THIN LAYER CHROMATOGRAPHY

INTRODUCTION

- **Thin-layer chromatography** (**TLC**) is a chromatography technique used to separate non-volatile mixtures.
- **principle** of separation depends on the relative affinity of compounds towards stationary and the mobile phase.

- A polar solvent will carry a polar compound farther while a non-polar solvent will carry a non-polar compound farther.

Thin layer chromatography can be used to:

- Identify compounds present in a given substance.
- Determine the purity of a substance.

-A plate of TLC can be made from aluminium or glass which is coated by a solid matter as a stationary phase.

- The coated material has 0.1-0.3mm in thickness

-some of them has been added by fluorescent indicator that will make it florescence during the UV light exposure.

STATIONARY PHASE

- Silica is commonly used as stationary phase
- The separation of sample mixture will be depent on the polarity of sample.

- Some modified silica is also used in certain purposes.
- EXAMPLES; Silicagel G , Cellulose.

MOBILE PHASE

- The ability of mobile phase to move up is depent on the polarity itself.
- Volatile organic solvents is preferably used as as mobile phase.
- Examples ; Chloroform , methanol.

MATERIALS

- TLC plate
- 'Developing container'
  - chamber/ jar/ glass beaker
- Pencil
- Ruler
- Capillary pipe
- Solvents / mobile phase
  - organic solvents
- UV lamp

METHOD OF PREPARATION

1.Developing Container Preparation

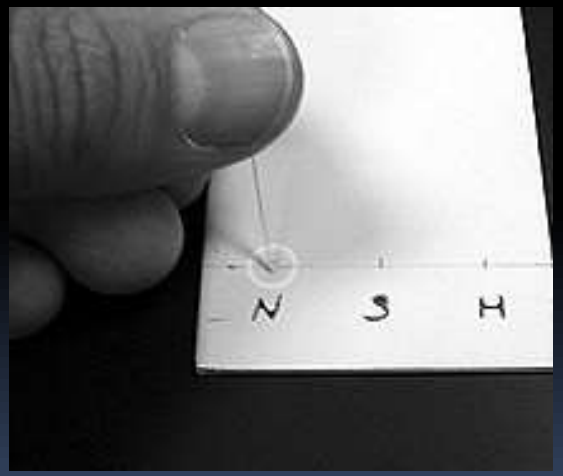
- Solvent is transferred into the container with 0.5-1cm in dept from the bottom

2. TLC Plate Preparation

- Commercially obtained with 5cm x 20cm in size
- Prepare your size when necessary
- Line 1 cm from the bottom with a pencil as a part should be spotted.

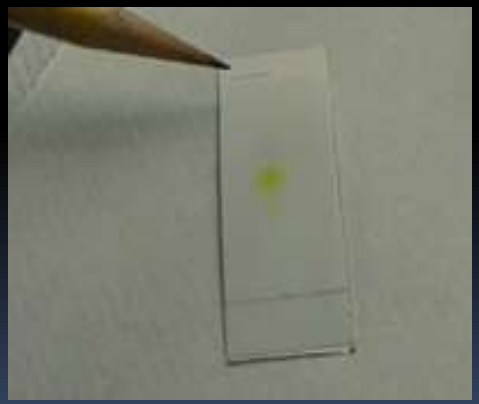
3.Spotting' TLC plates

- Make sure that your sample is liquified already.
- stick it using capillary pipe & spot onto the line you have made.



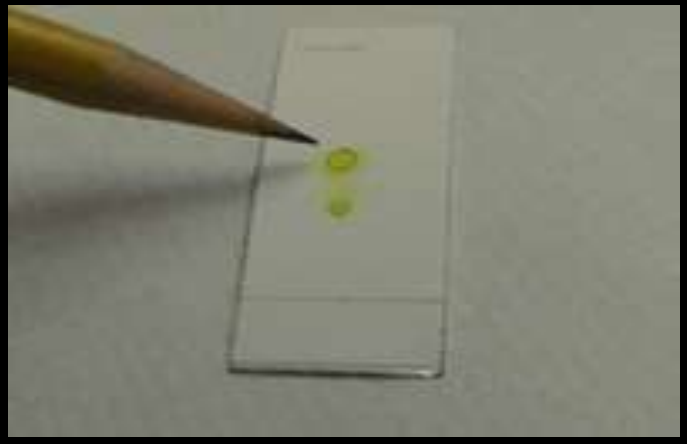
4.'Develop the plate'

