Thin Layer Chromatography

Chromatography is a group of techniques used to separate compounds. Separation is achieved by partitioning the substrates between a mobile phase and a stationary phase. The mobile and stationary phases will have different affinities for different substrates. As the substrates and the mobile phase move though the stationary phase, some substrates will stick to the solid phase more and a separation will be achieved.
Please visit the following web sites for more information on chromatography.
http://www.doggedresearch.com/chromo/chromatography.htm
http://www2.volstate.edu/chem/1120/Labs/TLC.htm


The TLC developing chamber

Thin Layer Chromatography
In thin layer chromatography a relatively thin layer of a solid stationary phase (typically silica gel or alumina) is spread over a solid support such as a glass plate or a plastic sheet. When I was a student, we had to make our own TLC plates using microscope slides and a silica gel slurry. We would spread he slurry over the plates and allow it to dry. When it was dry, we would hopefully be left with a thin even layer of gel spread across the glass slide. In reality, the layer was often not perfectly even but the results were still good. In this class, I buy our TLC plates pre made and our plates have a UV sensitive dye in them. Substrates that have aromatic or other UV sensitive functional groups will show up under UV light.

The purpose of our experiment will be to identify the contents of an unknown analgesic tablet. Please bring in an analgesic tablet to give to another group as their unknown. Please stick to analgesics with aspirin, caffeine, ibuprofen, or acetaminophen. Please prepare you notebook to take data.

Procedure
1. Obtain a capillary tube for use as a TLC spotter. You may have to create your own by "pulling" a borosilicate glass pipette. Obtain a TLC plate from your instructor.
2. Draw a very light line in pencil approximately 1 cm from the bottom of your TLC plate. Be careful not to damage the silica gel on the plate. This is where you will be spotting your compounds. Make spots along this line for each of the samples you are testing. In this case five.

3. Dissolve some of your mixture to be analyzed in a small test tube with a little solvent. (You want to choose a solvent which is relatively volatile and in which your compound is readily soluble. Your TLC solvent can be a good choice.) Dip your TLC spotter into the solution and allow capillary action to draw some of the solution into it. Remove any large drops of solution hanging from the edge and quickly touch the spotter to the TLC plate. The idea is to create a very small spot. In order to get the correct amount of material, I often apply material to the same place more than once. I dry the first spot by blowing gently and then spot again. The number of times you have to spot depends on the concentration of the sample, the type of TLC plate, the diameter of the spotter and probably a whole host of other things. I usually spot three times as a general rule.

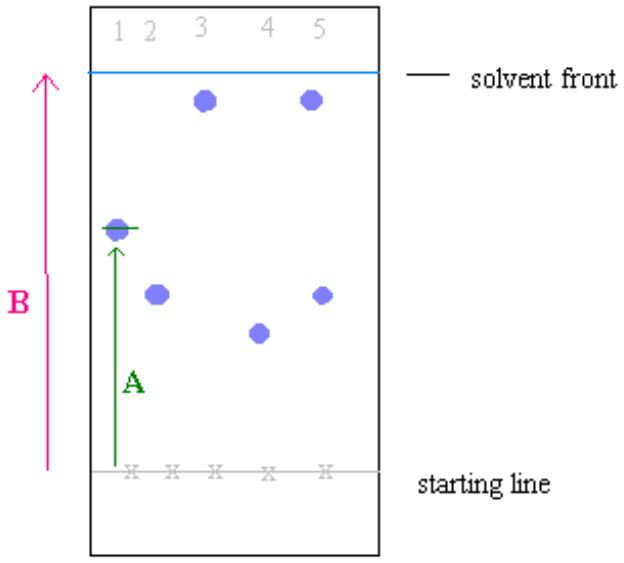
4. Setup your developing chamber by adding your developing solvent (In this case, 95% Ethyl acetate: 5% acetic acid) to a depth just below 1 cm (approximately 3/4 of a cm). The filter paper on the side of the chamber helps keep the atmosphere on the chamber saturated with the solvent and decreases the effects of evaporation.

5. Put your TLC plate in the developing chamber with the top not touching the filter paper on the side. Let the solvent rise to the within a centimeter of the top of the plate. Do not let the solvent front reach the top of the plate.

Remove the TLC plate from the chamber and mark the solvent front with your pencil. Allow the plate to dry. (Let the solvent evaporate)

6. How can we see the spots? In this case we are using a UV dye in the silica gel. The aromatic groups in the painkillers will absorb the UV and so in the light box we should be able to see the spots. Take your plates to the light box and mark the spots with a pencil.

7. Measure the distance from the starting line to each spot. Measure the distance from the starting line to the line where the solvent traveled. Calculate and record the Rf for all spots you observed on the plate. Rf is the distance the spot traveled/distance the solvent traveled. Always include Rf values in your lab report. Draw a picture in your notebook of the TLC plate with the spots.



In this sample chromatogram, The Rf for the first spot is the distance A divided by the distance B. When measuring the spots, you measure to the center of the spot. If the spot is tailing, you measure to the center of the darkest area of the spot. If you spots are too large, you may have to repeat the TLV, spotting a lesser amount. In this sample, 5 is the unknown and clearly is a mixture of compounds 2 & 3.

Post lab questions:

1) Arrange the following in order of increasing Rf in TLC? 3-pentanone, 3-pentanol, pentane.
2) If all your Rfs were too large, what would you do to your solvent system to bring them down?
   - What solvent(s) would you try next?
3) What would happen if you spotted too much compound on your TLC plate?
4) One of the compounds we are examining today has a chiral center.
   - Which one has a chiral center?
   - Identify it.

Other Reading

A background on Analgesics:
http://www.chemheritage.org/EducationalServices/pharm/asp/asp08.htm

Mode of Action:
http://catsis.weber.edu/ewalker/Medicinal_Chemistry/topics/Analgesia_antiinflam/Analgesics_anti-inflamitory.htm