Thin Layer Chromatography

Thin-layer chromatography (TLC) is a simple, inexpensive separation technique that is used frequently in organic chemistry. It is often used as a diagnostic tool to answer questions such as whether the unknown sample on the table contains one single compound or more than one compound, whether the product has started appearing in the organic reaction, whether the reaction has been completed or not, etc. It is generally used as a qualitative tool. TLC can also be used to identify the compounds in a mixture. This is done by comparison of TLC of an unknown sample with known materials. For separating components of a mixture by column chromatography, often appropriate conditions for separations can be identified by first doing TLC of the same mixture.

In TLC, there is a **mobile** phase, that is, liquid (either one single solvent or often mixture of solvents) and the **stationary** phase, that is, a solid **adsorbent**. The adsorbent (the stationary phase) is a powder that is coated onto a solid support, as a thin layer (about 0.25 mm thick). Smooth surface of glass, plastic, and aluminium are common solid support materials.

In most cases, the stationary phase (adsorbent) is highly polar, and the mobile phase (eluant) is fairly non-polar. Molecules that are more polar stick to the polar stationary phase more than fairly non-polar molecules which are carried along in the mobile phase (this is an equilibrium process, and all molecules undergo this process). Separation occurs because some molecules spend a greater percentage of time with the adsorbent (in other words, are retained by the adsorbent) than others.

Several factors determine the efficiency of chromatographic separation. For efficient separation, the adsorbent and the solvent system can be changed. Silica gel (SiO_2) is a very commonly used, strongly polar adsorbent material. Other common polar adsorbents include alumina, charcoal etc.

In practice, rather than adsorbent the most common factor that is adjusted to achieve good separation are the solvents used in the mobile phase. The best solvent for a particular application is often determined by experimental trials. The following table states a list of some of the common solvents. The "eluting power" of a solvent is largely a measure of how well it can carry along a compound which is adsorbed onto the stationary phase, often this happens when the solvent molecules displace the compound molecules from the stationary phase.

Commonly used eluting solvents for chromatography

1. Hexane or Pentane (*least eluting power*)
2. Cyclohexane
3. Benzene
4. Dichloromethane
5. Chloroform
6. Ether (anhydrous)
7. Ethyl acetate (anhydrous)
8. Acetone (anhydrous)
9. Ethanol

10. Methanol
11. Water
12. Pyridine
13. Acetic acid (*greatest eluting power*)

Reference:

1. A. Ault, *Techniques and experiments for organic chemistry*, 6th edition, University Science Books, California, 1998
2. Thin Layer Chromatography: A Complete Guide to TLC. *Chemistry Hall*. https://chemistryhall.com/thin-layer-chromatography/ (accessed on 26-04-2022).

While preparing solvent systems, the non-polar solvents mentioned at the top of the above list are often used as major component and a small proportion of a stronger eluting, more polar solvent is added to it. As the eluting power of the added solvent increases, the amount of solvent that need to be generally added (or used) decreases. "Medium polar" solvents like ethyl acetate may be used as 1%-50% combination with hexane making these mixtures very tunable and common. Stronger eluting solvents like methanol are generally not used more than 10% in a mixture. More than 1% of pyridine or acetic acid isn't often necessary, (a drop or two is more common); while these two additives are next to each other on the list, they can have very different effects on a separation depending on the functional groups in the molecules being separated. Water is very strongly eluting and its presence as an impurity in the solvent can be problematic.

The functional groups of the molecules in the mixture affect how strongly they are adsorbed by the stationary phase. Very "greasy" non-polar substructures, usually made entirely of carbon and hydrogen, are hardly adsorbed by silica gel at all. Polar groups, with oxygen and nitrogen are more strongly adsorbed. Alcohols, carboxylic acids, and amines form hydrogen bonds with the silica gel which leads to a strong adsorbing interaction.

