LAB 1:

THIN LAYER CHROMATOGRAPHY

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Submission Due: January 16th, 2019

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CHM1321 - Z05

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Procedure
See lab manual Experiment 1: Thin Layer Chromatography (pg 1-6) .

Qualitative Observations

- ➢ The TLC plate was composed of silica which was a white layer and an aluminum backing which was a silver colour. The compounds were all spotted on the white silica layer.
- ➢ Both benzophenone and biphenyl were transparent solutions. The unknown sample provided (#43) was composed of opaque white crystals. When dissolved in the transparent dichloromethane, the resulting unknown solution was also transparent.
- ➢ The solvent systems used were also all transparent and clear. They all had a strong odour.
- ➢ Ortho, meta and para bromonitrobenzene were transparent solutions.
- ➢ When adding the solvents with the capillary it was observed that there was a small wet circular spot for a second and then it would quickly disappear.
- ➢ When the prepared TLC plates were put into the jar with solvent, the solvent line would immediately climb up the plate.
- ➢ Within two minutes the solvent line would be within a centimeter of the top of the TLC plate.
- ➢ It was difficult to remove the TLC plate without touching the silica.
- ➢ After the removal of the TLC plate from the solvent jar the solvent would quickly dry and to the human eye it looked like nothing had changed.
- ➢ Under a UV light the TLC plates showed dark coloured spots which could be used to find the Rf values of both the references and unknowns in parts A, B and C.

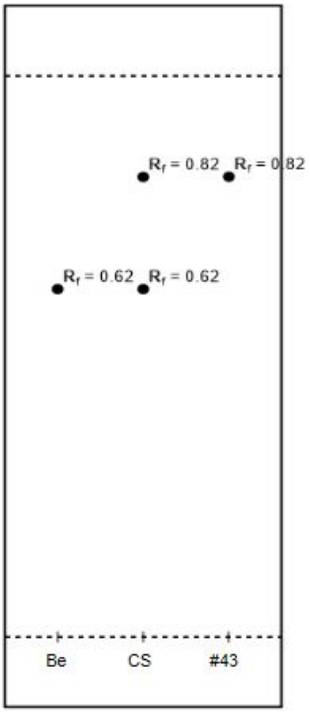
Quantitative Observations

Legend:

L = lowest Spot (Vertically)
M = Middle Spot (Vertically)
T = Highest Spot (Vertically)
d_s = displacement between former and new solvent line
d_X= displacement between starting solvent line and molecule
Ⓞ = 2 Spots
● = 1 Spot

Quantitative Observations: Part A

Figure 1.1. TLC of unknown substance #43 using benzophenone as reference

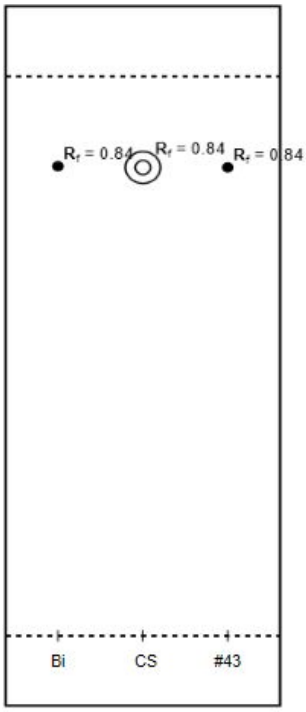


Solvent System:
EtOAc:hex
2:8

displacements:
d_s= 5.0 cm
d_{Be}= 3.1 cm
$d_{CS(L)}$= 3.1 cm
$d_{CS(T)}$= 4.1 cm
d_{43}= 4.1 cm

