University of Ottawa
CHM 1321 B

Experiment 1:
Thin Layer Chromatography

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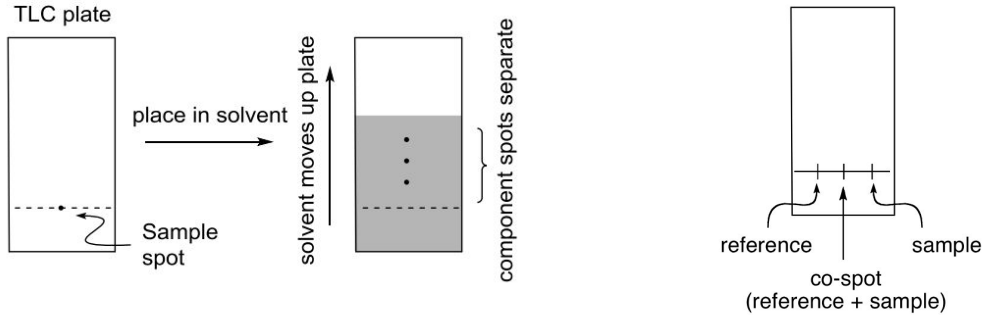
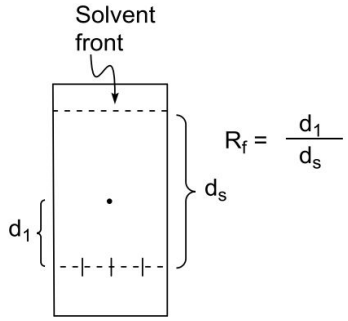
Introduction

According to the Lab Manual for Experiment 1 on Brightspace, Chromatography is a reliable method to prepare organic samples and to understand a product's affinity and ensure that the reaction reaches completion. Chromatography often uses a method known as Thin Layer Chromatography (TLC) that is used to monitor reactions. Usually this is done by preparing a plate and placing different compounds on the plate and see how far they reach the top of the plate.



The image above is obtained from the Lab Manual for Experiment 1 on Brightspace, it shows how samples are prepared onto a TLC plate and how they seperate after absorbing a solvent and viewed under a UV light.

When using this technique, it can be determine the polarity of the samples used in the TLC plate based on the distance the spot has travelled to the top of the plate. A mathematical relationship is used to determine the sample's polarity after it seperates



The image above is obtained from the Lab Manual for Experiment 1 on Brightspace, as stated above, sample's polarity is determined by calculating the difference in distance the sample has moved. This is represented by an R_f value. The R_f value is used to determine how polar, and therefore soluble, a sample is. The higher the sample moves onto the plate, the more polar the sample will be, whereas the lower the sample moes onto the plate, the less polar it will be.

Objective

-In this experiment, our first objective is to determine the R_f value of each TLC plate used in the experiment to determine which sample used in that part of the experiment to determine the polar properties of the compound as well as the unknown compounds used in the experiment.

-Our second objective is to determine the relative polarity of the unknown compounds that has been assigned using what is we know about TLC.
-In addition, this lab also serves as an introduction into chromatography to understand how the technique works and why it is used, therefore we also aim to understand how TLC works and the properties used to carry out a process using TLC.

Procedure

Part A.- Identifying Components of an Unknown Mixture using TLC

1. 5 ml of a 2:8 ethyl acetate and hexane solution is placed into two, medium-sized jars with covered lids.
2. Two TLC plates are prepared, a line is drawn at the bottom of the plate and three ticks are marked at the line (All TLC plates in this experiment are prepared this way).
3. A small tablespoon of our crystalline, unknown compound is dissolved into a solution of 2 mL dichloromethane in a small jar with a lid. A drop of this solution is carefully placed on both of the TLC plates' spot and the co-spot.
4. 1 mL of benzophenone is placed into a small jar, a drop of this compound is placed on the reference spot and the co-spot of ONE of the TLC plates
5. On the other TLC plate, 1 mL of biphenyl solution is placed into a small jar, and a drop of this compound is placed onto the reference spot on the OTHER TLC plate.
6. After placing drops of the benzophenone, biphenyl and the unknown compound onto the TLC plates, the two plate are carefully placed into seperate, medium-sized jars with the 2:8 solution and developed for 4 minutes.
7. The TLC plates are removed from their jars and dried for about 30 seconds.
8. The spots on the plates are observed with a UV ray light, a picture and drawing is taken, as well as the Rf value calculated for both these plates.
9. The medium-sized and small-sized jars are washed thoroughly to prepare for the next part of the experiment.

