Organic Chemistry Lab 1:
Thin Layer Chromatography

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Procedure

The procedure is as described in the lab manual (CHM1321 Introductory Organic Chemistry Laboratory Manual 2018, Dr. Tony Durst, Dr. Tito Scaiano, Dr. William Ogilvie, and Dr. Alison Flynn, 2018, Exp. 1, p 13 to 19).

However, one minor modification was made to the procedure.

> 8. Carefully place the TLC plates into the developing jar, ensuring that the pencil line is above the top of the solvent level. **If it would appear that the eluting solvent level will be higher than the pencil line, pour 1-2 mL out because it will wash away your spots.** Make sure that the silica layer faces up. Place the cover on the developing jar. Allow the solvent to elute until the solvent front is approximately 1 cm from the top of the plate (3–5 min).

In addition, we also conducted part A of the experiment twice because there was no visible spot on the TLC plate after visualizing plate 2 (biphenyl) in the sample lane.

Observations

- The unknown compound was a mixture that would separate when left sitting
- The unknown substance #89 was a crystallized powder
- The benzophenone in part A took 16 minutes to run the first time and 8 minutes the second
- The biphenyl in part A took 14 minutes to run the first time and 18 minutes the second
- In part B, both benzophenone plates in both Ethyl acetone and hexanes took a relatively short time, 9 and 10 minutes respectively
- In part B, the biphenyl plate in Ethyl acetone and hexanes took a relatively long time, 20 min, and a relatively short time in hexanes, 7 min
- Both Ortho- bromonitrobenzene and Meta- bromonitrobenzene took 7 minutes to run in part C and Para- bromonitrobenzene took slightly shorter at 6 minutes

Data Tables

Note: The experiment was preformed using unknown compound number 89.

Table 1: Part A - TLC Plates with 2:8 mixture of Ethyl Acetate as Elutant - Trial 1

Plate	Elutant	Spots/Lanes			Rf Values			
		Reference	Co-Spot	Sample	R	C		S
1	Ethyl Acetate	Benzophenone	- Benzophenone - Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	0.86	C1 0.23	C2 0.55	1.05
2	Ethyl Acetate	Biphenyl	- Biphenyl - Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	0.88	0.85		None

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Table 2 : Part A - TLC Plates with 2:8 mixture of Ethyl Acetate as Elutant - Trial 2

Plate	Elutant	Spots/Lanes			Rf Values		
		Reference	Co-Spot	Sample	R	C	S
1	Ethyl Acetate	Benzophenone	- Benzophenone - Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	0.72	0.70	0.74
2	Ethyl Acetate	Biphenyl	- Biphenyl - Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	1.10	1.18	1.10

Table 3: Part B - TLC plates with pure Ethyl Acetate as Elutant

Plate	Elutant	Spots/Lanes			Rf Values		
		Reference	Co-Spot	Sample	R	C	S
1	Ethyl Acetate	Benzophenone	- Benzophenone Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	0.88	0.93	0.96
2	Ethyl Acetate	Biphenyl	- Biphenyl - Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	0.93	0.96	0.96

Table 4: Part B - TLC Plates with Hexanes as Elutant

Plate	Elutant	Spots/Lanes			Rf Values			
		Reference	Co-Spot	Sample	R	C		S
3	Hexanes	Benzophenone	-Benzophenone - Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	0.03	0.02		0.02
4	Hexanes	Biphenyl	- Biphenyl Dichloromethane and Unknown #89	Dichloromethane and Unknown #89	0.45	C1 0.02	C2 0.43	0.00

