Experiment 1:

Thin Layer Chromatography (TLC)

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I.) Introduction

TLC also known as thin layer chromatography is a quick method used in separating organic materials as well as identifying organic compounds in solutions or mixtures and testing these organic compounds to see the extent of the completeness of its reaction, this method of separating organic compound is considered very efficient .

The experiment consists of a plate which in this experiment was made out of aluminium on one side silica gel on the other side. The silica gel side is the one used for separation of the organic compounds. This silica gel acts as a stationary phase in which the sample will dissolve in.

This experiment is divided into three parts. The purpose of Part A is to learn to identify the components of an unknown mixture, using Thin Layer Chromatography (TLC). We can thus observe the compounds that have migrated with the solvent by placing the TLC plate under a UV light, and ultimately identify each compound referring to their Rf value. In part B, we observe what happens when a different solvent is used to separate the same solution. In part C, we identify a new unknown mixture, this time containing two different compounds, which leads us to identifying the ratio of both compounds in the unknown solution with the help of image J.

II.) Procedures and Observations

Part A: Identifying the components of an unknown mixture using TLC:

Procedure:

- First off, we prepared two TLC plates using the suggested method in the protocol (refer to step 2 in part A). We drew a line approximately 0.5 cm or more from the bottom of our plates.
- Having placed three ticks on the drawn line, we then proceeded to use capillaries to spot each TLC plate with appropriate substances. (Refer to steps 5 to 7).
- The identification number of the unknown sample obtained is **sample #92**, and the two reference compounds we compared it to were benzophenone and biphenyl.
- After having spotted each TLC plate, we placed the TLC plates in separate jars contain the solution of 2:8 ETOaAc and hexanes (refer to step 8).
- However, we used 8ml of the 2:8 mixture of ethyl acetate (EtOAc) and hexanes in the developing jar rather than the 10ml suggested by the protocol in step 1, a precaution to assure that the solvent wouldn't pass the line.
- After having removed the plates and drawn the solvent front, we placed them under the UV light to circle the spots. (refer to step 9)

Observations:

Figure 1: Solvent system 2:8 mixture of ethyl acetate and hexanes with ref Benzophenone