Be = Benzophenone
CS = Co-Spot
#43 = Unknown Sample 43

Figure 1.2. TLC of unknown substance #43 using biphenyl as reference



Solvent System:
EtOAc:hex
2:8

displacements:
d_s= 4.5 cm
d_{Bi}= 3.8 cm
d_{CS}=3.8 cm
d_{43}=3.8 cm

Bi = Biphenyl
CS = Co-Spot
#43 = Unknown Sample 43

Quantitative Observations: Part B

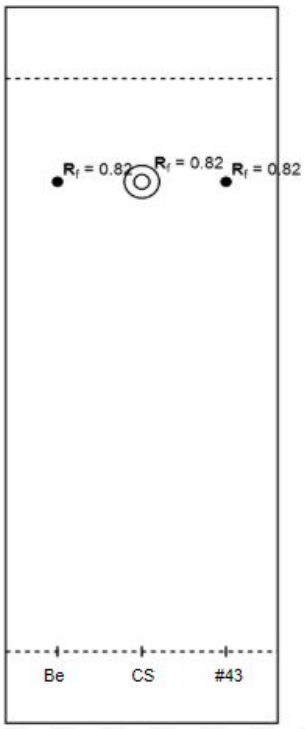
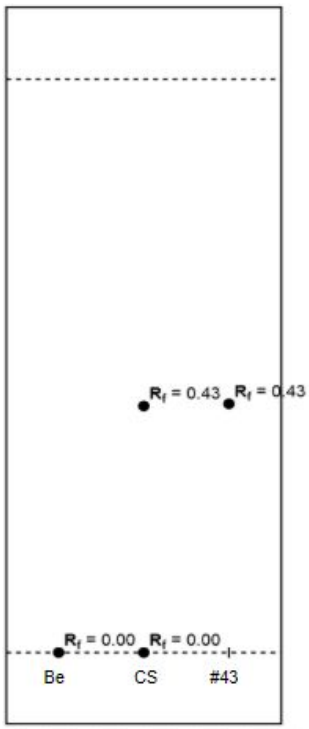
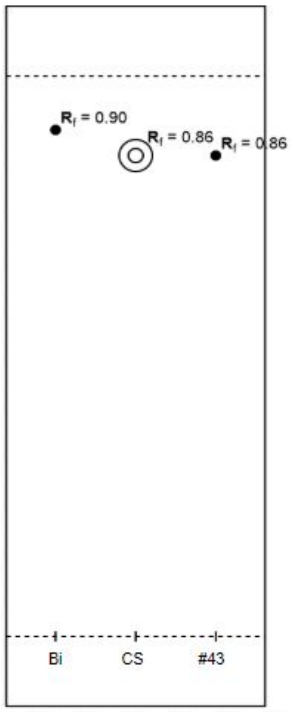
Figure 2.1. TLC of unknown substance #43 using benzophenone as reference	**Figure 2.2.** TLC of unknown substance #43 using benzophenone as reference
 Solvent System: Ethyl Acetate *displacements*: d_s= 5.0 cm d_{Be}= 4.1 cm $d_{CS(L)}$= 4.1 cm d_{43}= 4.1 cm Be = Benzophenone CS = Co-Spot #43 = Unknown Sample 43	 Solvent System: Hexane *displacements*: d_s= 4.9 cm d_{Be}= 0 cm $d_{CS(L)}$= 0 cm $d_{CS(T)}$= 2.1 cm d_{43}= 2.1 cm Be = Benzophenone CS = Co-Spot #43 = Unknown Sample 43

