Thin Layer Chromatography

Experiment # 1

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CHM1321 Section Z09

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Procedure:

As outlined in the Organic Chemistry Laboratory Manual, 2020, Dr. Tony Durst, Dr. Tito Scaiano, Dr. William Ogilvie, Dr. Alison Flynn. (Exp.1, p. 5-7)

Discussion:

- TLC plates are used as a reaction monitoring and sample purification system, using polarities of different known compounds to determine unknown compounds.
- The Stationary phase used is silica gel.
    - The silica gel (SiO_2) is polar due to its OH bonds on the surface of its structure as it contains a layer of water, where its oxygen atoms are attracted to protons forming these hydroxyl groups. The change in electronegativity between O and H form polar bonds as well as the separation of positive and negative charges form a dipole moment, thus the substance is highly polar.
- Organic solvents used to develop the plates are ethyl acetate: hexane 2:8, used in part A, ethyl acetate in part B and hexane: ethyl acetate 9:1 in part C.
    - This is the mobile phase on a TLC plate.
    - With capillary action, the mobile phase is drawn up through the stationary silica gel, pulling the sample along with it in order to separate the components of the sample based on its affinity for each phase.
    - The polarities of the organic solvents used are different, in order to demonstrate the effects of different polarities of a mobile phase on the compounds being tested.

    - This is shown when comparing the TLC plates using ethyl acetate: hexane 2:8 in Figure 1 to Figure 2 where the mobile phase used is ethyl acetate. The final displacement of the dots is shown to be greater in figure 2 compared to figure 1.
- The polarity of the organic solvents is what determines the Rf value of the compounds being measured
    - The polarity of the compound being measured will also determine how quickly it moves up the TLC plate, shown by how far each spot has travelled after a constant period of time.
    - Each spot that is used is measured after having travelled up the TLC plate
        - By measuring the distance from the initial spot location to the new spot location and from the initial spot location to the end-point that the mobile phase reached, an Rf value can be calculated.
        - The least polar of the solvents used in the mobile phase will move the quickest.
            - The polar compounds being used will travel at a set speed, though the mobile phase will reach the top quicker if it is less polar.
            - Since the time taken to reach the top of the TLC plate will vary depending on the mobile phase, the Rf value will change as well.

        - The organic compounds used are ethyl acetate and hexane in different concentrations.
            - Ethyl acetate is a polar compound because of the two polar CO ester bonds.
            - Using a polar molecule as the mobile phase changes the Rf value of the compound being analyzed as the attraction between the compound, the mobile phase and the stationary phase will change based on the polarities of each substance.
            - Hexane is a nonpolar molecule as the only intermolecular forces present are London dispersion forces.
            - Since there are no hydrogen bonds, it is not polar.
            - When combined the result is polar since the hydrogen bonds from the ethyl acetate still exist. Despite being mixed with a non-polar substance, the hydrogen bonds still exist there are just fewer opportunities overall, making it less polar.
- The 3 spots used during the experiment are the reference spot, co- spot and sample spot.
    - The reference spot is used to compare the polarities of the known compound (reference solution) to the unknown compound that is being determined.
        - In figure 1 benzophenone and biphenyl are used as reference solutions to determine what the unknown substance is. If the spots are in line with each other then the unknown substance is likely the reference solution, as seen on the benzophenone TLC plate.

- The sample spot is a mixture of the unknown compound and dichloromethane.
    - The unknown compound used is #46
    - Dichloromethane is used as it is a polar molecule that mixes well with organic compounds. Because of this, the overall polarity of the substance used will not be significantly affected, providing the most accurate results.
- The co-spot is a mix of both compounds if one dot appears than the reference and sample solutions will be of the same polarity, however, if two dots appear this demonstrates a difference in polarity between the reference solution and the sample solution.
    - This occurred when sample #46 was being tested with biphenyl as a reference. Since there were two co-spots at the end, there are two different affinities and thus two different compounds. Whereas when the sample was being tested with benzophenone, there was only one co-spot.
        - This happened because the polarities were the same, since they travelled the same distance, meaning the sample was benzophenone.
    - The co-spot is done last in order to avoid contamination of the sample that would then alter the polarity of the results, thus altering the Rf values determined.
    - In part C of the experiment, our dots were pushed slightly over due to the spots not being perfectly in line. This may have caused an error in the results as it may have been contaminated.

