INTRODUCTION

- TLC is one of the **simplest, fastest, easiest**, **and least expensive** of several chromatographic techniques used in qualitative analysis to separate organic compounds and to test the purity of compounds.

TLC is a form of liquid chromatography consisting of :

- **A mobile phase** (N-Butanol, Glacial acetic acid and water)

- **A stationary phase** (a plate or strip coated with silica gel)

Principle of TLC

- It is mainly based on the principle of partition chromatography.
- The components with more affinity towards stationary phase travels at slower pace compare to those with less affinity towards stationary phase.

Reagents

- 1. A plate or a strip coated with silica gel

2. Solvent

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

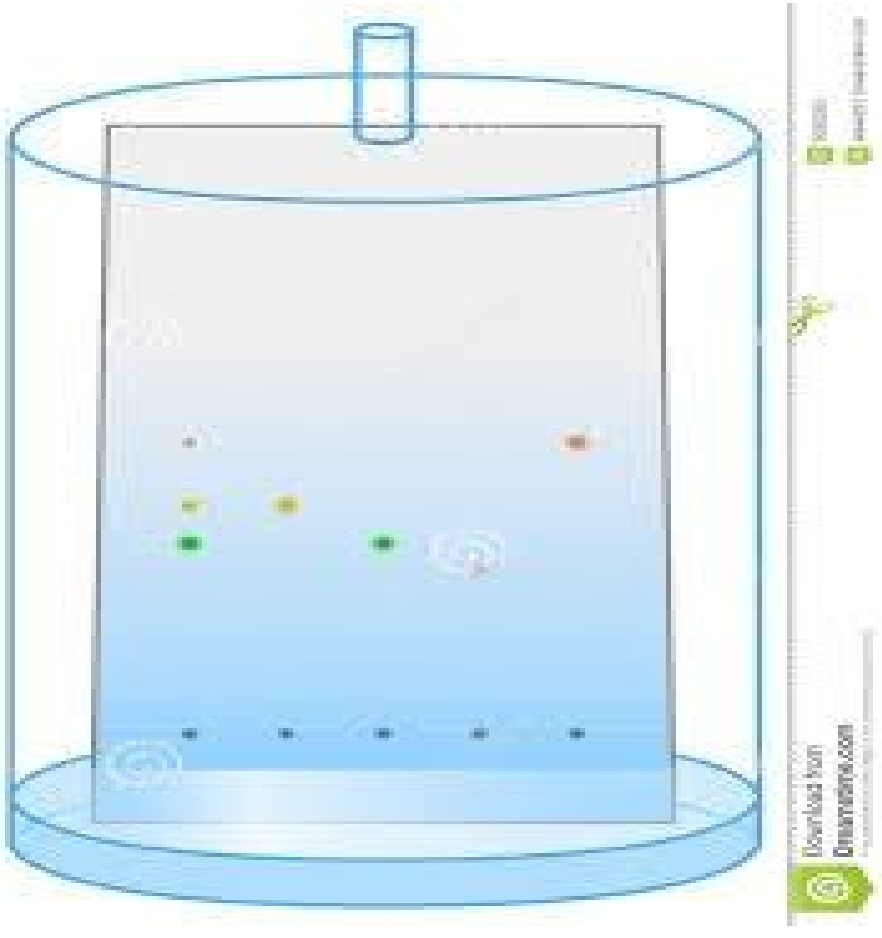
3. Visualizing agent

- 0.2% Ninhydrin

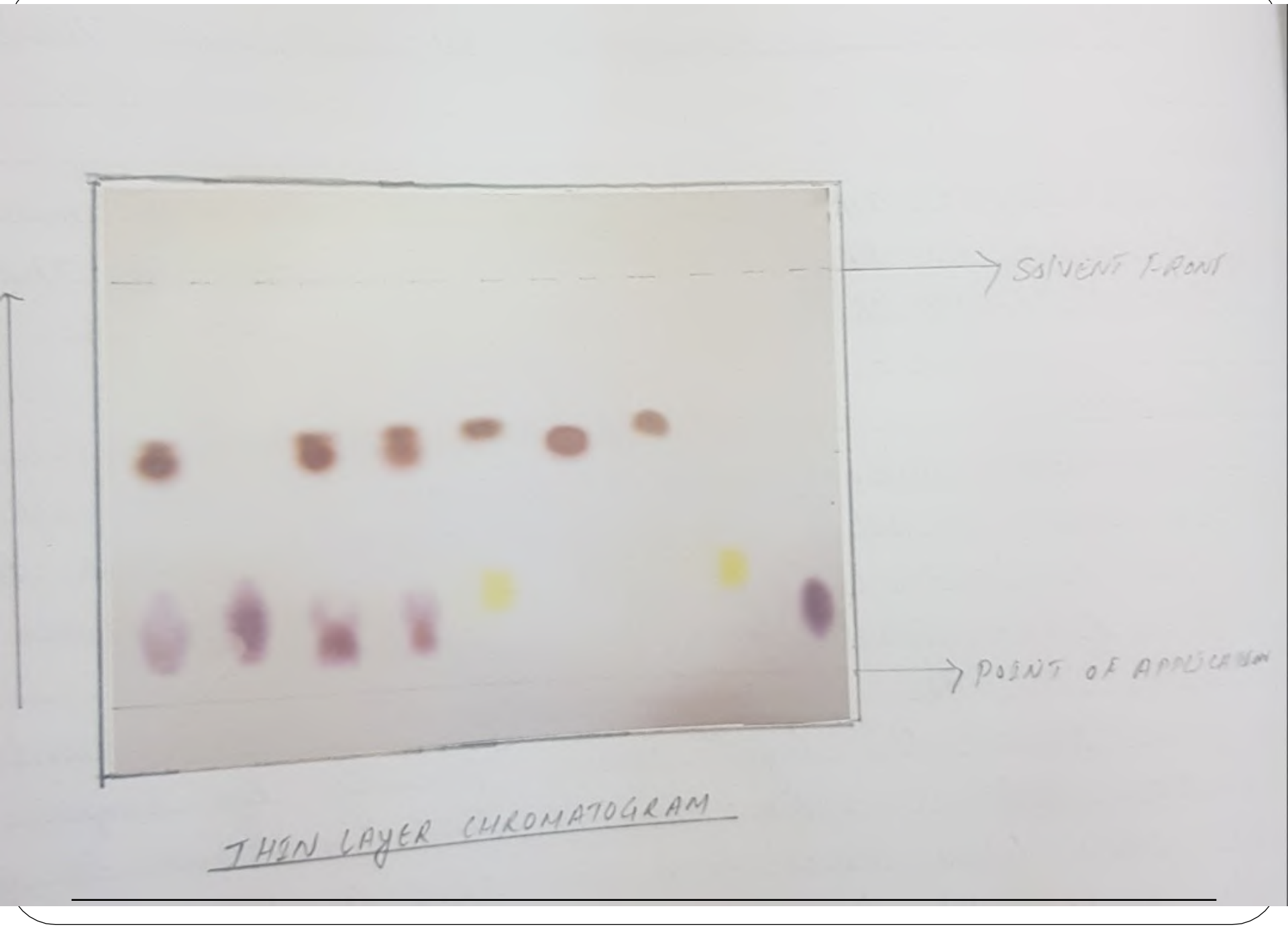
- 4. Standard amino acids and sample containing unknown amino acid

Procedure

- The mobile phase is added to TLC tank so that it is about 5mm in height.
- The lid of the chamber is closed for half an hour for saturation
- Use a soft pencil to mark the origin line 2cm from the bottom of silica gel coated strip.
- Spot is marked at least with 1 cm distance from each other
- Add 5µl of standard amino acid solutions and sample on the marked spot.
- Dry the spot with hair drier



- Place the silica gel coated strip in to TLC tank filled with solvent
- Close the TLC tank lid and the solvent is allowed to ascend
- The run is continued till the solvent reaches 2/3rd of the strip which approximately takes 1 hour
- The silica gel coated strip is removed and solvent front is marked.
- Dry the wet silica gel coated strip with hair dryer
- Spray ninhydrin solution carefully to identify the spot
- Calculate the Rf value



THIN LAYER CHROMATOGRAM

Chromatography (TLC)

Amino acid	Distance travelled by solute from origin (cm)	Distance travelled by solvent from origin (cm)	R_f
Proline	3.9	12.9	0.30
Histidine	1.9	12.9	0.14
Cryptophan	8.2	12.9	0.63
Unknown (i)	8.2	12.9	0.63
ii)	1.9	12.9	0.14

Clinical Application of TLC

- Screening of inborn errors of metabolism like Phenylketonuria, Maple syrup urine disease, Cystinuria etc.
- Identification, purity testing and determination of active ingredients in drugs.
- Determination of pesticides and fungicides in drinking water