Figure 2.3. TLC of unknown substance #43 using biphenyl as reference

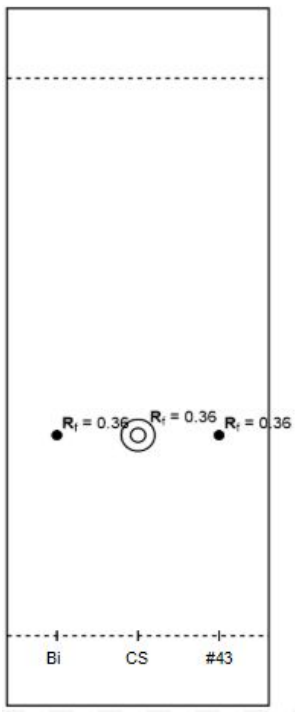


Solvent System:
Ethyl Acetate

displacements:
d_s= 4.9 cm
d_{Bi}= 4.4 cm
d_{CS}= 4.2 cm
d_{43}= 4.2 cm

Bi = Biphenyl
CS = Co-Spot
#43 = Unknown
Sample 43

Figure 2.4. TLC of unknown substance #43 using biphenyl as reference



Solvent System:
Hexane

displacements:
d_s= 4.7 cm
d_{Bi}= 1.7 cm
d_{CS}= 1.7 cm
d_{43}= 1.7 cm

Bi = Biphenyl
CS = Co-Spot
#43 = Unknown
Sample 43

Quantitative Observations: Part C

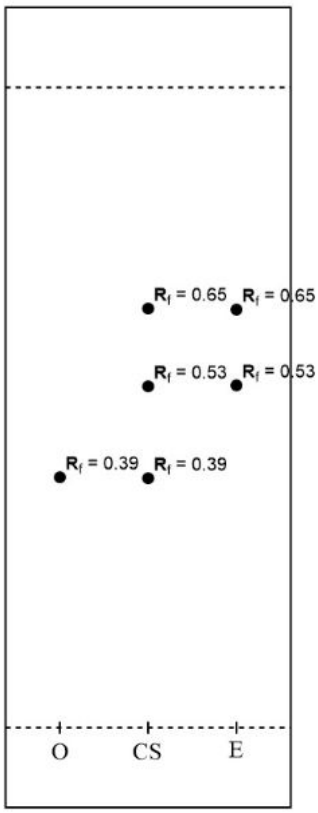
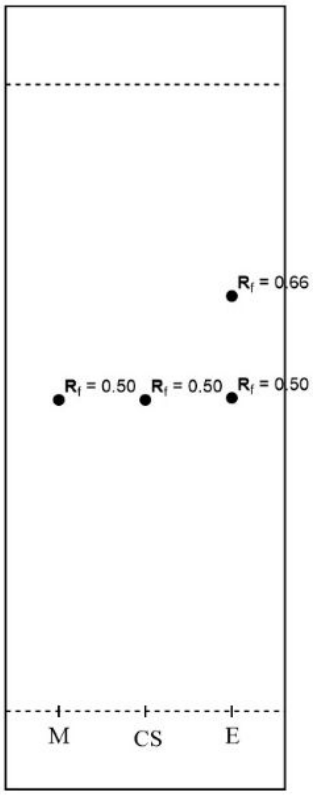
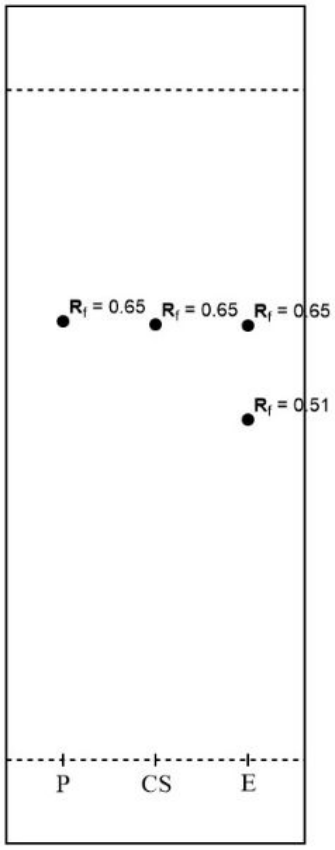
Figure 3.1. TLC of unknown substance E using ortho-bromonitrobenzene as reference	**Figure 3.2.** TLC of unknown substance E using meta-bromonitrobenzene as reference
 Solvent System: Hex:EtOAc 9:1 *displacements*: d_s= 5.1 cm d_O= 2.0 cm $d_{CS(L)}$= 2.1 cm $d_{CS(M))}$= 2.7 cm $d_{CS(T)}$= 3.3 cm $d_{E(L)}$= 2.7 cm $d_{E(T)}$= 3.3 cm O = ortho - bromonitrobenzene CS = Co-Spot E = Unknown Sample E	 Solvent System: Hex:EtOAc 9:1 *displacements*: d_s= 5.0 cm d_M= 2.5 cm $d_{CS)}$= 2.5 cm $d_{E(L)}$= 2.5 cm $d_{E(T)}$= 3.3 cm M = meta - bromonitrobenzene CS = Co-Spot E = Unknown Sample E