Table 5: Part C - TLC Plates with 1:9 Mixture of Hexanes:Ethyl Acetate

Plate	Elutant	Spots/Lanes			Rf Values					
		Reference	Co-Spot	Sample	R	C 1	C 2	C 3	S 1	S 2
1	1:9 Mixture of Hexanes:Ethyl Acetate	A (Ortho-bromonitrobenzene)	A and ZZ	ZZ	0.38	0.38	0.50	n/a	0.38	0.50
2	1:9 Mixture of Hexanes:Ethyl Acetate	B (Meta-bromonitrobenzene)	B and ZZ	ZZ	0.50	0.35	0.50	n/a	0.31	0.48
3	1:9 Mixture of Hexanes:Ethyl Acetate	C (Para-bromonitrobenzene)	C and ZZ	ZZ	0.56	0.32	0.44	0.54	0.30	0.44

TLC Plate Figures

Note: In all figures, R stands for reference, C stands for Co-Spot, and S stands for sample.

Figure 1: Part A - TLC plates in 2:8 Ethyl Acetate, sample is unknown #89, reference for 1 and 2-1 is benzophenone, reference for 2 and 2-2 is biphenyl.

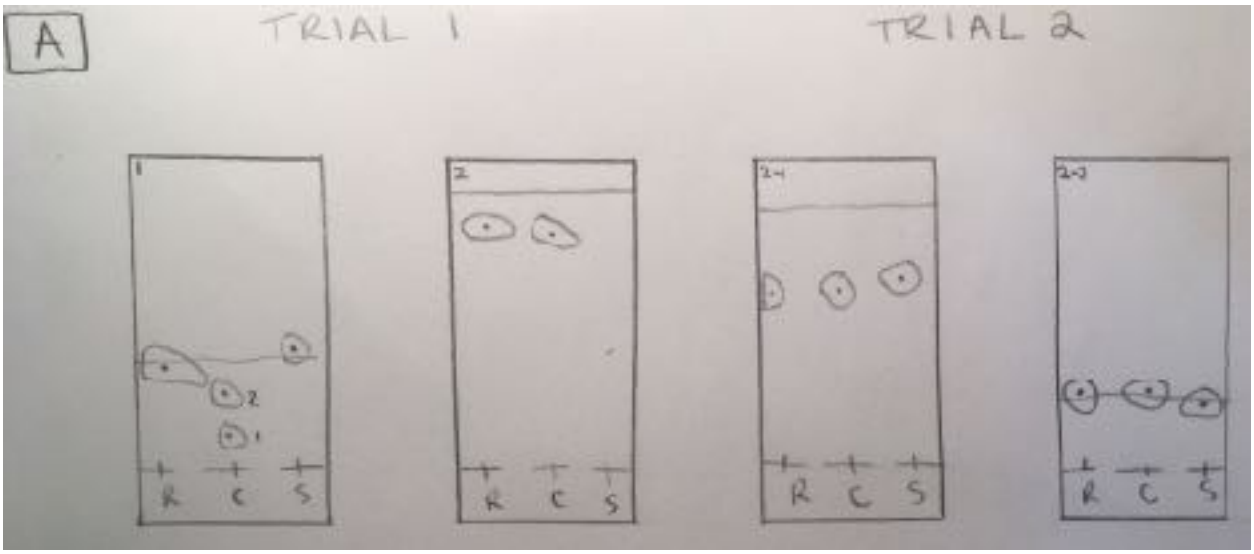


Figure 2: Part B - TLC plates 1 and 2 in Ethyl Acetate, 3 and 4 in Hexanes. Sample is unknown #89, reference for 1 and 3 is benzophenone, reference for 2 and 4 is biphenyl.

