Organic Chemistry Lab 1:
Thin Layer Chromatography

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Date performed: January 10, 2017
Date submitted: January 17, 2017

University of Ottawa

Procedure

The procedure is as described in the lab manual (CHM 1321 Introductory Organic Chemistry Laboratory Manual 2017, Dr. William Ogilvie and Dr. Tony Durst, 2017, Exp.1, p. 13 to 19).

However, a couple of modifications were made to the procedure.

- A modification was made to **Step 4** of **Part A** of the procedure and it is as follows:
  > **4.** In a small test tube, dissolve 30.0 mg of the sample in 2 mL of dichloromethane. Label the test tube to remember the contents of it.
- Another modification was made to **Step 9** of **Part A** of the procedure and it is as follows:
  > **9.** Place 2 mL of the reference solution of biphenyl in a separate small test tube. Spot this sample solution onto the other plate that you prepared in Step 2, on the reference lane and co-spot lane. Carefully place the TLC plate under the UV light to make sure there are 3 visible spots on the silica - one per lane on each tick mark. If there aren't 3 visible spots on the silica under the UV light, spot the lanes again (these visible spots should look bright pink under the UV light).
- A last modification was to **Step 1** of **Part C** of the procedure and it is as follows:
  > **1.** Choose the unknown XX found in the burette in the fume hood of the lab. Using a 50 ml beaker obtain 2 ml of the unknown XX.

Observations

- The dichloromethane is a transparent colourless liquid substance, with a strong odour.
- The unknown compound #33 is a white crystallized powder, with no odour.
- The 8:2 mixture of ethyl acetate is a transparent colourless liquid substance, with a strong odour.
- The pure ethyl acetate is a transparent colourless substance, with a sharp sweet odour, like the odour of nail polish.
- The 9:1 mixture of ethyl acetate is a transparent colourless liquid substance, with a strong odour.
- The pure hexane solvent is a transparent colourless liquid substance, with a light odour, like the faint odour of gasoline.
- The "A" substance (ortho-bromonitrobenzene) is a yellow, transparent liquid substance.
- The "B" substance (meta-bromonitrobenzene) is a colourless, transparent liquid substance.
- The "C" substance (para-bromonitrobenzene) is a colourless, transparent liquid substance.
- The UV lamp displayed that the spots on the plates eluted in ethyl acetate had traveled further than the spots on the plates eluted in hexanes.
- Separation only occurred on the biphenyl plate eluted in the hexanes solvent system.
- Slightly more extensive separation was noted on the co-spot lane on the meta-bromonitrobenzene TLC plate.

Data Tables

Note: My lab partner and I were assigned unknown compound #33.

Table 1: Part A TLC Plates with 2:8 Mixture of Ethyl Acetate Eluant

Eluant	**Spots / Lanes**			**Rf values**	
	Reference	Co-Spot	Sample	R	S
2:8 mixture of ethyl acetate (EtOAc)	Benzophenone	- Benzophenone - Dichloromethane and unknown compound #33	Dichloromethane and unknown compound #33	0.57	0.56
2:8 mixture of ethyl acetate (EtOAc)	Biphenyl	- Biphenyl - Dichloromethane and unknown compound #33	Dichloromethane and unknown compound #33	0.79	0.58

Table 2: Part B TLC Plates with Pure Ethyl Acetate Eluant

Eluant	**Spots / Lanes**			**Rf values**	
	Reference	Co-Spot	Sample	R	S
Pure ethyl acetate (EtOAc)	Benzophenone	- Benzophenone - Dichloromethane and unknown compound #33	Dichloromethane and unknown compound #33	0.84	0.87
Pure ethyl acetate (EtOAc)	Biphenyl	- Biphenyl - Dichloromethane and unknown compound #33	Dichloromethane and unknown compound #33	0.77	0.75

Table 3: Part B TLC Plates with Hexane Eluant

Eluant	**Spots / Lanes**			**Rf values**	
	Reference	Co-Spot	Sample	R	S
Hexanes	Benzophenone	- Benzophenone - Dichloromethane and unknown compound #33	Dichloromethane and unknown compound #33	0.29	0.05
Hexanes	Biphenyl	- Biphenyl - Dichloromethane and unknown compound #33	Dichloromethane and unknown compound #33	0.12	0.02

Table 4: Part C TLC Plates with 1:9 Mixture of Ethyl Acetate Eluant

Eluant	**Spots / Lanes**			**Rf values**		
	Reference	Co-Spot	Sample	R	S (2 spots appeared)	
1:9 mixture of ethyl acetate	A (ortho-bromonitrobenzene)	XX and A	XX	0.35	0.56	0.35
1:9 mixture of ethyl acetate	B (meta-bromonitrobenzene)	XX and B	XX	0.57	0.62	0.43
1:9 mixture of ethyl acetate	C (para-bromonitrobenzene)	XX and C	XX	0.57	0.57	0.36