Part B.- Effect of a Solvent on TLC

1. 5 mL of 100% ethyl acetate is prepared into two, medium-sized jars.
2. Two new TLC plates are prepared, one TLC plate has the unknown compound placed onto the sample spot and co-spot, and benzophenone is placed onto the reference spot and co-spot. While another TLC plate has the unknown compound placed onto the sample-spot and co-spot and biphenyl placed onto the reference spot and co-spot.
3. The two TLC plates are then placed into two separate jars with 100% ethyl acetate solution and developed for 4 minutes
4. The TLC Plates are then removed from the jars and dried for 30 seconds, then observed under a UV ray light to obtain an image and drawing of the plates
5. The jars are washed to prepare for the next phase of the experiment

6. 5 mL of 100% hexane is prepared into two-medium-sized jars.
7. Steps 2-5 are repeated using 100% hexane instead of 100% ethyl acetate.

Part C.- Identification and Separation of Unknowns

1. An unknown compound is assigned to us to prepare a TLC plate for.
2. 5 mL of a 9:1 hexane and ethyl acetate solution is prepared into a jar.
3. Three TLC plates are prepared, the unknown compound is placed on all three of the plate's sample and co-spot, while the 3 different reference solutions are placed onto the TLC plate's reference spot spot and co-spot. In order, the reference solutions are o-bromonitrobenzene, m-bromonitrobenzene, and p-bromonitrobenzene.
4. All three TLC plates are developed in 3 separate jars for 4 minutes. A picture and drawing is taken for each one.
5. All materials used are cleaned and returned to proper place after experiment is complete.

Observations

Part A

- The assigned compound is known as #78. It is a solid, crystalline substance that was dissolved in water in order to perform chromatography
- The solvent solutions used to develop the TLC plates have a strong odor to them and are very volatile, they evaporate very quickly.
- The benzophenone and biphenyl solutions are also volatile, have a strong smelling odor, and evaporate quickly when placed on the spots of the TLC plate.
- The TLC plate with benzophenone as a reference had all the dots positioned at the same level near the top of the plate using a UV light, indicating a non-polar substance.
- The TLC plate with biphenyl as a reference had dots positioned at different levels but located near the top of the plate, which still indicated a nonpolar substance.

Part B

- The TLC plates developed in the 100% ethyl acetate solution were visualized using a UV light, the dots were positioned near the top of the plate.
- The TLC plate with benzophenone as a reference had all the dots positioned at the same level near the top of the plate.
- The TLC plate with biphenyl as a reference had all the dots positioned at the same level and near the top of the plate.
- The TLC plates developed in the 100% hexane solution were visualized using a UV light, the dots were positioned near the bottom of the plate.
- The TLC plate with benzophenone as a reference had all the dots positioned at the same level but at near the bottom of the plate.
- The TLC plate with biphenyl as a reference had the dots positioned at different levels but all appear near the bottom of the plate.

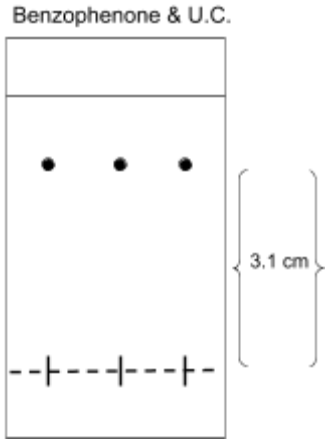
- In the pure hexane solution using the biphenyl as a reference, the solvent was absorbed significantly by the TLC plate, which placed the solvent line higher than where the dots are located.

