CHROMATOGRAPHY

Main Components of Chromatography

Chromatography is a separation technique used to separate the components of a mixture based on their different affinities for two phases. It involves **two main phases**:

- **Stationary phase**
- **Mobile phase**

1. Stationary Phase

The **stationary phase** is the phase that **does not move** during the chromatographic process. It provides a surface on which separation occurs.

- It may be a **solid** (e.g., silica gel, alumina, chromatography paper) or a **liquid coated on a solid support**.
- In **column chromatography**, the stationary phase is packed inside a glass column.
- In **paper chromatography**, the paper itself acts as the stationary phase.

Example:
In column chromatography, silica gel is packed into a column, and the solvent flows through it.

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2. Mobile Phase

The **mobile phase** is the phase that **moves over the stationary phase**, carrying the components of the mixture with it.

- It can be a **liquid** (liquid chromatography) or a **gas** (gas chromatography).
- In **column chromatography**, the mobile phase passes through the column.
- In **paper chromatography**, the solvent rises up the paper by capillary action.

Different components move at different speeds depending on how strongly they interact with the stationary phase.

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3. Rf (Retention or Retardation Factor) Value

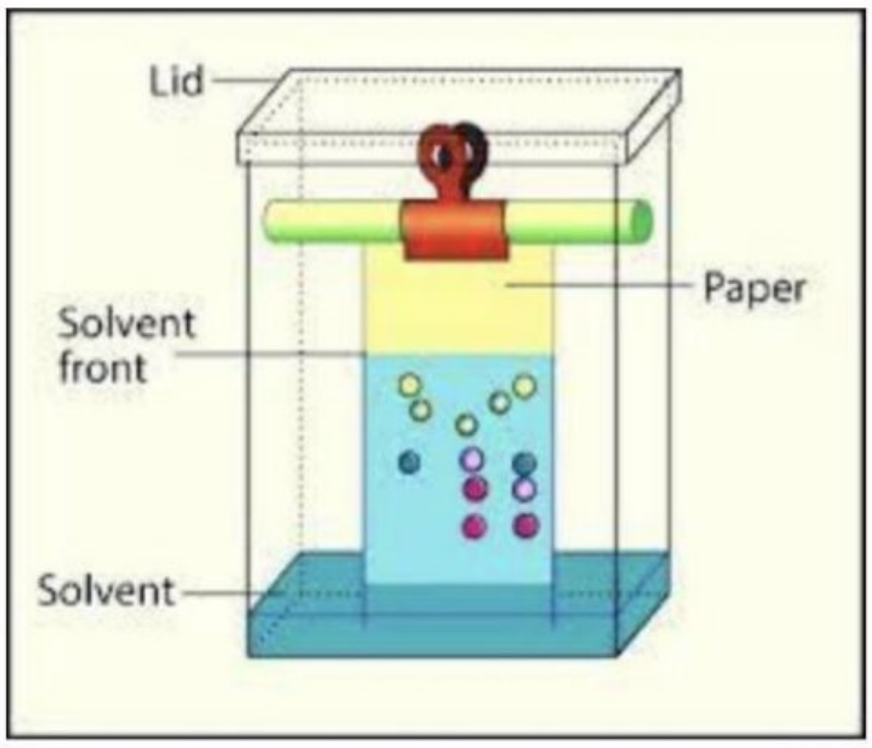
The **Rf value** is a numerical value used to identify compounds in chromatography.