TLC Plate Figures

Figure 1: Part A TLC Plate #1

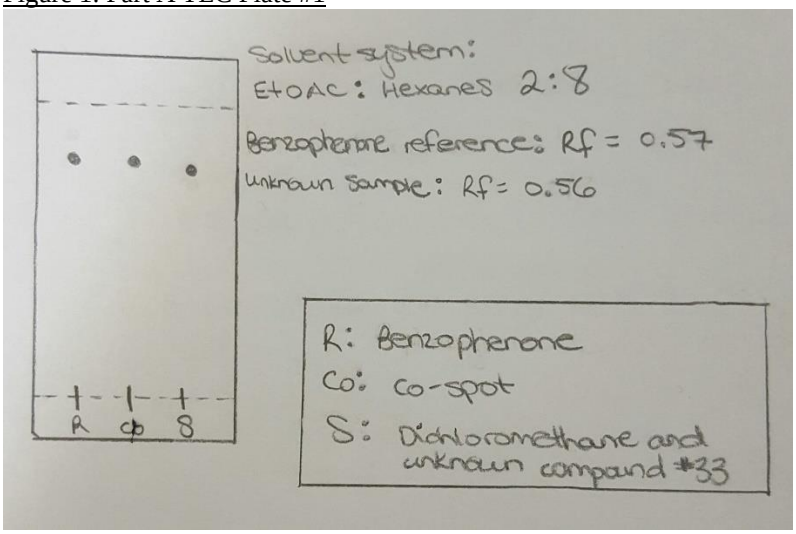


Figure 2: Part A TLC Plate #2

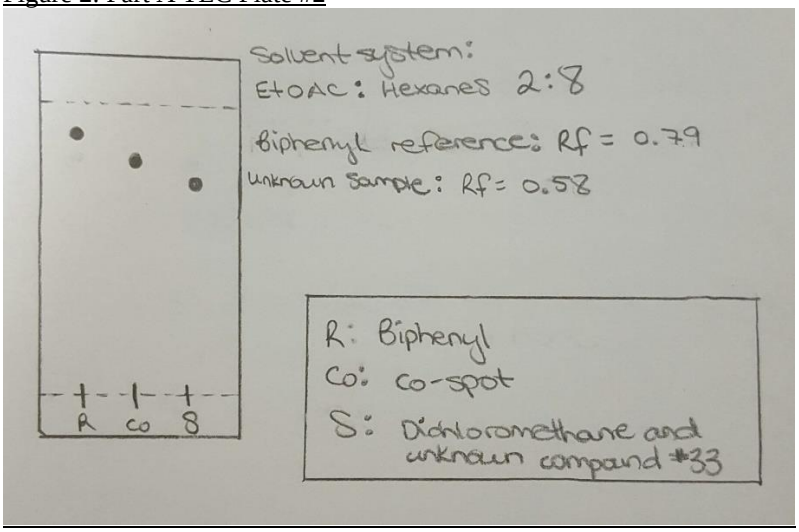


Figure 3: Part B TLC Plate #1

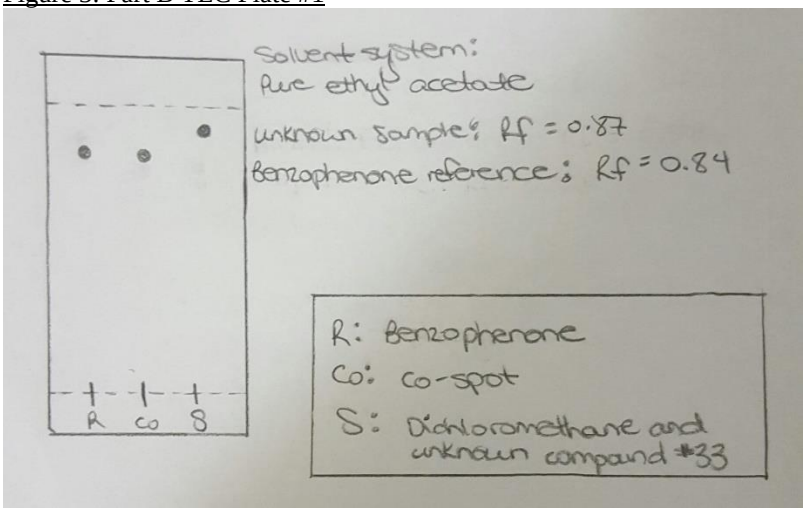


Figure 4: Part B TLC Plate #2

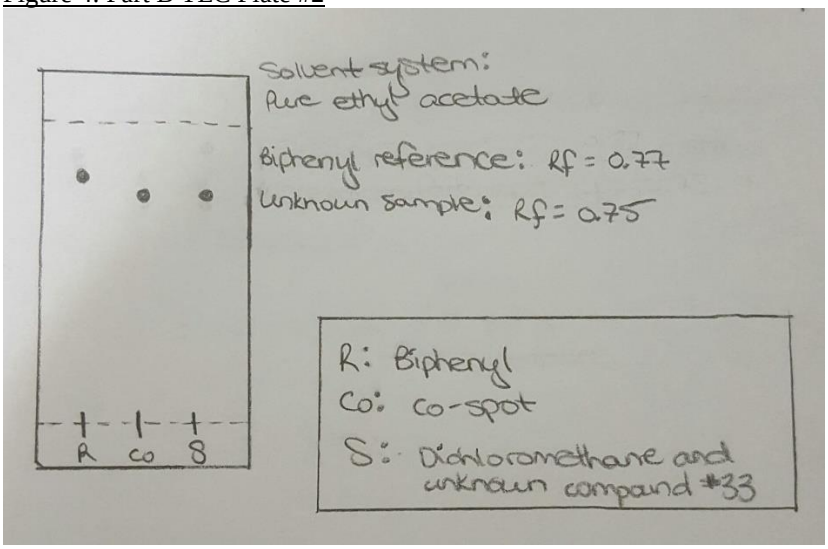


