CHROMATOGRAPHY

- Chromatography is the technique of separation of mixtures in to individual component by using **stationary** and **mobile phase**

- The word chromatography has been derived from greek word **'Chroma'** meaning color and **'graphein'** meaning writing.

- Tswet, Russian botanist is credited for development of chromatography

Chromatography	Stationary phase used	Property used for separation
• **Paper chromatography**	• Water held on a solid support of filter paper (or Cellulose)	• Based on the polarity • Least Polar moves faster
• **Thin layer chromatography**	• Silica gel (Kieselguhr) spread on a glass plate or a plastic sheet or aluminium sheet.	• Based on Polarity • Least Polar moves faster

PAPER CHROMATOGRAPHY

- Paper chromatography is a partition chromatography.

- It is technique to resolve a mixture of substances by continuous distribution between the stationary and mobile phase.

- The mobile phase, which is the solvent, has tendency to carry the substance with it while the stationary phase, which is hydrated cellulose fibres have a tendency to retard the movement of substances relatively as a result of various interactive forces between it and the substance.

Reagents

1. Solvent

- N-Butanol,Glacial acetic acid and water are mixed in the ratio of 4:1:5

2. Visualizing Agent

- Ninhydrin solution

3. Standard Amino Acid solution and unknown sample

<u>Types of paper chromatography</u>

- 1. Descending paper chromatography

- 2. Ascending paper chromatography

- 3. Circular paper chromatography

Procedure

- A circular whatmann number 1 filter paper is taken and from the centre of circular paper a radius of 1 cm is drawn and application points are marked.

- A hole is made in the centre portion with the help of compass.

- Equal volume of standard solution along with sample is applied on the marked spot using capillary tube

- Two petri plate of equal circumference are taken so that each plate fit over one another

- 3/4th of one of the petri plate is filled with the solvent

- Now circular paper is placed on the petri plate such that wick dips within the solvent.

- The other petri plate is used to cover, without disturbing the paper.

- The run is continued till the solvent reaches the rim of the petridish.

- Solvent front is marked and paper is removed from the solvent and dried

- Spray the paper with ninhydrin solution and dry it with the help of hair drier.

- Calculate the retardation factor value
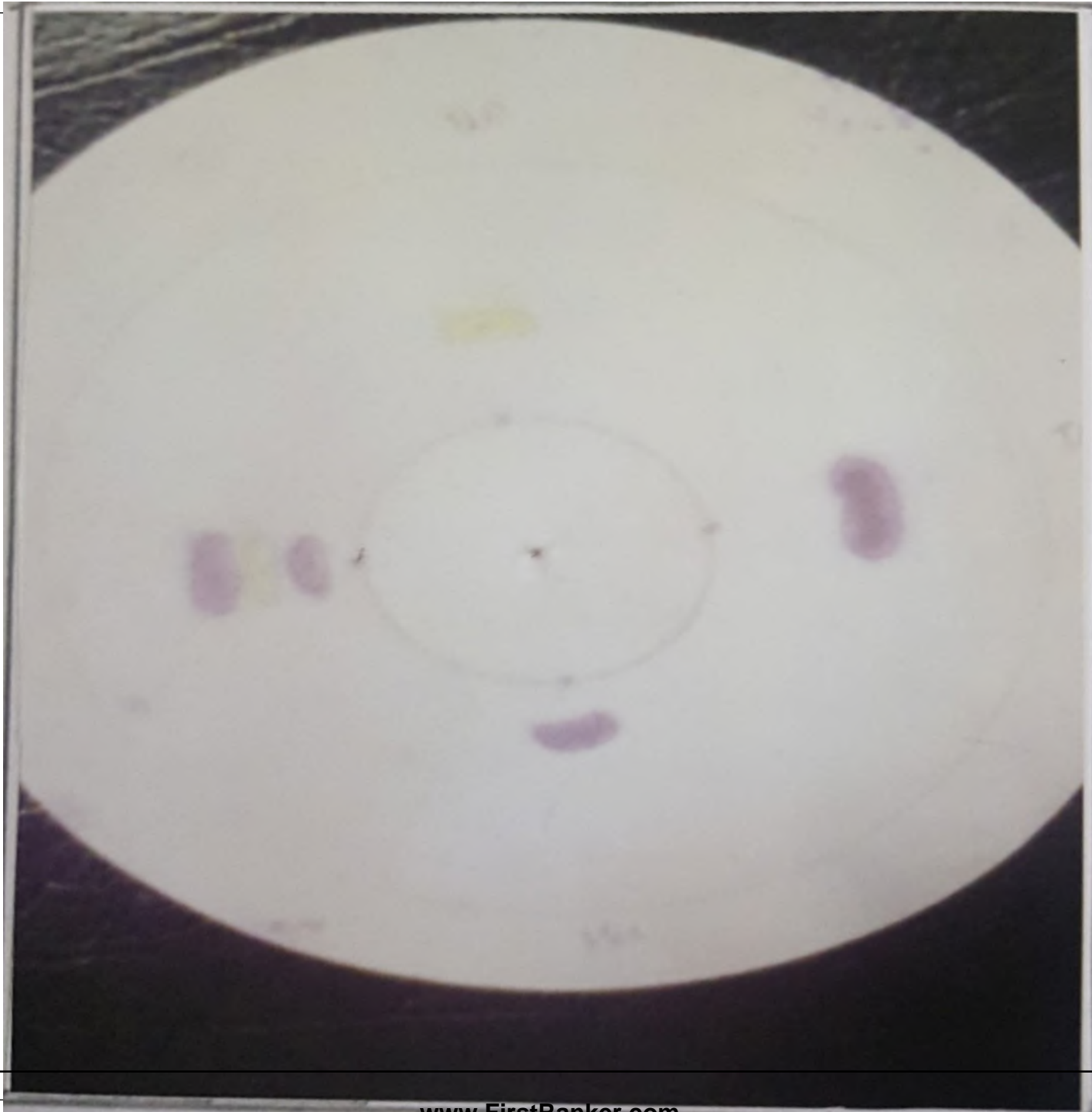
Amino acid	Distance travelled by solute from origin (cm)	Distance travelled by Solvent from origin (cm)	Rf
Proline	3.2	7.6	0.42
Tyrosine	3.4	7.6	0.44
Histidine	2.4	7.6	0.3L
Unknown I	3.4	7.6	0.44
Unknown II	2.4	7.6	0.3L

CLINICAL APPLICATIONS

- Screening of inborn errors of amino acid metabolism like **phenylketonuria, maple syrup urine disease**.

- To analyze different compounds in drugs.

- It is also used in testing of antibiotics