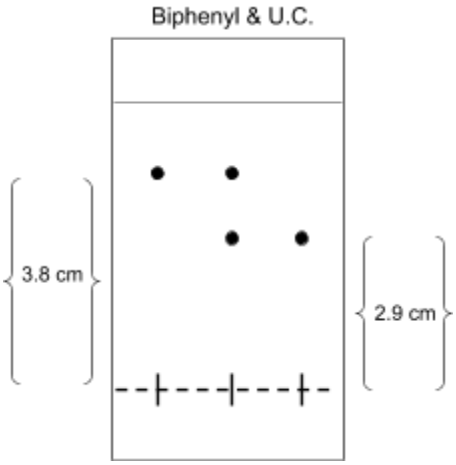
Part C

- The unknown compound assigned to us was known as compound D.
- 3 TLC plates were developed and visualized using a UV ray light.
- The TLC plate with p-bromonitrobenzene had the dots positioned near the top of the plate.
- The TLC plate with o-bromonitrobenzene had the dots positioned slightly lower than that of p-bromonitrobenzene, as indicated by a lower R_f value under Raw Data.
- The TLC plate with m-bromonitrobenzene had the dots positioned closer to the bottom of the plate.
- Comparing the R_f values of each of these bromonitrobenzene solutions, p, m, and o all share nearly the same R_f values,