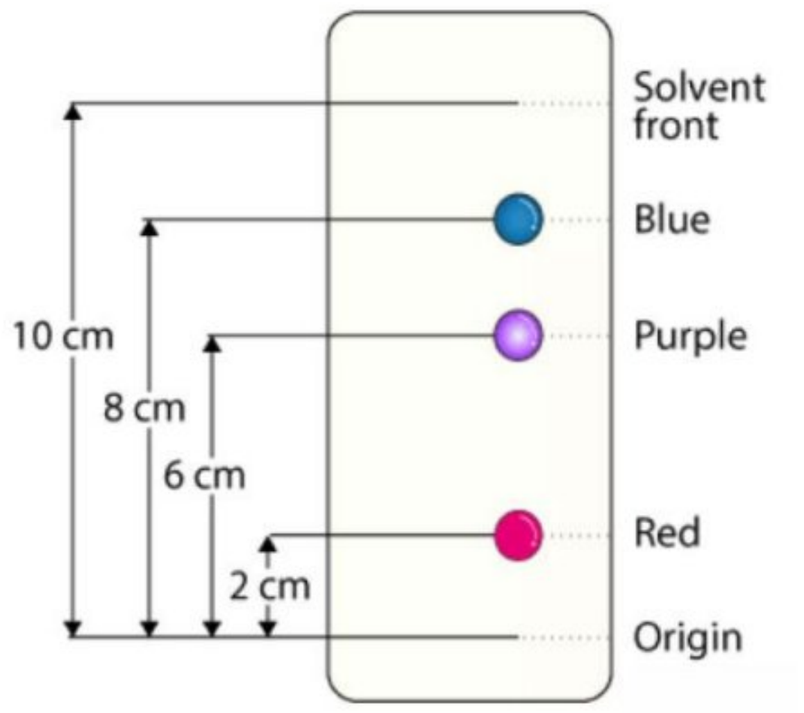
It is defined as:

$$\text{Rf value} = \frac{\text{Distance travelled by solute (compound)}}{\text{Distance travelled by solvent front}}$$

- The distances are measured from the **baseline**.
- The Rf value has **no units**.
- Each compound has a characteristic Rf value under the same experimental conditions.

Rf values help in **identification and comparison of compounds**.





4. Solvent Front

The **solvent front** is the **furthest point reached by the mobile phase** on the chromatogram.

- It shows how far the solvent has travelled.
- The solvent front must be marked immediately after removing the chromatogram from the solvent.
- Separation occurs due to a **dynamic equilibrium** between the stationary and mobile phases.

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5. Baseline

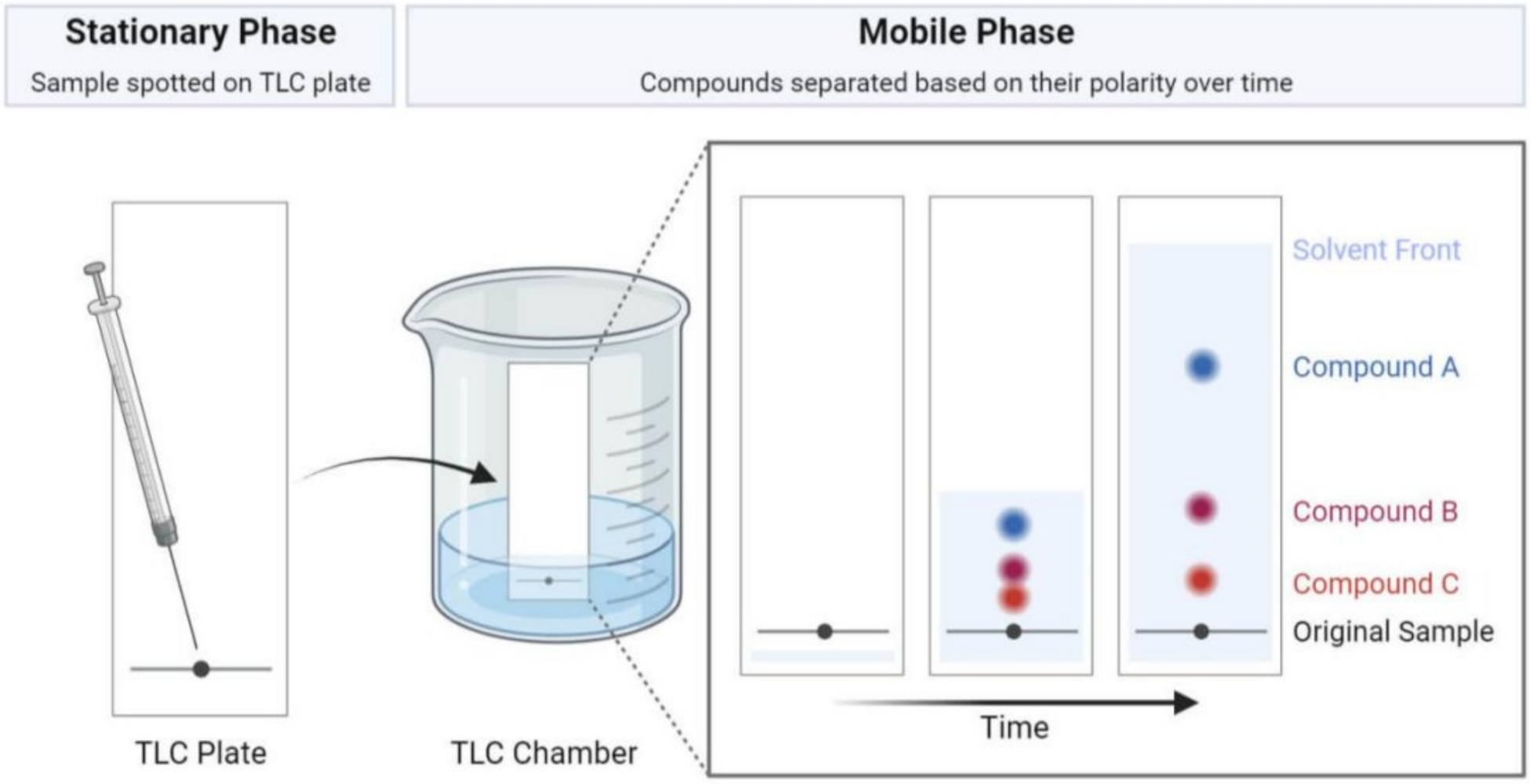
The **baseline** is the **starting line** drawn near the bottom of the chromatographic paper or plate.

