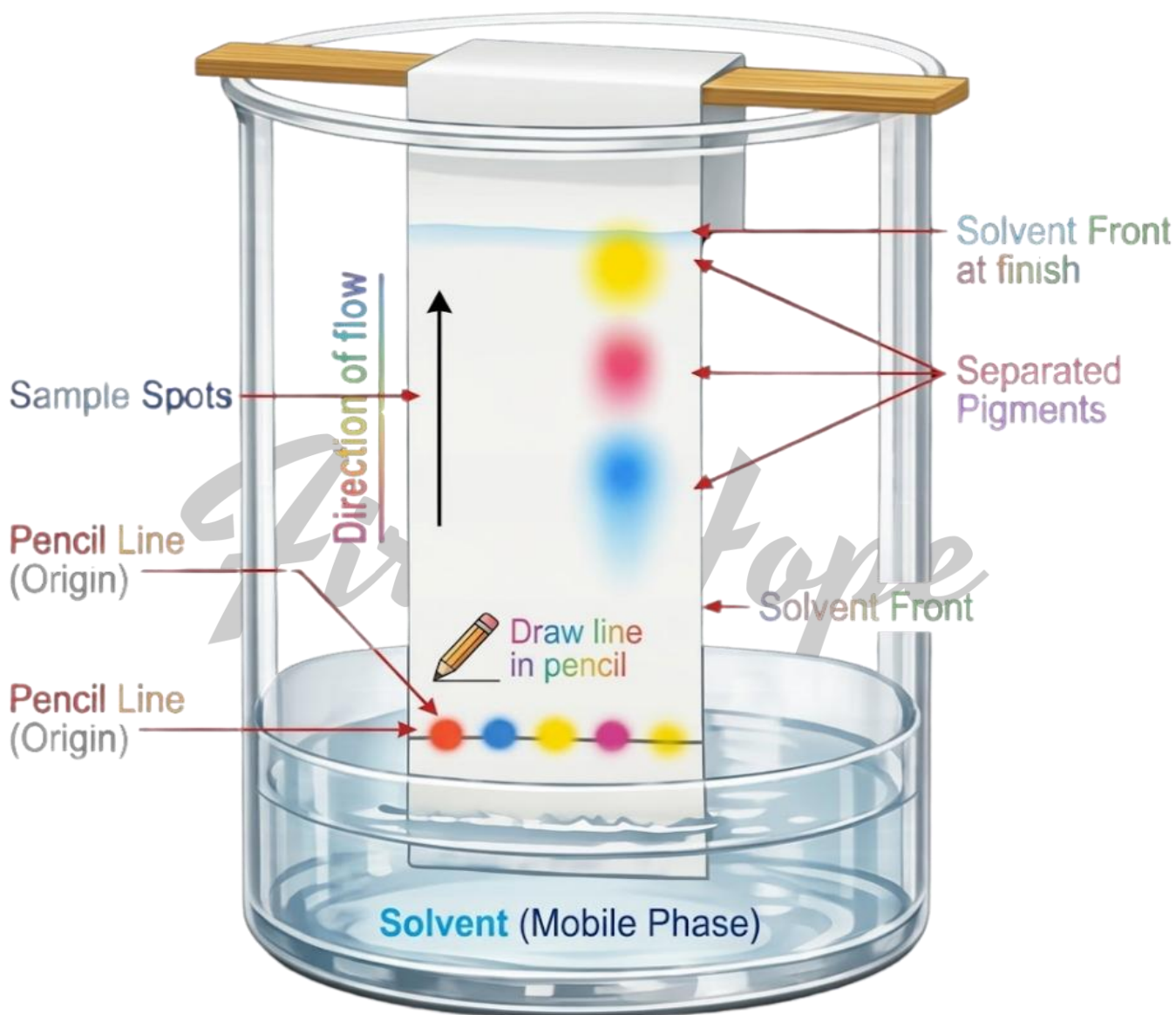


Chromatography

Paper Chromatography

Principle

- Paper chromatography works on the **partition principle**, which means a substance distributes itself between two different phases.