Raw Data

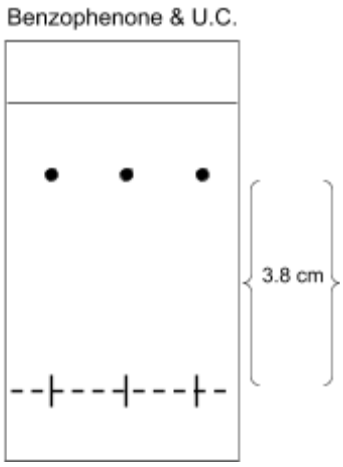
Part A

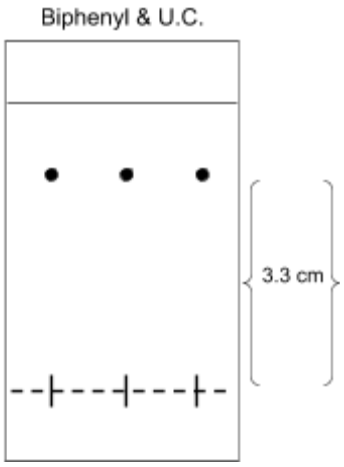




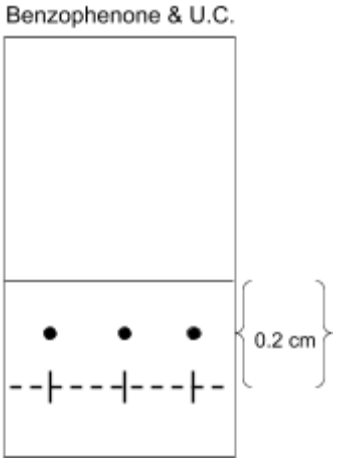
Part B

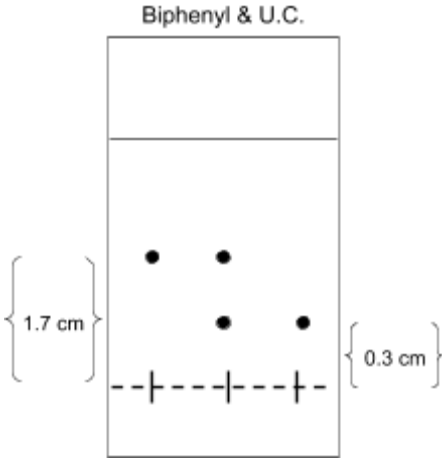
100% Ethyl Acetate



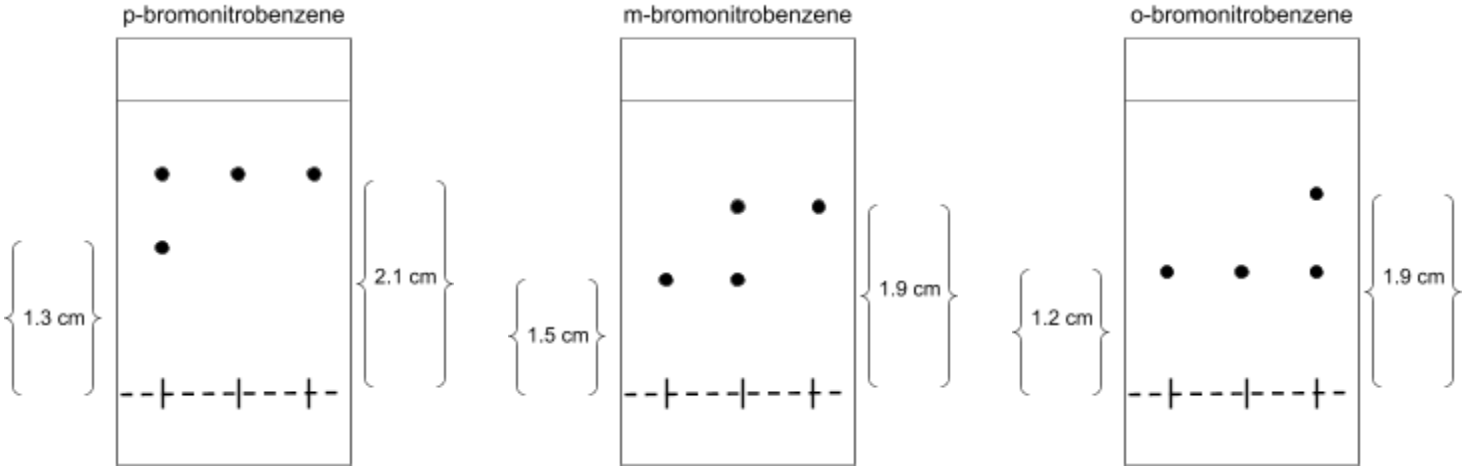


100% Hexane





Part C



Discussion

In Part A of the experiment it is observed that the unknown compound appears to be a non-polar compound, as indicated by a large R_f value of 3.1 for benzophenone and 2.9 for biphenyl. The position of the dots appear near the top of the two TLC plates, therefore, it is visualized that this compound would not have a high reactivity and be unable to bond to hydrogen due to being non-polar.

In Part B of the experiment, the use of 100% ethyl acetate, a polar solvent, enabled the unknown compound to move further up the plate, the same matter also occurred with the two reference compounds, benzophenone and biphenyl. The use of a polar solvent allowed us to visualize the unknown and reference compounds as very non-polar. The R_f value obtained for benzophenone was 3.8, and the biphenyl was 3.3.

Whereas the use of 100% hexane, a non-polar solvent, enabled the unknown compound to move further down the TLC plate, the same also occurring with the two reference compounds. The use of a non-polar solvent allows us to visualize the unknown and reference compounds as very polar, which alters the position of the unknown and reference compounds to appear as polar compounds when visualizing them. This likely indicates a great difference in polarity between the solvent and the unknown compounds. The R_f value obtained for the unknown for benzophenone was 0.2, and for biphenyl it was 0.3.

It is also important to note how in Part A and Part B of the experiment, the unknown compound and the benzophenone reference share similar properties in terms of polarity, their dots are at the same level in Part A and in Part B 100% ethyl acetate and 100% hexane. Whereas in comparison to the use of the unknown compound and the biphenyl reference, the biphenyl is always positioned lower than the unknown, which indicates that the biphenyl is more polar than the unknown compound. However, this is not observed in Part B with 100% ethyl acetate, likely because the polar solvent enabled the unknown compound or the reference compound to be less polar and match each other's dot position.

In Part C of the experiment, unknown compound (D) is positioned relatively near the top of the plate in each of the three TLC plates, indicating that it is a nonpolar compound as well. The solvent used (9:1 hexane: ethyl acetate) is relatively non-polar as well, which indicates that the dots were positioned further up the plate due the non-polarity. The R_f value in all three are very close, taking an average from all of them (1.9, 2.1, 1.9) obtains an R_f value of 1.9. This likely indicates that the three reference compounds are similar in terms of polarity, and this reflects the unknown compound as well.