- The Rf value is the measure of the polarity of each spot, determined by the displacement of the compound divided by the displacement of the solvent
    - A small Rf value indicates a slow-moving substance, thus the displacement of the compound would be smaller.
    - The displacement depends on the polarity of the substance.
    - More polar compounds would be strongly attracted to the silica gel, and stay closer to the bottom.
    - Small Rf values thus indicate a polar compound
        - As seen in Figure 3, when sample I was being tested with 3 compounds, the Rf values of the sample were lower than in parts A and B (fig.1 and fig.2). Since there was a lower Rf value, the compound is more polar.
    - A larger Rf value has a greater displacement, indicating the substance would have moved quicker, drawn with the mobile phase due to the attraction of non-polar molecules to other non-polar molecules, due to London dispersion forces, greater in larger molecules.
        - As seen in Figure 1 and Figure 2, there is a difference in the Rf values between each TLC plate. Benzophenone had a lower Rf value meaning it was the most polar of the two substances compared.
    - The reference formula was used to determine the polarity of the unknown samples compared to the polarity of the known reference. Ideally, the Rf value of the reference should be 0.5 to 0.6, however, changing the polarity of the mobile

phase has an effect on the Rf values of the reference solution as the attraction between substances will be altered in strength.

  - This can be seen when comparing the Rf values of the reference dots from figure 1 and figure 2 where the mobile phases have been changed. In figure 1, the Rf value of benzophenone in EtOAc hexane is 0.73, and when used as a reference solution with the mobile phase of EtOAc, the Rf value is shown to have increased to 0.89, demonstrating that the spot moves faster in the EtOAc due to the greater quantity of hydrogen bonds being formed between the reference and more polar mobile phase. This is also shown when comparing the Rf values of the biphenyl reference spot from figure 1 to figure 2.
  - Due to benzophenone and biphenyl being very high in figure 2 EtOAc, rather than 0.5-0.6, EtOAc is not an ideal mobile phase and a non-polar mobile phase is more efficient when determining the unknown sample.
  - This also may have been a source of error as both figure 1 and figure 2 did not contain reference dots of 0.5-0.6.

- In part C the reference solutions used are m-bromonitrobenzene, o-bromonitrobenzene and p-bromonitrobenzene. The solutions are all benzenes, the prefix indicating which carbon the NO_2 group is attached to.
  - Depending on where the NO_2 is located can change the polarity of the substance. o-bromonitrobenzene is more polar than para, due to the location of its bromine on carbon 1 and its NO_2 on carbon 2, whereas

p-bromonitrobenzene has its NO_2 on carbon 4 which slightly cancels out its dipole, thus making it less polar than ortho. This is similar for meta, thus demonstrating why sample I is a combination of meta and ortho bromonitrobenzene. The reference spots are also shown to be lower in figure 3 for o-bromonitrobenzene and m-bromonitrobenzene, and p-bromonitrobenzene contained the highest reference spot (largest Rf), demonstrating its non-polar properties.

- The mobile phase used is hexane EtOAc 9:1. This mobile phase is ideal as it is non-polar, allowing the movement of non-polar substances further up the TLC plates, with the polar substances remaining closer to the bottom. The Rf values of the reference solutions are also close to 0.5-0.6.
- Substance I is a combination of m-bromonitrobenzene and o-bromonitrobenzene as the Rf values are the same as the reference. Since there are two molecules in the substance, there are two sample dots and three co-spots at the end of the reaction. By examining each plate after the reaction (fig. 3), one sample and one co-spot will be in line with the reference and its co-spot if the reference solution is present in the sample solution. As seen in figure 3, one of the sample spots and its co-spot line up with the reference for both m-bromonitrobenzene and o-bromonitrobenzene but not p-bromonitrobenzene, proving the combination of molecules in substance I.