Figure 5: Part B TLC Plate #3

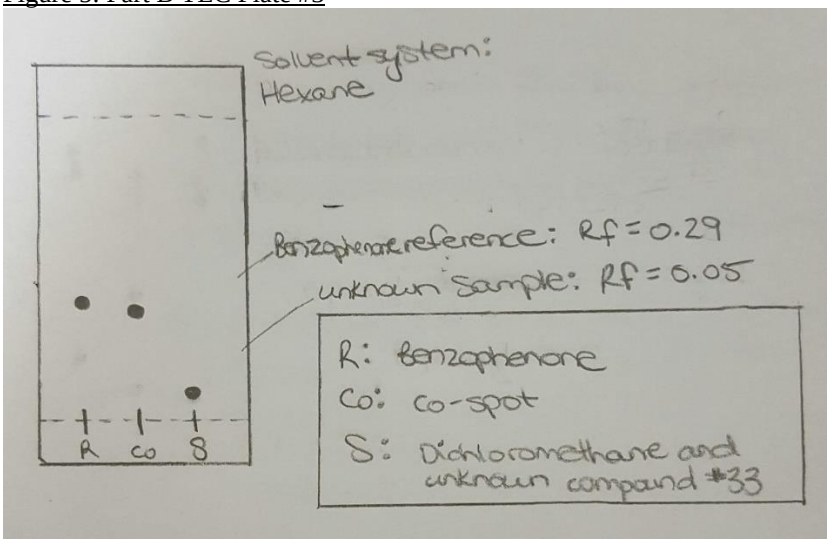


Figure 6: Part B TLC Plate #4

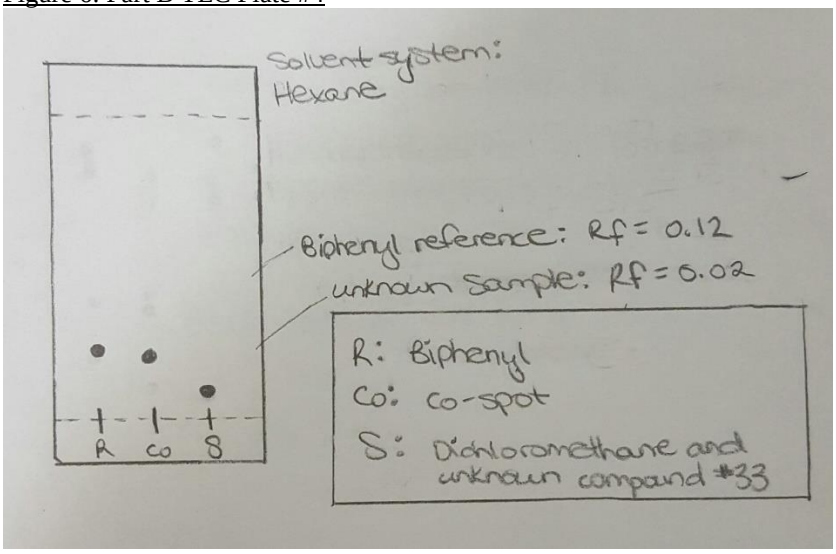


Figure 7: Part C TLC Plate #1

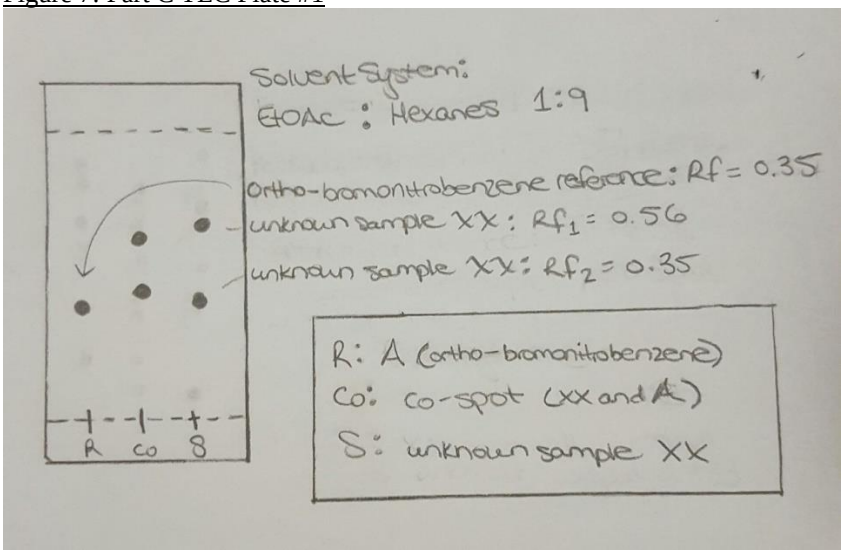


Figure 8: Part C TLC Plate #2

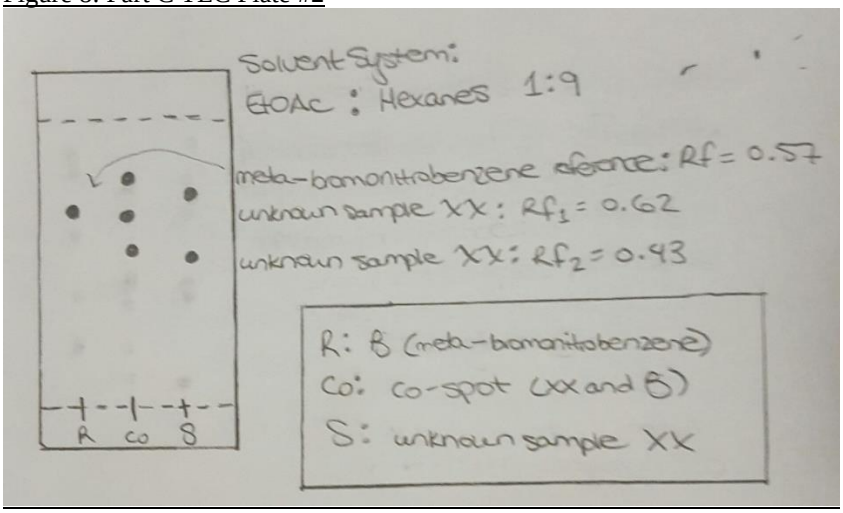