Conclusion

Overall, we were able to obtain rather conclusive results that clearly reflect our understanding into Thin Line Chromatography in the form of R_f values that reflect the polarity of the compound as well as the compound ability to bond to hydrogen. The polarity of the reference and the unknown compounds used in the experiment were identified and allowed us to postulate onto why their position appears on the TLC plate as discussed in the Discussion. Lastly, we were also able to obtain a better understanding of chromatography in the process and how it works.

In terms of uncertainty in the data, since the only method to calculate the R_f value was visually and by the use of a ruler, it would be difficult to try and reduce this uncertainty. However, the qualitative observations gathered allow for more conclusive results that were discussed.

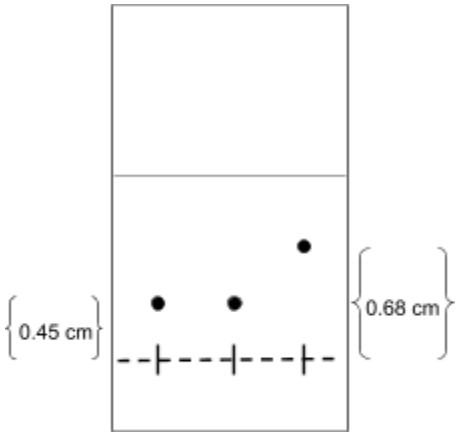
Answers to Questions

1. It is important to place the co-spot last after putting the sample and reference because a reaction may take place between the two mixtures in the co-spot that we would need to observe change positions.

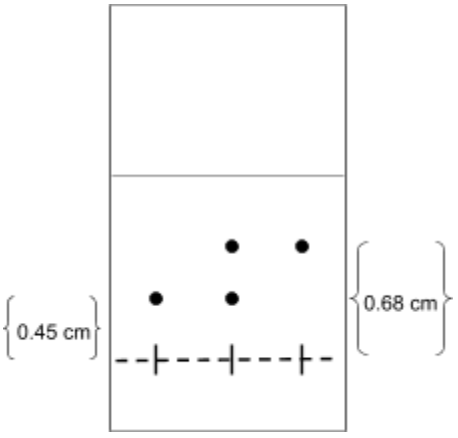
2. The increasing polarity of a solvent causes the compounds spotted on the TLC plate to be positioned further up the plate than where they originally where, making the compounds appear more non-polar due to the high polarity of the solvent.

3.

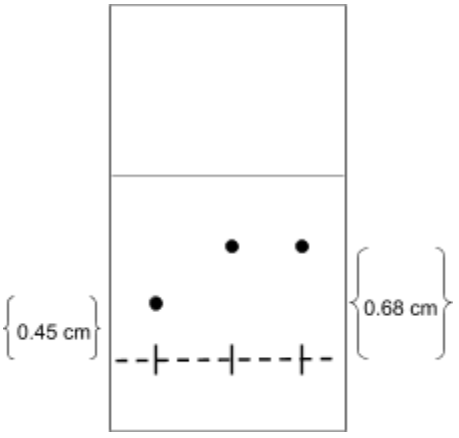
a. *Draw a picture of her TLC plate at the beginning of the reaction.*



b. *Draw a picture of her TLC plate after 50% completion (50% of the A molecules have been converted to B molecules).*



c. *Draw a picture of her TLC plate at the end of the reaction (all of the A molecules have been converted to B molecules).*

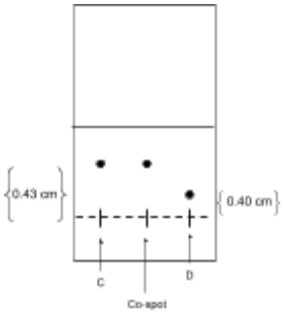


d. *Why is it better to use a sample of molecule A rather than molecule B to follow the reaction?*