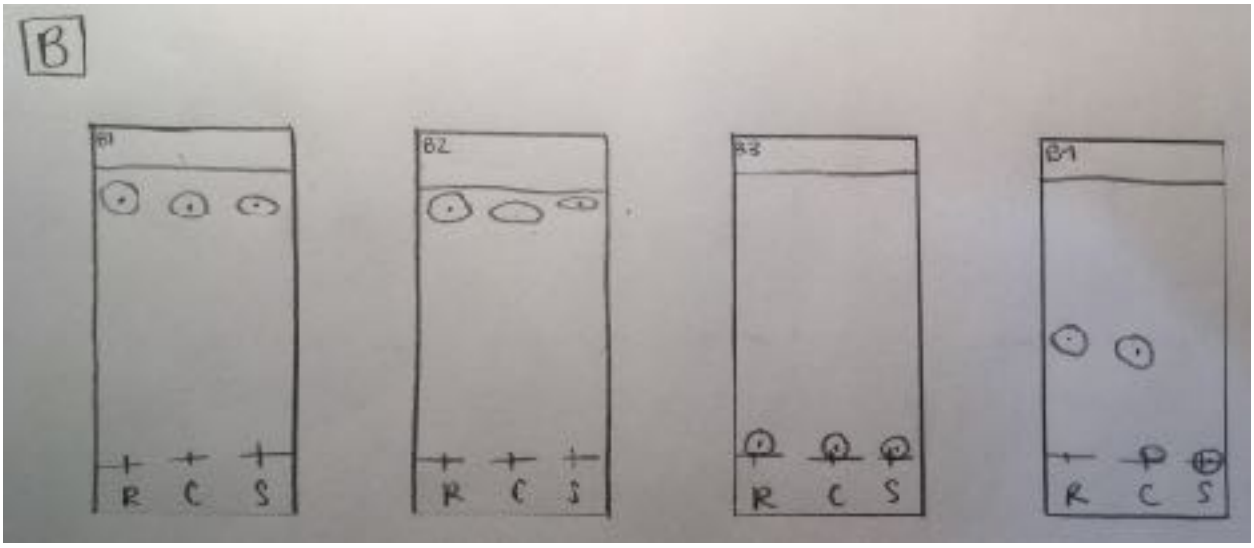


Figure 3: Part C - TLC plates in 9:1 Hexanes:Ethyl Acetate. Sample is unknown ZZ, reference in plate 1 is ortho- bromonitrobenzene, plate 2 is meta- bromonitrobenzene, and plate 3 is para- bromonitrobenzene.