Figure 9: Part C TLC Plate #3

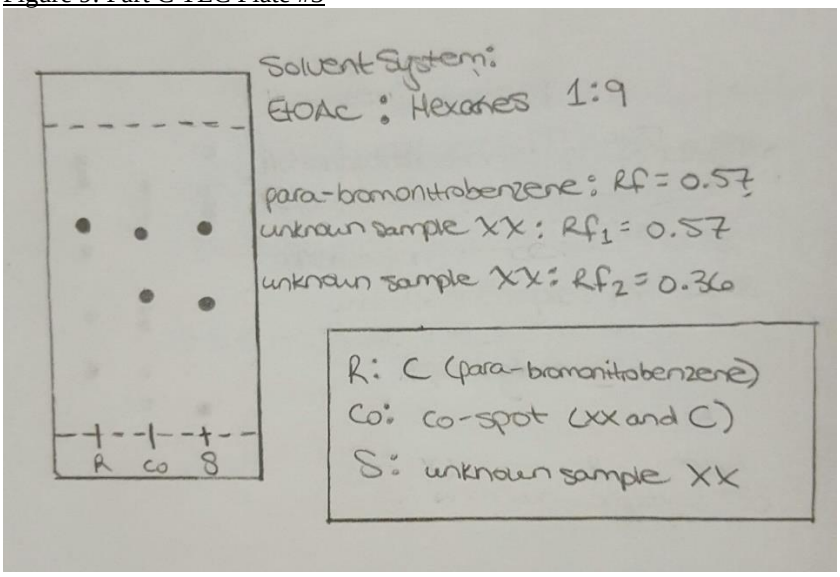


Figure 10: Part C TLC Plates spotted with ortho-bromonitrobenzene, meta-bromonitrobenzene and para-bromonitrobenzene respectively, under UV light

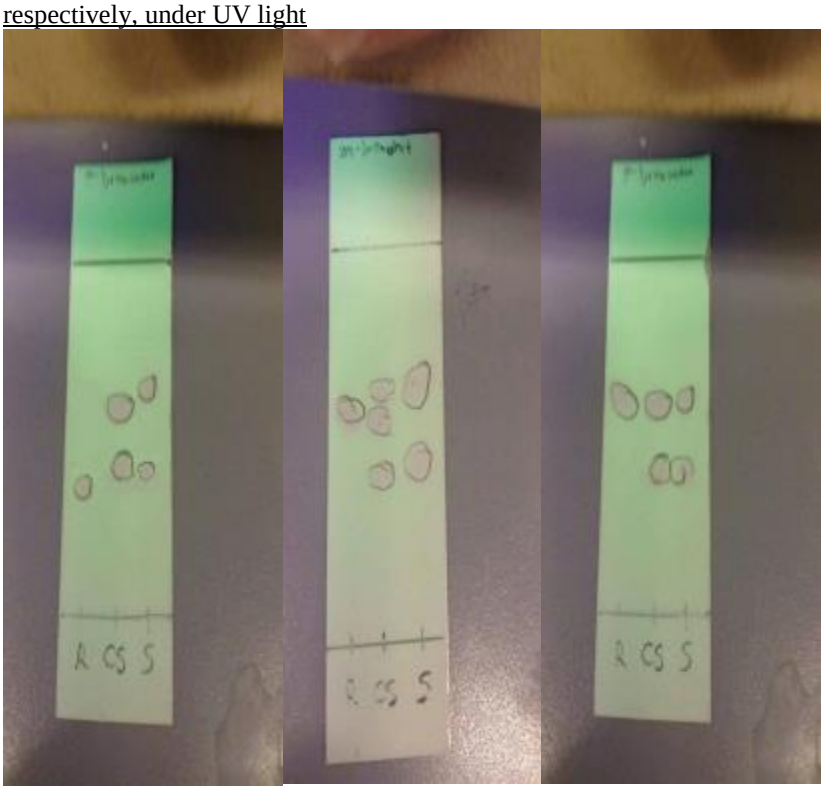
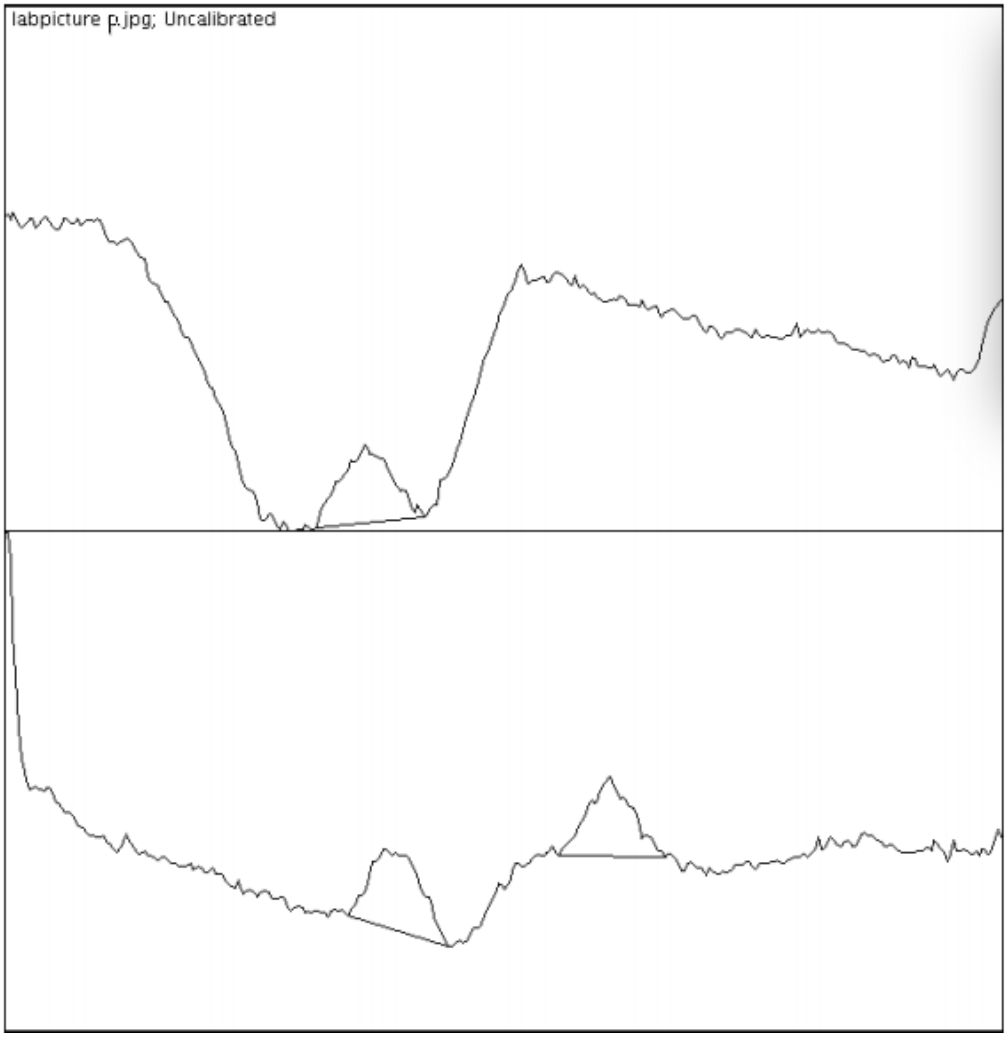