- The sample mixture is applied as small spots on this line.
- All distances for Rf calculation are measured from the baseline.
- It serves as a **reference point** for analyzing movement of components.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a chromatographic technique used to separate and identify components of a mixture based on their **different affinities for the stationary and mobile phases**.

In TLC, substances move across a **thin layer of adsorbent** coated on a solid support. Some substances adhere strongly to the stationary phase and therefore move slowly, while others interact more with the mobile phase and move faster. As a result, the components of the mixture separate and appear as **distinct spots** on the plate.



Principle of Thin Layer Chromatography

TLC works on the principle of **adsorption and differential migration**. The separation depends on:

- Nature of the stationary phase
- Nature of the mobile phase
- Polarity of the substances

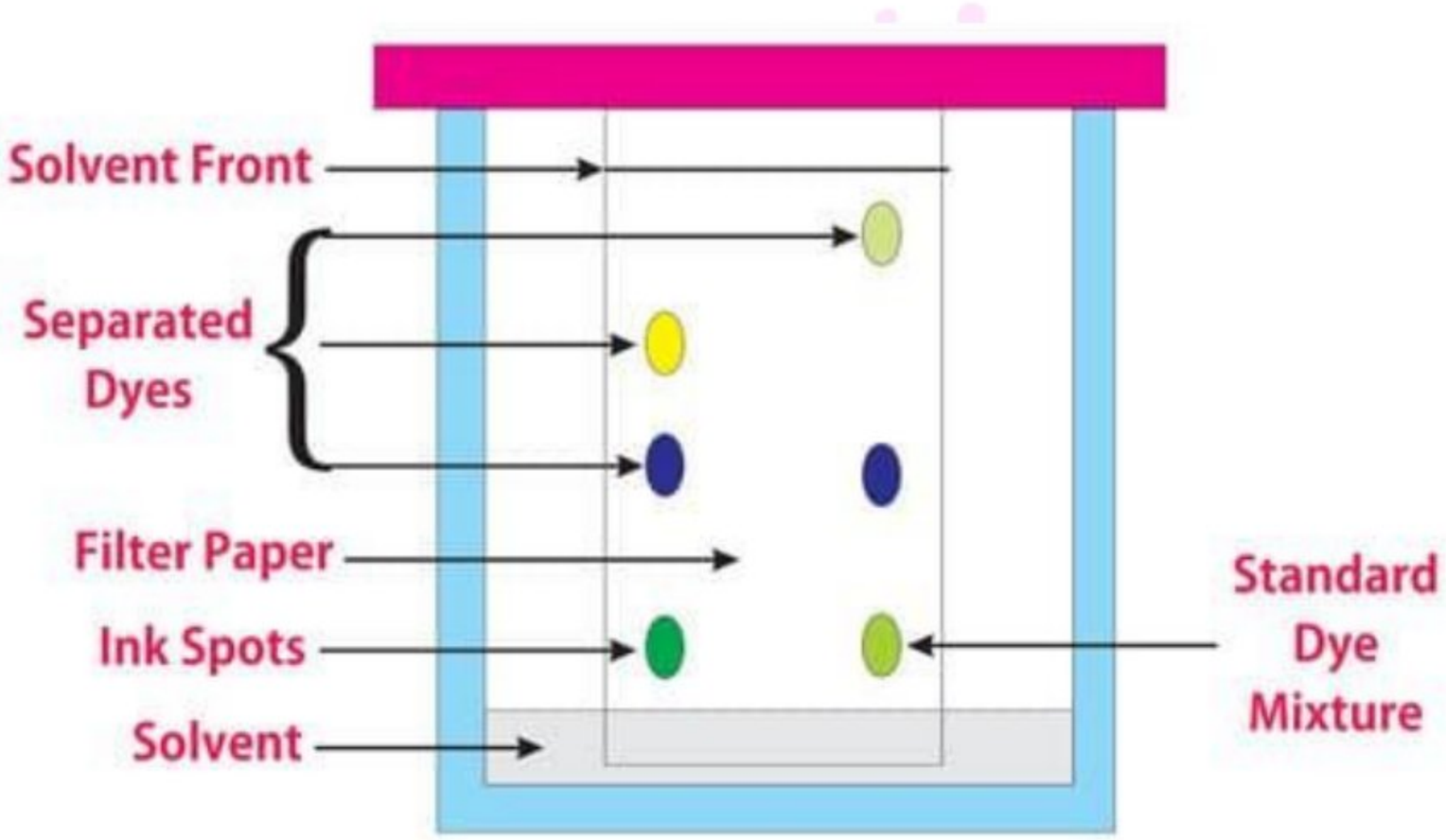
Substances that are strongly adsorbed on the stationary phase move a shorter distance, while weakly adsorbed substances move farther.

Components of TLC

- **Stationary Phase:** A thin layer of adsorbent such as **silica gel or alumina** coated on a glass, plastic, or aluminium plate.
- **Mobile Phase (Eluent):** A pure solvent or mixture of solvents that rises up the plate by capillary action.
- **TLC Plate:** A chemically inert and stable plate with a uniform thin layer of stationary phase.
- **Developing Chamber:** A closed container that maintains a saturated solvent atmosphere, prevents evaporation, and protects from dust.