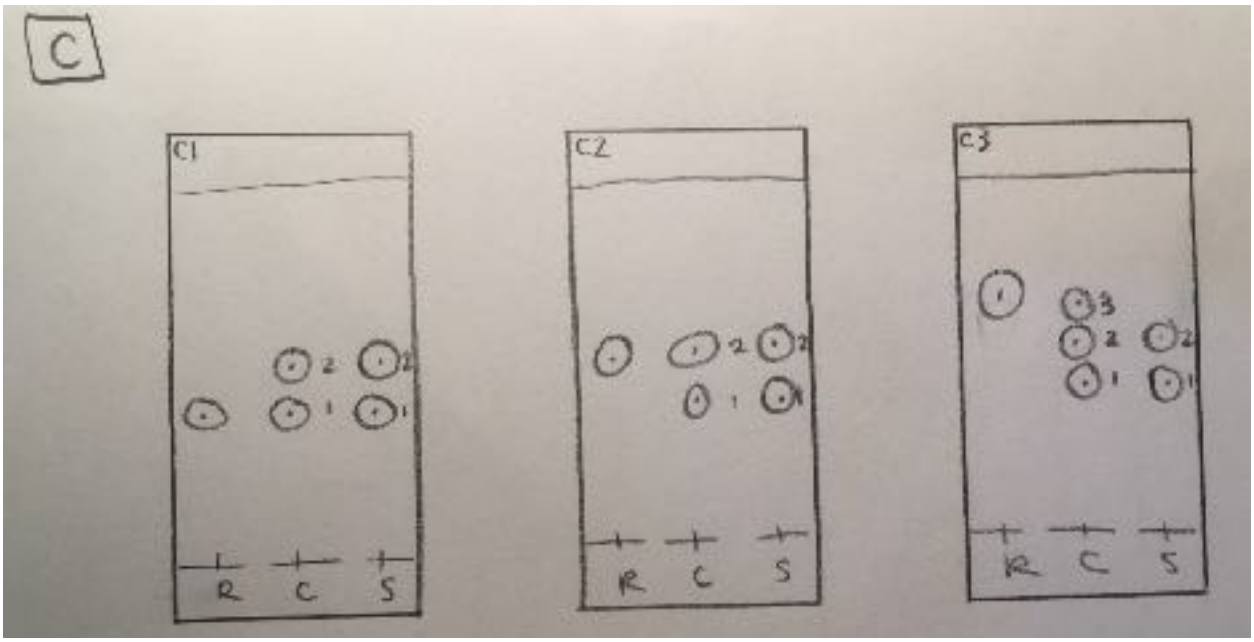


Figure 4: Part C TLC plates spotted with ortho-, para-, and meta- bromonitrobenzene respectively visualized under the UV light.

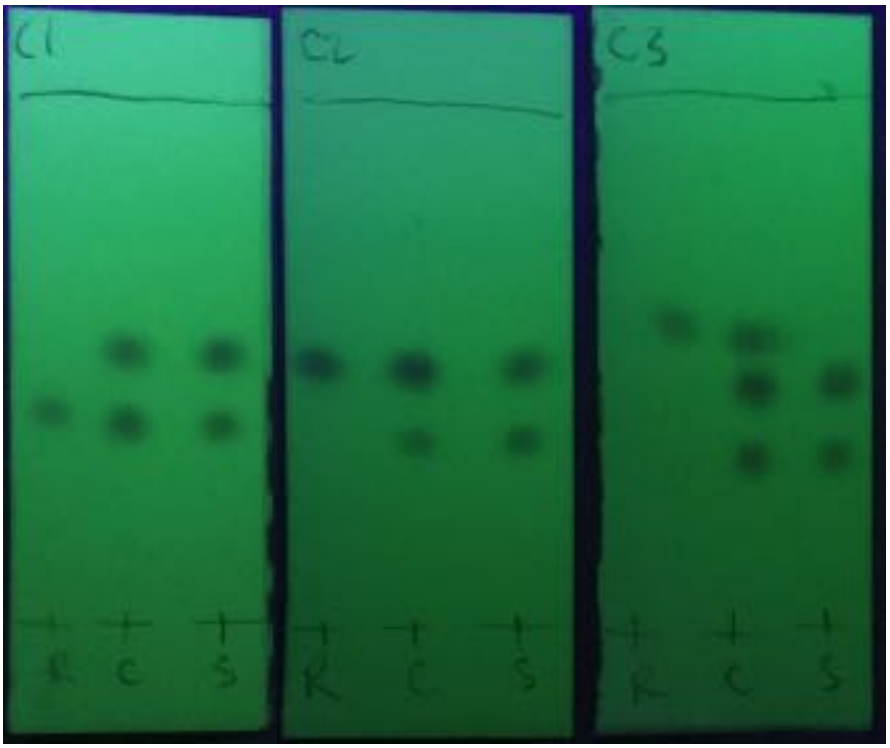


Figure 5: Part C TLC plate spotted with Ortho- bromonitrobenzene with lanes indicated with Image J

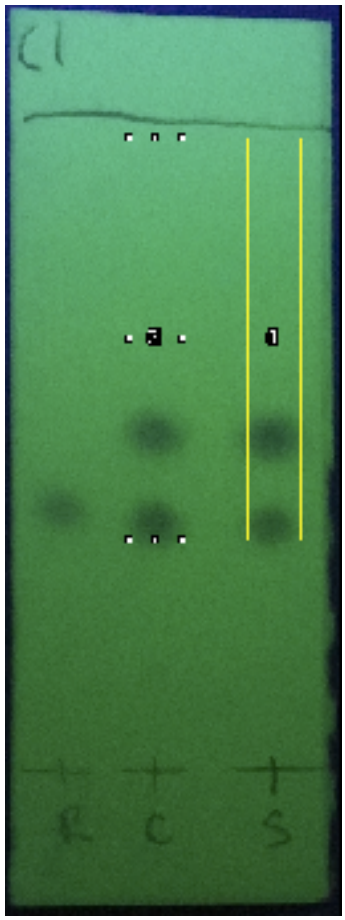


Figure 6: Lane Profile for the Ortho-bromonitrobenzene TLC Plate (top = sample, bottom = co-spot)

