THIN LAYER CHROMATOGRAPHY

Dr. R.T. Patil
Department of Botany
Gopal Krishna Gokhale College, Kolhapur

Learning Objective

1. State the definition of TLC
2. Explain the phases used in TLC
3. List the materials & methods used in TLC
4. List the application of TLC
5. List the advanteges & disadavantages of TLC

Thin Layer Chromatography

- **What is TLC?...**

  One of analysis method that is used to identify the unkonwn compounds and to determine the purity of mixture.

- This method is simple, rapid and cheap
- Widely used in pharmaceutical & food stuff industry.

-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 depend on the polarity of sample.

- Some modified silica is also used in certain purposes.

Stationery phase	Description	Application
Silica gel G	Silica gel with average particle size 15µm containing ca 13% calcium sulfate binding agent	Used in wide range pharmacopoeial test
Silica gel G_{254}	Silica gel G with fluorescence added	Same application with Silica gel G where visualization is to be carried out under UV light.
Cellulose	Cellulose powder of less than 30µm particle size.	Identification of tetracyclines

MOBILE PHASE

- The ability of mobile phase to move up is depend on the polarity itself
- Volatile organic solvents is preferably used as mobile phase.

MOBILE PHASE

SOLVENT	POLARITY INDEX
Heksana	0
Butanol	3.9
Chloroform	4.1
Methanol	5.1
Ethanol	5.1
Acetonitrile	5.8
Air	9.0

MATERIALS

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

METHOD

1. Developing Container Preparation

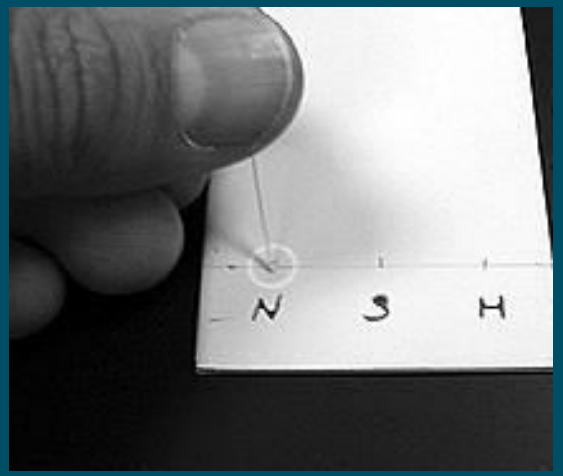
Solvent is transferred into the container with 0.5-1cm in depth from the bottom

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.

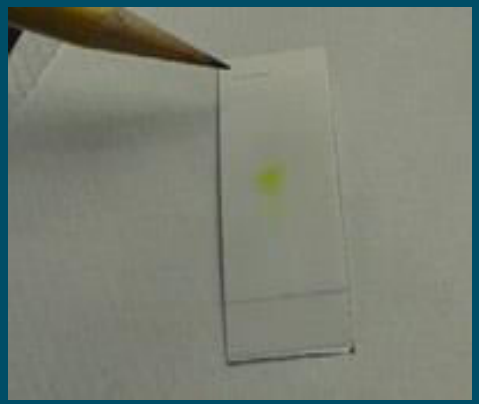
3.Spotting' TLC plates

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



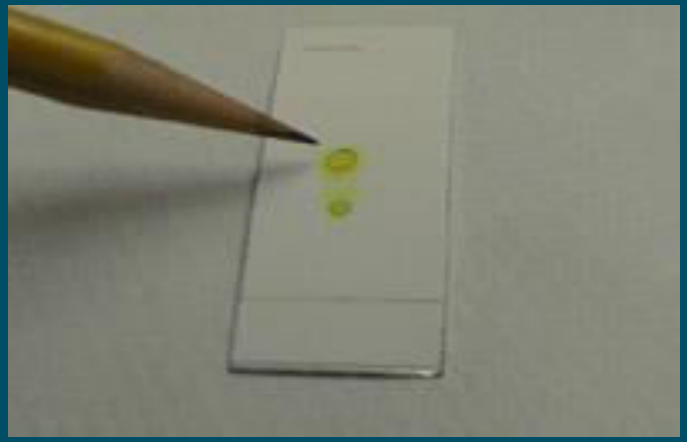
4.'Develop the plate'

- 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) Iodination-put the plate in which the spots face to the iodine crystal and see what is the spot color changing
2) Ninhydrin:

   -specific identification of amino acid compounds.