Procedure of Thin Layer Chromatography

The mixture to be separated is dissolved in a suitable solvent and applied as a **small spot** on the TLC plate at least **2 cm above the baseline** using a capillary tube. The plate is then placed in a developing chamber containing the mobile phase. The solvent rises up the plate and carries the components of the mixture to different heights, resulting in separation.



Working of Thin Layer Chromatography

1. The stationary phase (silica gel or aluminium oxide) is coated on the plate and allowed to dry.
2. A pencil is used to draw a baseline near the bottom of the plate.
3. Small spots of the sample solution are applied on the baseline using a capillary tube.
4. The mobile phase is poured into the TLC chamber, and moist filter paper is placed inside to maintain constant humidity.
5. The TLC plate is placed carefully inside the chamber and covered with a lid.
6. The solvent rises up the plate by capillary action, separating the components.
7. When the solvent front reaches near the top, the plate is removed and dried.
8. The separated spots are observed under **UV light** or using suitable detecting agents.

Applications of Thin Layer Chromatography

1. Testing and analysis of medicines such as sedatives and local anesthetics.
2. Biochemical analysis of substances obtained from food.
3. Identification of natural products like essential oils and alkaloids.
4. Purification and comparison of samples with standard compounds.
5. Separation and identification of colours, sweeteners, and preservatives in the food industry.
6. Used in the cosmetic industry for quality control.
7. Useful in organic synthesis to monitor chemical reactions.