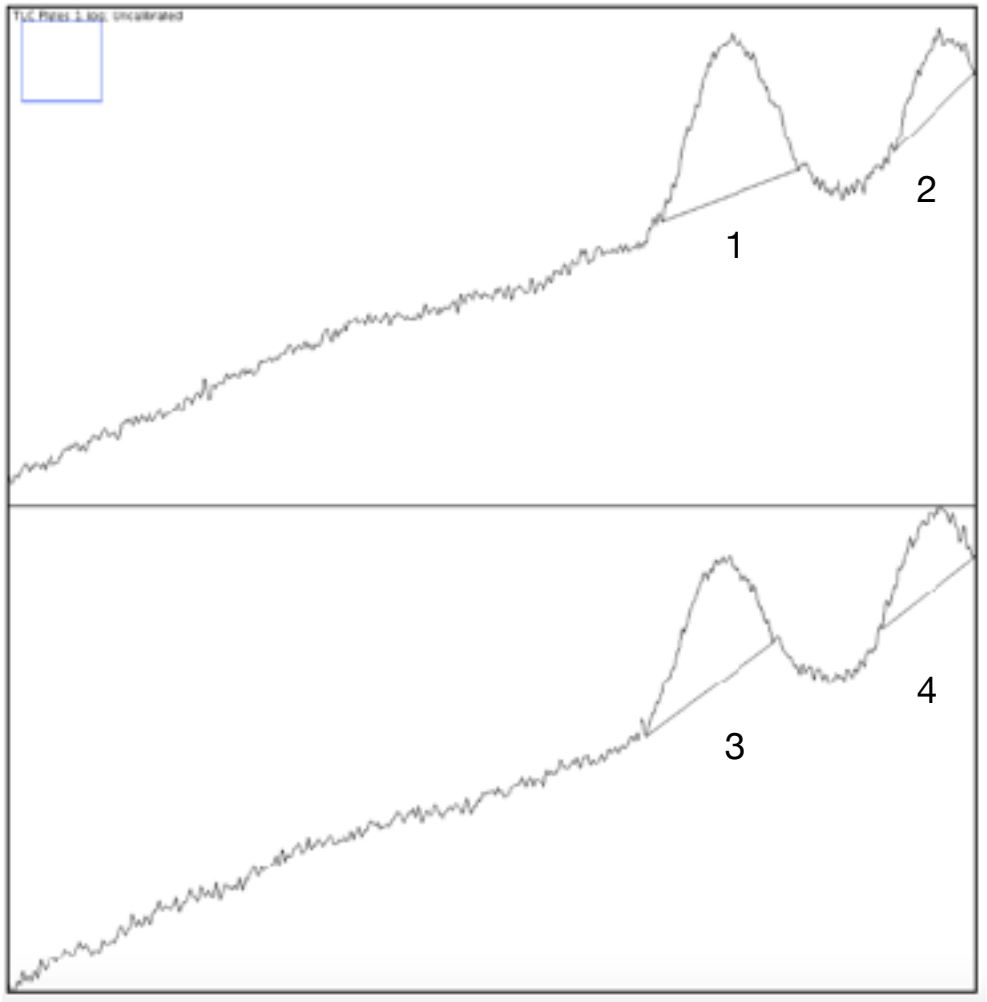


Figure 7: The Areas Between the Peaks and Horizontal Lines

	Area
1	13661.643
2	3924.309
3	10065.099
4	4974.673

Calculations

1. **Example Calculation of an Rf Value: The Rf value for the Ortho-bromonitrobenzene reference spot of Part C TLC plate C1.** This Value can be found in Table 5.

