

THIN LAYER CHROMATOGRAPHY



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Learning Objective



- 1. State the definition of TLC**
- 2. Principle of TLC**
- 3. Explain the phases used in TLC**
- 4. List the materials & methods used in TLC**
- 5. List the application of TLC**
- 6. List the advantages & disadvantages of TLC**

Thin Layer Chromatography

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 fluorescence during the UV light exposure.

PRINCIPLE OF TLC



The principle of separation is adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through due to capillary action (which occurs against gravitational force).

The components are moving according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase **travels slower. The component with lesser affinity towards the stationary phase **travels faster**.**

Thus the components are separated on the TLC plate based on the affinity of the components towards the stationary phase.

STATIONARY PHASE

- Silica is commonly used as stationary phase
- The separation of sample mixture will be dependent on the polarity of sample.
- Some modified silica is also used in certain purposes.



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

MOBILE PHASE

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

MOBILE PHASE



SOLVENT	POLARITY INDEX
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 dept from the bottom



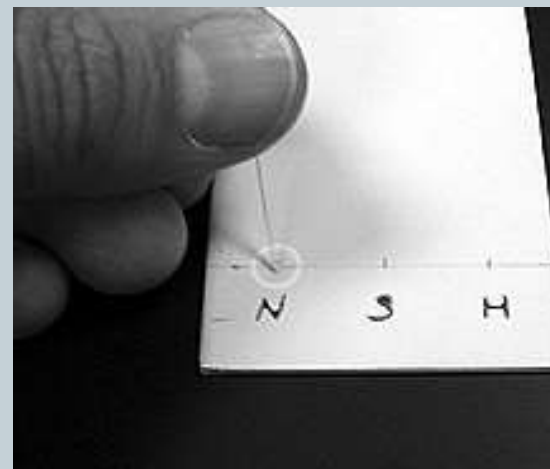
2. TLC Plate Preparation

- **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.**



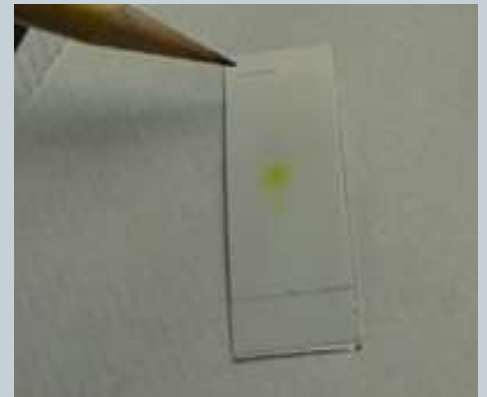
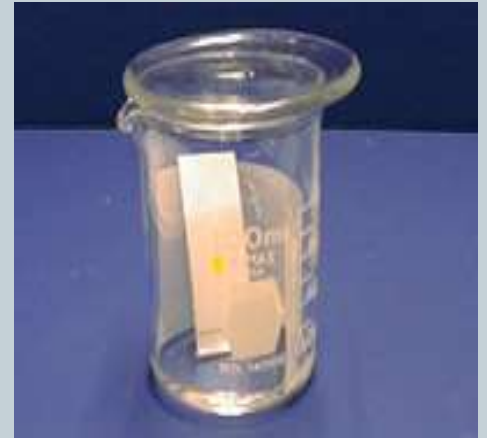
3. Spotting' TLC plates

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



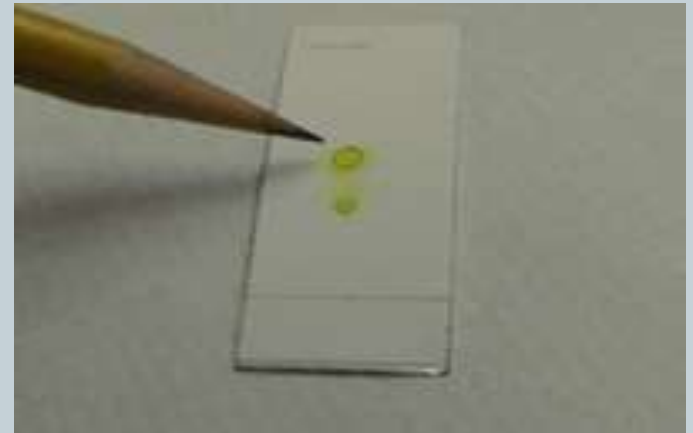
4. 'Develop the plate'

