.

**📖 Theory:**

Paper chromatography is primarily based on **partition chromatography**. The cellulose paper holds a layer of water molecules (stationary phase), and the mobile phase is an organic solvent. Components migrate based on their **solubility in the mobile phase** and **affinity toward the stationary phase**.

**🔎 Types of Paper Chromatography:**

1. **Ascending Chromatography:** Solvent moves upward on the paper.
2. **Descending Chromatography:** Solvent moves downward due to gravity.
3. **Radial or Circular Chromatography:** Solvent moves outward in a circular pattern.
4. **Two-dimensional Chromatography:** Separation is done in two directions using two different solvents.

**⚙️ Procedure:**

1. **Preparation of Paper:** Use Whatman filter paper.
2. **Sample Application:** Apply small spots of sample mixture near one edge.
3. **Development:** Place the paper in a chamber with the mobile phase solvent. Ensure the spot is above the solvent level.
4. **Separation:** Allow solvent to travel by capillary action, carrying the components.
5. **Drying:** Remove the paper and allow it to dry.
6. **Detection:** Use visualizing agents (e.g., ninhydrin for amino acids, UV light for fluorescent compounds).

**📈 Factors Affecting Separation:**

* Type of solvent used (polarity)
* Temperature and humidity
* Nature of sample
* Quality of filter paper
* Time of development

**📝 Advantages:**

* Simple and cost-effective
* Requires minimal equipment
* Suitable for small-scale and qualitative analysis
* Ideal for teaching and preliminary compound screening

**⚠️ Limitations:**

* Not suitable for volatile or large molecules
* Limited quantitative precision
* Less efficient compared to HPLC or GC
* Time-consuming compared to modern methods

**🌍 Applications:**

* Identification of drugs in forensic samples
* Separation of amino acids and nucleotides
* Detection of adulterants in food and pharmaceuticals
* Analysis of plant pigments and herbal formulations
* Monitoring of synthetic reactions and stability studies