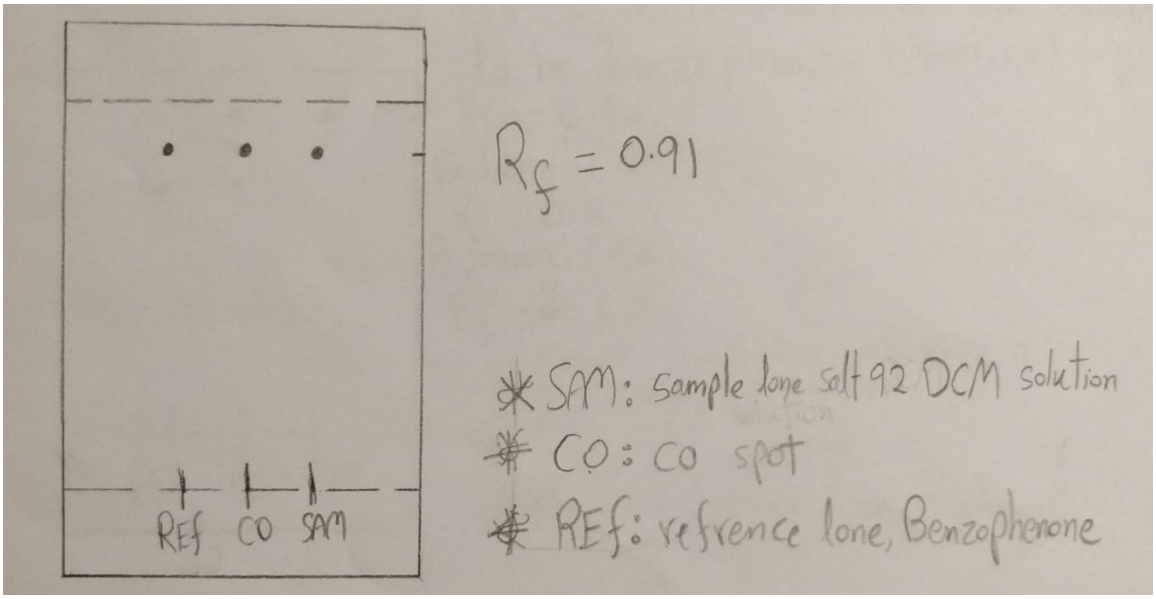
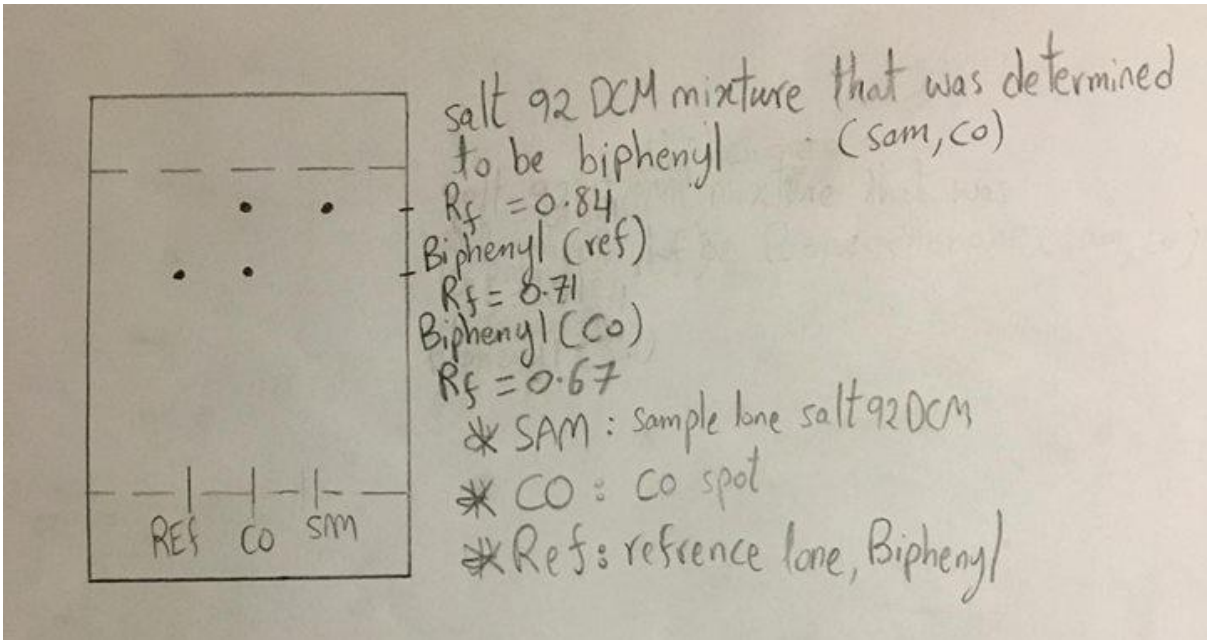


Figure 2: Solvent system 2:8 mixture of ethyl acetate and hexanes with ref Biphenyl



We then were able to compare the unknown solvent to the reference compound by comparing their Rf values:

For the plate with benzophenone as the reference compound:

- Sample: $R_{funkown}$= distance travelled by sample in cm (sample)/distance travelled by solvent in cm = 0.91
- Benzophenone: $R_{fbenzophenone}$ = 0.84

For plate with Biphenyl as the reference compound:

- Unknown substance: $R_{funkown}$ = 0.71
- Biphenyl: $R_{fbiphenyl}$ = 0.67

- The salt #92 obtained from TA was white in colour and had a crystal form
- The solution had a strong smell.
- When identifying the salt using TLC we noticed that the Biphenyl with the salt went higher than the benzophenone.
- The biphenyl reference also matched the sample Rf value in terms of height suggesting that the unkown compound is Biphenyl.