- after spotting, put the plate inside the chamber in the ascendant position
- Make sure that the dept 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 crystall 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) **KMnO₄**
 - used to identify a reducing agent such as glucose, fructose, vitamin C and others.
- 4) ***Alkaline tetrazolium blue***
 - specifically used for corticosteroid identification

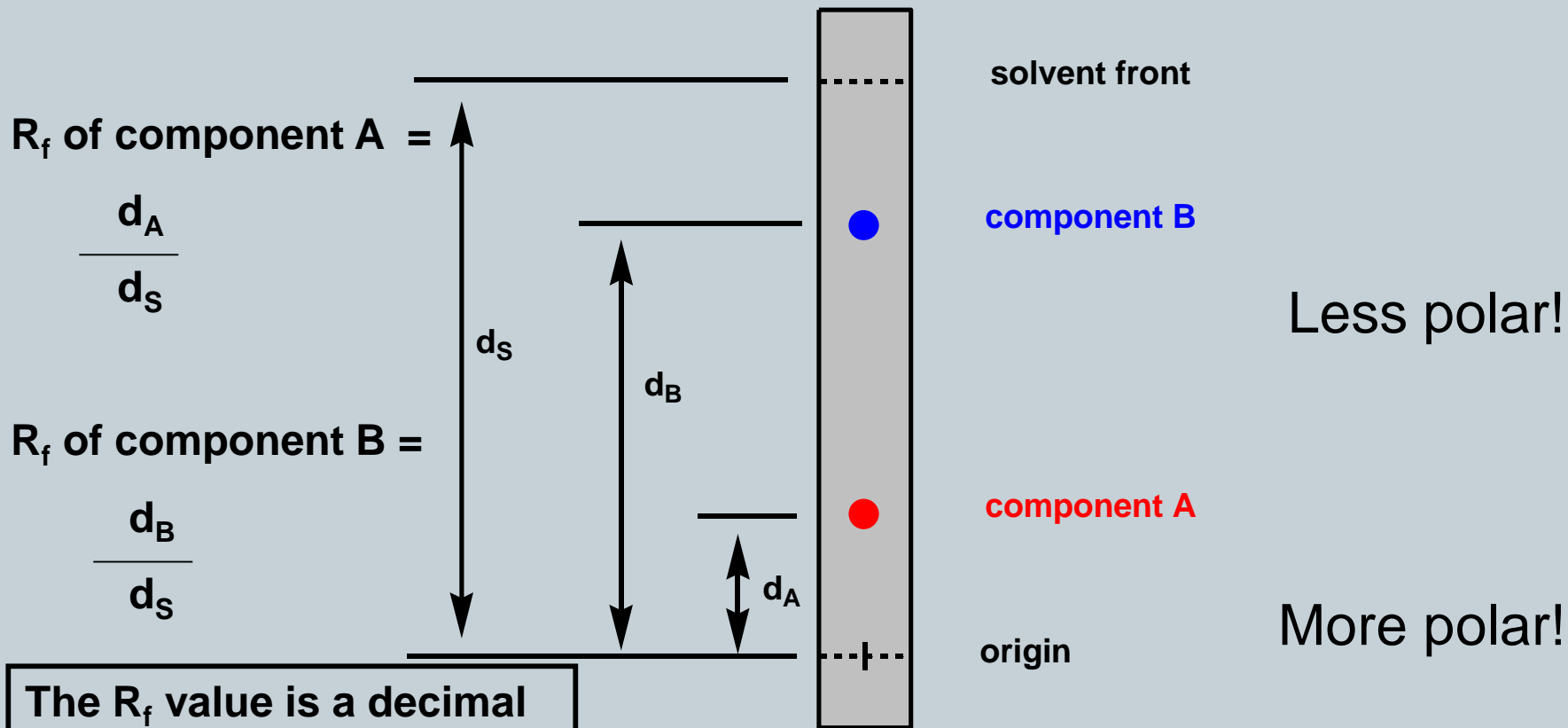
The use of R_f as separation parameter

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

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

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Thin-Layer Chromatography: Determination of R_f Values



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



- 1. -Quantitative determination of known sample**
- 2. Done by scratching the spot using spatula, and extract the compound using the suitable solvent**
- 3. 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 **< 1-2 mm**

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 and its applications

- 1. Precise and effective**
- 2. More stable toward various organic solvents**
- 3. Examination of reactions and also useful for separational and purification process like distillation, molecular distillation.**
- 4. Biochemical Analysis like from body fluids like blood plasma, serum, urine e.t.c.**
- 5. Separation of multicomponent pharma formulations.**

