CH 2252 Instrumental Methods of Analysis

Unit – V

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Planar Chromatography

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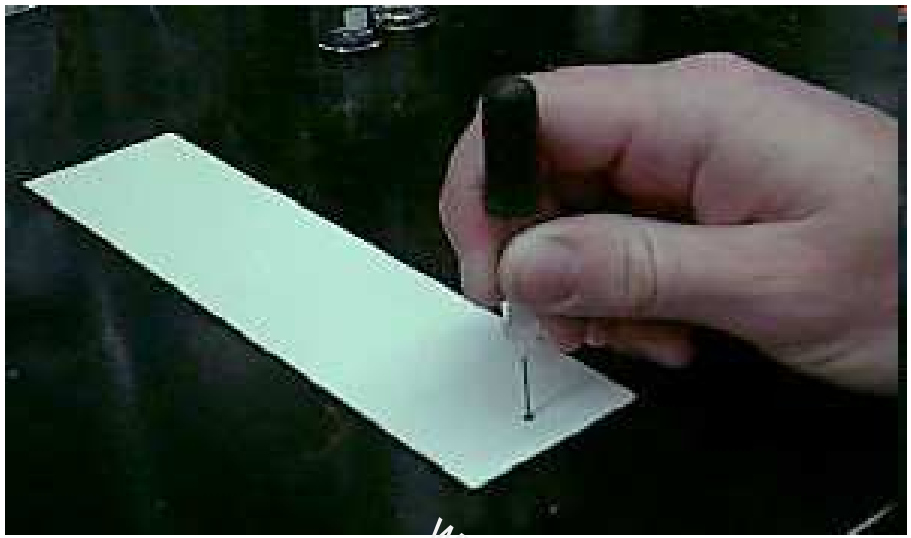
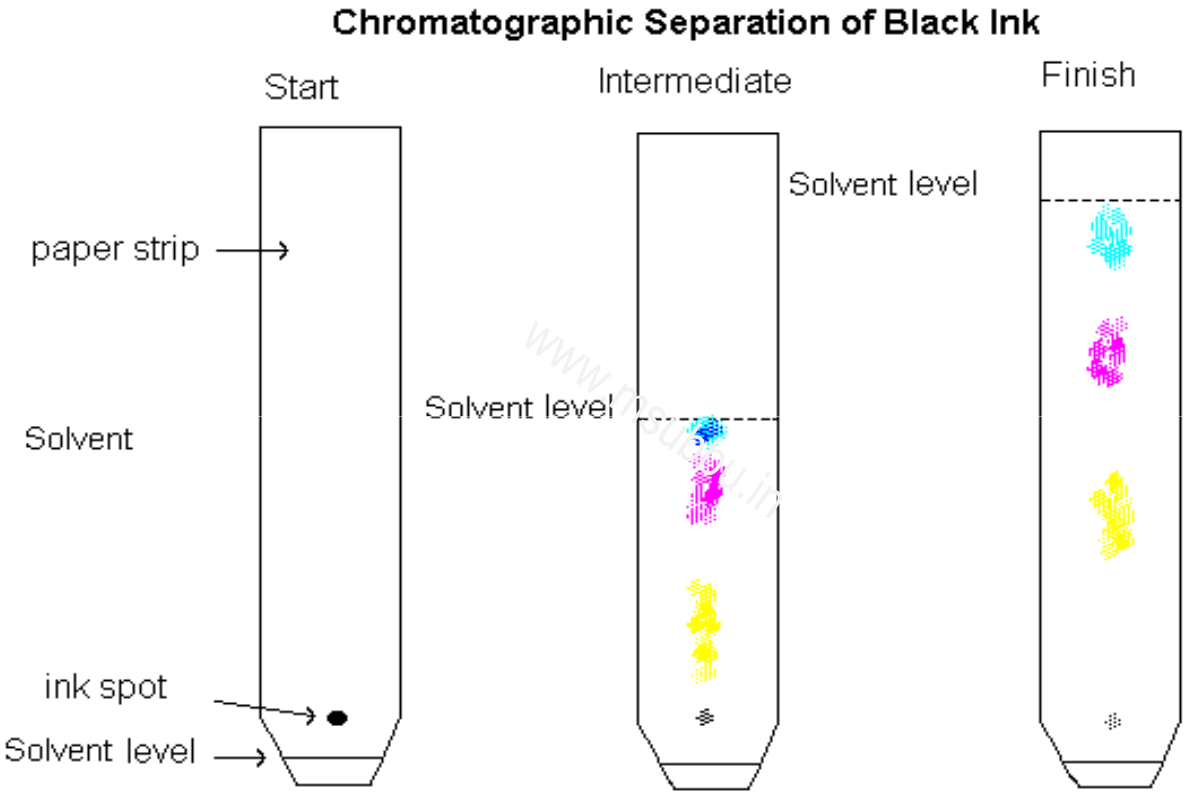
Planar Chromatography

- A separation technique in which the stationary phase is present as or on a plane.
- The plane can be a paper, serving as such or impregnated by a substrate as the stationary bed (***paper chromatography***, PC) or a layer of solid particles spread on a support e.g. a glass plate (***thin layer chromatography***, TLC).
- Sometimes planar chromatography is also termed open-bed chromatography