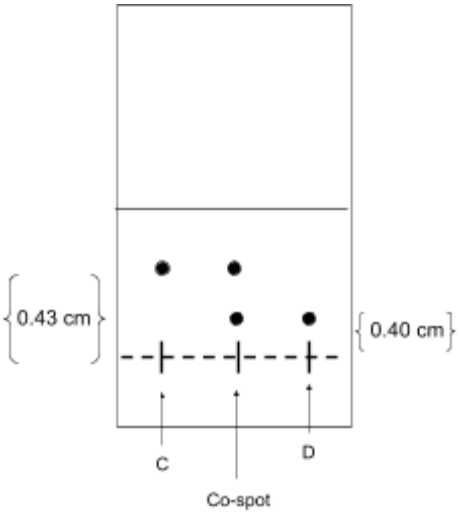
Molecule A is the reactant while Molecule B is the product, therefore it will be easier to follow a forward reaction (reactants -> products) and to observe how A forms into B.

4.

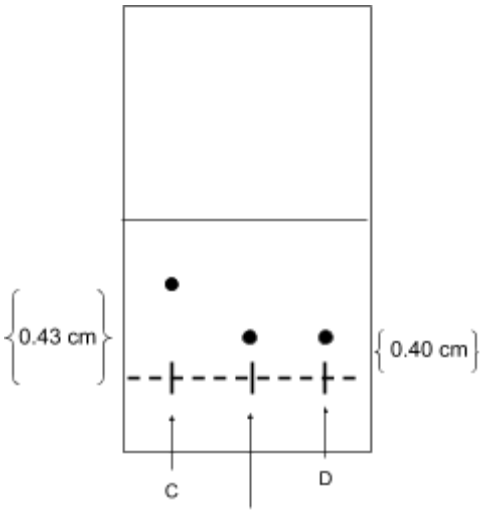
a.



b.



c.



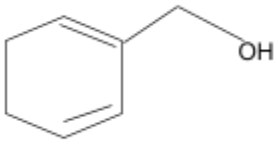
d.

The co-spot is important in order to differentiate the reference compound and the sample compound R_f values in order to compare the two if the dots were positioned close together, making visualizing difficult.

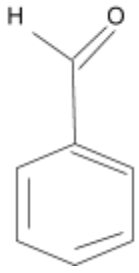
5. **For each of the following sets of compounds perform the following:**
    a. **Draw the line structure of each molecule.**
    b. **Arrange them in order of increasing polarity.**
    c. **Explain your reasoning in part b.**
        i. *Benzyl alcohol, benzaldehyde, benzyl acetate*

a.

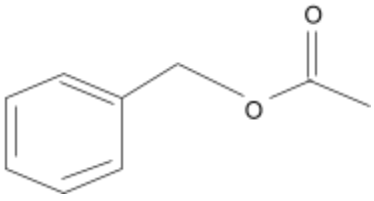
Benzyl Alcohol



Benzaldehyde



Benzyl Acetate



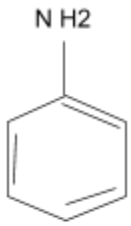
b. - Benzaldehyde, Benzyl alcohol, Benzyl acetate

c. -The polarity is ordered this way due to the number of hydrogen atoms attached to the carbon atoms of these structures. The Benzyl acetate has 9 carbons, and a total of 11 hydrogens. Benzyl alcohol has 7 carbons and 9 carbons and Benzaldehyde has only 7 carbons and 7 hydrogens

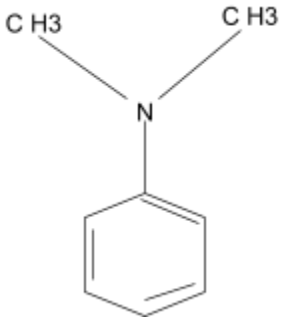
ii. *Aniline, N,N-dimethylaniline, naphthalene*

a.

