

Paper Chromatography and Thin Layer Chromatography

Paper chromatography, thin layer chromatography, and dye electrophoresis are used to separate solutions into their components. Electrophoresis uses electricity whereas paper and thin layer chromatography use the relationship between molecules and how much they want to be with the mobile or stationary phases. In the paper and thin layer chromatography lab, we will be doing similar exercises with different stationary phases.

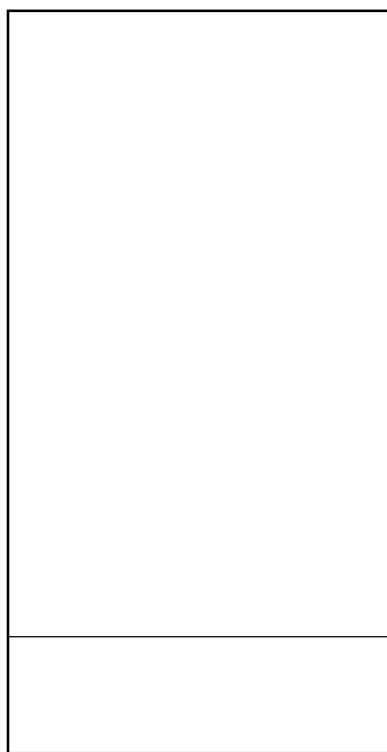
To do:

1. Get a piece of silica gel TLC plate and a piece of filter paper.
2. Measure about 1 cm from the bottom of the TLC plate and the paper. Lightly draw a line. Be careful to not chip the TLC plate. The line is just a guide- it is not required for the dyes to be separated; it is there so that you start each dye at the same distance from the bottom of the plate or paper. Make a very light dot or X so you can plan where you will put your dots. You'll be loading 5 samples- try to space them out equally along the line.
3. Load a microcapillary tube with a sample. You should practice releasing the sample before you actually do it on the real lab materials. There are extra filter paper circles you can use to practice.
4. Gently tap the microcapillary tube onto a spot on the line you drew on the TLC plate or filter paper. Do not hold the microcapillary tube there too long because you want to make as small of a dot as possible.
5. Wait a moment for the spot to dry. Reapply the sample to the same dot. We are trying to make a very small, concentrated dot. If you can, dot the same spot 3 times.
6. Switch people in the lab group so another person has a chance to do this. You should be marking a TLC plate and a piece of filter paper the same way.
7. Continue adding dots until you have loaded the following samples on your TLC plate and filter paper:
 - a. Sanfranin O, SO
 - b. Orange G, OG
 - c. Xylene Cyanol, XC
 - d. Bromophenol blue, bpb
 - e. Torani syrup, berry
8. Each person in the lab group should load at least one dye to the TLC plate and to the filter paper
9. Put some 70% isopropanol in a beaker. Make sure the height of the alcohol is not above the line you drew and therefore not above the spots you put on your TLC plate or filter paper.
10. The TLC plate will support itself in the beaker- it is ok to let it tilt slightly
11. In a second beaker, repeat putting in the alcohol. This beaker will be for the filter paper. If the filter paper is not supported it will slide into the alcohol and you will lose all of your samples to the alcohol. There are a few ways to support the filter paper: put a hole in the top of the paper, put a pencil through the hole, and hang the paper in the beaker; you may be able to put paper clips on the sides of the filter paper and support it in the beaker by putting the paper clips on the edges of the

beaker; you may be able to problem solve something that works better than either of these two ideas.

12. Do not put the TLC plate and the filter paper in the same beaker because the filter paper will wick itself to the TLC plate.
13. Let the alcohol and the samples migrate as long as possible. When you remove the plate or paper from the beaker, lightly draw a line where the edge of the solvent ran. If you forget to do this right when you remove the strips, you may lose the opportunity because the alcohol evaporates fairly quickly.
14. To clean up: if there is no color in your alcohol, please put it in the used 70% isopropanol bottle. Wash out the beakers and dry them. Put the microcapillary tubes in the "used microcapillary tube" beaker. If we can reuse them, we will in future years.
15. While the dyes are migrating, you can make the 1x TAE buffer. You can also weigh out the agarose so you can then make the 50 mL of 0.7% agarose solution (in 1x TAE). Do NOT put the agarose in water- make sure you use buffer!

This end is toward the solvent front gets up of the solvent.



top of the container- when the here, take the paper / TLC plate out

Don't put alcohol samples to go

above the line- you do not want your swimming in the alcohol.

This part of the paper

or TLC plate goes in the alcohol.

The line at the bottom represents the 1 cm line. You'd put the 5 samples on that line- evenly spaced out.