   - Ninhydrin solution will show a purple spot when it is sprayed to the amino acid spot.
3) $KMnO_4$

   used to identify a reducing agent such as glucose, fructose, vitamin C and others.
4) *Alkaline tetrazolium blue*

   specifically used for corticosteroid identification

The use of Rf as separation parameter

- The distance taken through by the solvent to move up will be assigned as solvent front
- The distance taken trough by the sample to move up will be assign as sample front
- Rf value is obtained by dividing the sample front toward solvent front

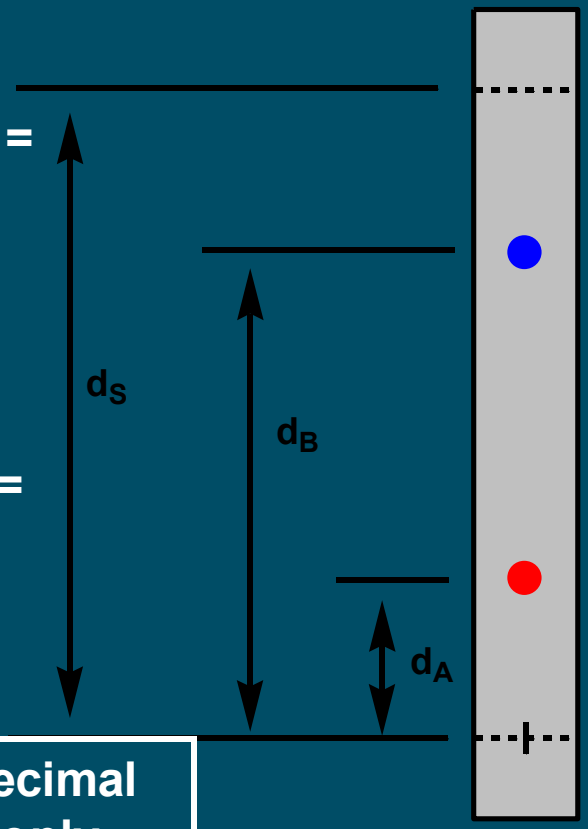
$$R_f = \frac{\text{sample front}}{\text{solvent front}}$$

-

Thin-Layer Chromatography: Determination of R_f Values

R_f of component A = $\frac{d_A}{d_S}$

R_f of component B = $\frac{d_B}{d_S}$



Less polar!

More polar!

The R_f value is a decimal fraction, generally only reported to two decimal places

7. Quantitative determination of known sample

- Done by scratching the spot using spatula, and extract the compound using the suitable solvent
- The liquid extract can be determined its content using other method such as spectroscopy.

Problems commonly occur in TLC and how to solve

a. The spot shape is too broad

- Diameter is supposed to be < 1-2mm

b. The movement of solvent

- should be straight up
- unproportionality in stationary phase surface will inhibit the movement of solvent

c. streaking formation

- caused by too concentrated sample

TLC Compared to Paper Chromatography

1. Precise and effective
2. More stable toward various organic solvents

Advantages

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

REFERENCES

1. R.A.Day, Jr. A.L.Undewood(1987). Analisis Kualititatif. Edisi ke Empat, ms: 474-529.
2. David G. Watson(2005). Pharmaceutical analysis. Edisi ke-2, ms 315-331
3. http//orgchem.colorado.edu/hndbooksuppor t/TLC/TLCprocedure.html

Thank You