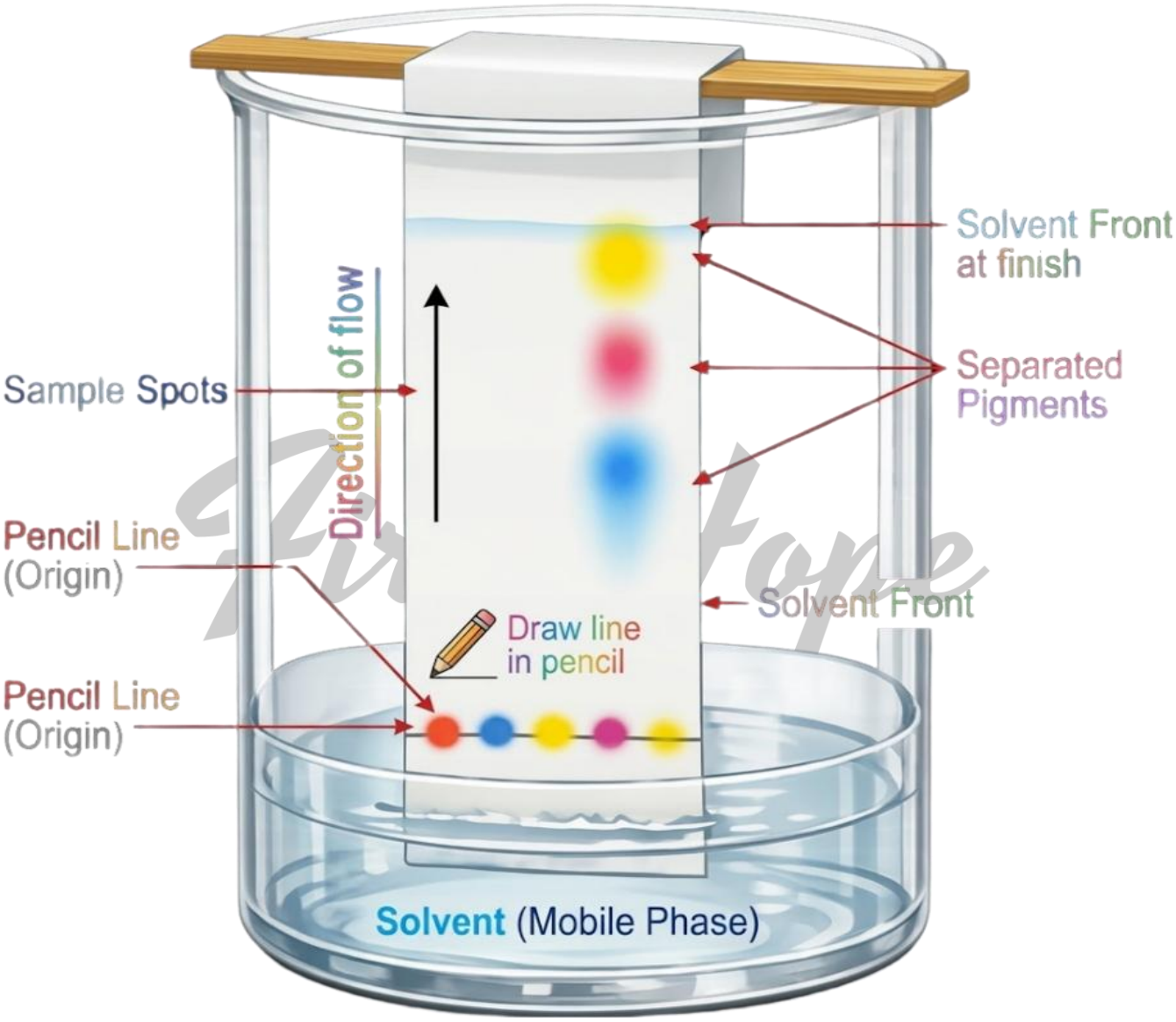
Chromatography

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:**

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- 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.