Phases involved:

- **Stationary phase:** Water molecules trapped inside the cellulose fibers of paper
- **Mobile phase:** A solvent that moves over the paper

Detailed mechanism:

- The paper contains tiny pores filled with water
- When the solvent moves, the sample components dissolve in it
- **Each component has:**

- Some attraction to water (stationary phase)
- Some attraction to solvent (mobile phase)

Separation occurs because:

- If a substance is more soluble in solvent → it travels further
- If a substance is more attracted to water → it stays behind

Important concept:

- This repeated process of dissolving and re-adsorbing causes separation

Partition coefficient:

$$K = \frac{\text{Concentration in stationary phase}}{\text{Concentration in mobile phase}}$$

- High K → more in paper → slow
- Low K → more in solvent → fast

Apparatus

Paper chromatography uses simple equipment but must be handled carefully.

1. Chromatography paper

- Made of pure cellulose
- Acts as stationary phase
- Must be uniform and clean

2. Developing chamber

- Airtight container (glass jar)
- Maintains solvent atmosphere

3. Solvent system

- Single solvent or mixture
- Choice depends on nature of sample

4. Capillary tube

- Used to apply very small amount of sample

5. Pencil

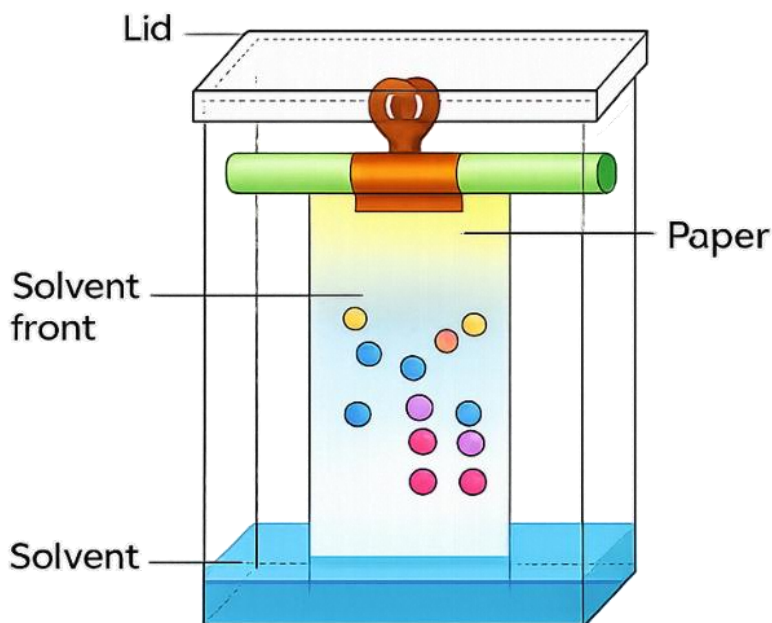
- Used to mark baseline (ink cannot be used)

6. Lid or cover

- Prevents evaporation and ensures saturation

Instrumentation (Setup and Working)

- Even though simple, correct procedure is important.



1. Preparation

- Draw a horizontal line (origin) near bottom of paper

2. Sample application

- Apply small, concentrated spot
- Dry the spot to prevent spreading

3. Placement in chamber

- Paper placed vertically in solvent
- Solvent level must be below the spot

4. Development

- Solvent rises due to capillary action
- Carries sample upward

5. Separation process

- Components move at different speeds
- From separate spots

6. Stopping

- Remove paper when solvent front reaches near top

7. Marking

- Mark solvent front immediately

8. Visualization

- Colored spots visible directly

➤ **Colorless spots detected using:**

- UV light
- Chemical reagents

Chromatographic Parameters

Important values used to analyze results.

Rf value (Retention factor)

$$R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

- Always between 0 and 1
- Used for identification

Example:

- Substance moves 4 cm
- Solvent moves 8 cm
→ $R_f = 4/8 = 0.5$

Properties:

- Same substance has same R_f under same conditions

Importance:

- Helps in identification of compounds

Factors Affecting Resolution

Resolution means how well two components are separated.