Observations:

Observation of TLC plates using **sample 46** and mobile phase **ethyl acetate + hexane 2:8**

Reference solution	benzophenone	biphenyl
Bottom to top line (cm)	4.9	5.4
Bottom to sample dot (cm)	3.6	3.9
Bottom to reference dot (cm)	3.6	4.6
Bottom to co-spot sample dot (cm)	3.6	3.9
Bottom to co-spot reference dot (cm)	3.6	4.6
Rf value sample dot (sample calculation A)	0.73	0.72
Rf value reference dot (sample calculation A)	0.73	0.85
Rf value co-spot sample dot (sample calculation A)	0.73	0.72
Rf value co-spot reference dot (sample calculation A)	0.73	0.85

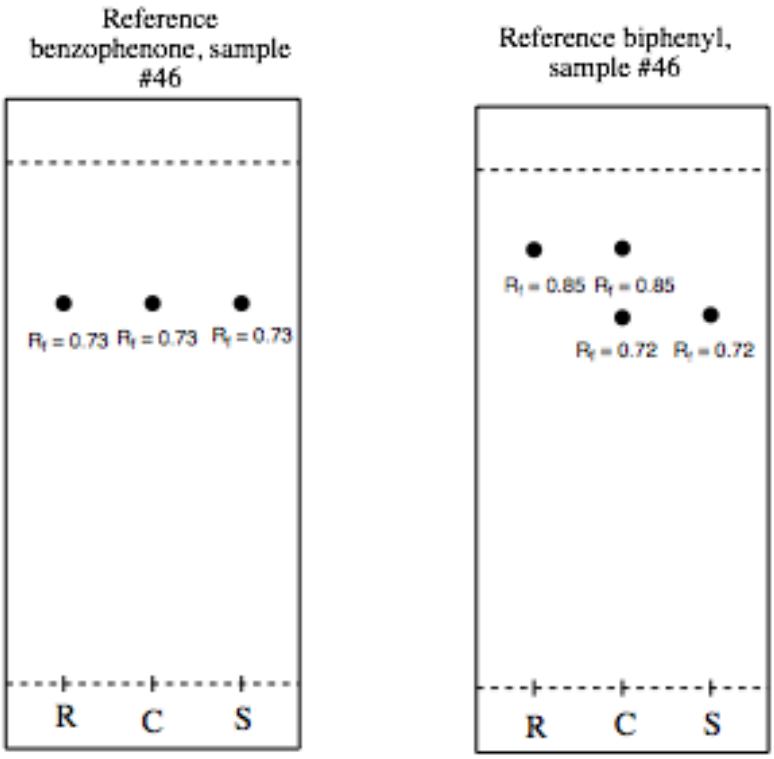


Figure 1: EtOAc hexane 2:8

Observation of TLC plates using **sample 46** and mobile phase **ethyl acetate**

Reference solution	Benzophenone	biphenyl
Bottom to top line (cm)	5.3	5.4
Bottom to sample dot (cm)	4.7	4.9
Bottom to reference dot (cm)	4.7	4.9
Bottom to co-spot dot (cm)	4.7	4.9
Rf value sample dot (sample calculation A)	0.89	0.91
Rf value reference dot (sample calculation A)	0.89	0.91
Rf value co-spot dot (sample calculation A)	0.89	0.91

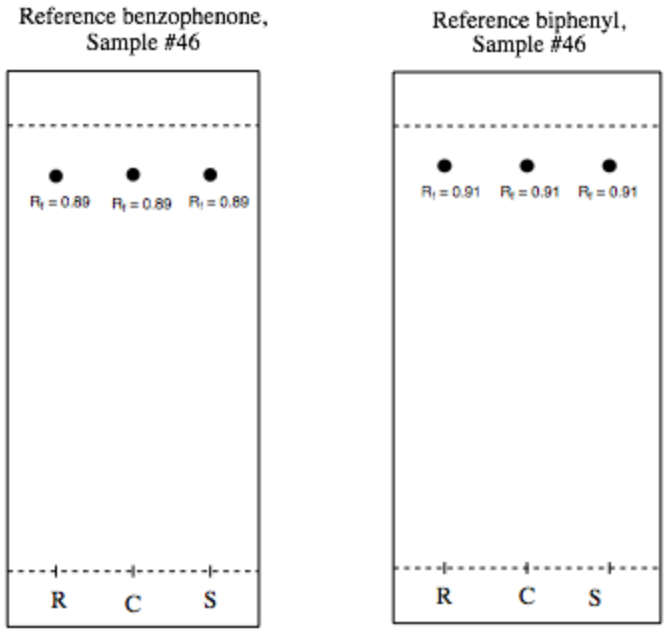


Figure 2: EtOAc

Observation of TLC plates using **sample I** and mobile phase **ethyl acetate + hexane 9:1**

