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THIN LAYER CHROMATOGRAPHY

Aim:

To identify the unknown molecule based using TLC Chromatography.

Objective:

After performing this practical, the learner will be able to:

PRO1: Understand the apparatus setup for TLC.
PRO2: Calculate the RF value for given samples.
PRO3: Identify the diffrent compound from the given mixture.

Apparatus:

- Nitrobenzene
- Phenol
- UV apparatus
- TLC plate
- Unknown solution
- Capillary tubes
- Pencil, etc.

Observation:

Sample	Distance travelled by solute(molecule)	Distance travelled by solvent (mobile phase)	Rf Value.
Nitrobenzene	1.15	3.25	0.35
Phenol	2.4	3.25	0.73
Unknown	2.4	3.25	0.73

Calculation:

$$R_f = \frac{\text{Distance travelled by molecule}}{\text{Distance moved by mobile phase}}$$

For Phenol = $\frac{1.15}{3.25} = 0.35$

For Nitrobenzene = $\frac{2.4}{3.25} = 0.73$

For Unknown = $\frac{2.4}{3.25} = 0.73$

Result & Discussion:

PRO1: Saturation of Mobile Phase in Developing Chamber Allows it to disperse Uniformly throughout resulting in an ideal setup to Perform Chromatography.

PRO2: Use of Origin Line on TLC Plate results in Precise Measurement Using Pencil for marking the Origin Line doesn't Hinder with the Experiment as Lead is an Inorganic Compound unlike Organic Pen Ink, which does not react with the Compounds as well as the Mobile Phase. Capillary tube helps spotting the solution in a unit amount.

PRO3: Solutions seperate & disperse their Components through the Mobile Phase supported by the solid phase, TLC Plate. The developing chamber is sealed until solution Reaches 75% of Plate Height Preventing any External Contact.

PRO4: UV Light provides a visual Representation of Dispersion of Given Solutions allowing further Calculations. It is cheap Alternative for Indication Chemicals like Ninhydrin Retention Factor being Molecular Specific can be used to identify the Unknown Solution.
Since Rf (Nitrobenzene) = Rf (Unknown Sample),
Hence the Unknown Sample is Nitrobenzene.

Conclusion:

TLC Method of Chromatography is Used for Identifying Unknown Compounds & Determining the Polarity of Substances.

Quiz:

1] Define and Explain Significance of Rf Value.

⇒ Retention Factor or Rf Value is the ratio of Distance Travelled by Solute to Distance Travelled by Solvent. Rf value has Many Significant Applications such as Identifying Unknown Compounds, Determining Polarity, Relative Masses, Relative Solubility, etc.

2] What is the Principle of Thin Layer Chromatography?

⇒ The Principle of Thin Layer Chromatography (TLC) is Seperation of Components in a mixture by Adsorption, According to their Affinity towards Stationary Phase & Mobile Phase.

3] Name the adsorbents used in Thin Layer Chromatography

⇒ TLC can be performed using inorganic adsorbents like Silica Gel (SiO_2), Alumina (Al_2O_3) or using Organic solvents like Cellulose, charcoal & activated Carbon, Ion exchange Resins, etc.

4] UV light is used as a detecting agent in TLC for indicating colourless substances like Alkaloids, Steroids & Amino Acids. Some Reagents can also be used as a detecting Agents for specific purposes like Ninhydrin, Aniline-Phtalate Reagent, etc.

5] Which solvent is used as a mobile phase in Thin Layer Chromatography?

⇒ Mixtures of Hexane (C_6H_{14}) or Chloroform ($CHCl_3$) or Acetone (C_3H_6O) are solvents generally used as Mobile Phase.