Figure 11: Lane Profile for the para-bromonitrobenzene TLC Plate



The Areas Between the Peaks and the Horizontal Lines

	Area
1	1925.205
2	2025.447
3	1722.376

Source: Image J

Note: The values above describe the areas under the peaks enclosed by horizontal lines. Peak 1 is the peak in the upper curve situated in the dip of the curve and enclosed by the horizontal line above it. Peak 2 is the left-hand peak in the bottom curve enclosed by the horizontal line below it. Peak 3 is the right-hand curve, adjacent to Peak 2, and enclosed by its horizontal line below it.

Calculations

Example Calculation of an Rf Value:

The Rf value for the benzophenone (reference) spot of the Part A TLC Plate #1:
Rf value = Distance traveled by the compound / Distance traveled by the solvent
= 2.7 cm / 4.7 cm
= 2.7 / 4.7
= 0.57

Therefore, the Rf value (retention factor) for the benzophenone spot of the Part A TLC Plate #1 is 0.57.

Determining the Component Ratio of the Unknown Mixture:

Area of Peak 2 = 2025.447

Area of Peak 2 + Peak 3 = 2025.447 + 1722.376
= 3747.823

Percent Peak 2 = (Area of Peak 2) / (Area of Peak 2 + Area of Peak 3) x 100%
= (2025.447 / 3747.823) x 100%
= 54.04329%

Percent Peak 3 = 100% - 54.04329%
= 49.95671%

The value for Percent Peak 3 is the percent of ortho-isomer in the ortho-para mixture of bromonitrobenzene. Thus, the percent of ortho-isomer in the mixture of bromonitrobenzene was calculated to be approximately 49.95671%.

Determining the Mole Percent of the Para-Isomer in the Mixture:

Substitute 49.95671% in for “y” into the calibration curve equation to determine the mole percent of the para-isomer in the mixture. In the equation, “x” represents the mole percent of the para-isomer in the ortho-para mixture of bromonitrobenzene.

The calibration curve equation:
$y = 1.0114x - 2.0208$

Sub in y = 49.95671 into the equation above
$49.95671 = 1.0114x - 2.0208$
$x = (49.95671 + 2.0208) / 1.0114$
$x = 51.31965\%$

The mole percent of the para-isomer in the ortho-para mixture of bromonitrobenzene was calculated to be approximately 51.31965%. Thus, the mole percent of the ortho-isomer in the ortho-para mixture of bromonitrobenzene is 100% - 51.31965%, which equals 48.68035%. Therefore, the ortho:para ratio in the mixture is approximately 49:51, which can be expressed as basically a 1:1 ratio.

