

## Thin Layer Chromatography Basics

It is expected that you will read up on Thin Layer Chromatography and Column Chromatography. The following is a reasonably good site for Thin Layer Chromatography.

<http://orgchem.colorado.edu/Technique/Procedures/TLC/TLC.html>

The following is a reasonably good site to get some background on Column Chromatography, which is a related topic and Column Chromatography may be carried out next week. .

<http://www.youtube.com/watch?v=EytuRMS1154>

You should record the chromatography techniques in your lab note book as well as the synthetic procedure you have researched. . Take good notes in lab lecture and when studying any references you use.

### **Thin Layer Chromatography - A Brief Description and General Instructions**

1. Obtain a Thin Layer Chromatography (TLC) sheet from the dessicator. This sheet will be about the size of an iphone, but much thinner. One side is a very thin coating of silica gel (a very polar stationary phase) that is in a binder. The silica gel is impregnated with a fluorescent compound that will glow green or orange depending on the compound impregnated. When exposed to UV light, the plates will look sort of like those glow sticks and necklaces kids have in the summer. One side is white and sort of rough, the back side is plastic or aluminum. You will do your work on the rough side.
2. You should draw a line with a pencil very lightly about 1 cm up from the lower edge of the TLC plate. This is the origin. See the diagram below.
3. You need to prepare standards. Take a spatula tip of each of the starting materials in a vial and add about 1 mL of dichloromethane. This is sort of an estimate of the solid and solvent. You should also make a small solution of your reaction mixture/crude product. If it is liquid, you should take a drop or two and dissolve it in about 1 mL of dichloromethane. Again, the solutions are just approximate. The goal is to study the components of the reaction vs. the starting materials. New spots observed after elution and visualization indicate product, whereas spots seen at the same location as the starting material standards indicate incomplete reaction.
4. The standards and the reaction mixture should be spotted on the TLC plate as indicated on the diagram below. The idea is to use a different capillary pipet (these will be available in the lab ) for each compound. You dip the capillary into the solution being spotted and it will enter the capillary via

capillary action (no pun intended) and then it is a good idea to blot it a bit on a paper towel so you don't get a big blob of liquid on the end of the capillary. Then, lightly spot the solution on its mark on the TLC plate. The idea is to spot it and get the smallest spot possible. Then reapply it several times going for a small concentrated spot. You need to let the dichloromethane evaporate between each application. You will do this for the standards and the reaction as diagramed. It may take you a couple plates to get the hang of it. It is important for all members of your group to get the hang of this technique.

5. Realize it is really important to spot about a cm up from the bottom of the plate, to keep the spots about a cm apart and to spot in from the edge of the plate about a cm as diagramed.
6. **Preparing the Eluting chamber.** We will use a makeshift eluting chamber that will be either a 250 mL or 400 mL beaker with about a 0.5-1.0 cm of dichloromethane with about 1.0 mL of methanol added (eluting solvent). You need to use a piece of filter paper as diagramed below to saturate the beaker's atmosphere with the eluting solvent. Use a watch glass to cover the chamber.
7. **Eluting the TLC plate.** Dip your TLC plate in the solvent. It is important that the solvent does not immediately touch your spots. If it does, you need to adjust the level of the eluting solvent or spot a new plate. The former is preferable. The plate should be dipped, but also resting against the wall of the beaker. The back side toward the beaker, but on the opposite side of the beaker from the filter paper. The solvent rises up the plate by capillary action. It will take about ten minutes. In this process, there is a competition for the compounds by the silica gel on the plate (very polar – the stationary phase) and the solvent system (varying degrees of polarity, but less polar than the stationary phase – this is the mobile phase). The compounds are in equilibrium between the stationary phase (the silica) and the mobile phase (the solvent). If the compound is extremely polar it will spend most of its time interacting with the silica gel and it won't move much. If it is of very low polarity (provided it is soluble in the eluting solvent), it will spend most its time in the solvent and will move way up the plate. Compounds move to varying degrees depending on their polarity. Compounds that are the same, will elute to the same point on the plate, so your standard lanes will help you identify compounds in the reaction mixture.
8. When the solvent is about a cm from the top of the plate, you should remove the plate and lightly mark the level of the solvent. This is called the solvent front and its level is significant. Let the solvent completely evaporate off in the hood. The spots are visualized (most of the compounds we are working with are white and are not readily visible) with a UV lamp. Be careful not to expose your hands or your neighbors to the UV lamp. The background will

fluoresce as previously described and your compounds should show up as blue or black spots in the background. Lightly circle them with a pencil. After this, you will more permanently visualize the compounds with the p-anisaldehyde stain. This entails dipping your plate into the stain with forceps and then, blotting the excess stain off with a towel. The plate then needs to be baked in an oven at about 100 °C for ten minutes or so. Be careful with the stain as it contains strong acids. Warfarin and derivatives stain purple, 4-phenyl-3-buten-2-one stains red and 4-hydroxycoumarin does not stain at all (white). This is very useful to tell what is going on. What would you expect on the first week? If it did not occur to you earlier, 4-hydroxycoumarin and 4-phenyl-3-buten-one are the starting materials that need to be spotted on the plate, flanking the reaction mixture. What would you expect next week?

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