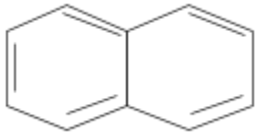
Amiline



N-N-dimethylaniline



Naphthalene



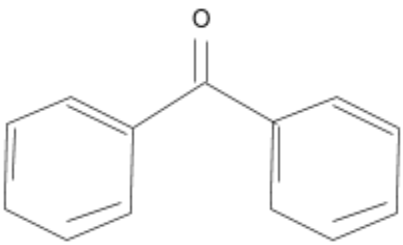
b. -Aniline, N-N-dimethylaniline, Naphthalene

c. -Aniline and N-N-dimethylaniline both have the least amount of hydrogen attached to the carbon ring, only 5. Whereas Naphthalene has the most attached to its two carbon rings, 8 hydrogen in total.

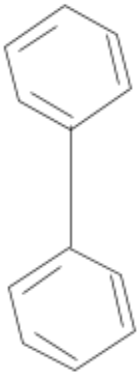
iii. Benzophenone, Biphenyl, Benzoic acid

a.

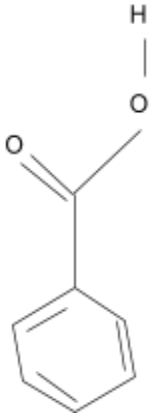
Benzophenone



Biphenyl



Benzoic Acid



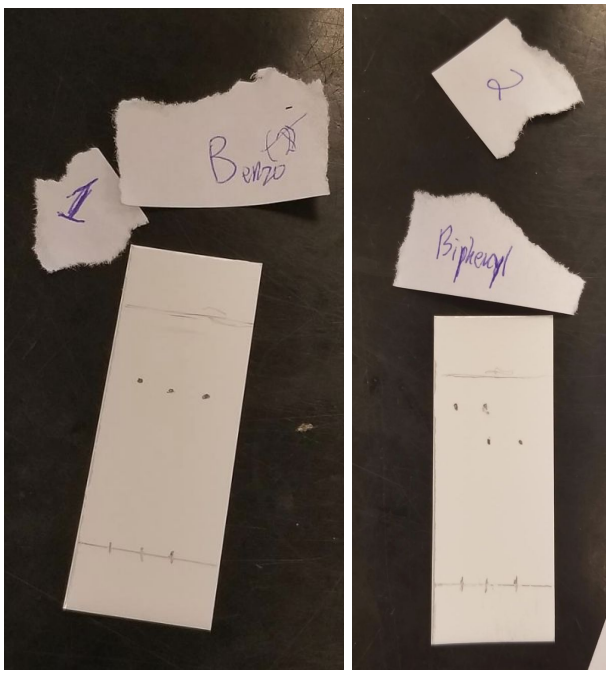
b. -Benzoic Acid is the least polar, the carbon ring has only 4 hydrogen. Benzophenone and Biphenyl are the most polar, as they have 2 carbon rings, and a total of 8 hydrogen.

c. -For each of the parts in question 5, the reason we are able to determine the polarity of these molecules is because of the number of hydrocarbons. The bonding of hydrogen to carbon is considered polar, so the more hydrogen is bonded to carbon, the more polar the molecule will be.

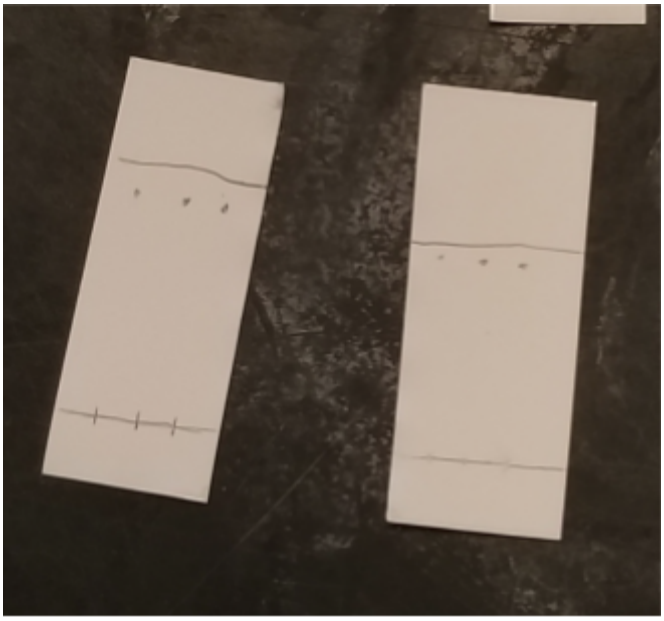
All of these molecules have benzene (a 6 carbon ring), each with 4-5 hydrogen on them that will aid in making the molecule polar. The more rings there are, or the less double and single bonds there are, allows for more hydrogen to bond and to achieve a higher polarity.