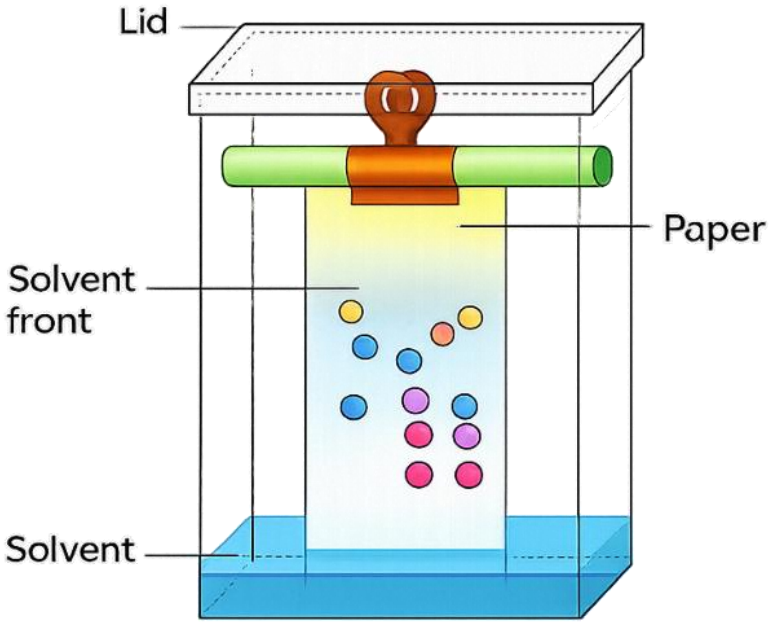
1. **Chromatography paper**
   - Made of pure cellulose
   - Acts as stationary phase
   - Must be uniform and clean
2. **Developing chamber**
   - Airtight container (glass jar)
   - Maintains solvent atmosphere
3. **Solvent system**
   - Single solvent or mixture
   - Choice depends on nature of sample
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)

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- 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

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- **Colorless spots detected using:**
  - UV light
  - Chemical reagents

Chromatographic Parameters

Important values used to analyze results.

Rf value (Retention factor)

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

- Always between 0 and 1
- Used for identification

Example:

- Substance moves 4 cm
- Solvent moves 8 cm
  → Rf = 4/8 = 0.5

Properties:

- Same substance has same Rf under same conditions

Importance:

- Helps in identification of compounds

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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

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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.

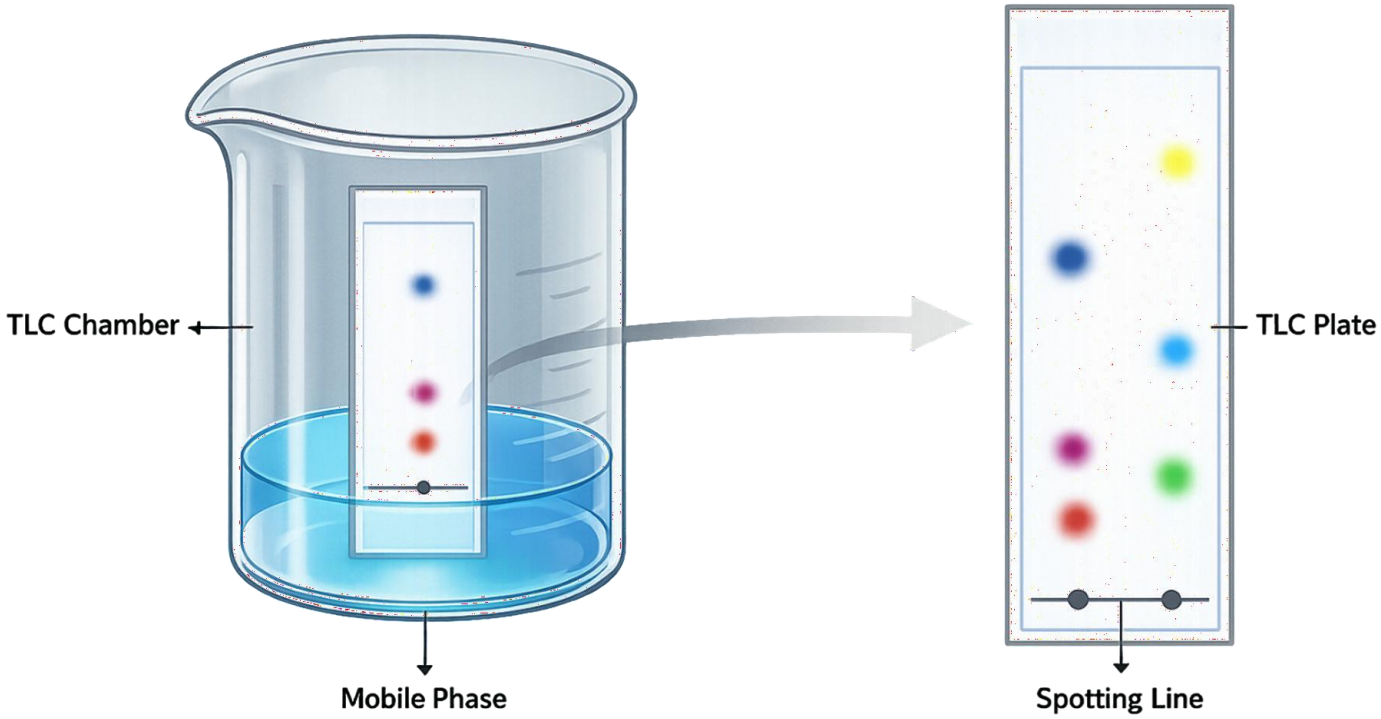
Uses:

- 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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