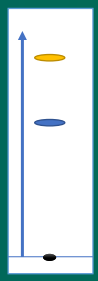
Thin Layer Chromatography



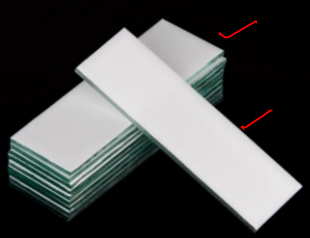
- ✓Basic Introduction
- ✓Principle
- ✓Procedure
- ✓Application
- ✓Retardation/Retention Factor

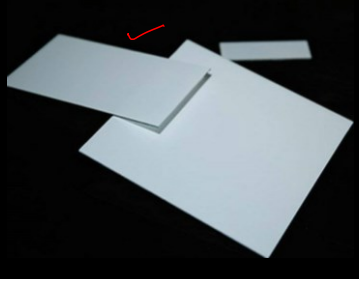
Chromatography
Instrumental Analysis

THIN LAYER CHROMATOGRAPHY

Introduction:

- Thin Layer Chromatography is a technique used to isolate non-volatile mixtures of different compounds by using a thin layer of adsorbent (stationary phase) coated in either glass plate (Slides), aluminum sheet, or plastics
- In 1938, Izrnailov and Shraiber separated the plant extract using 2 mm thick and film layer of alumina glass plate





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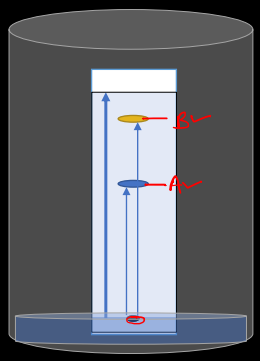
Principle:



- Adsorption Chromatography involves the separation of a chemical mixture in the TLC
- When the mobile phase (liquid) run over the TLC plate (coated with adsorbent; Stationary phase; Solid) by the capillary action, the analyte mixture is separated out due to adsorption phenomenon and give the different Rf (Retardation factor) values for each component present on the analyte sample

Rf = Distance travel by solute (spot)/ Distance travel by Solvent

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Types & Techniques:

SN	Technique	Stationary Phase	Mobile Phase
1	Adsorption	Silica gel, Alumina, Charcoal	Non-Polar/Polar solvent
2	Partition	Cellulose, Silica Gel	Mixed aqueous, organic solvent
3	Reverse Phase	ODS silica gel, coated silica, acetylated cellulose	Mixed aqueous, polar solvent
4	Ion Exchange	Ion exchange resins, CM cellulose	Buffered aqueous solution
5	Size Exclusion	Dextran gels	Buffered aqueous solution

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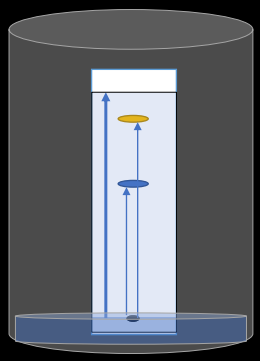
Stationary Phase:



- **Silica Gel:**
- it is a an amorphous and porous form of silicon dioxide (SiO_2) and acidic in nature
- most commonly used in the TLC
- Average particle size- 15 um
- Silica Gel G (50% slurry) mainly used in TLC contains- SiO_2 + $CaSO_4$ or Gypsum (10%)
- Silica Gel GF254 (50% slurry) is more useful in TLC, it contains SiO_2 + $CaSO_4$ or Gypsum + $Zn_4Si_2O_7(OH)_2$ or Zinc Silicate
- Gypsum is used as a binder and Zinc silicate used as a fluorescent material.

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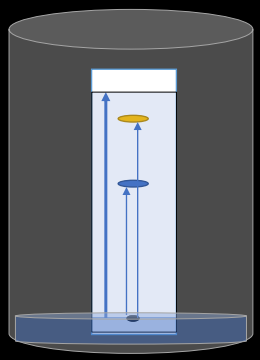
Stationary Phase:



- **Alumina:**
- Alumina (Al_2O_3) is second most widely used in the TLC
- It is prepared from $Al(OH)_3$ by calcination process at 500 C
- Avg particle size- 12 um
- Unlike the Silica Gel G binder is not required
- Basis of nature there are three types of alumina- acidic, basic, and neutral
- **Diatomaceous Earth (Kieselguhr):**
- Naturally occurring, soft, siliceous sedimentary rock,
- Composition- 80-90% Silica, 2-4% Alumina, and 0.5-2% Iron oxide
- Particle size range- 10-200 um