Adsorbance of organic compounds (by organic functional group)

1. Saturated hydrocarbons, alkyl halides (*least strongly adsorbed*)
2. Unsaturated hydrocarbons, alkenyl halides
3. Aromatic hydrocarbons, aryl halides
4. Polyhalogenated hydrocarbons
5. Ethers
6. Esters
7. Aldehydes and ketones
8. Alcohols
9. Acids and amines (*most strongly adsorbed*)

Reference:

1. A. Ault, *Techniques and experiments for organic chemistry*, 6th edition, University Science Books, California, 1998.

2. Thin Layer Chromatography, *LibreTexts* (accessed on 26-04-2022) https://chem.libretexts.org/Ancillary_Materials/Demos_Techniques_and_Experiments/General_Lab_Techniques/Thin_Layer_Chromatography

TLC is useful because it is reproducible. For a particular adsorbent/solvent/compound combination, the ratio of the distance the compound spot travels to the distance the solvent front travels remain constant. This ratio is called the R_f value.

$$R_f = \frac{\text{distance travelled by substance}}{\text{distance travelled by solvent front}}$$

Two substances having the same R_f value (under the same conditions) does not **conclusively** prove that the two substances are the same.

Techniques for Thin Layer Chromatography

Spotting of the sample

We can obtain a pre-coated TLC plate from the laboratory instructor. The adsorbent that is **silica gel** is already coated on this plate. First, a light pencil line is drawn leaving 0.5 to 1 cm distance from the edge of the plate (**Caution!!** pencil should not be used forcefully otherwise the pre-coated silica gel will come out). This is the baseline on which we will spot the sample. A very small quantity of compound is used for spotting. A small amount of the sample is dissolved in the appropriate solvent (that will be informed by the laboratory instructor) in a sodium fusion tube or an Eppendorf vial. A thin capillary is used to put a tiny amount of sample solution on the baseline. Generally, a single sample spot is spotted at the center of the baseline. If spotting is done for more than one sample spots, then an appropriate distance between the spots should be kept so that the spots do not merge during elution (see the diagram below). **Caution!!** it is advisable to keep the spot small, just 1–2 mm only in diameter. If a heavier spot is needed, the solvent is allowed to evaporate, and then spotted again.

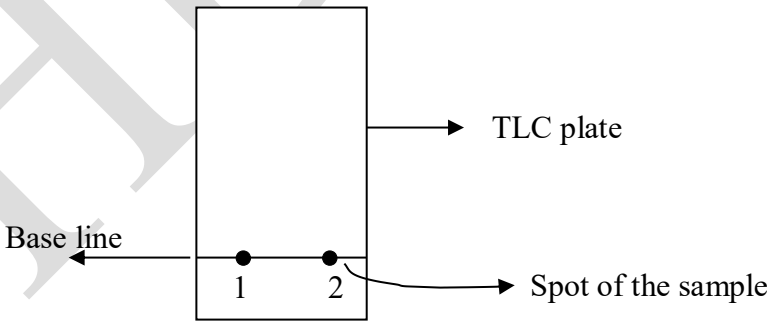

Figure 1: TLC plate with two sample spots

Preparation of container for developing TLC

A dry beaker (100 to 150 mL capacity or a TLC chamber) is taken. If it isn't dry, it is then rinsed with the solvent that will be put in it. **Caution!!** *Do not rinse with any other solvent, especially with water.* A clean dry piece of filter paper (can be cut if necessary) is placed standing up against the wall of the beaker. The paper will soak up some of the solvent and help to saturate the atmosphere inside the beaker with solvent vapor. This will make the plate run

faster by slowing the evaporation of solvent from the plate.
A small amount of solvent is poured into the beaker just a few millimeters in height from the base of the beaker and the beaker is covered with a watch glass or Petri dish so that there is not much evaporation of solvent/s.
In case too much solvent is used, then its level will be above the baseline on the TLC plate and thus it will drain the spot. The height of the solvent in the beaker **should not** be above the baseline on the TLC plate.

Running of a TLC Plate

The TLC plate is placed gently into the beaker (forceps can be used to insert the TLC plate) and the beaker is covered with a watch glass. **Check that the baseline is not immersed in the solvent system. T**he solvent front is monitored travelling up the plate which could take a while (especially if we have a less volatile solvent). When the solvent front is 0.5-1.0 cm away from the **top edge** of the plate, the plate is taken out with forceps and the solvent front is marked immediately with a pencil. This marking is required for calculating the R_f values.