Discussion

In Part A of the experiment, we compared the polarities of a sample of the unknown solution (#33) to two reference solutions, benzophenone and biphenyl, on a silica gel TLC plate to determine the unknown compound. While benzophenone is a polar compound, biphenyl does not exhibit polarity due to its symmetrical structure and the lack of any polar functional groups. On the benzophenone TLC plate, the sample and reference spots were not separated, indicating that both the sample compound and reference compound exhibited the same equal polarities and levels of adsorption. The relatively neutral 2:8 ethyl acetate to hexanes solvent system allowed the unknown compound and benzophenone compound to travel across the plate based on their affinity for the silica gel adsorbent stationary phase. There was minimal interaction between the solvent and adsorbent and between the solvent and the compound. The retention factor (Rf) values of the reference solution and sample solution were experimentally determined to be nearly matching at 0.57 and 0.56 respectively. This confirms that the unknown sample compound was the reference compound, benzophenone. The Rf value found for biphenyl, 0.79, was different than the unknown compound sample's Rf value, 0.58. This reveals that the unknown compound is not biphenyl. This lower Rf value of the unknown compound indicates that it has a stronger affinity for the silica gel adsorbent on the TLC plate than the biphenyl reference compound. Thus, the unknown compound adsorbed to the silica gel and did not travel as far as biphenyl did on the silica gel, resulting in the separation of the compounds and the conclusion that it is not biphenyl and is more polar than it.

In Part B of the experiment, we examined the effect of two different solvent systems on the development of a TLC plate. Based on the polarity scale for solvents in Table 2 of the lab manual, ethyl acetate (EtOAc) exhibits a greater polarity than hexanes. Due to its high polarity, the ethyl acetate solvent strongly interacted with the silica gel, interacting more with the silica gel than the benzophenone sample to the silica gel which is also a compound with high polarity.

In the elution of the biphenyl TLC plate in the ethyl acetate solvent system, the high polarity of the pure ethyl acetate caused the benzophenone to travel a greater distance than the biphenyl up the silica gel TLC plate, but they both had high Rf values. This was the opposite result of the outcome we expected. It was expected that separation would occur but that the biphenyl would travel further up the plate than benzophenone due to its nonpolarity. This opposite outcome could have occurred because of possible sources of error, explained later in this discussion, and also because the high polarity of the ethyl acetate solvent caused it to interact very strongly with the silica gel adsorbent. The interactions of biphenyl and benzophenone with the solvent and the silica gel were weak in comparison and thus, the attraction of the ethyl acetate to the silica gel forced both the biphenyl and benzophenone spots far up the plate. It was inferred that the extremely high polarity of ethyl acetate forced even the polar benzophenone up the plate by again interfering with any possible attraction between the spotted solutions and the silica gel adsorbent.

In the elution of the TLC plates by the hexanes solvent system, the plates displayed that the reference solutions and sample solutions both remained very close to the starting point, with very low Rf values. Hexane is a less polar solvent than the ethyl acetate so this solvent system did not interfere with the attraction of the reference compounds to the silica gel adsorbent. The unknown sample compound, found in Part A to be benzophenone, also depicted very little movement from the starting point. The reference solutions basically adhered to the starting point and did not dissolve in the mobile phase of the hexanes solvent as would be expected since benzophenone solution is highly polar compound, while the hexanes solvent system is nonpolar.

Finally, an unknown mixture, labelled XX, was spotted in the sample lanes of three TLC plates and visualized against three reference compounds, ortho-bromonitrobenzene, meta-bromonitrobenzene and para-bromonitrobenzene, one on each plate. The visualization was performed to determine the mole percent of the compounds in the unknown mixture and then determine the relative ratio of those compounds. Development of the TLC plates in the relatively nonpolar solvent system of 1:9 EtOAc to hexanes created minimal interaction between the solvent and the compounds and silica gel. On the para-bromonitrobenzene TLC plate, separation occurred in the unknown mixture where one of unknown sample's spots had the exact same Rf value as the reference spot of para-bromonitrobenzene, which was a value of 0.57. The other spot was more polar and had a lower Rf value of just 0.38. On the ortho-bromonitrobenzene TLC plate, the unknown sample spot in this case also had the exact same Rf value as the reference spot of ortho-bromonitrobenzene, which was a value of 0.35. These matching sample spot Rf values to the reference compound's Rf values leads to the conclusion that the unknown mixture XX contained ortho and para isomers. No matching occurred on the meta-bromonitrobenzene TLC plate because the sample Rf values of 0.62 and 0.43 and the reference Rf value of 0.57 differed. After analysis with ImageJ and some consequent calculations (see "Calcuations"), the components of the unknown mixture were determined to consist strictly of ortho-bromonitrobenzene and para-bromonitrobenzene and the ratio of ortho-para was determined to be approximately 1:1. The mole percent of the para-isomer was found to be 51.31965% and the mole percent of the para-isomer was found to be 48.68035%.

Possible sources of error for the experiment may have been due to human error. The line indicating the level of solvent absorption in some cases was not quickly enough drawn, resulting in a flawed measurement of solvent because it dries and becomes not visible, which would alter the Rf value for some spots. This error could have been avoided by more quickly drawing the line or by having the line drawn by a device that would precisely sense when the solvent elution neared the end of the plate and draw the line for us. Also, two of the TLC plates in Part C were accidentally left a slightly longer time in the solvent, which caused the length of solvent absorption and the resulting Rf value to be perhaps greater than normal and very near the top of the plate. Another source of error for