Appendices

1. TLC Plates for Part A - 2:8 Ethyl Acetate : Hexane Solvent

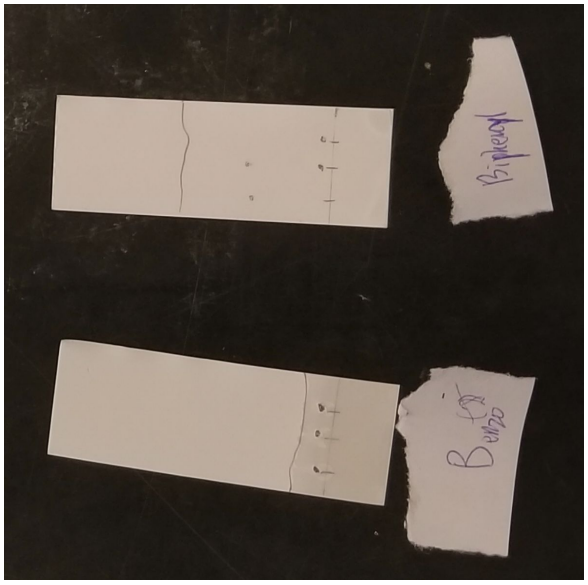


2. TLC Plates for Part B - 100% Ethyl Acetate Solvent

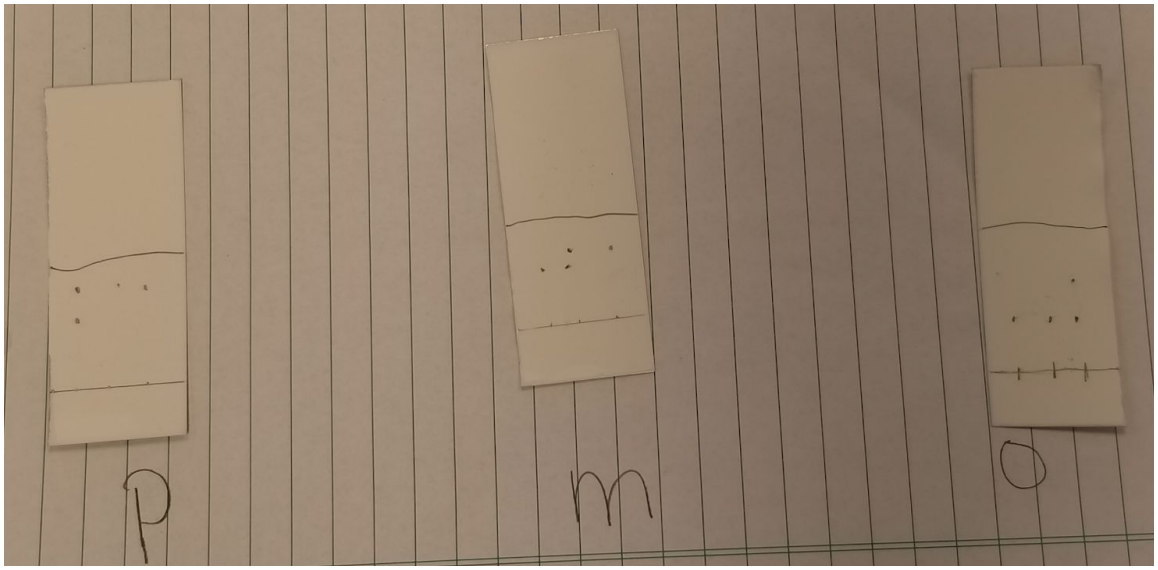


Benzophenone Biphenyl

3. TLC Plates for Part B - 100% Hexane Solvent



4. TLC plates for Part C - 9:1 Hexane : Ethyl Acetate Solvent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m
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