SOP: THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures containing different polarities. This method can be used toquantitatively identify the components in a mixture. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose – this is called the TLC plate. This layer of adsorbent is known as the stationary phase.

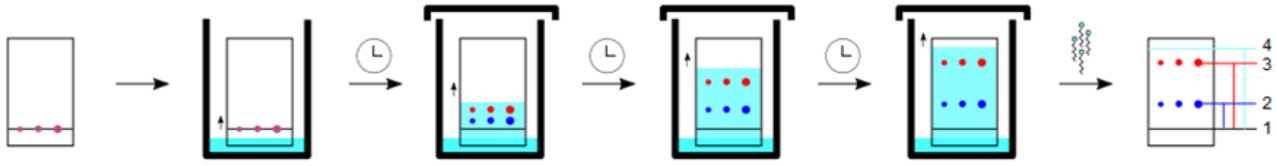
After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. The mobile phase has different properties from the stationary phase. For example, with **silica gel**, a **very polar substance**, non-polar mobile phases such as heptane are used. The polar silica gel will want to hang onto more polar substances than less polar ones. The mobile phase may be a mixture, allowing chemists to fine-tune the bulk properties of the mobile phase.

After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light onto the sheet; the sheets are treated with a phosphor, and dark spots appear on the sheet where compounds absorb the light impinging on a certain area. Chemical processes can also be used to visualize spots; anisaldehyde, for example, forms coloured adducts with many compounds, and sulfuric acid will char most organic compounds, leaving a dark spot on the sheet.

To quantify the results, the distance travelled by the substance being considered is divided by the total distance travelled by the mobile phase. (The mobile phase must not be allowed to reach the end of the stationary phase.) This ratio is called the retention factor or Rf. In general a substance whose structure resembles the stationary phase will have low Rf, while one that has a similar structure to the mobile phase will have high retention factor. Retention factors are characteristic, but will change depending on the exact condition of the mobile and stationary phase. For this reason, chemists usually apply a sample of a known compound (called standards) to the sheet before running the experiment.

Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance.

Figure 1: Development of a TLC plate, a purple spot separates into a red and blue spot.



TLC Plate

- TLC plates can be cut to size needed. Glass cut with glass cutter.
- Only handle the TLC plate by its edges, using e.g. a tong or tweezers, wear clean latex gloves – this prevents contaminating the TLC plate's surface.
- Draw a light line (the origin) in pencil 1.5cm from bottom edge of plate. Try not to damage layer.
- Make 1cm spaced apart vertical markers along the origin. These markers are used at points of reference. Mark the markers at top of TLC plate with pencil.

Spotting of Plate

- Using a capillary tubes, a small spot of the standard and sample solutions are applied as a spot to the markers made at bottom of plate. For every solution/standard use a new clean capillary tube to avoid cross contamination.
- Can use any solvent for sample & standard as long as it is a volatile solution and cause standard and/or sample to dissolve fully. Methanol is usually a good solvent to use.
- The spots are allowed to completely evaporate off to prevent it from interfering with the sample's 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.
- This step is often repeated to ensure there is enough analyte 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 (origin).

Developing Chamber

- 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.
- The chamber 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. (Failure to saturate the chamber will result in poor separation and non-reproducible results).

The Front

- The TLC plate is then placed in the chamber so that the spot(s) of the sample & standards are to the bottom, BUT the spots/origin must not touch the eluent at bottom of chamber.
- Close the chamber lid again.
- The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample).
- It usually takes a few minutes and one will easily see the "wet" solvent front of the eluent ascending the silica surface.
- The plate is removed from the chamber before the solvent front reaches the top (about 1cm from top) of the stationary phase. Continuation of the elution will give a misleading result.
- Without delay, the solvent front, the furthest extent of solvent up the plate, is marked.

Visualizing

- As some plates are pre-coated with a phosphor such as zinc sulphide, allowing many compounds to be visualized by using ultraviolet light; dark spots appear where the 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 results.
- Under UV outline all the spots with a pencil so it is visible under visible light.
- Remove plate from light box.
- Index the reference spots/standards and compare unknown with these reference spots.

Rf Values

Rf = Distance moved by a Spot (mm) / Distance moved by Solvent (mm)
Spot moved = 28.0mm & Solvent moved = 80.0mm to front
28mm/80mm = 0.35

Report Rf values to 2 decimal places.
The closer the Rf value is to that of a standard the larger the chance that the unknown is the same as the standard.
Rf values are always less than 1.

Seldom will the Rf values be the same in the second decimal place, due to experimental mistakes and inconsistences. But one can with great certainly confirm with TLC plates that a compound is absent or present in the solution tested by comparing it to a standard/s Rf values in same TLC plate.

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase and because of differences in 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. (A compound elutes from a column when the amount of solvent collected is equal to 1/Rf). Chemists often use TLC to develop a protocol for separation by chromatography and they use TLC to determine which fractions contain the desired compounds.

Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal-phase silica gel is used as the stationary phase, it can be considered polar. Given two compounds that differ in polarity, the more polar compound has a stronger interaction with the silica and is, therefore, more capable to dispel the mobile phase from the binding places. As a consequence, the less polar compound moves higher up the plate (resulting in a higher Rf value).

If the mobile phase is changed to a more polar solvent or mixture of solvents, it is more capable of dispelling solutes from the silica binding places, and all compounds on the TLC plate will move higher up the plate. It is commonly said that "strong" solvents (eluents) push the analyzed compounds up the plate, whereas "weak" eluents barely move them. The order of strength/weakness depends on the coating (stationary phase) of the TLC plate.
For silica gel-coated TLC plates, the eluent strength increases in the following order: perfluoroalkane (weakest), hexane, pentane, carbon tetrachloride, benzene/toluene, dichloromethane, diethyl ether, ethyl acetate, acetonitrile, acetone, 2-propanol/n-butanol, water, methanol, triethylamine, acetic acid, formic acid (strongest).

HEALTH & SAFETY ISSUES

1) Always work in a fume hood as solvents are usually very flammable and may cause health effects if breathed in.
2) Ensure there are no heat sources close to chamber.
3) When cutting glass plates, be careful that you do not inhale silica dust or when breaking plate it does not break and stick into hand.
4) Always cap chemicals/solvents immediately after use.
5) Ensure TLC plate waste is discarded of in the TLC waste container.
6) Only place hands in UV light box when shielded & do not leave hands to long in box, can cause sunburn.
7) Do not look directly into UV box can cause eye damage.

OTHER ISSUES

1) Make marks on TLC plate with pencil ONLY. Stay away from edges of plate & do not scrape or dig into plate.
2) Do not make the spot on TLC plate to big. Only a small spot is necessary.
3) Use as low as possible concentration of sample/standard as needed otherwise one do not get good separation.
4) Filter paper at bottom of chamber assist that TLC plate does not slip around in chamber.