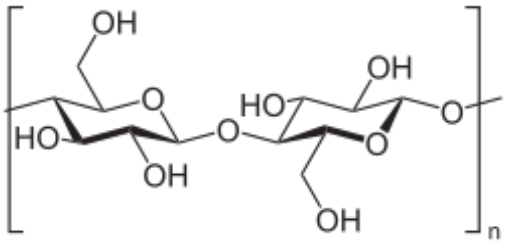
12AL Experiment 3 (3 days): Chromatography (Paper & Thin-Layer, TLC)

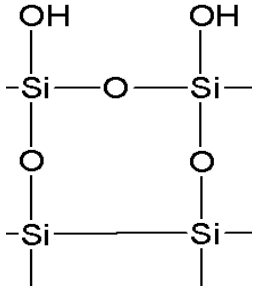
Safety: Proper lab goggles/glasses must be worn (even over prescription glasses). In addition, gloves are advised due to the use of organic solvents and handling of chromatography paper.

Background:

Chromatography is a physical separation of mixtures, utilizing the intermolecular attractions between molecules, a mobile phase, and a stationary phase. (*Review your General Chemistry 1A: dispersion, dipole-dipole, H-bonding). Paper, Thin-Layer, Gas, & Column Chromatography are some of the most popular forms of separation and you will gain experience with the first three over the course of the semester. Chromatography is a powerful form of organic chemistry as the components of mixtures, from simple mixtures of dyes & inks to more complex mixtures that constitute medicines & other drugs, can be easily separated and further identified.

In paper and thin-layer chromatography, mixtures are spotted onto a chromatography "plate" otherwise known as the stationary phase. The paper plate is composed of cellulose, while the TLC plate is composed of silica bonded to a plastic backing. Upon inspection of the structures of cellulose and silica respectively below, you should notice that they are both highly polar substances.





The plates are then "developed" by placing them in a beaker containing an appropriate eluting solvent, the mobile phase. The solvent travels up the plate via capillary action. When the solvent reaches the "spots" on the plate, those molecules that are more attracted to the solvent will travel with this mobile phase toward the top of the plate; thus separation is taking place. The eluting solvent is always less polar than the stationary phase – some solvents used may also be nonpolar. Your solvent choice depends on the mixtures to be separated – today the eluting solvents are already prepared for you eliminating the need for trial and error.

The idea is that the stationary phase and the mobile phase are of differing degrees of polarity – thus molecules will be separated over the plate surface. Highly polar molecules will be attracted greatly to the highly polar plate and will not move much from the origin line, resulting in little distance traveled, a low Rf value. In contrast,

less polar molecules will travel with the less polar solvent up the plate, resulting in more distance traveled, a high Rf value.

The plates must be removed from the solvent before the solvent reaches the end of the plate (about 1cm from the top). If the solvent travels to the very edge, molecules will continue to be pulled up and will begin to collide with each other.

The plates are then visualized in a variety of manners; in paper chromatography with felt pens, the individual molecules seen will be colored; in TLC chromatography with drugs, the experiment will utilize UV light and Iodine Chambers to view the individual molecules present in the drug mixtures.

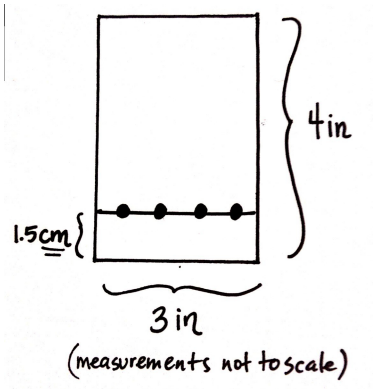
Objective: 1. To learn all aspects of paper and TLC chromatography. 2. To separate felt-pen inks into their individual colored components (different manufacturers use different combinations of dye molecules to create their colored felt-pens.) 3. To separate known drugs into their individual active components. 4. To determine the identity of an unknown drug mixture by matching its chromatogram with one of your known chromatograms.

Procedure/Data:

Paper Chromatography

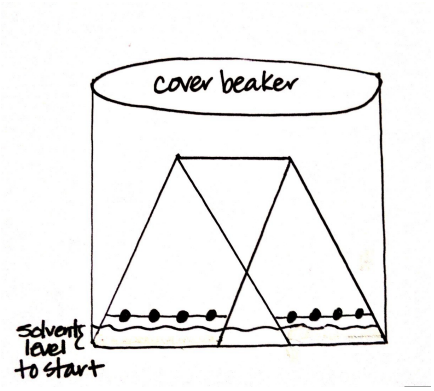
1. Obtain two paper chromatograms (~size = 4in x 3in); using a PENCIL, lightly draw an origin line 1.5cm from bottom of chromatogram

2. Spot the felt pens (ROYGBP, Blk1, Blk2) onto the paper (4 pens per chromatogram) – be careful to make spots that are "not too light", and "not too concentrated", as well as spots that are evenly spaced and of equivalent size.