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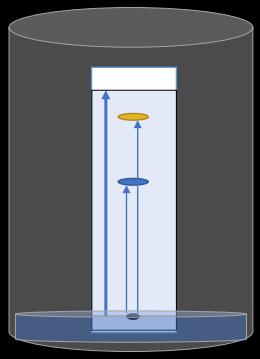
Stationary Phase:

- **Cellulose**
- Modified cellulose powders are used to obtain ion-exchange separation in TLC
- It can be used with or without binder
- Separation by partition mechanism
- It commonly used to separate the hydrophilic substance like amino acid, sugers, etc,



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Stationary Phase:

Table: Stationary phase and mode of separation

Stationary Phase	Chromatographic Mechanism	Typical Application
Silica Gel	adsorption	steroids, amino acids, alcohols, hydrocarbons, lipids, aflaxtoxin, bile, acids, vitamins, alkaloids
Silica Gel RP	reversed phase	fatty acids, vitamins, steroids, hormones, carotenoids
Cellulose, kieselguhr	partition	carbohydrates, sugars, alcohols, amino acids, carboxylic acids, fatty acids
Aluminum oxide	adsorption	amines, alcohols, steroids, lipids, aflatoxins, bile acids, vitamins, alkaloids
PEI cellulose	ion exchange	nucleic acids, nucleotides, nucelosides, purines, pyrimidines
Magnesium silicate	adsorption	steroids, pesticides, lipids, alkaloids

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Solvent Polarity Index

Solvent Polarities for Various Liquids

Solvent	Solvent Polarity Index, *P*
Hexane	0.1
Carbon tetrachloride	1.56
Isopropyl ether	1.83
Toluene	2.4
Methyl-*t*-butyl ether	2.4
Chloroform	2.7
Diethyl ether	2.8
Dichloromethane	3.1
Isopropanol	3.92
Tetrahydrofuran	4.0
Ethyl Acetate	4.4
Methanol	5.1
Acetone	5.1
Dioxane	5.27
Acetonitrile	5.8
Water	10.2

Mobile Phase:

- Proper solvent selection is the most important aspect of TLC, and determining the best solvent may require a degree of trial and error.
- As with plate selection, keep in mind the chemical properties of the analytes. A common starting solvent is 1:1 hexane:ethyl acetate.

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Development of Solvent System

Phytochemical	Mobile phase	Confirmatory test	Extract	R_F Value
Alkaloids	Acetone:water:26% ammonia (90:7:3)	Dragendorff reagent	1 ml HCL+9 ml water	0.96
Flavonoides	Chloroform: Ethyl acetate (6:4)	Aluminum chloride reagent	70% ethanol	0.97
Tannins	Chloroform: Ethyl acetate (6:4)	10% $FeCl_3$ reagent	25ml water	0.99
Phenols	Toluene: Acetone: Formic acid (60:60:10)	10% KOH reagent	Methanol	0.97
Saponins	Ethyl acetate	Vanillin sulfuric acid reagent	Methanol	0.99

Sr. No.	Elutant (mobile phase combination)	Ratio	5 ml Volume of mobile phase	Remark
1	Hexane : Ethyl Acetate	100:0	5:0	increasing polarity according to constituent's nature
2	Hexane : Ethyl Acetate	90:10	4.5:0.5	
3	Hexane : Ethyl Acetate	70:30	3.5:1.5	
4	Hexane : Ethyl Acetate	50:50	2.5:2.5	
5	Hexane : Ethyl Acetate	0:100	00:5	
6	Dichloromethane : Acetone	90:10	4.5:0.5	
7	Dichloromethane : Acetone	70:30	3.5:1.5	
8	Dichloromethane : Acetone	00:100	00:5	
9	Dichloromethane : Methanol	90:10	4.5:0.5	
10	Dichloromethane : Methanol	70:30	3.5:1.5	
11	Dichloromethane : Methanol	50:50	2.5:2.5	
12	Dichloromethane : Methanol	00:100	00:5	