Rf Value = $\frac{\text{Distance Traveled by the Compound}}{\text{Distance Traveled by the Solvent}}$
= 2.4 cm / 4.7 cm
= 0.50

Therefore, the retention factor, or Rf value, for the Ortho-bromonitrobenzene spot of the Part C TLC plate C1 is 0.50. All Rf values can be found in tables 1 through 5.

2. **Example Calculation of the percentage of absorbance of the Unknown Mixture:**

Area of peak 1 : 13661.643
Area of peak 2 : 3924.309
Total Area of peak 1+2: 17585.952

% peak 1 = (area of peak 1 / Total area of Peak 1+2) x 100%
= 3924.309 / 17585.952 x 100%
= 77.68%

% peak 2 = 22.32%

% peak 3 = 66.92%

% peak 4 = 33.08%

Peaks 2-4 calculated with same method with values from figure 7 calculated with the image J software using figure 6.

3. **Calculation of determining the mole percent of the Unknown Mixture:**

Peak 2 is one of the ortho isomer peaks because its sample spot, sample spot 1 (lower) has the same Rf value as the reference spot for ortho-bromonitrobenzene.

Y = % absorbance (ortho) = 22.32
Y = 1.0114x - 2.0208 (from given ortho-meta calibration curve)
22.32 + 2.0208 = 1.0114x
24.32 = 1.0114x.
x = 24.05

% ortho = 24.05 %
Therefore % meta = 100% - 24.05% = 75.95%

Discussion

Part A

- we compared the polarities of unknown solution #89 on a silica gel TLC plate with 2 reference solutions, benzophenone and biphenyl to determine the unknown compound
- Benzophenone is a polar compound
- Biphenyl is non-polar, it has no polar functional group and is symmetrical
- Silica is very polar
- Ethyl Acetate and Hexanes (2:8) is relatively neutral which allowed the reference, compound and co-spot to travel on the TLC plate based on their affinity for silica
- In our benzophenone TLC plate the reference, co-spot, and sample spot stayed level the whole time causing me hypothesize our unknown was benzophenone
- The biphenyl plate did not travel very far or very quickly which was not expected due to its non-polar attributes
- Despite this flaw it is still likely that benzophenone is the unknown because the retention factor (Rf) values were relatively the same across the plate, being 0.72, 0.70, and 0.74 for the reference, co-spot, and sample respectively
- Had the sample been biphenyl the sample spot would have had a Rf differing from the reference and the co-spot would have had 2 spots
- The biphenyl trial is being ignored due to clear error, possible sources explained later in the discussion
- All data is from part A trial 2

Part B

- the polarities of the same references from part A, Biphenyl and Benzophenone, were compared to the polarity of the unknown solution #89 in 2 different eluting solutions
  - Ethyl Acetate
  - Hexanes
- According to the polarity scale found in Table 2 in the lab manual, Ethyl Acetate is more polar than Hexanes (CHM1321 Introductory Organic Chemistry Laboratory Manual 2018, Dr. Tony Durst, Dr. Tito Scaiano, Dr. William Ogilvie, and Dr. Alison Flynn, 2018, Exp. 1, p 13 to 19)
- Ethyl Acetate
  - I would expect the polar Ethyl Acetate to strongly interact with the polar silica
  - I would expect the biphenyl to travel further up the plate than the benzophenone due to it’s non polarity