1. Solvent system

- Most important factor
- Wrong solvent → poor separation

2. Paper quality

- Thickness and uniformity matter

3. Sample size

- Large spot → spreading → poor separation

4. Temperature

- Affects solvent movement and solubility

5. Humidity

- Changes water content in paper

6. Development time

- Too short → incomplete separation
- Too long → diffusion of spots

7. Nature of substance

- Polarity affects movement

Applications

Paper chromatography is widely used for simple separations.

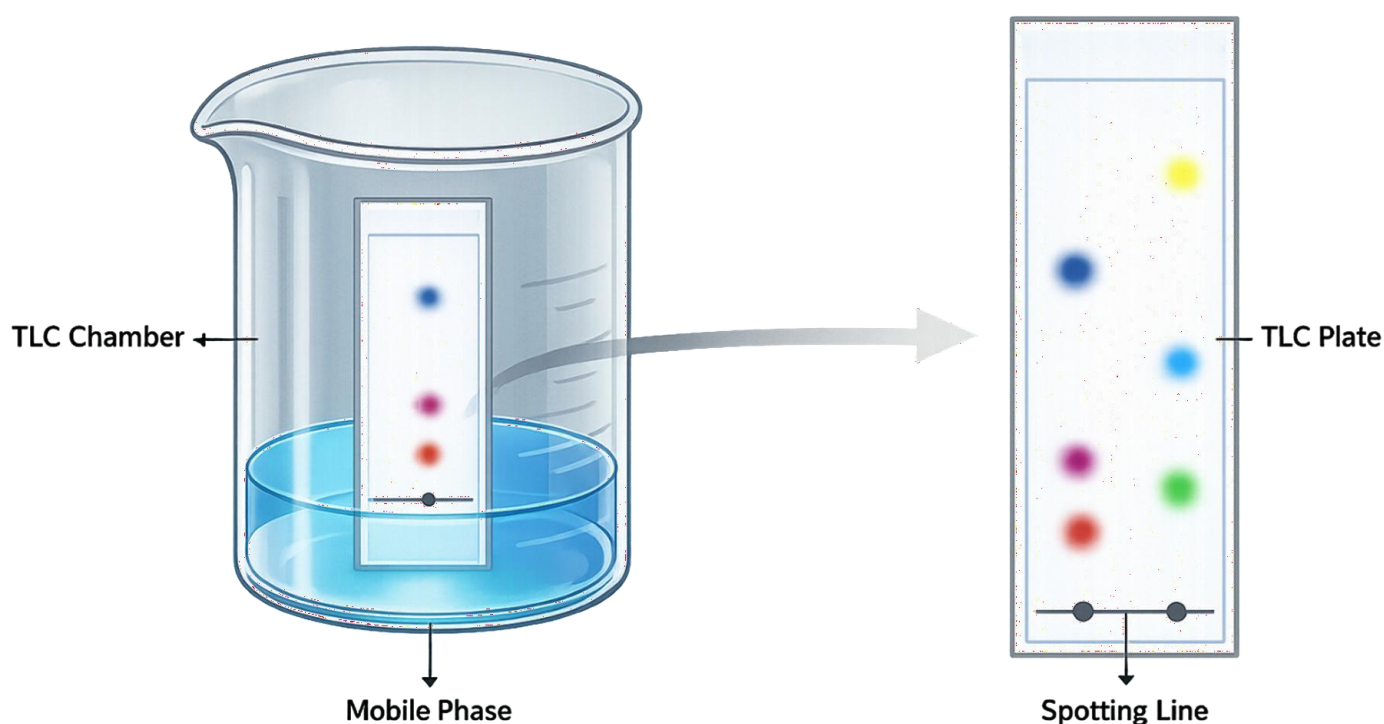
Uses:

- Separation of amino acids
- Identification of sugars
- Analysis of inks and dyes
- Plant pigments (chlorophyll, carotenoids)
- Drug analysis (basic level)
- Food analysis (additives, colors)

Thin Layer Chromatography (TLC)

Principle

- Thin Layer Chromatography is based mainly on the **adsorption principle**, although sometimes partition also plays a role.





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