Reference solution	M		P		O	
Bottom to top line (cm)	5.5		5.6		6.7	
Bottom to sample dot (cm)	2.1	3.0	2.3	3.0	2.9	3.2
Bottom to reference dot (cm)	3.2		3.6		2.9	
Bottom to co-spot sample dot (cm)	2.1	3.0	2.3	3.0	2.9	3.2
Bottom to co-spot reference dot (cm)	3.2		3.6		2.9	
Rf value sample dot (sample calculation A)	0.38	0.55	0.41	0.54	0.43	0.48
Rf value	0.55		0.64		0.43	

reference dot (sample calculation A)						
Rf value co-spot sample dot (sample calculation A)	0.38	0.55	0.41	0.54	0.43	0.48
Rf value co-spot reference dot (sample calculation A)	0.55		0.64		0.43	

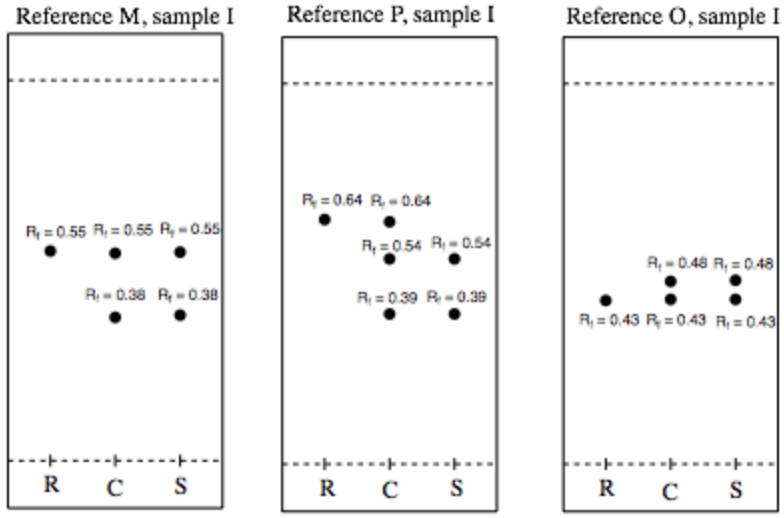
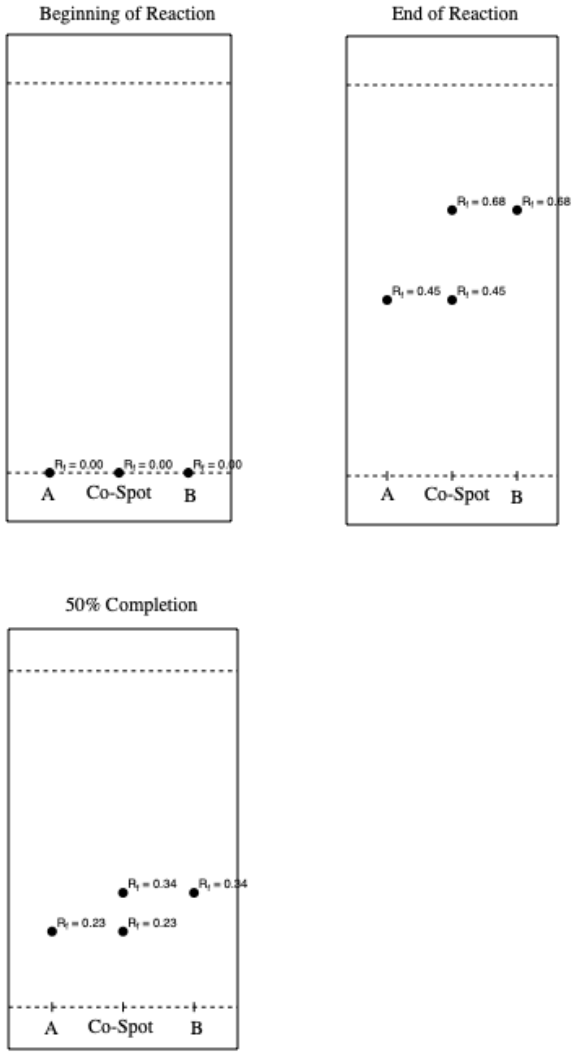


