Paper Chromatography

For S.Y. B.Sc. Sem-IV

By Dr Vipul B. Kataria

Always keep in mind

- **It is partition chromatography**
- **The stationary phase is liquid**
- **The mobile phase is liquid**

Introduction

- The paper chromatography includes a specially designed filter paper on which solvent flows and the migration of different substance is observed.
- One of the two solvents is immiscible or partially miscible with other solvent.
- The separation is dependent upon differential migration of mixture of substances that occurs due to difference in partition co-efficient.
- The components of mixture to be separated migrate at different rates and appear as spot on filter paper.

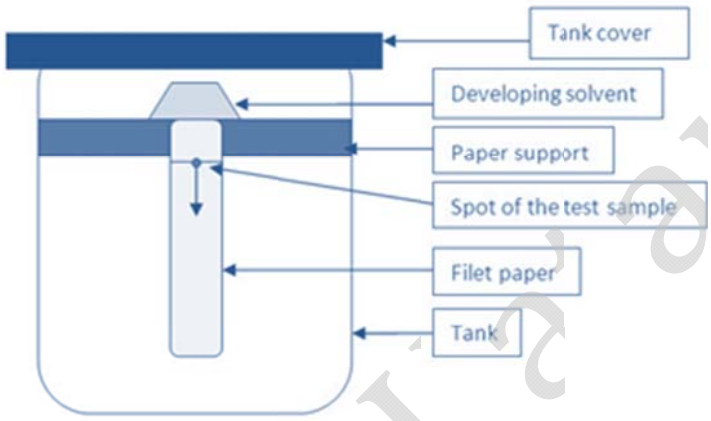
Migration Parameters

- $R_f = \frac{Distance\ travelled\ by\ solute\ from\ the\ origin\ line}{Distance\ travelled\ by\ solvent\ from\ origin\ line}$
- $R_x = \frac{Distance\ travelled\ by\ solute\ from\ the\ origin\ line}{Distance\ travelled\ by\ standard\ substance\ from\ origin\ line}$
- $R_M = \log[{}^{1}/_{R_f - 1}]$
- R is function of partition co-efficient.
- R is constant for a substance for a constant chromatography conditions (Paper, Temperature, duration and direction of development, humidity, size of vessel etc.)
- Rx is used when the solvent runs off the paper. In such cases movement of substance is denoted by Rx instead of Rf.
- R_M is additive term.

Types of Paper Chromatography

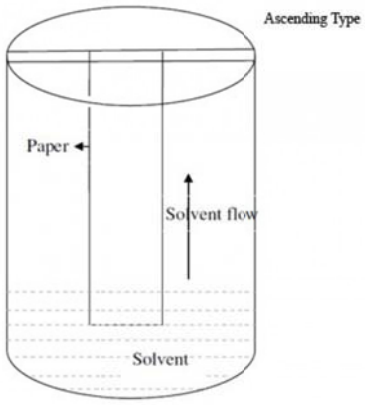
The paper chromatography can be classified into following techniques.

1. Descending Chromatography



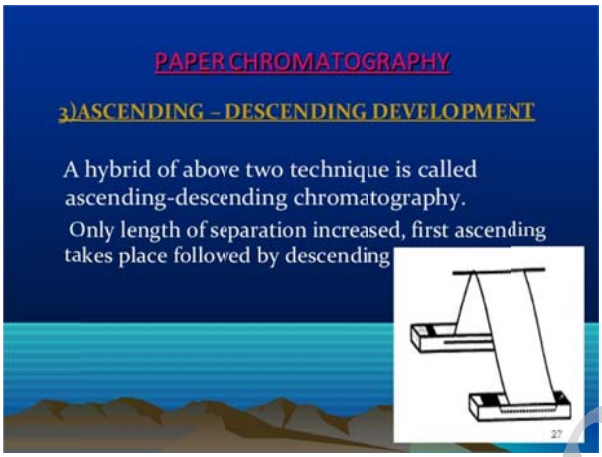
- In such chromatography technique, solvent travels down the filter paper.
- It is advantageous technique as it is continuous development technique.
- It is fast.