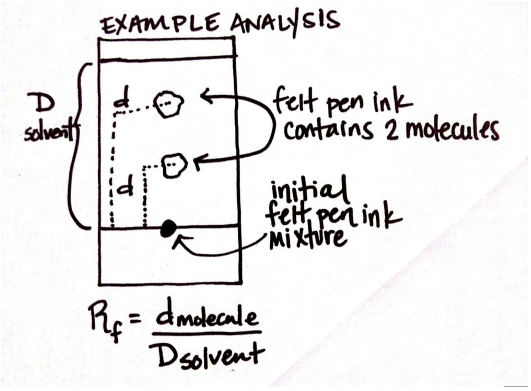
3. Use your pencil to label the identity of each spot.

4. "Tee-pee" your chromatograms in a beaker of appropriate size – chromatograms should not touch sides of beaker.



5. Very carefully, pipette in the eluting solvent (**1-butanol/water/ethanol/acetic acid; 120:40:20:1)** so that the SOLVENT height does NOT TOUCH THE SPOTS. It should be capillary action that draws the solvent up towards the origin where it will come in contact with the spots in order to start the migration upwards.

6. Remove your chromatograms when the solvent line (you will see the "wetness") is about 1cm from the top of the chromatogram – MARK line with a pencil.



7. Of all the molecules on the chromatogram, ONLY calculate the highest and lowest Rf values. (also write H and L on chromatogram as asked in postlab)

<u>Show the calculation here:</u>

High Rf Calculation: **Low Rf Calculation:**

8. Complete the following:

Red Ink Mixture	Number of Molecules in the ink?
Orange Ink Mixture	Number of Molecules in the ink?
Yellow Ink Mixture	Number of Molecules in the ink?
Green Ink Mixture	Number of Molecules in the ink?
Blue Ink Mixture	Number of Molecules in the ink?
Purple Ink Mixture	Number of Molecules in the ink?
Black #1 Mixture	Number of Molecules in the ink?
Black #2 Mixture	Number of Molecules in the ink?

9. Attach paper chromatogram to this page.

TLC Chromatography

1. List names of all drugs spotted and their active ingredients before beginning.

Drug Name	**Active Ingredients**
1.	
2.	
3.	
4.	
5.	
6. Unknown Mixture	n/a

1. TOUCHING ONLY THE SIDES OF THE TLC SILICA PLATES, obtain two chromatograms (~size = 4in x 3in); using a PENCIL, very lightly draw an origin line 1.5cm from bottom of chromatogram. DO NOT MAKE A GROOVE IN THE SILICA!

2. You will be using double open-end glass capillary tubes to spot the known drug mixtures + unknown onto your TLC plates (the drugs have already been dissolved in a solvent to make spotting easier for you). Use a new capillary tube for each new drug mixture.

3. Dip the end of the tube into the liquid (capillary action draws a small amount of liquid up the tube), and GENTLY TOUCH the TLC plate to spot the liquid onto the silica. Again, do not press into the silica as it will cause an indentation! Your spots should not be overly large, and should be evenly spaced as in paper chromatography.

4. "Tee-pee" your chromatograms in a beaker of appropriate size – chromatograms should not touch sides of beaker.

5. Very carefully, pipette in the eluting solvent (**ethyl acetate/acetic acid; 200:1)** so that the SOLVENT height does NOT TOUCH THE SPOTS.

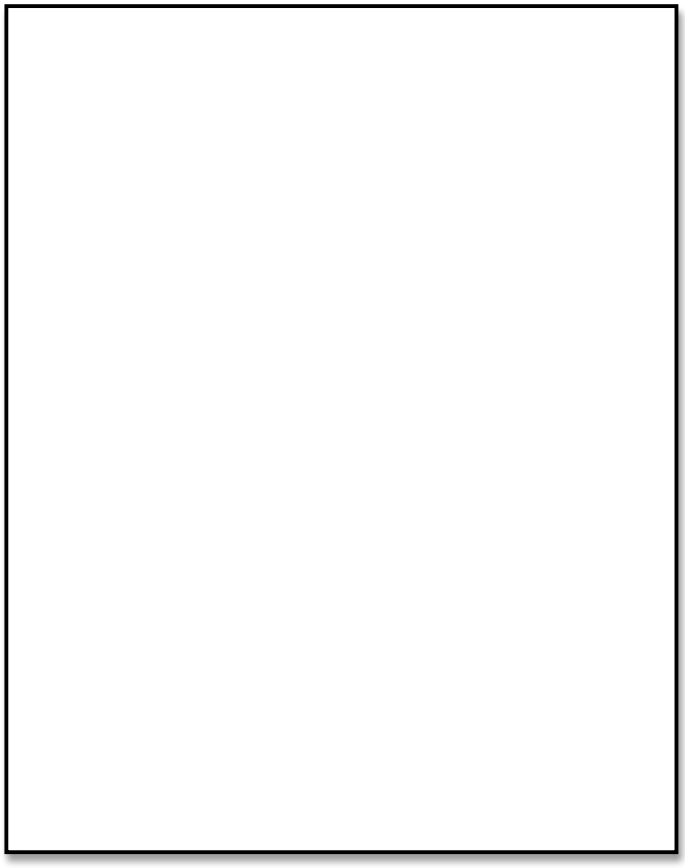
6. Remember, the movement of molecules will NOT BE VISIBLE like in the colored felt pen chromatography. You will visualize your molecules after developing the plates.