Figure 3: EtOAc hexane 9:1

Questions

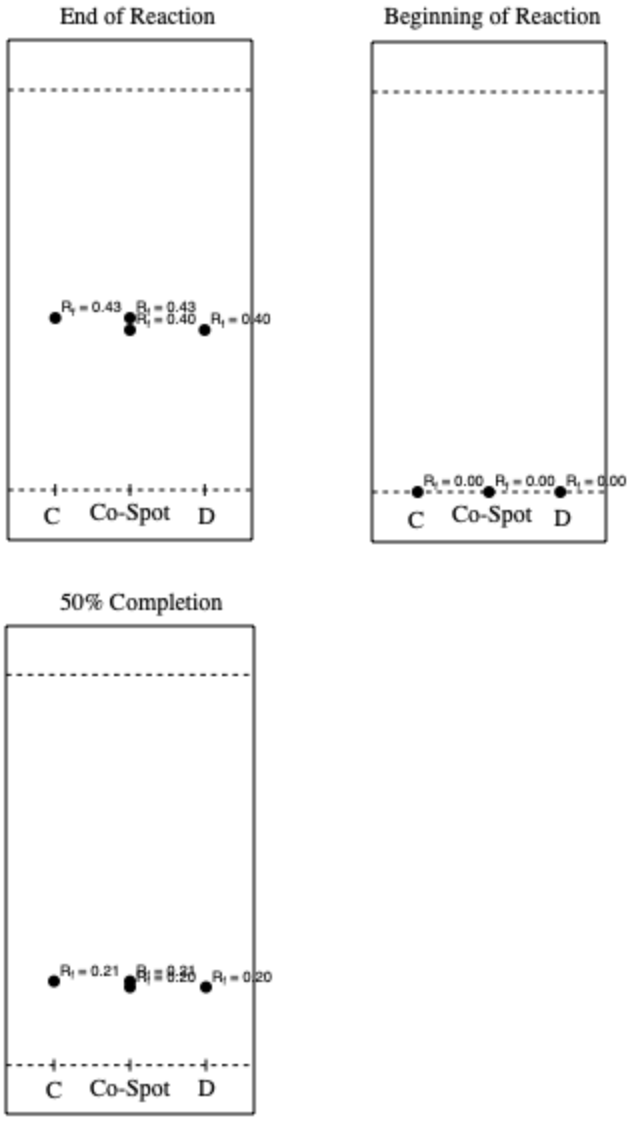
1. It is important to make the co-spot last to avoid contamination. If the co-spot is done first, the second solution added will have touched the first solution, resulting in a mixture of the two compounds. If the compounds mix the results will not be accurate.
2. Increasing the polarity of the substance used will change the speed at which it moves across the TLC plate. As the polarity of the compound increases, the attraction to the silica gel on the TLC plate increases. Because of this, the compound will move more quickly across the TLC plate, overall, speeding up the lab

3.



d. Molecule A is better to use as it has more hydrogen bonds to the silica gel, making it more polar, providing a lower Rf value. Since it moves slower, the reaction is easier to observe.

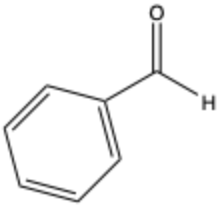
4.



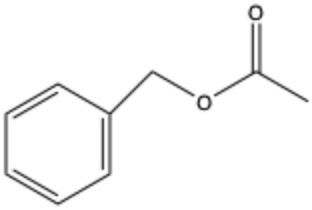
d. It is important to use a co-spot because it confirms if the unknown substance is the same as the known substance. If there is only one spot on the co-spot lane, the unknown molecule is the same as the reference, whereas if two spots appear, the unknown molecule is different from the reference molecule.