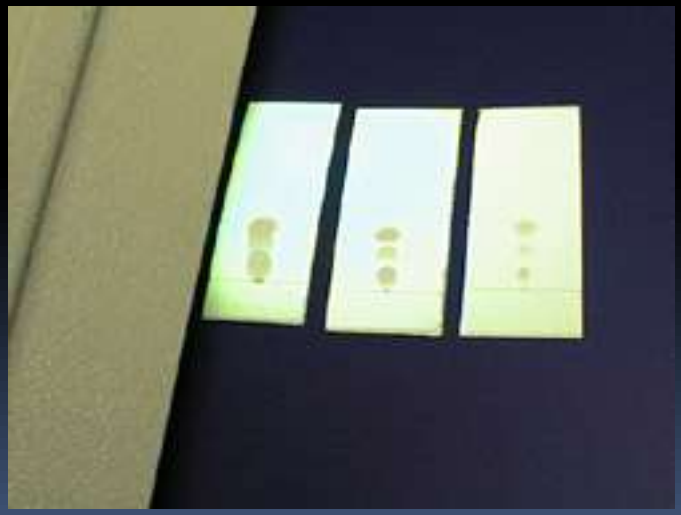
- after spotting, put the plate inside the chamber in the ascendant position
- Make sure that the depth of solvent doesn't touch the spots.
- Let it develop up to the 1cm from the top of plate.
- After that, pull out the plate from the chamber and let the solvent be vaporized.



5. Detection of spots

- The color samples are easy to be seen and no need to use UV lamp to detect them





6. DETECTION OF SPOT

1) Ninhydrin :

   - spesific identification of amino acid compounds.

   - Ninhydrin solution will show a purple spot when it is sprayed to the amino acid spot.

2) $KMnO_4$:

   used to identify a reducing agent such as glucose, fructose, vitamin C and others.

- R_f value is the ratio of the distance the spot travels from the origin to the distance the solvent travels.

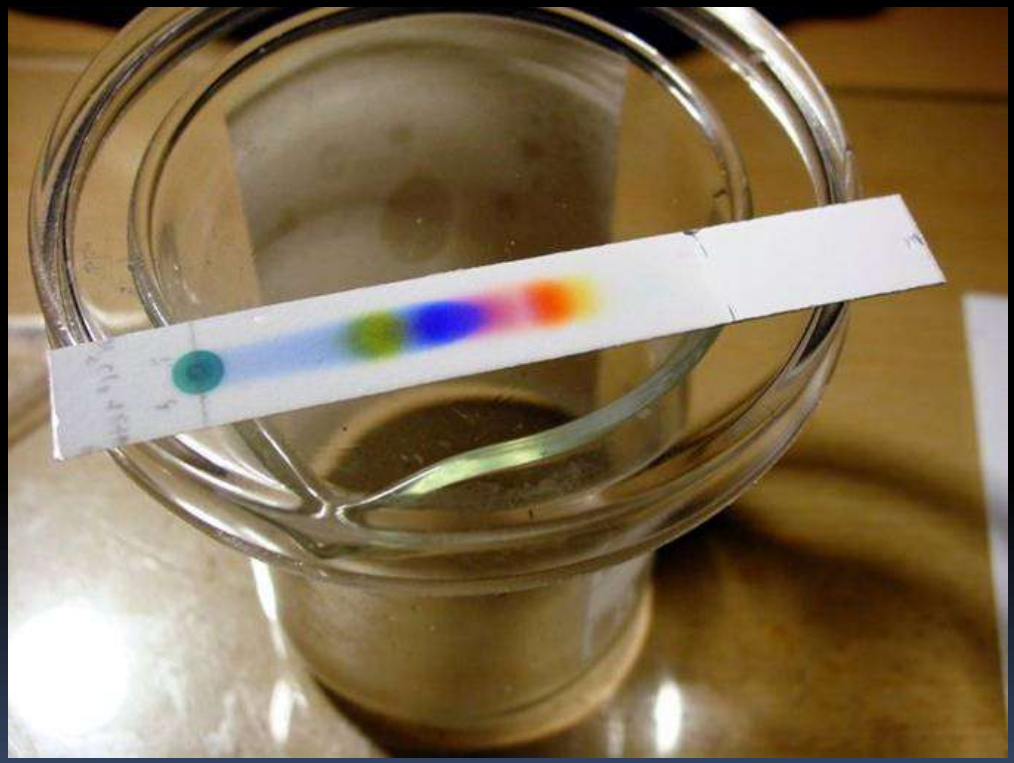
$$R_f = \frac{\text{Distance from centre of solute spot (cm) to the baseline}}{\text{Distance from solvent front to baseline (cm)}}$$

Advantages

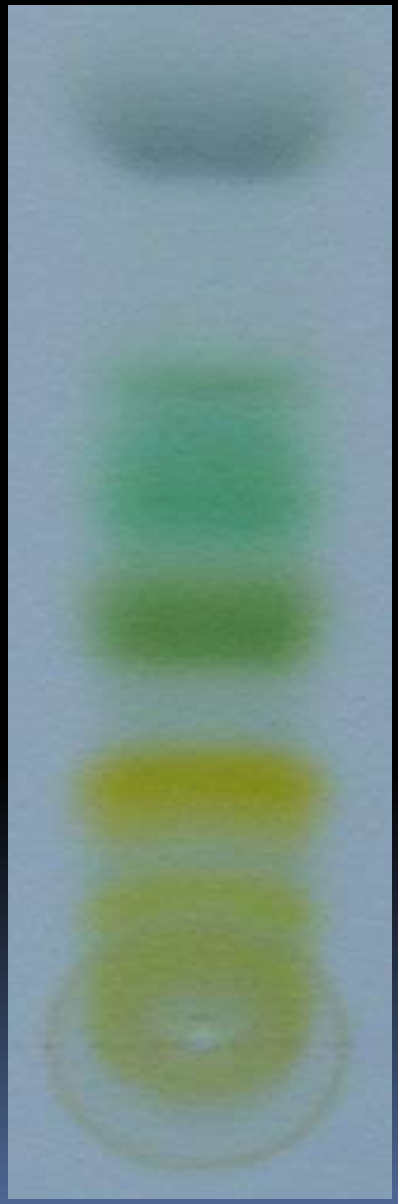
- Cheap
- Simple
- The developing can be monitored visually
- Able to use various chemical as a detector