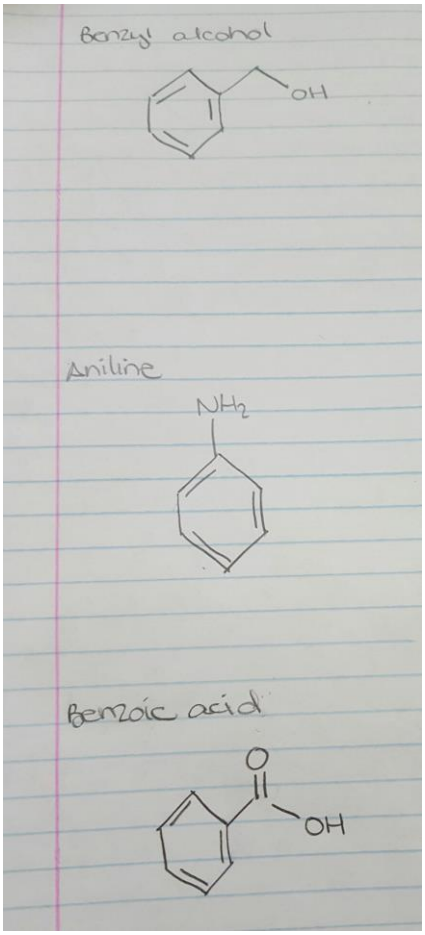
this experiment is that the elution of two TLC plates was performed at the same time and in the same container. This could have caused cross-contamination between the two plates, if some of the organic compounds touched the other plate. This could have caused more spots to appear of a TLC plate or the lack of a spot appearing at all. Another source of error could have been measuring error since in some cases, the spot appeared with a faint smudge below it, making it hard to decide where to measure the distance traveled from. Also, sometimes the solvent line 1 cm below the top of the TLC plate was not straight across, but actually a bit slanted. This made it hard to decide which point to measure the distance traveled by the solvent from.

A possible amelioration for this experiment could be make sure to draw the solvent line quicker right after the TLC plate is removed from the eluant before it dries and disappears. Also, another is to make sure to watch the elution more carefully and remove the TLC plates a bit earlier before the eluant gets too close to the top of the plate which could perhaps cause flawed Rf values. Another amelioration is to make sure to check each TLC plate under the UV light every time before each elution to make sure the spots at the starting points are visible before eluting them. The spots after the elution will not show up if they were not even visible beforehand.

Questions

1. The polarity of the solvent interferes with the molecular interaction of the silica gel with the compound, increasing the distance the compound travels up the plate. An increased solvent polarity allows both polar and nonpolar compounds to travel a greater length up the TLC plate. Increasing the polarity of a solvent may hinder separation of the compounds as the polarity of the solvent may interact so strongly with the polar silica gel adsorbent that the compound experiences a relatively small force of attraction and could move quickly up the plate. Polar solvents interact better with the silica gel. Thus, it causes higher Rf values to occur as the interaction pulls the solvent higher up the plate as well as the polar compound. As an example, when the percentage of the polar EtOAc solvent system was increased, the spots traveled up the silica gel TLC plate a greater length. When the percentage of the hexanes solvent system was increased, and when we used just a pure hexane solvent, the compounds only traveled only slightly, or not at all, up the silica gel TLC plate.

2. When a compound is polar, more forces and hydrogen bonding occurs with the silica gel on the TLC plate, which is a polar substance as well. Therefore, as the polarity of a compound increases, the less distance the compound will travel up the plate pulled by the solvent because it is attracted more to the silica gel and remains unmoved or close to the starting point. Therefore, the Rf values will be smaller.