Part B: Effect of solvent on TLC

Procedure:

- After cleaning the two jars, we repeated the process of part A using the same sample and references compounds, but this time using pure **EtOAc** as the eluant. (Refer to steps 1 to 4 part B)
- Again, we repeat this process but using pure **Hexanes** as the eluant. (Refer to steps 5 to 7)

Observations:

Using pure ethyl acetate:

Figure 3: Biphenyl pure ethyl acetate solvent system

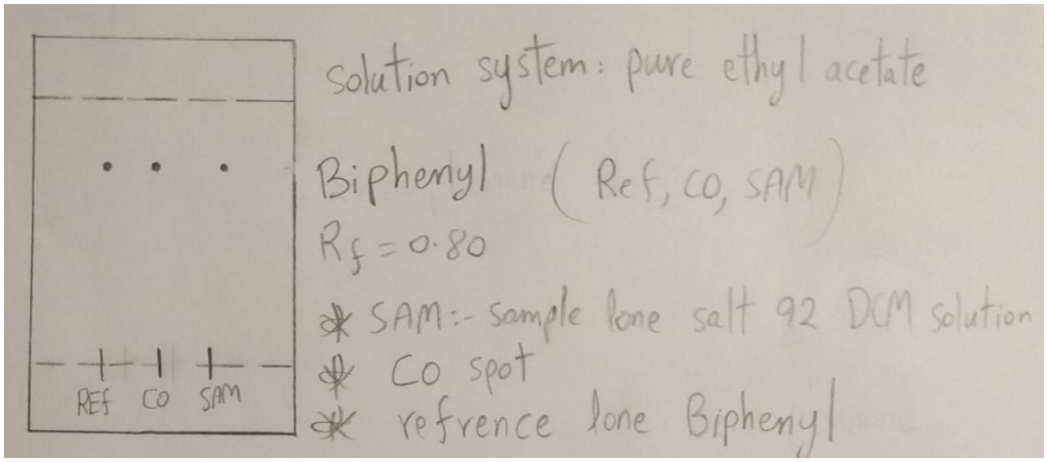
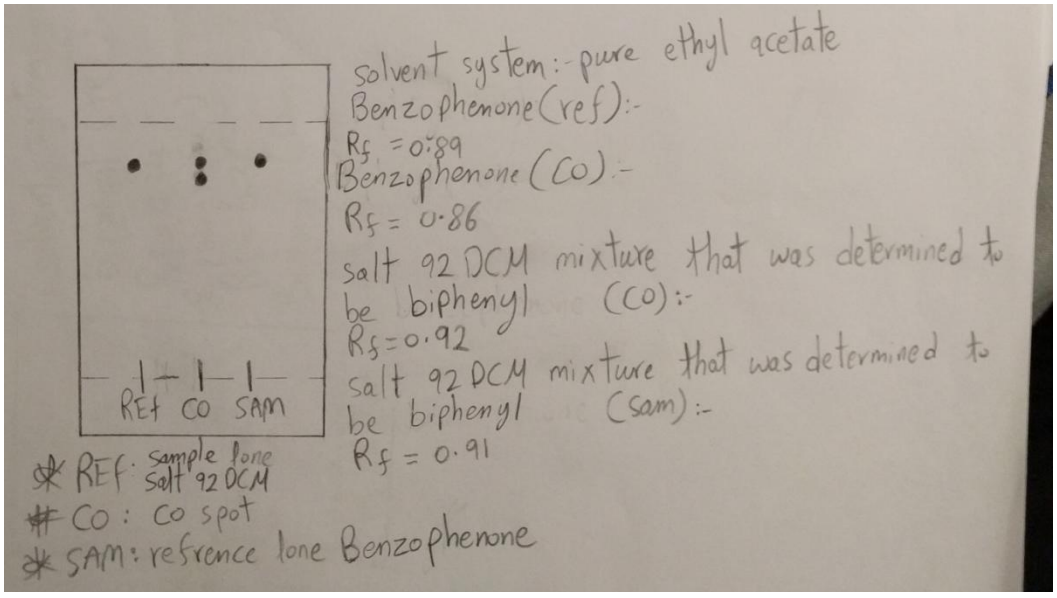


Figure 4: Benzophenone pure ethyl acetate solvent system



Observations:

Using pure Hexanes:

Figure 5: Biphenyl pure hexanes solvent system

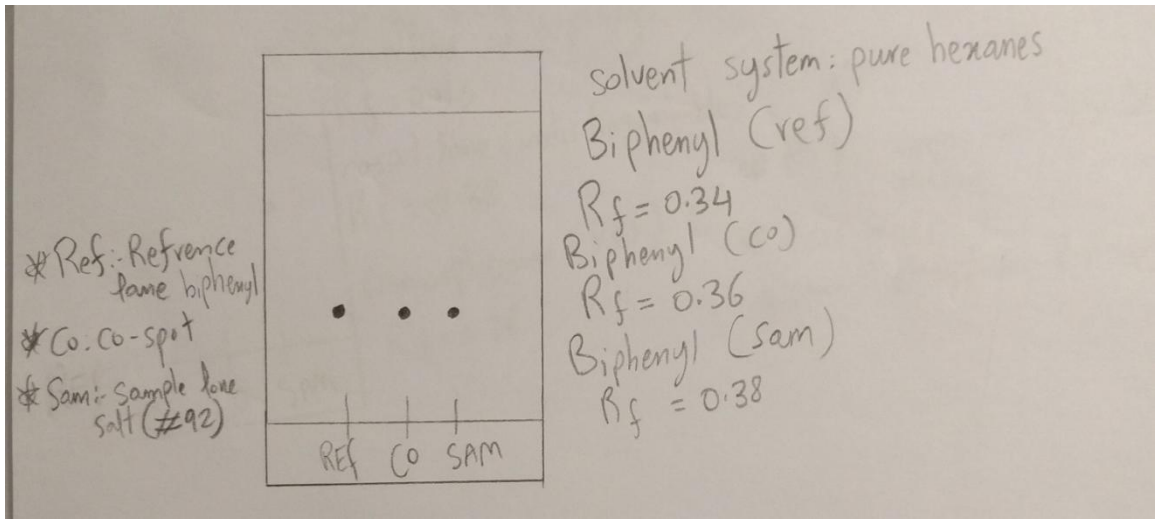
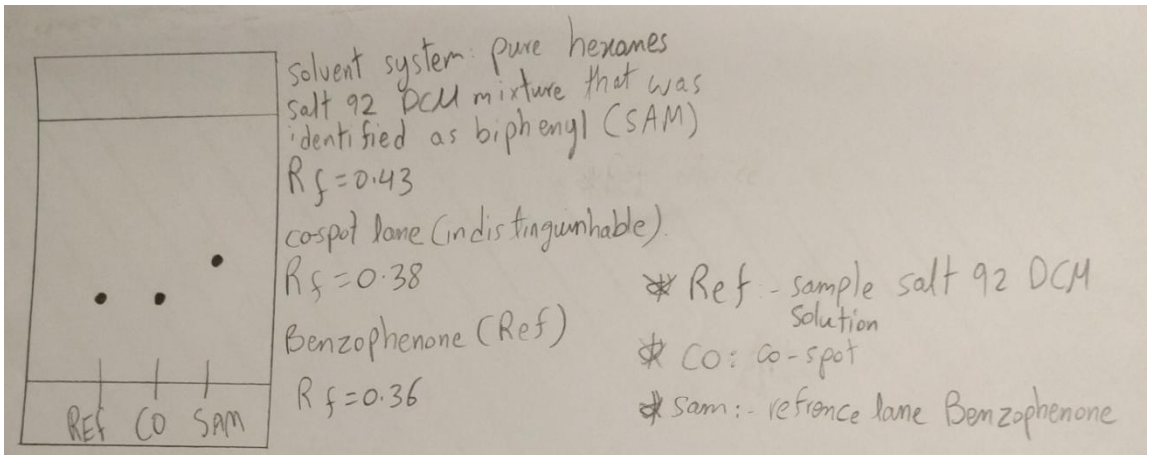


Figure 6: Benzophenone pure hexanes solvent system



For the plate with Benzophenone with EtOAc as the eluant:

- Sample: R_f = 0.91
- Benzophenone: R_f = 0.86

For the plate with Biphenyl with EtOAc as the eluant:

- Sample: R_f = 0.80
- Biphenyl: R_f = 0.80

For the plate with Benzophenone with Hexanes as the eluant:

- Sample: R_f = 0.43
- Benzophenone: R_f = 0.36

For the plate with Biphenyl with Hexanes as the eluant:

- Sample: R_f = 0.38
- Biphenyl: R_f = 0.34

- Both solutions are colourless and have a strong smell
- When dealing with pure ethyl acetate the biphenyl were all (sam co ref) similar to each other while in the benzophenone there was a separation of the compounds in the co-spot (lane).
- When dealing with pure hexanes the biphenyl had all similar spots in Rf values however in benzophenone there were different plots to each sample in the Rf values.

Part C: Ratio of organic compounds

Procedure:

- We repeat the process using different substances: we had the vial labeled **YY** as our sample, and we have three new refrence compounds: o-bromonitrobenzene, m-bromonitrobenzene, and p-bromonirtobenzene, and using 9:1 Hexanes and EtOAc as the solvent. (refer to step 1 and 2 of part C)
- Since there are two compounds in the new mixture, we will have two spots for the sample lane, each one corresponding to either: o-bromonitrobenzene, m-bromonitrobenzene, or p-bromonitrobenzene.
- Then using the UV light to detect the spots and mark them
- We then analyze the data using image J to calculate the area under the graph and find the percentage composition of the compound YY.

Observations:

- The YY solution was colourless and odour less.
- The bromonitrobenze's are considered toxic since they were kept under the fume hood
- After using the YY solutions and comparing it to the reference sample we noticed that m and p bromonirtobenze matched together at the same height while O-bromonitrobenze had a lower Rf value.
- This can indicate that O-bromonitrobenze is not a component of the YY mixture.

III.) Discussion:

In part A, we were able to deduce that salt #92 is crystallised Biphenyl. Referring back to figure 1, both spots on the sample and reference lane have the same Rf values of 0.91. This indicates that the sample lane has the same polarity as of that of Biphenyl (the reference) which can be used to conclude that salt #92 is crystallised Biphenyl. In addition, the co-spot lane is used to identify close spots on the sample and co-spot. It is made by marking the sample and reference on the middle lane, this can tells that if there is only one spot after the TLC process then it means that the sample and reference compound are the same. Also we can refer to figure 2 to see that the Rf values of the sample and reference (Benzophenone) are different since there are two different points which indicates that they are not the same substances. Furthermore, we can compare the polarities of Benzopheneone and Biphenyl to support the idea. Biphenyl has an Rf of 0.84 on the TLC plate while Benzophenone has an Rf of 0.71. This indicates that Biphenyl is less polar than Benzophenone. Since compound with less polarity go up further on TLC plate gives them lower Rf values. Looking at the molecular structure can give an idea about the polarity of Biphenyl and Benzophenone. Referring to figure 7 it can be noticed that there is an oxygen atom which is a considered a high electronegative atom and causes the molecule to be polar when compared to Biphenyl which is a perfect non polar molecule in which the dipoles cancel out each other.

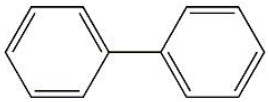


Figure 8: molecular structure of Biphenyl

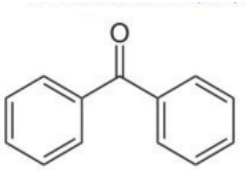


Figure 7: Molecular structure of Benzophenone

In part B, the procedure is the same, however we place our plates in a pure Ethyl acetate eluant and then a pure Hexanes eluant. According to the lab manual, ethyl Acetate is more polar than Hexanes. In fact, we learn that non-polar compounds don't interact with the silica gel and travel with the eluant, thus travelling up the TLC plate much faster. In contrast, polar compounds interact better with the silica and thus stick to it, travelling up the TLC much slower.

Using ETOAc as the eluant, we can see in our observations that the Rf values are all almost the same around 0.8, for both the benzophenone, the biphenyl and the sample. This suggests that the solvent, EtOAc is very polar, to the point where both reference compounds want to stick it instead of sticking to the silica(also very polar) and thus travel very fast and almost reach the solvent front, no matter the polarity of the compounds.