Advantages

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

Identification unknown drugs using standard Reference

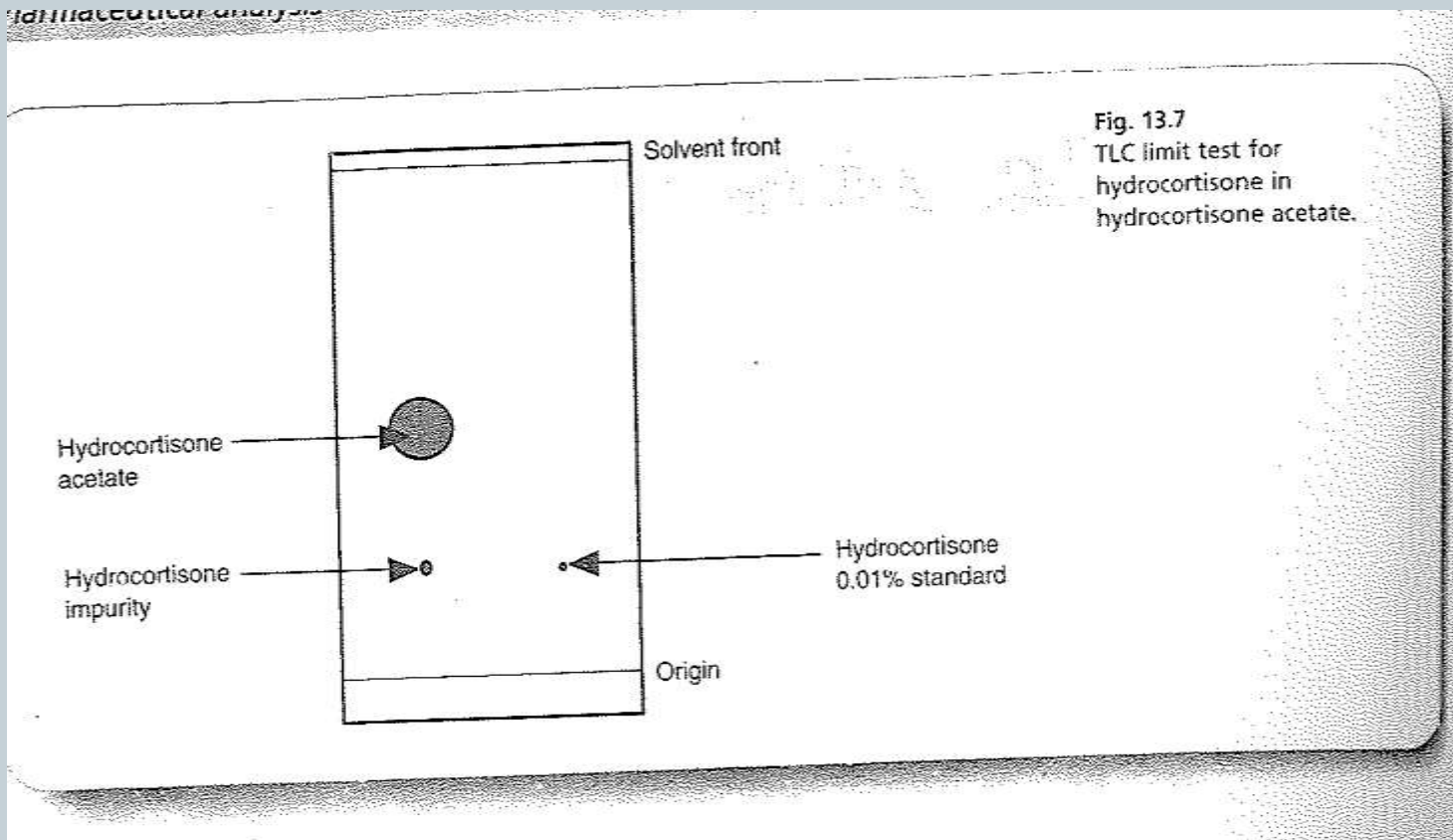


Fig. 13.7
TLC limit test for
hydrocortisone in
hydrocortisone acetate.



- **Also useful in food and cosmetic industries.**
- **Analysis various medicines like hypnotics, sedatives, anti histaminics, anti convulsant etc.**



THANK YOU