5. i.

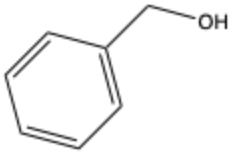
Benzaldehyde



Benzyl Acetate



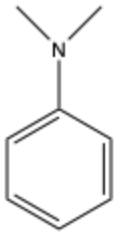
Benzyl Alcohol



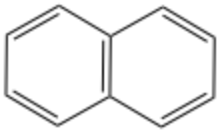
Benzaldehyde is the least polar since aldehydes have less potential for hydrogen bonding, therefore reducing the polarity. Alcohols have the most opportunities of the three for hydrogen bonding, through the OH group, meaning benzyl alcohol is the most polar.

ii.

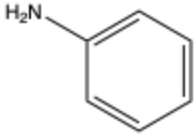
N,N-dimethylaniline



Naphthalene



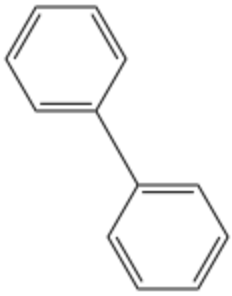
Aniline



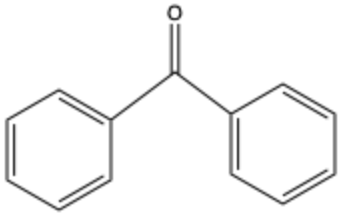
Aniline is the most polar since the N group has the most potential to hydrogen bond. N, N-dimethylaniline is the least polar since there are fewer opportunities to hydrogen bond than the other molecules.

iii.

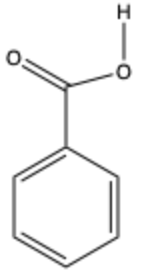
Biphenyl



Benzophenone



Benzoic Acid



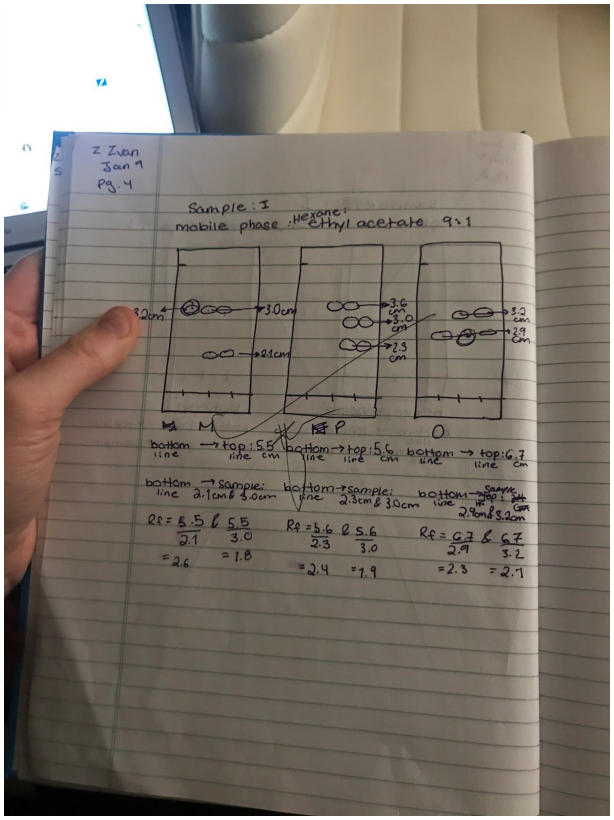
Benzoic acid is the most polar since it has both a carbonyl group and a hydroxide, giving it more opportunities to hydrogen bond. Biphenyl is less polar than benzophenone since benzophenone has a carbonyl group that is able to hydrogen bond, whereas the biphenyl does not.