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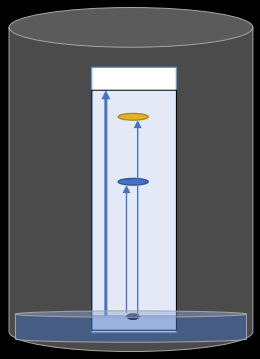
Procedure:

- **Preparation of TLC plate**
- First prepare the slurry of adsorbent, e.g., Silica Gel G (50 % w/v)
- TLC glass pate is dried in oven at 1100 C
- Then prepare the TLC plate (Thickness 250 um) by different methods:
  - Pouring
  - Dipping
  - Spraying
  - Spreading



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Procedure:

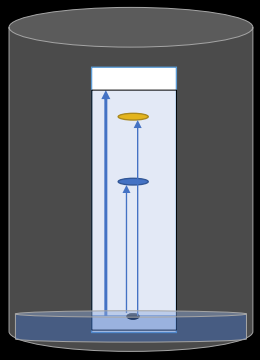
- **Preparation of TLC plate**

Layers have been classified into two types :

(A) Solid Layers

(B) Loose Layers

(A)Solid layers : For solid layers, a uniform layer of the adsorbent material is applied to a lean glass plate with the help of an applicator (***Stahl's applicator***). The most popular thickness of layer is 0.25 mm and the layers thinner than this are avoided. With the help of ***Stahl's Model II-S*** it is possible to prepare layers of thickness **0.25 – 2 mm.**



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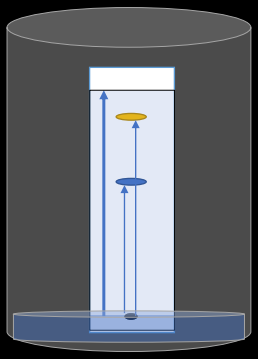
Procedure:

- **Preparation of TLC plate**
- (B) **Loose layers :** Loose layers may be prepared by any of the following methods :
  - (i) Pouring of suspension onto the plate (aqueous slurry of adsorbent).
  - (ii) Dipping of plates in the suspension
  - (iii) Spraying with a thin suspension.



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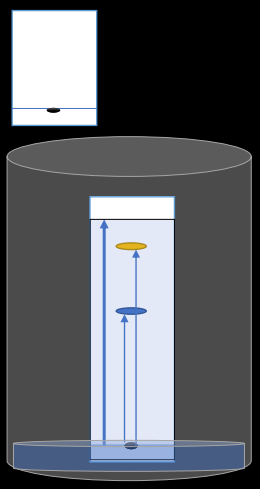
Procedure:

- apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil.
- Apply sample solutions to the marked spots.
- Pour the mobile phase into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase.
- Place the plate in the TLC chamber and close it with a lid. It is kept in such a way that the sample faces the mobile phase.
- Immerse the plate for development. Remember to keep the sample spots well above the level of the mobile phase. Do not immerse it in the solvent.
- Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber.



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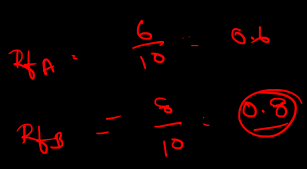
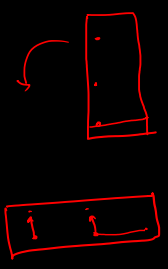
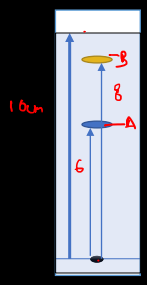
Analysis:

- **Calculate The Rf Value**

Rf = (distance covered by the sample) / (distance covered by the solvent)







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Application:

- Separation of the components from mixture
- The qualitative analysis of the components.
- TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.
- It is used for the purification of samples and direct comparison is done between the sample and the authentic sample.
- It is used in the food industry, to separate and identify colours, sweetening agent, and preservatives
- It is used in the cosmetic industry.
- It is used to study if a reaction is complete.

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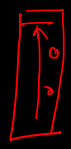
Advantages:

- Easy to analyze
- Easily visualize
- Inexpensive
- Quicker
- Several compounds can easily get isolated through TLC



Disadvantages

- The TLC procedure can not be used for lower detection limit experiments because it has a high detection limit.
- Result reproduction is challenging in TLC.
- TLC is limited to qualitative analysis
- The separation length is also restricted as compared to other chromatography methods.

PC

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