Analyzing a Mixture by Thin Layer Chromatography (TLC)

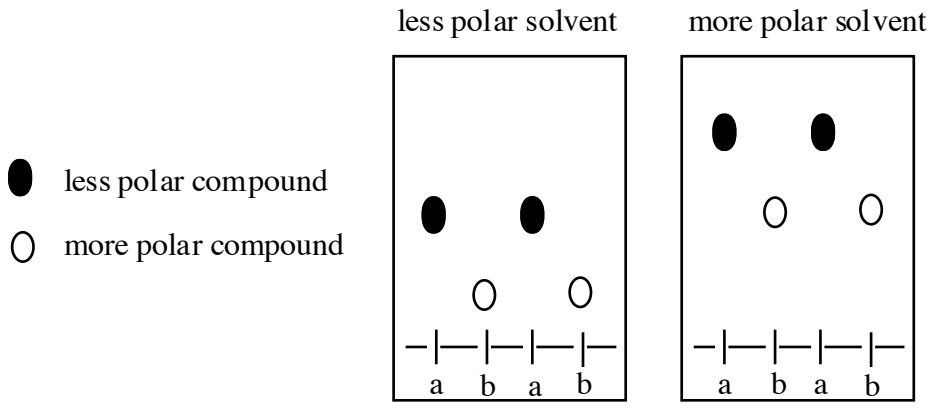
Thin layer chromatography is a method for analyzing what compounds are present in a mixture. For example, it can be used to see whether a starting material has been converted to product during a reaction, to see what compounds are present in an unknown mixture, or to check the identity and purity of a final product.

Chromatography in general is a separation technique in which different compounds in a mixture are carried by a mobile phase at different rates along a stationary phase. In TLC, the stationary phase is a thin piece of plastic or aluminum coated with silica gel powder, and the mobile phase is a solvent, called the developing solvent, which moves up the plate by capillary action. Compounds move at different rates on a TLC plate because of the proportion of time spent absorbed on the plate vs. dissolved in the solvent. The more time they spend dissolved in the solvent, the faster they will move; the more time they spend absorbed on the plate, the slower they will move.

The characteristic that determines this ratio is polarity. The silica gel plate is very polar. Compounds which are also polar will tend to stick more to the plate, and will move more slowly. Compounds which are less polar will tend to dissolve in the solvent, and will move faster. If two different samples run the same height on a plate, this is good evidence that they are the same compound; if they move differently, they are different.

The polarity of the solvent used to carry the spots up the plate will also affect the distance the spots travel. A more polar solvent will be able to compete with the silica gel on the plate, and all spots will be carried correspondingly higher than they would with a less polar solvent.

In practice, the developing solvent is chosen so that the spots go to more or less in the middle of the plate. The closer they are to the top or the bottom, the more difficult it is to tell them apart. If all of the spots run to the top, a less polar solvent should be used; if they all stick to the baseline, more polar solvent should be used. Mixtures of two solvents (such as hexane and ethyl acetate) are often used so that small changes in the polarity can be made by changing the ratio of the two solvents.



Procedure:

- In order to get the compound onto the TLC plate, it needs to be in solution. If the compound you want to analyze is not in solution, you will need to make one using one of the small bottles in your

drawer. It doesn't matter what solvent is used to make this solution, since it evaporates within seconds of applying the spot to the plate – the only important thing is that it dissolves the compound. Only a small amount is needed, so the solution should be fairly dilute. One or two drops of a liquid compound or the end of a spatula of a solid compound mixed with 2/3 of a bottle of solvent usually makes a solution of appropriate concentration. If the solution is too weak, you won't be able to see the spots well. If the solution is too strong, the spots will form a long streak that will be difficult to interpret.

- TLC plates can be obtained from the box on my desk. Hold the plates by the edges – the coating flakes off easily, and fingerprints are visible under UV light. With a pencil (not a pen) lightly draw a line across the bottom of the plate, about a centimeter from the edge. Don't press too hard or you may scrape off the coating and ruin the plate. Also, if you make the line too close to the bottom you won't be able to add enough solvent for a good analysis. Make 2-4 small hash marks on your line depending on how many spots you want to analyze. They should be evenly spaced and not too close to the edges. Label them so you know which is which.

- Then take your 50 ml beaker and add about 5 ml of the developing solvent. Before spotting the plate, you may want to stick it into the beaker to check your solvent level; the solvent should come about half way to the line you have drawn – if not take some out or add some more. Also, the TLC plate should not touch the sides except at the very top – if it does, use your 100 ml beaker. Remove the plate and cover the beaker with a watch glass to slow the evaporation of the solvent. (Once you gain experience, you probably won't need to check the height of the solvent every time.)

- Take a capillary tube from the beaker and remov the cleaning solvent by spotting it on the paper towel. Then dip it into the solution you want to analyze, and then touch it quickly to the plate where your hash mark crosses the line to make a spot. The smaller the spot, the better it will be. Clean the capillary by spotting it on a paper towel two or three times, dipping it into the beaker of cleaning solvent, and spotting it again. (Don't hold the capillary tube in the solvent - you want the compound to end up on the towel, not in the beaker).

- There are a few compounds which are colored – you should be able to see these spots under ordinary light. Many other organic compounds absorb UV light. The property which usually determines this is the number of conjugated C=C. The more conjugated C=C, the lower the wavelength of light will be absorbed – those with at least three absorb UV light, while those with many absorb visible light. Our TLC plates have been treated so that you can see compounds which absorb UV light when the plate is placed under a UV lamp. After you have spotted your plate, look at it under the lamp (on the short wave setting) to make sure that you can see the spots. (UV light causes sunburn – don't look into the light or let it shine on your fingers for an extended period of time, and turn it off when you're done.)

- Now it is time to develop the plate. Place it in the beaker and cover the top with a watch glass. Do not pick up or jostle the beaker while the plate is developing. The solvent should flow up the plate by capillary action. It should go up in a straight line - if you observe it going up one side faster than the other, your plate may be difficult to interpret. It will carry the compounds in the spots with it – but the less polar spots will travel faster than the more polar spots. When the solvent is nearly at the top, remove the plate and draw a line showing how far up the solvent went.

- Visualize the plate under UV light. Draw a light pencil outline of each of the spots, following the actual outlines as best you can. Then take the plate away from the light and analyze the spots to see what you can determine.

- Whenever you take a TLC, draw a copy of it in your lab notebook. Lay it on the book and copy it as accurately as you can, the same size as the real TLC plate. Label the spots, and write down the solvent that you used to develop the plate. Then analyze what the plate is telling you.

A quick version of TLC steps :

1 - Make a solution of the compound (if it isn't already in solution)
2 - Draw a line on the bottom of the plate; draw hash marks and label them
3 - Add developing solvent to the beaker; check the level of the liquid against the plate
4 - Spot the plate; check the spots under the UV lamp
5 - Develop the plate with the watch glass covering the jar without moving the jar
6 - Visualize the plate under UV light; draw an outline of all spots
7 - Analyze the plate; copy it into your notebook