Class – 6th Semester (Botany Hons)

Paper DSE-4:- Analytical techniques in plant

Unit 5:- Chromatography

TIC (Thin Layer Chromatography)

Definition:-

It is a chromatographic technique which is used to separate non volatile mixture. Thin layer chromatography is preformed on a sheet of glass, plastic or aluminium foil which is coated with a thin layer of absorbent material usually silica gel, aluminium oxide or cellulose.

Principle:-

Similar to the other chromatography methods thin layer chromatography is also based on the following principle –

1. The separation depends on the relative affinity of compounds towards stationary and the mobile phone.
2. The compounds under the influence of the mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved.
3. Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate, the nature of character are identified by means of suitable detection techniques.

System Components:-

TLC system components consists of –

1. TLC plate preferably readymade with a stationary phase. There are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in affine particle size. The thickness of the absorbent layer is typically around 0.1-0.25mm for analytical purposes and around 0.5-2mm for preparative TLC. The TLC plate is dried and activated by heating in an oven for 30 minutes at 110^{o}c.
2. **TLC chamber :-**

   This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots it also prevents the evaporation of solvents and keeps the process dust free.

3. **Mobile Phase :-**

   This comprises of a solvent or solvent mixture. The mobile phase used should be particulate free and of the highest purity for proper development of spots. The solvents recommended are chemically inert with the sample, a stationary phase.

4. **A filter paper :-**

   This is moistened in the mobile phase, to be placed inside the chamber. This helper develops a uniform rise in a mobile phase over the length of the stationary phase.

5. **Capillary tube :-**

Technique:-

To run a thin layer chromatography plate, the following procedure is carried out –

1. With a pencil a thin mark is made at the bottom of the plate to apply the sample spots. Using a capillary, a small spot of solution containing the sample is applied to a plate, about 1.5 centimetres from the bottom edge. The solvent is allowed to completely evaporate off to prevent it from interfering with samples interactions with the mobile phase in the next step. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber. The step is often repeated to ensure there is enough analyse at the starting spot. On the plate to obtain a visible result. Different samples can be placed in a row of spots the same distance from the bottom edge, each of which will move in its own adjacent lane from its own starting point.

2. A small amount of an appropriate solvent (eluent) is poured into a glass beaker or any other suitable transparent container (separation chamber) to a depth of less than 1cm. A strip of filter paper (aka “wick”) is put into the chamber so that its bottom touches the solvent and the paper lies on the chamber wall and reaches almost to the top of the container is closed with a cover glass or any other lid and is left for a few minutes to let the solvent vapours ascend the filter paper and saturate the air in the chamber.

3. The TLC plate is then placed in the chamber so that the spot (s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The solvent moves up the plate by capillary action,

meets the sample mixture and carries it up the plate (elutes the sample). The plate should be removed from the chamber before the solvent from reaches the top of the stationary phase (continuation of the elution will give a misleading result) and dried.

4. Without delay the solvent front, the furthest extent of solvent up the plate, is marked.

5. The plate is visualized. As some plates are pre-coated with a phosphor such as zinc sulphide, allowing many compounds block the UV light from striking the plate. Alternatively, plates can be sprayed or immersed in chemicals after elution. Various visualising agents react with the spots to produce visible react with the spots to product visible results.

Analysis:-

As the chemicals being separated may be colourless, several methods exist to visualized the spots –

- Fluorescent analyses like quinine may be detected under black light (366nm)
- Often a small amount of fluorescent compound, usually manganese activated zinc silicate, is added to the adsorbent that allows the visualization of spots under UV.C light (254nm). The adsorbent layer will thus fluoresces light green by itself, but spots of analyse quench this fluorescence.
- Iodine vapours are a general unspecific colour reagent.
- Specific colour reagents into which the TLC plate is dipped or which are sprayed on to the plate exist –
  - Potassium permanganate oxidation
  - Bromine
  - Acidic Vanillin
  - Phosphomolybdic acid.
- In the case of lipid, the chromatogram may be transferred to a polyvinylidene fluoride membrane and then subjected to further analysis, i.e – mass spectrometry –(eastern bolt technique).