Sample Calculations:

A. $Rf = \frac{displacement\ of\ compund\ (d1)}{displacement\ of\ the\ solvent\ front\ (ds)}$

$= \frac{3.6cm}{4.9cm}$

$= 0.73$



Z Ivan
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Pg. 4

Sample: I
mobile phase: Hexane: ethyl acetate 9:1

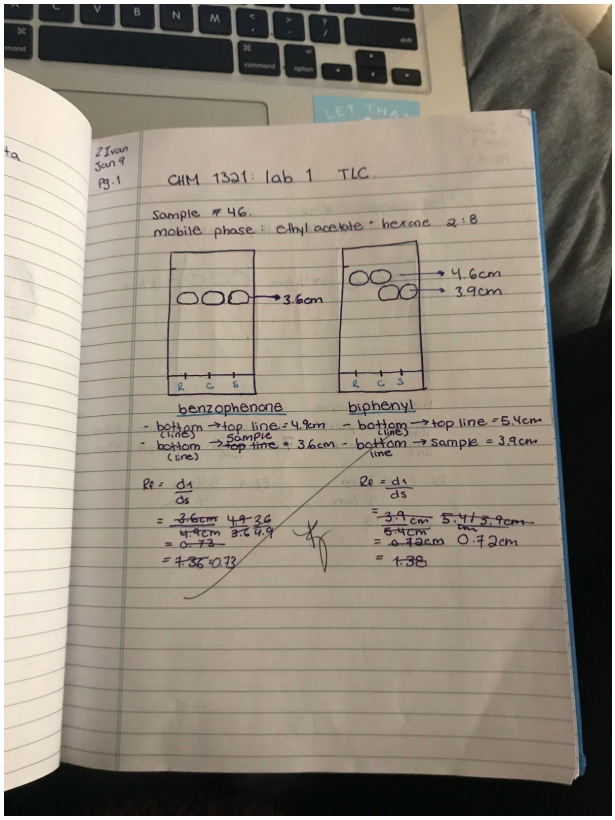
bottom line → top: 5.5 cm | bottom line → top: 5.6 cm | bottom line → top: 6.7 cm

bottom line → sample: 2.1cm & 3.0cm | bottom line → sample: 2.3cm & 3.0cm | bottom line → sample: 2.9cm & 3.2cm

$Rf = \frac{5.5}{2.1} = 2.6$ & $\frac{5.5}{3.0} = 1.8$

$Rf = \frac{5.6}{2.3} = 2.4$ & $\frac{5.6}{3.0} = 1.9$

$Rf = \frac{6.7}{2.9} = 2.3$ & $\frac{6.7}{3.2} = 2.1$



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Pg. 1

CHM 1321: lab 1 TLC

Sample # 46.
mobile phase: ethyl acetate + hexane 2:8

benzophenone

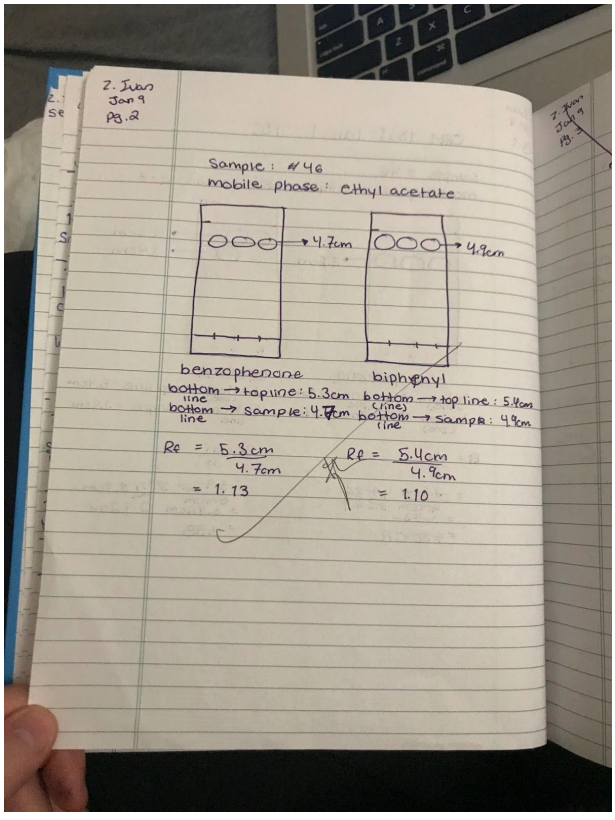
- bottom (line) → top line = 4.9cm
- bottom (line) → sample → top line = 3.6cm