    - We saw that both traveled to the top of the TLC plate and had similar Rf values but biphenyl took twice as long to do so which was the opposite of what was expected
    - The interactions between the references and their solvent Ethyl Acetate and the silica gel are weak when compare to that of the interactions between the Ethyl Acetate and silica gel so the tide of the solvent traveling up the plate carried all the spots with it
- Hexanes
    - Being a less-polar solution I would expect that the spots would not travel as far up the plate because the attraction between silica and hexanes is not as strong
    - I would expect and I saw that biphenyl would move further up the plate because of it's non-polar nature (plate B4)
    - It has less of an attraction to the polar silica gel so would not resist the tide of the solution
    - These points, reference and co-spot point 2, had higher Rf values
    - Due to the polar nature of the benzophenone and the sample which we determined to be benzophenone, I would expect and I saw that the spot would not move far
    - This is because of the polar attractions to the silica gel
    - These points had low Rf values

Part C

- we were given an unknown mixture "ZZ"
- This was spotted on three TLC plates and visualized against three reference compounds
    - A - Ortho- bromonitrobenzene
    - B - Meta- bromonitrobenzene
    - C - Para- bromonitrobenzene
- The plates were developed in relatively non-polar solvent system Ethyl Acetate and Hexanes (1:9) due to it's large component of hexanes
- On the Ortho- bromonitrobenzene plate one of the sample spots, the one closer to the spotting line, had the same Rf value as the Ortho- bromonitrobenzene reference
- On the Meta-bromonitrobenzene plate one of the sample spots, the one further from the spotting line, had the same Rf value as the Meta- bromonitrobenzene reference
- On the Para- bromonitrobenzene plate none of the sample spots had the same Rf value as the reference spot and the co-spot split into three spots
- This leads me to believe that ZZ is composed of
    - Ortho- bromonitrobenzene
    - Meta- bromonitrobenzene
- With this reasoning I used TLC plate C1 to make my graph analysis with Image J
- The mole percent of the components of the compound was found to be:
    - 24.05% Ortho- bromonitrobenzene
    - 75.95% Meta- bromonitrobenzene
    - See "calculations" for steps and sample calculation

Error

- In trial 1 of part A when we were still getting used to putting the TLC plates into the jars, plate A2 was dropped diagonally with the silica side facing into the eluting liquid
- It was quickly rescued but may have been the cause for the missing spot in the solution lane
- In trial 1 and 2 of part A we left in plate 1 (trial 1) and 2 (trial 2) in for a very long time trying to get the solution to travel 1cm from the top
- This caused error in calculating the Rf values because the solution began traveling back down and the solution line was no longer accurate
- Some of the spots showed up above solution line giving Rf values greater than 1
- We also noticed the solution would evaporate quickly forth top portions and if you didn't draw the solution line quickly, it may have been inaccurate and affected Rf values
- Finally, the ruler used to measure the plates to find Rf values was divided into mm
- I believe the calculation of Rf value could have been more accurate had the ruler had finer divisions because some spots were as small as 2mm wide