Spotting a TLC plate with sample

Running the TLC plate in solvent

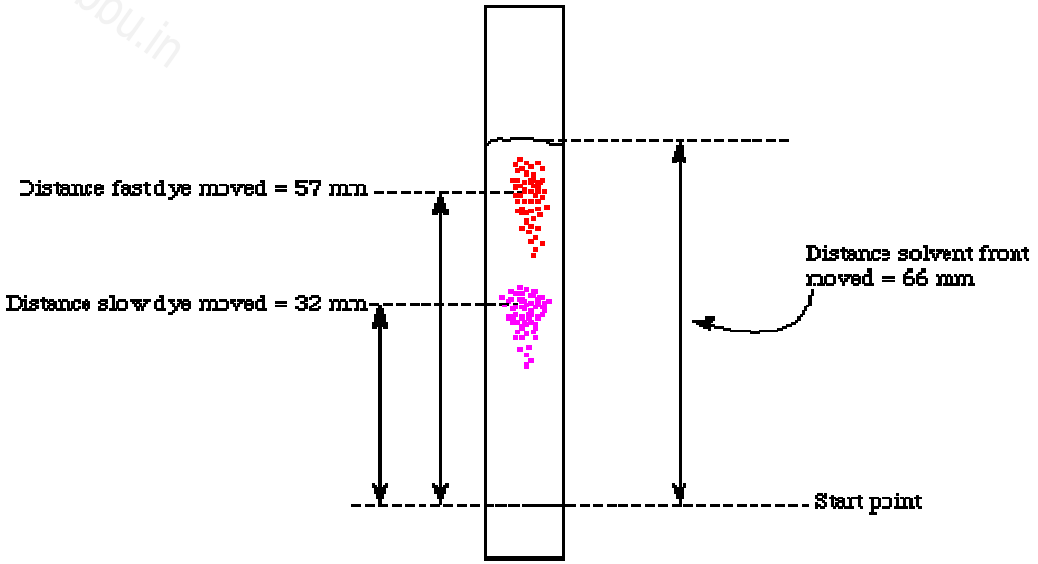

Chromatographic Separation of Black Ink
Start
Intermediate
Finish
paper strip
Solvent
ink spot
Solvent level
Solvent level
Solvent level

Retardation factor (Rf)

$$R_f = \frac{\text{Distance compound has moved from origin}}{\text{Distance of solvent front from origin}}$$

In paper chromatography when the conditions are kept constant, a particular compound always travels a fixed percentage of the distance traveled by the solvent front. The symbol Rf stands for "retardation factor" or "ratio-to-front". It is expressed as a decimal fraction. The Rf value is a constant for a given compound.

For best results, Rf values should be between 0.3 and 0.7



$$R_f = \frac{\text{Distance solute center of gravity moved}}{\text{Distance solvent front moved}}$$

$$R_{f\ \text{fast dye}} = \frac{57\ \text{mm}}{66\ \text{mm}} = 0.86 \qquad R_{f\ \text{slow dye}} = \frac{32\ \text{mm}}{66\ \text{mm}} = 0.48$$

Thin Layer Chromatography (TLC)

- Thin-layer chromatography (TLC) is one of the most popular and widely used separation techniques because of its ease of use, cost effectiveness, high sensitivity, speed of separation, as well as its capacity to analyze multiple samples simultaneously

- TLC is related to paper chromatography (PC) as both use a stationary phase and a liquid phase to move the sample

Operation of TLC

- In TLC, the sample is applied as a small spot or streak to the marked origin of stationary phase supported on a glass, plastic, or metal plate.
- The sample solvent is allowed to evaporate from the plate that is then placed in a closed chamber containing a shallow pool of mobile phase at the bottom.
- The mobile phase moves through the stationary phase by capillary forces. The components of the sample mixture migrate at different rates during movement of the mobile phased through the stationary phase.
- The migration of each component in a mixture during TLC is a result of two opposing forces: capillary action of the mobile phase and retardation action of the stationary phase. Both forces contribute to achieve differential migration of each component.

Operation of TLC (contd.)

- When the mobile phase has moved an appropriate distance, the plate is removed from the chamber and the solvent front is marked.

- Developed TLC plates can be detected by various means, based on the nature of the sample. They could be nondestructive (UV densitometer), destructive (derivatizing agents), or the combination of both. The results can be documented by photography and saved electronically for archiving and future reference.

Sorbents for TLC

- Silica gel, cellulose, alumina, polyamides, ion exchangers, chemically modified silica gel, and mixed layers of two or more materials, coated on a suitable support.

- Silica gel is by far the most commonly used sorbent supported on a glass plate

Mobile phase in TLC

- The choices of mobile phase range from single component solvent systems to multiple-component solvent systems with the latter being the most common (hexane, ethyl acetate, methanol)

Least polar

petroleum ether

cyclohexane

CCl_4

tricholoethane

toluene

benzene

dichloromethane

chloroform

diethyl ether

ethyl acetate

acetone

n-propanol

ethanol

methanol

water

pyridine

Most polar

Important Features of TLC over HPLC

- While HPLC is widely used for separation and quantification, TLC remains a valuable and commonly used separation technique because of its features that are complementary to HPLC. The majority of TLC applications use normal-phase methods for separation, whereas reversed-phase methods dominate in HPLC. Some of the most important features of TLC compared to HPLC are:
  - Open format of stationary phase and evaluation of the whole sample
  - Simple sample preparation: Samples for TLC separation often involve fewer cleanup steps because every sample is separated on fresh stationary phase, without cross-contamination or carryover
  - High sample throughput: The simultaneous but independent application and separation of multiple samples in TLC results in higher sample throughout and less time consumption, as well as lower costs
  - Flexible and versatile dissolving solvent and mobile phase