$Rf = \frac{d_1}{d_s} = \frac{3.6cm}{4.9cm} = 0.73$

biphenyl

- bottom (line) → top line = 5.4cm
- bottom (line) → sample = 3.9cm

$Rf = \frac{d_1}{d_s} = \frac{3.9cm}{5.4cm} = 0.72cm$



2. Ivan
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Pg. 2

Sample: # 46
mobile phase: ethyl acetate

benzophenone

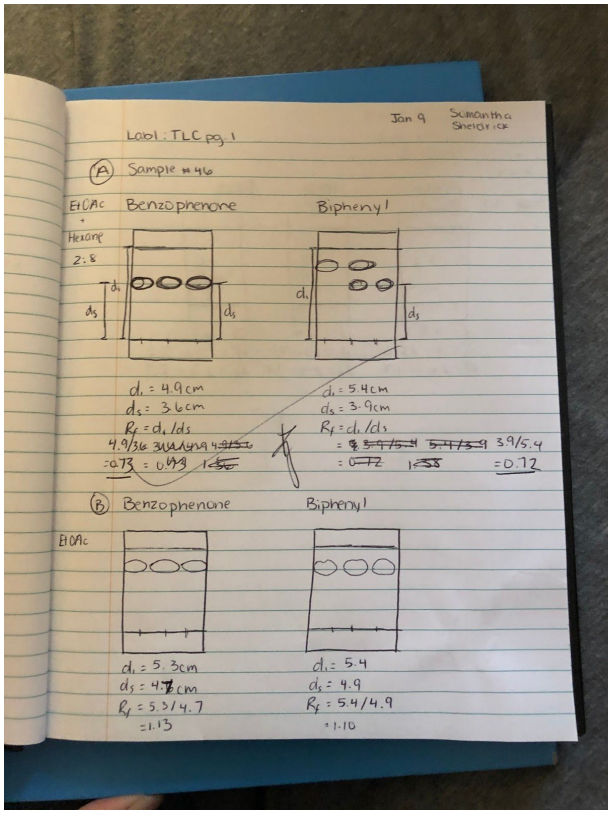
bottom line → top line: 5.3cm
bottom line → sample: 4.7cm

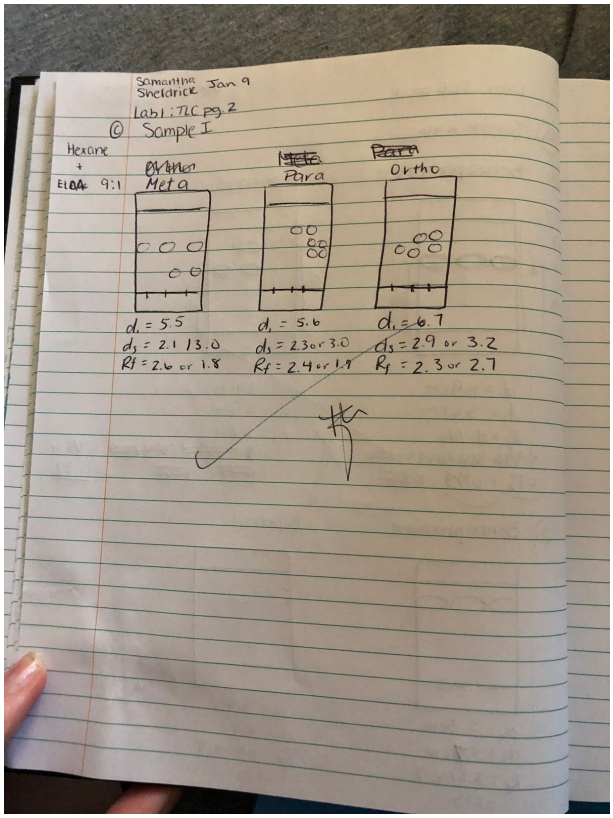
$Rf = \frac{5.3cm}{4.7cm} = 1.13$

biphenyl

bottom (line) → top line: 5.4cm
bottom line → sample: 4.9cm

$Rf = \frac{5.4cm}{4.9cm} = 1.10$





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