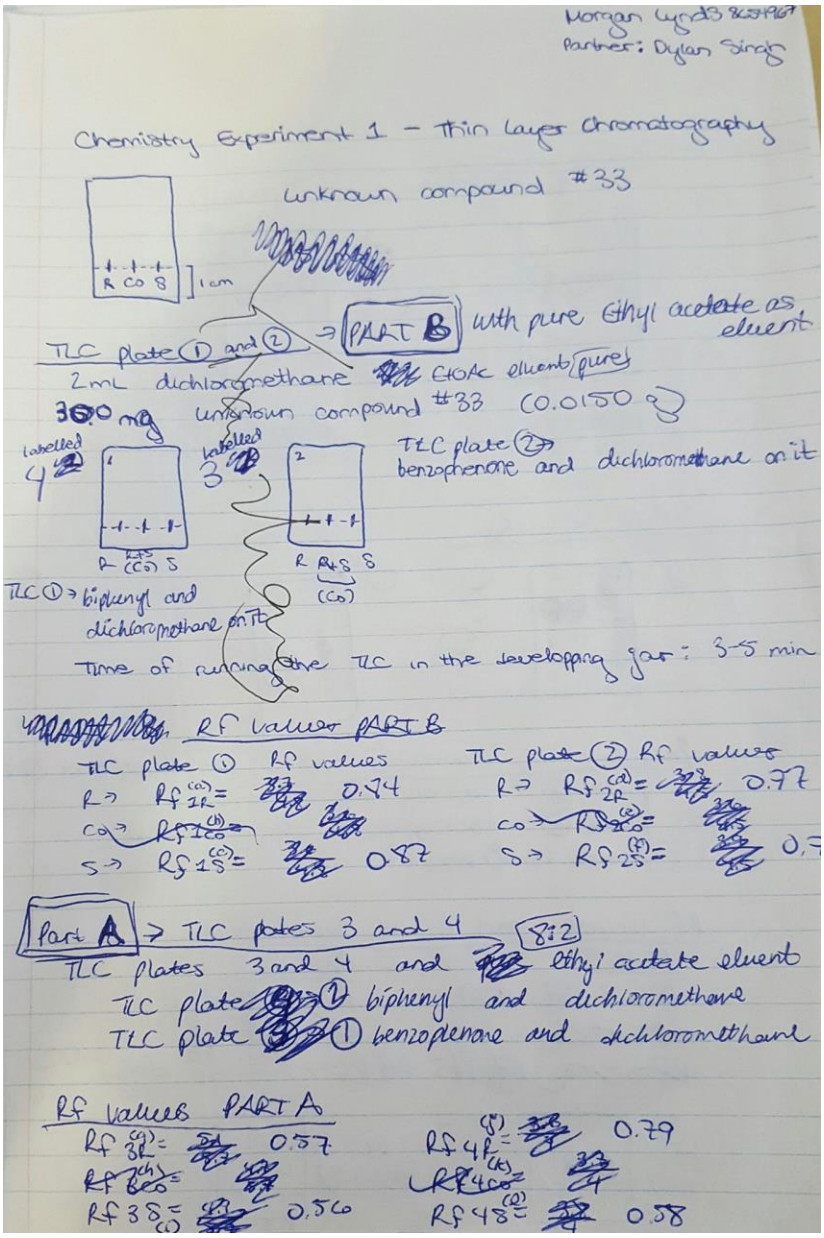


a) As explained above, the lowest Rf value will occur for the most polar compound. Out of the three given compounds, benzyl alcohol is the most polar because it contains a hydroxyl group, and this functional group creates a dipole moment and a hydrogen bond can form.

b) Out of the three given compounds, aniline would be the most polar molecule and therefore have the smallest Rf value; this is because hydrogen bonds can form with the 2 hydrogen atoms and it contains one electron lone pair on the nitrogen atom where bonding can occur.

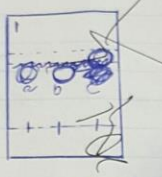
c) Out of the three given compounds, benzoic acid would have the smallest RF value as it is a highly polar substance, which increases its tendency to form a hydrogen bond with the functional hydroxyl OH group.

Raw Data

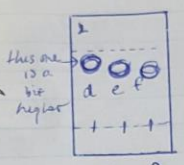

Morgan Lynch
Partner: Dylan Singh
Chemistry Experiment 1 – Thin Layer Chromatography
Unknown compound #33
R Co S
1cm
PART B with pure Ethyl acetate as eluent
TLC plate ① and ② → 2mL dichloromethane
EtOAc eluent pure
300 mg unknown compound #33 (0.0150 g)
labelled 4
labelled 3
TLC plate ② benzophenone and dichloromethane on it
TLC ① → biphenyl and dichloromethane on it
Time of running the TLC in the developing jar: 3-5 min
Rf values PART B
TLC plate ① Rf values
R → Rf 1R = 0.74
Co → Rf 1Co =
S → Rf 1S = 0.87
TLC plate ② Rf values
R → Rf 2R = 0.77
Co → Rf 2Co =
S → Rf 2S =
Part A → TLC plates 3 and 4 8:2
TLC plates 3 and 4 and Ethyl acetate eluent
TLC plate ③ ② biphenyl and dichloromethane
TLC plate ④ ① benzophenone and dichloromethane
Rf values PART A
Rf 3R = 0.57
Rf 3Co =
Rf 3S = 0.56
Rf 4R = 0.79
Rf 4Co =
Rf 4S = 0.58

Part B drawings

TLC plate ①



TLC plate ②

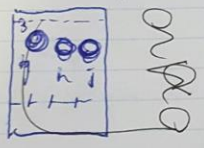


Benzophenone dichloromethane

Biphenyl dichloromethane

Part A drawings

③



④

Part B – TLC plates ⑤ and ⑥ with Hexanes as eluent

plate ⑤ ⇒ biphenyl and dichloromethane

plate ⑥ ⇒ benzophenone dichloromethane

Hexane (8mL) eluent

Rf values part B with hexanes eluent

Plate 5

Rf m = 0.29

Rf o = Rf 5 = 0.05

Plate 6

Rf p = 0.12

Rf q = 0.02

Rf r =

⑤ (Part B 3)

⑥ (Part B 4)

Part C unknown sample XX in 9:1 hexane eluent

Plate 7 ⇒ ref: A orthoaminobenzene and sample XX

Plate 8 ⇒ ref: B para-aminobenzene and sample XX

Plate 9 ⇒ ref: C meta-aminobenzene and sample XX

Rf values

Plate ⑦

Rf s = 0/5.6

Rf t = 0/5.6

Rf u = 0/5.6

Orange moved up

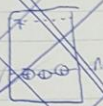


Plate ⑧

Rf v = 3.6/4.6

Rf w = 2.8/4.6

Rf x = 3.0/4.6

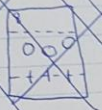
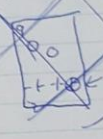


Plate ⑨

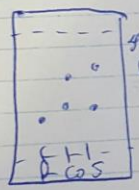
Rf y = 2.5/3.2

Rf z = 3.2/3.2

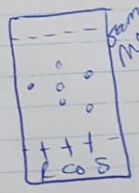
Rf aa = 0/3.2 = 0



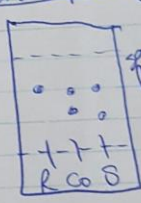
Part C Plate ①



Part C Plate ②



Part C Plate ③



Rf values

R = 0.35

S_1 = 0.56

S_2 = 0.35

R = 0.57

S_1 = 0.62

S_2 = 0.43

R = 0.57

S_1 = 0.57

S_2 = 0.36