Using Hexanes as the eluant this time, we get different results. The benzophenone has an Rf of 0.36 and the sample an Rf of 0.43 on that TLC, and on the second TLC Biphenyl has an Rf of 0.38 and the sample an Rf of 0.34 as well. Thus Biphenyl travelled faster (with the solvent), but benzophenone travels slower, and is lower than the sample. This leads us to conclude that Biphenyl is less polar than Benzophenone, and so sticks less to the silica gel, moving up the plate slower. The hexanes eluant is not as polar as the EtOAc and so could not get the benzophenone to move higher up and the faster like the EtOAc did. These deductions are consistent with the molecule structures of benzophenone and biphenyl. In fact, benzophenone has more lone pairs which are located on the oxygen atom, which means it has a better capability of creating hydrogen bonds, making it more polar.

In part C, we first must determine the two compounds that are found in YY. First of all, we notice that the Rf values match in p-bromonitrobenzene and m-bromonitrobenze . since o-bromonitrobenze has a lower Rf value we can exclude it since it doesn't match the other spots.

Next, we determine the ratio of the compounds in YY using the program image J we used the lane of the sample on one of our TLC's, ultimately chossing the best photo, and obtained two peaks and calculated their areas. This was, we can find the percentage of each compound in YY using the following:

<u>For p-bromonitrobenzene:</u>

% peak 2= $\frac{area\ of\ peak\ 2}{area\ of\ peak\ 2+area\ of\ peak\ 3} x100 = \frac{8758.974}{8616.510+8758.974} x100$ = 50.4%

% peak 3= $\frac{area\ of\ peak\ 3}{area\ of\ peak\ 2+area\ of\ peak\ 3} x100 = \frac{8616.510}{8616.510+8758.974} x100$ = 49.6%

<u>For m-bromonirtobenze:</u>

% peak 2= $\frac{area\ of\ peak\ 2}{area\ of\ peak\ 2+area\ of\ peak\ 3} x100 = \frac{3084.891}{7525.154+3084.891} x100$ = 29.07%

% peak 3= $\frac{area\ of\ peak\ 3}{area\ of\ peak\ 2+area\ of\ peak\ 3} x100 = \frac{7525.154}{7525.154+3084.891} x100$ = 70.93%

Therefore the percentage of p-bromonitrobenze is therefore 100-50.4 = 49.6%

According to the calibration curve for the Amount of the meta isomer in an metapara mixture of bromonitrobenze, the curve's equation is y=0.923x + 0.786. thus, we replace the percentage value of m-bromonitrobenze as our unknown y to find x

Which is the mole% of the meta isomer.

Y = 0.923x + 0.786

X_{meta}= $\frac{y-0.786}{0.923}$ = $\frac{50.4-0.786}{0.923}$ = 53.753 % mole

The mole % of p-bromonitrobenze:

X_{para}= 100-53.753 = 46.24 mole

Sources of error:

Our results may not be accurate due to various factors that could have potentially affected and altered them such as:

- The accuracy of the lines created on Image J to calculate the areas.
- The size of our spots, which did not overlap but could have had an effect on the results obtained on image J.
- The use of a ruler to measure the distance travelled by the compounds and the solvent front is imprecise.
- The estimation of the center of the spot we drew to identify the spots on the TLC plates.

Questions:

1.) Increasing the polarity of the solvent system affects the results of a TLC because the compound, no matter if there are very or a little but polar, will be more easily attracted to the polar solvent rather than the silica gel (which itself is also very polar). The fact that the compound interact better with the solvent system with the increased polarity makes the compounds move up with the solvent and not stick to the plate, and thus move up faster and travel a longer distance on the TLC plate.