Examples on TLC separations

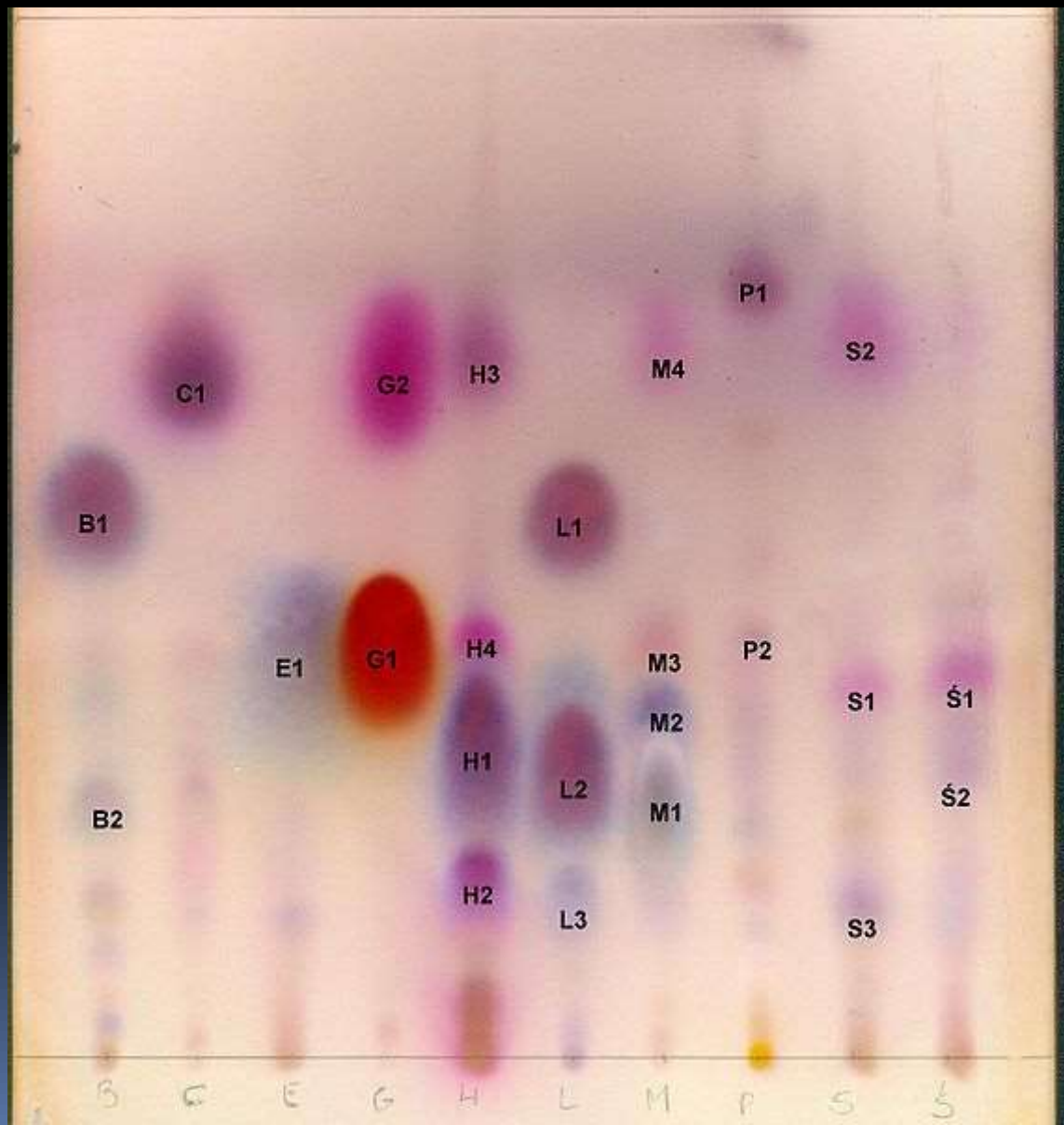
1 .Separation of black ink on a TLC



2. The chromatography of an extract of green leaves (for example spinach)



3. The Chromatogram of 10 essential oils colored with vanillin reagent.



- THANK YOU