Separation process:-

Different compound in the sample mixture travel at different rather due to the differences in their attraction to the stationary phase and because of differences in the solubility in the solvent. By changing the

solvent, or perhaps using a mixture, the separation of components (measured by the Rf value) can be adjusted. Also the separation achieved with a TLC plate can be used to estimate the separation of a flash chromatography column. Chemists often use TLC to develop a protocol for separation by chromatography and use TLC to determine which fractions contain the desired compounds.

Separation of compound is based on the completion of the solute and the mobile phase for binding sites on the stationary phase. For example, it normal phase silica gel is used as the stationary phase, it can be considered polar. Given two compounder that differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore, the better able to displace the mobile phase from the available binding sites. As a consequence, the less polar compound mover higher up to the plate resulting in a higher Rf value.

If the moble phase is changed to a more polar solvent it becomes better at binding to the polar plate and therefore displacing solutes from it, so all compounds on the TLC plate will move higher up to the plate. So, it is commonly said that 'strong' solvents push the analyzed compounds up the plate, whereas 'weak' solvents barely move them.

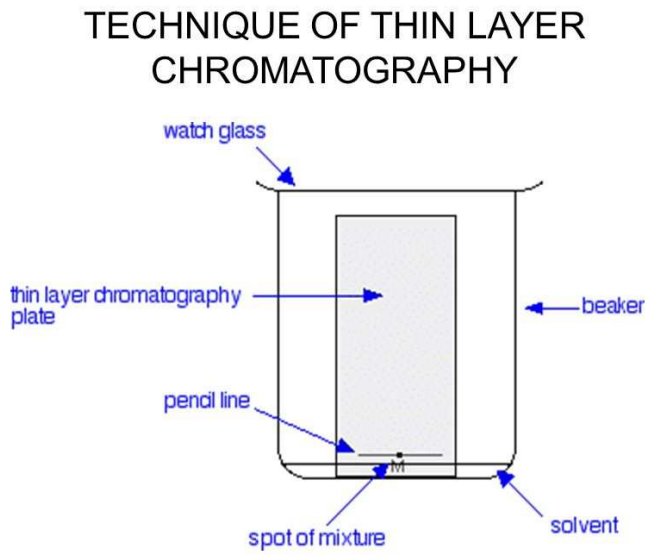
What is Rf value:-

The relationship between the distance travelled by the solvent front and the compound is usually expressed as Rf value

$$Rf\ value = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

Higher the Rf value lesser the polarity of the substance. Lower the Rf value higher is the polarity of the substance.

Fig:-



Applications:-

1. ***Purity of any sample*:**
   Purity of sample can be carried out with TLC. Direct comparison is done between the sample and the standard or authentic sample; if any impurity is detected, and then it shows extra spots and this can be detected easily.
2. ***Identification of compounds:***
   Thin layer chromatography can be employed in purification, isolation and identification of natural products like volatile oil or essential oil, fixed oil, waxes, alkaloids, glycosides, steroids etc.
3. ***Examination of reactions:***
   Reaction mixture can be examined by Thin layer chromatography to access whether the reaction is complete or not. This method is also used in checking other separation processes and purification processes like distillation, molecular distillation etc.
4. ***Biochemical analysis:***
   TLC is extremely useful in isolation or separation of biochemical metabolites or constituent from its body fluids, blood plasma, serum, urine etc.
5. ***In chemistry:***
   TLC methodology is increasingly used in chemistry for the separation and identification of compounds which are closely related to each other. It is also used for identification of cations and anions in inorganic chemistry.
6. ***In pharmaceutical industry:***
   Various pharmacopoeias have adopted TLC technique for detection of impurity in a pharmacopoeial chemical.
7. Various medicines like hypnotics, sedatives, anticonvulsant tranquillizers, antihistaminics, analgesics, local anaesthetics, steroids have been tested qualitatively by TLC method.
8. One of the most important applications of TLC is in separation of multicomponent pharmaceutical formulations.
9. *In food and cosmetic industry*, TLC method is used for separation and identification of colours, preservatives, sweetening agent, and various cosmetic products.

Dr. B.D.Guria
- Asst. Prof. in Botany