2.) a.) Benzyl alcohol, benzaldehyde, benzyl acetate: between the three, the compound that would have the smallest Rf value on silica gel is the one that would travel the least far would be the benzyl alcohol because it is the most polar. In fact, in comparison with the two other compounds, benzyl alcohol has the -OH group. This means that this group not only accept hydrogen but also donate. Also, the electronegativity of the Oxygen is much higher than the Hydrogen; therefore the bond is very polar. The benzyl aldehyde does not have a Hydrogen, and the electronegativity of the Carbon is less than of the Oxygen, therefore the bond is less polar. The same goes for the benzyl acetate, which has no Hydrogen, only bonds between Carbon and Oxygen in the functional group.

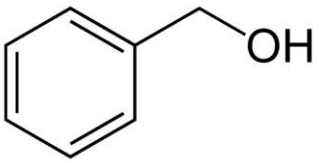


Figure 9: Benzyl alchol

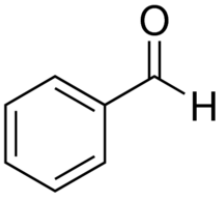


Figure 10: Benzaldehyde

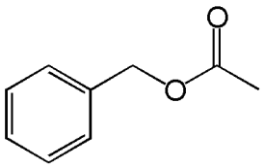


Figure 11: Benzyl acetate

b.) Aniline, N,N-dimethylaniline, and naphthalene: First off, the least polar molecule is naphthalene because of the absence of a polar functional group (only composed of C-H double and single bonds). The difference between the N,N-dimethylaniline and the aniline is that the N,N-dimethylaninlne has a nitrogen atom connected to two Carbons, whereas the aniline has a nitrogen atom connected to two H's, which indicates that the analine can both donate and accept a hydrogen. The difference in electronegativity between the Nirtogen and the Hydrogen is superior to the difference between the Nitrogen and the Carbon, and therefore the aniline molecule is more polar and will have a smaller Rf value.

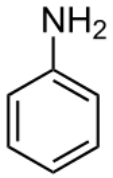


Figure 12: Aniline

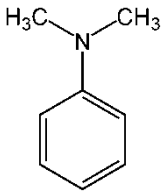


Figure 13: N,N-dimethylaniline

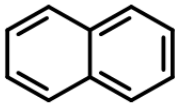


Figure 14: Naphthalene

c.) Benzophenone(refer back to figure 7), biphenyl (refer back to figure 8) and benzoic acid: The least polar molecule out of these three is the biphenyl, because of the absence of a polar functional group. The benzophenone has a carbonyl group, however, the carbonyl group is less polar than the alcohol group on the benzoic acid, which has a hydrogen atom connected to the Oxygen rather than a carbon atom, and as mentioned in question 1, the difference is electronegativity is higher between those two atoms, and therefore the benzoic acid is more polar, and will therefore have a smaller Rf value.

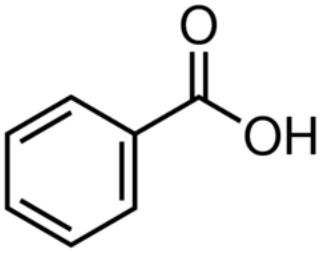


Figure 15: Benzoic acid

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Experiment 1 Lab 1

observations:-
. salt is white and had a crystalline form (the dichloromethane)
. strong smell similar to benzene. The benzophenone.
which also had a strong smell.
benzophenone D_1 D_2 D_3 52 cm

$D_3 = 5.7$ cm

biphenyl $D_1 = 3.9$ cm D_2 4.9 cm $D_3 = 4.9$ $D_4 = 4.1$ cm

$D_5 = 5.8$ cm

part B:

observations-
• smell of EtOAC was extremely strong ILC's were prepared in the same way.
• Henanes also had a strong smell

same y ethyl acetate

Benzophenone: D_1 D_2 $D_3 = 4.7$

$D_4 = 5.9$ cm

Biphenyl: $D_1 = 5.2$ $D_2 = 4.9$ $D_3 = 5.3$ $D_4 = 5.1$ $D_5 = 5.7$

Henanes:
Benzophen $D_1 = 2$ cm $D_2 = 1.9$ cm $D_3 = 1.8$ cm
Biphenyl a accurate due to system solvent
$D_1 = 1.9$ cm $D_2 = 2$ cm $D_3 = 2.3$ cm $D_4 = 5.3$