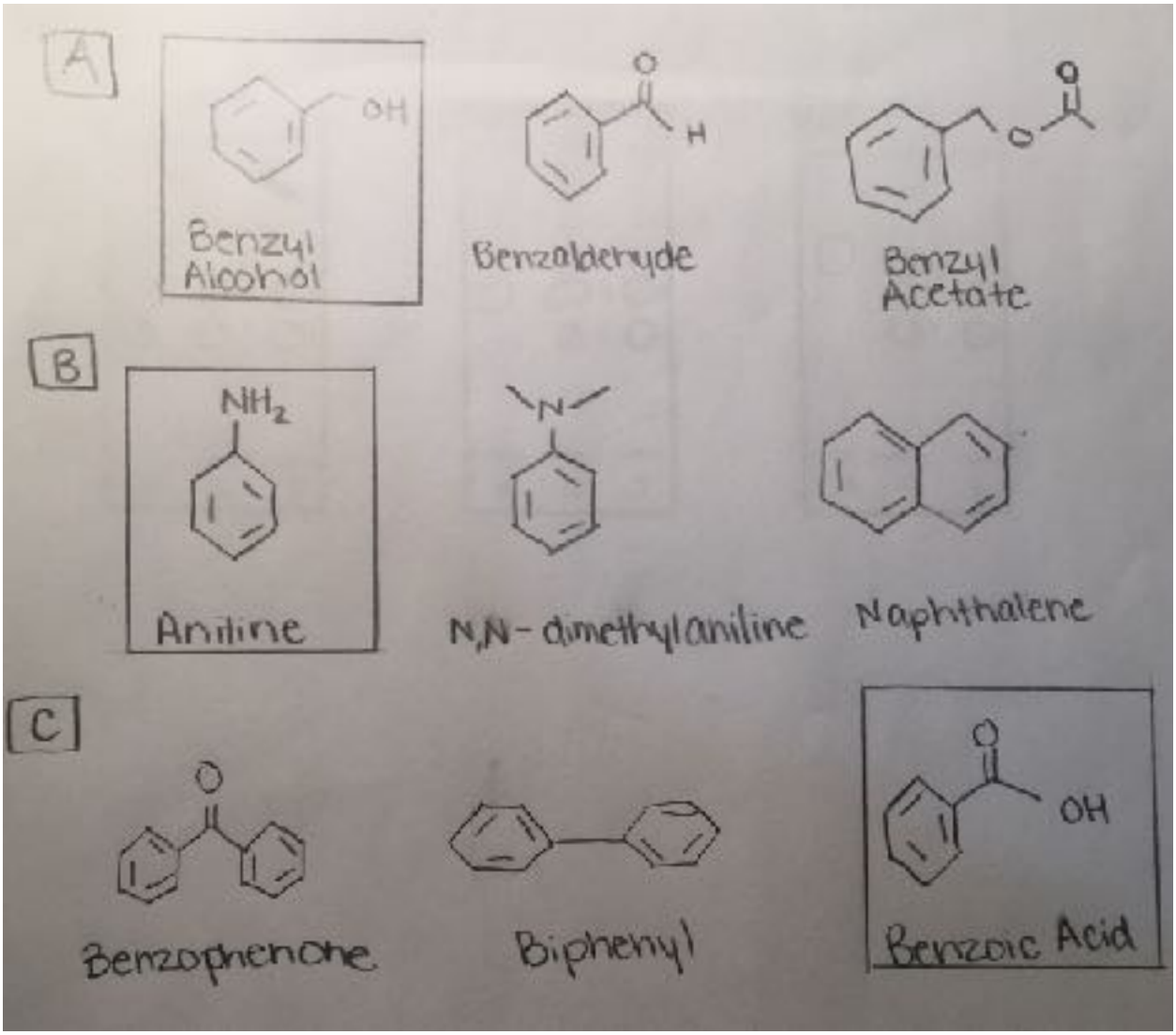
Questions

1.
- The increasing polarity of the solvent system affects the result of the TLC
- the intermolecular forces between the solvent and the silica, the solvent and the compound, as well as the silica and the compound, are determined by the polarity
- Increased polarity of the solvent would cause higher attraction between the solvent and the very polar silica on the TLC plate causing the spots to be swept quickly up the plate
  - Example: ethyl acetate
- Very polar compounds would have a high attraction to the plate and would not move very far up it when the eluting solvent was absorbed due to the higher level of attraction
- Solvents with low polarity would also have the same effect
  - Example: hexanes

2.
- Rf values are calculated by dividing the distance a spot traveled by the distance the solvent traveled
- This means a spot that traveled far up a TLC plate would have a large Rf value, close to 1
- Polar compounds are attracted to the silica and don't travel as far, meaning the most polar in the set of compounds would have the smallest Rf value.

A. In this set of compounds, benzyl alcohol is the most polar because it has a hydroxyl group which can form a hydrogen bond and creates a dipole moment

B. Out of these compounds, aniline would be the most polar because hydrogen bonds can form with the 2 Hydrogen atoms and it contains one lone electron pair which leaves the potential for bonding
C. Benzoic acid would be the most polar out of the three given compounds. It has an OH which means it has the tendency to hydrogen pond and is highly polar.



Raw Data