Limitations of Thin Layer Chromatography

1. Separation length is shorter compared to other chromatographic techniques.
2. Being an open system, humidity and temperature can affect results.
3. TLC is mainly suitable for detecting small quantities of substances.
4. Quantitative analysis is less accurate compared to advanced methods.

Application of Thin Layer Chromatography in Forensic Chemistry

Thin Layer Chromatography plays an important role in **forensic chemistry** by enabling rapid and reliable separation and identification of substances in complex samples.

Major Forensic Applications:

1. **Drug Analysis:**
   TLC is widely used to separate and identify illicit drugs such as cocaine and heroin by comparing Rf values with known standards.
2. **Poison Detection (Poison Report):**
   In suspected poisoning cases, TLC helps analyze biological samples such as blood, urine, and tissues to identify toxic substances.
3. **Trace Evidence Analysis:**
   TLC is used to analyze fibers, paints, and dyes found at crime scenes and compare them with reference samples.
4. **Gunshot Residue (GSR) Analysis:**
   It helps detect chemical residues produced by firearms.
5. **Explosives Detection:**
   TLC is used to identify explosive residues such as nitrates and nitroaromatic compounds.
6. **Document Examination:**
   TLC helps analyze inks and paper coatings to detect forgery and authenticate documents.

7. **Preliminary Toxicological Screening:**
   TLC serves as a screening tool to identify drugs before confirmatory analysis using techniques such as **GC-MS** or **HPLC**.

Selection of Mobile and Stationary Phase

The effectiveness of chromatography largely depends on the **proper selection of the stationary phase and the mobile phase**. Appropriate selection ensures efficient separation, good resolution, and reliable results.

Selection of Stationary Phase

In chromatography, an **adsorbent** is commonly used as the stationary phase. The stationary phase must possess the following characteristics:

1. **High and selective adsorption capacity**
   It should selectively adsorb different components of the mixture to varying extents.
2. **Large surface area**
   The stationary phase should be finely divided to maximize surface area for better adsorption.
3. **High mechanical stability**
   It should resist breakdown and minimize dust formation during handling and flow of the mobile phase.
4. **Chemical inertness**
   It must not react chemically with the sample components or the mobile phase.
5. **High purity**
   Impurities can interfere with separation and affect accuracy.
6. **Easy availability and cost-effectiveness**
   It should be readily available and economical.

The stationary phase is selected so that the components of the sample have **different affinities** for it. Due to these differences, components move through the stationary phase at **different rates**, leading to separation.

Resolution Considerations

The degree of separation (resolution) required also affects the choice of stationary phase.

- For separating **closely related compounds**, a stationary phase with **small particle size** is preferred.
- Smaller particles provide greater surface area and reduce diffusion, resulting in **sharper bands and better separation**.

Selection of Mobile Phase in Chromatography

The **mobile phase** is chosen to ensure effective movement and separation of sample components. Selection depends more on **chemical and physical properties** than on the manufacturer; however, high-quality solvents from reputable suppliers are essential.

Important Factors in Mobile Phase Selection

1. Solvent Purity

- High-purity solvents reduce interference and background noise.
- **HPLC-grade or MS-grade solvents** are preferred for accurate and reproducible results.
- Impurities in solvents can affect retention times and peak shapes.

2. Polarity

- The polarity of the mobile phase should be compatible with the polarity of the analytes.
- Proper polarity ensures optimal interaction between analyte, stationary phase, and mobile phase.

3. Viscosity

- The mobile phase should have suitable viscosity to allow smooth flow.
- Low viscosity helps maintain a proper flow rate and good peak shape.

4. Chemical Stability

- The solvent must be chemically stable.
- It should not react with the analyte or damage the stationary phase.

5. UV Transparency

- If UV detection is used, the mobile phase should not absorb UV radiation at the detection wavelength.

6. pH Control

- For **ionizable or charged compounds**, controlling the pH of the mobile phase is essential.
- Proper pH prevents unwanted ionization and improves reproducibility and resolution.

For More Information:

Online and physical tuition is available

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