7.3. Thin layer Chromatography (TLC) Guide

Overview:

Thin Layer Chromatography (TLC) is an extremely useful technique for monitoring reactions. It is also used to determine the proper solvent system for performing separations using column chromatography. TLC uses a stationary phase, usually alumina or silica, that is highly polar (standard) or non-polar (reverse phase). The mobile phase is a solvent whose polarity you will choose. In 5.301, and in most lab applications, you will use standard phase silica plates. You will apply your reaction mixture in solution to the plate and then "run" the plate by allowing a solvent (or combination of solvents) to move up the plate by capillary action. Depending on the polarity of the components of the mixture, different compounds will travel different distances up the plate. More polar compounds will "stick" to the polar silica gel and travel short distances on the plate. Non-polar substances will spend more time in the mobile solvent phase and travel larger distances on the plate. The measure of the distance a compound travels is called the R_f value. This number, between zero and one, is defined as the distance the compound moved from the baseline (where it was originally spotted) divided by the distance the solvent front moved from the baseline.

Reference:

For a thorough discussion see LLP pages 145-152.

Steps for TLC:

1) Cut TLC plates. Usually silica plates are bought as square glass pieces that must be cut using a diamond tipped glass cutter and following a template. Before scoring the glass, use a ruler and a pencil to lightly mark baselines on the silica side of the plate (be careful not to remove any silica from the plate). Using a sharp glass cutter and a ruler as a guide, you should have no problem scoring the glass. Once the entire plate is scored, you can then break the glass into individual pieces. (In the beginning this may be frustrating, but after some practice, you should become comfortable with this technique.)

2) Determine an appropriate solvent system. Your compounds will travel different distances up the plate depending on the solvent you choose. In non-polar solvents like pentane and hexane, most polar compounds will not move, while non-polar compounds will travel some distance up the plate. In contrast, polar solvents will usually move non-polar compounds to the solvent front and push the polar compounds off of the baseline. A good solvent system is one that moves all components of your mixture off the baseline, but does not put anything on the solvent front - R_f values between 0.15 and 0.85. This is not always

possible, but should be your goal when running a TLC. (For column chromatography the correct solvent system should give an R_f between 0.2 and 0.3.) Now, which solvents to pick? Here is a list of some standard solvents and their relative polarity (from LLP):

Very polar additives:
Methanol > Ethanol > Isopropanol
Moderately polar additives:
Acetonitrile > Ethyl Acetate > Chloroform > Dichloromethane > Diethyl Ether > Toluene
Non-polar additives:
Cyclohexane, Petroleum Ether, Hexane, Pentane
Common solvent combinations:
Ethyl Acetate/Hexane : 0–30% most popular combination, sometimes tough to remove solvents completely on rotary evaporator
Ether/Pentane: 0–40% very popular, easy to remove on the rotary evaporator
Ethanol/Hexane or Pentane: 5–30% useful for very polar compounds
Dichloromethane/Hexane or Pentane : 5–30% sometimes useful when other mixtures fail

3) Fill TLC chamber with 1–2 mL of the desired solvent system. Place a large piece of cut filter paper in the chamber as well.

4) Spot the compound on the baseline of the TLC plate. We will use commercial spotters, but spotters can be pulled from hot Pasteur pipets (you may see this in your UROP). If you are monitoring a reaction, make sure to spot the starting material, the reaction mixture, and a co-spot of both.

5) Run the TLC. Let the solvent go about 90% of the way up the plate.

6) Remove the plate from the chamber and mark the solvent front immediately with a pencil. You will use this to calculate the R_f.

7) Let the solvent dry off of the plate.

8) Visualize the TLC using non-destructive technique(s). The best non-destructive method is the UV lamp. Place your plate under the UV lamp and circle any UV active spots with your pencil. Although we won't do this in 5.301, another popular non-destructive method is staining with iodine. (You might see this in your UROP.)

9) Visualize the TLC using a destructive method. This will be critical for compounds that are not UV-active. There are several varieties of stains that are very useful and will be available to you in 5.301. To use the stain, pick up the dried TLC plate with a pair of tweezers and dip it into the stain, making sure to cover the area from the baseline to the solvent front. Completely dry the back of the plate with a paper towel. Place on a hot plate and watch the development of the spots. Remove the TLC plate from the heat once the spots are visible and before the background color obscures the spots.

9) Revise your choice of solvent system based on the results of your initial TLC. Make the solvent system more polar if you want a larger R_f or make it less polar if you want to decrease the R_f. Also, if there is "streaking" of your compound on the plate - basically you see large streaks instead of sharp circles - your sample is probably too concentrated. Try diluting your sample and running the TLC again. If this doesn't work, you will have to move to a different solvent system.

10) Label your TLC, calculate the R_f for each spot and draw a picture of it in your notebook.