2. Ascending Chromatography



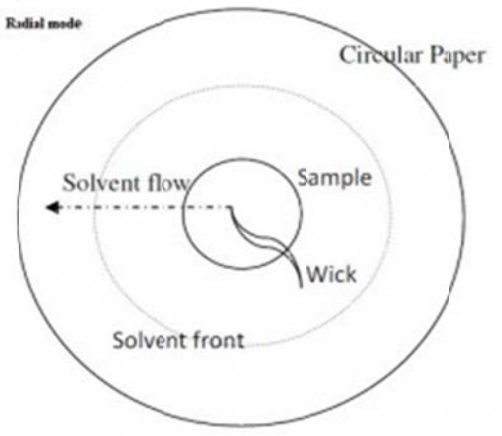
- In such chromatography technique solvent travels up the filter paper.
- It is routine technique and employed when Rf value is quite different for components of mixture.

3. Ascending-Descending Chromatography



- It is hybrid technique comprise of ascending as well as descending chromatography.
- First, solvent travels up the paper and at edge the paper is bended with the help of support (glass road) and from that point solvent travels down the filter paper.

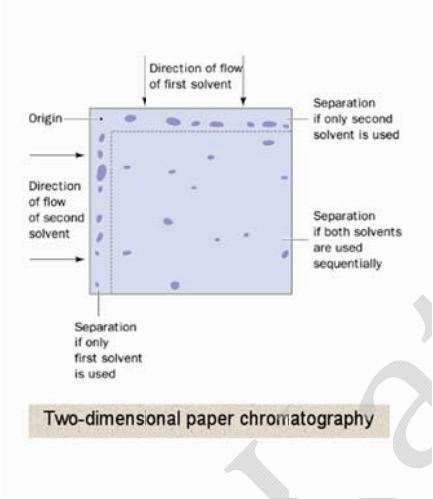
4. Radial Paper Chromatography



- It is also known as circular paper chromatography.
- In this technique, circular shape paper is used.
- The spots of mixture employed in circular shape.
- The solvent travels through paper via a wick dipped in solvent and attached with paper in the middle.

- The solvent travels horizontally.
- After sufficient travelling, it is allowed to dry and spot can be visualize by appropriate visualizing agent.

5. Two Dimensional Chromatography



- In such technique, square rectangular paper is used.
- The sample is employed in corner and allowed to run with solvent in both directions one by one.

Experimental details of paper chromatography

1. Choice of paper chromatographic technique

- The choice of technique depends upon nature of substance to be separated.
- The type of technique determines the efficiency and speediness of results.

2. Choice of filter paper

- It is dependent upon technique (either qualitative or quantitative)
- Nature of substance (hydrophilic or lipophilic)

3. Proper developing solvent

- The choice of developing solvent is dependent upon the Rf values of substance to be separated.
- A solvent or mixture of solvent, which gives Rf value 0.2 – 0.8 for sample should be selected.

4. Preparation of samples

- It is impossible to decide standard procedure of preparation of sample.
- The sample having trace amount of substance (10-20 Ng) can be identified easily.

5. Spotting

- A horizontal line is drawn on the paper by a pencil.
- The sample solution is spotted on that line (origin line) and allowed to dry.

6. Drying the chromatograms

- The wet chromatograms are allowed to dry in drying cabinet.

7. Visualization

- It can be done by either chemical or physical means.
- Chemical Detection: Various chemicals are used to visualize spots on colourless chromatogram. The visualizing agents are sprayed or the paper is dipped into them.
- Physical Detection: UV lamp is used to visualize spots.

Applications of Paper Chromatography

- It is widely used for qualitative and quantitative analysis of organic, inorganic and biochemical substances.
- It is used for separation of amino acids.
- It is also used for separation of sugars.