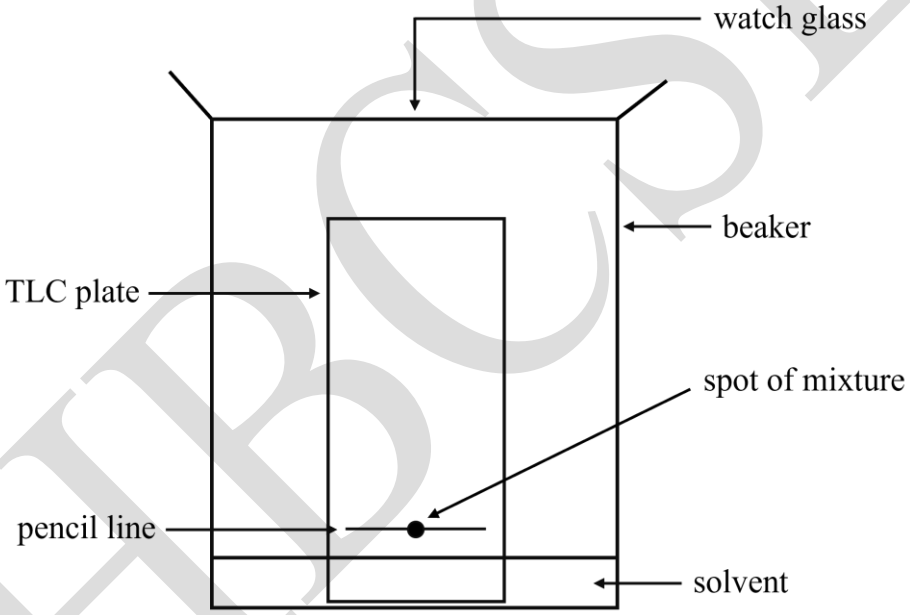

Figure 2: TLC set-up

Visualization

If the component(s) that is/are present in the sample is/are colored, then the spots will be visible as such. In case the substances are not colored, these can be visualized using UV light. The aromatic rings in the molecules of organic compounds absorb UV light, causing dark spots to appear where the compound is present. The plate is watched under UV lamp and any spots seen under UV light are circled so that their positions are known after the plate is removed from the UV chamber.
Compounds that are not UV active can be seen by appropriate staining of the TLC plate. Sometimes chamber containing iodine is used or otherwise, the plate is sprayed with an appropriate reagent. Reactions that cause a color change occur with the compound on the plate,

and then the spots become visible.
For any TLC experiments, it is recommended to draw the diagram of each plate in the notebook as part of the record of the experiment followed by reporting the R_f values of the spots. In addition, a photo can also be taken using a mobile phone and included in the report.
Note for the instructor: For the following TLC experiments, 3 to 4 solvents can be given to students. Students should be allowed to run the TLC in these solvents and then should decide the best solvent system. Open discussion with students will direct them to understand how to select solvent or solvent systems and also make them aware of the errors they make while carrying out the actual experiment.

TLC experiments:
Any of the following activities can be given to the students.

I. Mixture of o-nitroaniline and p-nitroaniline

Solvent for dissolution: methylene chloride or acetone
Solvent for TLC: plain hexane and ethyl acetate and a combination of both solvents in different proportions
Visualization of spots: by naked eyes

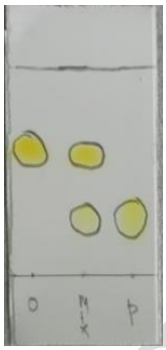

Figure 3: An example of TLC of o- and p-nitroaniline along with their mixture has shown here.

'o' is o-nitroaniline, 'p' is p-nitroaniline at extreme left and right sides respectively and the middle spot is a mixture of both o- and p-nitro aniline.

II. Mixture of phenol and resorcinol

Solvent for dissolution: acetone

Solvent for TLC: hexane and ethyl acetate with 5: 2 volume ratio.

Visualization of spots: by UV chamber

Figure 4: An example of TLC of phenol and resorcinol along with their mixture has shown here.

This TLC plate is showing the spots for phenol (P) and resorcinol (R) at extreme left- and right sides in the UV chamber. The middle spot is a mixture of both 'P' and 'R'.

III. Turmeric powder

1 g of Turmeric powder

Solvent for dissolution: ethanol (5 mL), place the turmeric and ethanol mixture in warm water and filtered it. Perform the TLC with the filtrate part.

Solvent for TLC: Chloroform: ethanol: acetic acid (94: 5: 1)

Visualization of spots: by naked eyes or iodine vapors or UV chamber (generally three bands are visible - curcumin (topmost), mono-methoxy curcumin (middle) and di-methoxy curcumin (last).

Figure 5: A TLC of ethanolic extract of turmeric

IV. Black or Red Ink (The sample ink is easily available and can be easily performed)

Solvents for TLC: plain ethanol, or hexane

Visualization of spots: by naked eyes