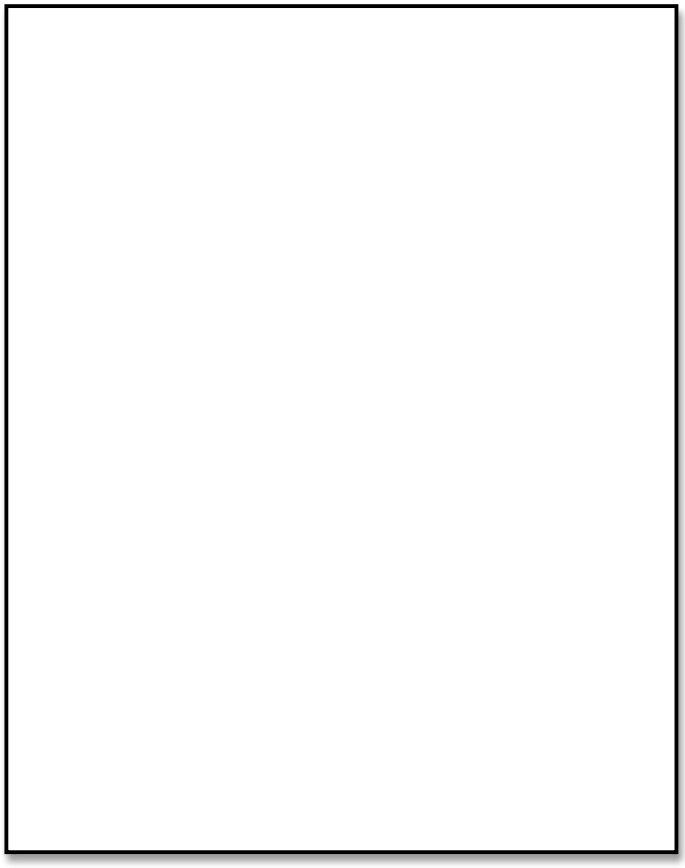
7. Remove your chromatograms when the solvent line (you will see the "wetness") is about 1cm from the top of the chromatogram – MARK line with a pencil.

8. Let your chromatograms dry.

9. To first visualize your dry plates, you are to put them under the UV lamp located in the DARK SCALEROOM (be careful!). Many compounds present in your drugs will glow. Mark them on your TLC plate with pencil. Do this AND also indicate the components on the diagrams below with a description of their glowing color – is it a bright blue? perhaps a dark blue?, etc… If you simply write they glow, then you aren't distinguishing different properties amongst chemicals and won't easily identify your unknown mixture.

10. After the UV lamp (*must be after – I_2 staining before will ruin the UV action), put your TLC plates in an Iodine chamber – the silica adsorbent should face down towards the subliming Iodine in order to stain it. After staining, record the color of staining on this sheet too. Again, is it a dark stain? Light stain? Size of stain? Etc…





11. What is the identity of your unknown?_____

12AL Prelab 3: Chromatography (Paper & Thin-Layer, TLC)

1. What is a mobile phase in Paper/TLC chromatography?

2. What is the stationary phase in Paper/TLC chromatography?

3. What is an adsorbent?

4. Silica plates are polar or nonpolar? Explain.

5. How do you calculate the Rf value of a spot on a chromatography plate?

6. Molecules with low Rfs are strongly polar or weakly polar? Explain why they have a low Rf (an explanation is not 'because it didn't move much')

7. What happens if you have too much solvent in your beaker and it covers the origin line? Be clear & specific.

12AL Postlab 3: Chromatography (Paper & Thin-Layer, TLC)

1. Which molecule in all your paper chromatography data had the highest Rf? Label it "H" on your chromatogram. (The Molecule is NOT the Ink – the original inks separate into individual colored molecules on the paper)

    a. What does this mean about its molecular polarity? Explain.

    b. What does this mean about its interactions with the paper? With the solvent? Explain.

2. Which molecule in all your paper chromatography data had the lowest Rf? Label it "L" on your chromatogram. (The Molecule is NOT the Ink – the original inks separate into individual colored molecules on the paper)

    a. What does this mean about its molecular polarity? Explain.

    b. What does this mean about its interactions with the paper? With the solvent? Explain.

3. Draw Lewis Structures (aromatic rings can be in line/bond form) and Label all Functional Groups (alcohol, carboxylic acid, ester, etc….) for the drugs: Aspirin, Acetaminophen, Ibuprofen, Salicylamide, and Caffeine.

4. Research and describe one simple chemical test (no litmus paper/ pH paper) that would distinguish between acetaminophen and ibuprofen. Explain.

5. Which of the molecules in question 3 would have the highest Rf. Explain.