Figure 3.3. TLC of unknown substance E using para-bromonitrobenzene as reference



Solvent System:
Hex:EtOAc
9:1

displacements:
d_s= 4.9 cm
d_M= 3.2 cm
d_{CS}= 3.2 cm
$d_{E(L)}$= 2.5 cm
$d_{E(T)}$= 3.2 cm

P = para - bromonitrobenzene
CS = Co-Spot
E = Unknown Sample E

Sample Calculation of Rf Value

From Part A, Figure 1.1

Given:	*Calculation:*
d_s= 5.0 cm d_{Be}= 3.1 cm $d_{CS(L)}$= 3.1 cm $d_{CS(T)}$= 4.1 cm d_{43}= 4.1 cm	**$Rf = d_x/d_s$** $Rf_{Be,CS(L)}$ = 3.1 cm /5.0 cm = 0.62 $Rf_{CS(T),\ 43}$ = 4.1 cm /5.0 cm = 0.82

Discussion

Part A

In this part of the experiment, the identity of sample #43 had to be obtained. In order to do so two TLCs were done each with a different reference of known compounds. The results of these TLCs are displayed in Figure 1.1, with benzophenone as the reference, and Figure 1.2, with biphenyl as the reference. In figure 1.2, the point made by the unknown shared the same R_f value with the point made by the reference, biphenyl. The co-spot which includes both biphenyl and the unknown also shares the same R_f value of 0.84. This means that the unknown sample provided must be biphenyl. This is because the R_f value is a measure of the polarity of a substance which is determined by how far a substance is able to move up the TLC plate ("Experiment 1 : Thin Layer Chromatography," 2014). Thus, substances that have a similar or equal R_f value are usually the same. It could not have been benzophenone nor could it contain benzophenone because the R_f value of the unknown does not match that of the reference, benzophenone, as it is seen that the points are not in alignment in figure 1.1. The R_f value of benzophenone in figure 1.1 is 0.62 and this is not at all close to the R_f value of the unknown (0.82). This R_f value is, in fact, much closer to that in figure 1.2 as previously stated, further solidifying that the unknown is biphenyl.

Part B

In this part of the experiment, the effects of the solvent on a TLC was tested or demonstrated. 4 TLCs were performed. 2 with Ethyl Acetate (EtOAc) as the eluant and 2 with Hexane as the eluant. When performing a TLC, the distance a compound moves, be it polar or nonpolar, is not only dictated by the compound's own polarity, but also by the polarity of the solvent system. The more polar the solvent is, the more it breaks down the intermolecular forces between the TLC plate (silica gel) and the compound ("Experiment 1 : Thin Layer Chromatography," 2014). This enables the compound to be more mobile and cover a farther distance which in turn gives it a larger R_f value. This difference can be seen in figure 2.1 and 2.2. Figure 2.1 represents a TLC done in a solvent system made of ethyl acetate and figure 2.2 represents a TLC done in a solvent system of hexanes. The R_f values for figure 2.1 are overall greater than the R_f values in figure 2.2. This shows that ethyl acetate is the more polar solvent. This makes sense because hexanes are mainly composed of carbon and hydrogen whereas ethyl acetate has oxygens which enable hydrogen bonding making this substance more polar. The same observation is also seen when comparing figures 2.3 (with EtOAc) and 2.4 (with Hexane). The R_f values for the plate run in the EtOAc eluant is greater than the plate run in the Hexane eluant because EtOAc is the more polar solvent.

Part C

In this part of the lab, an unknown sample (E) was provided and its components needed to be determined using TLCs and 3 different references, o-bromonitrobenzene, m-bromonitrobenzene, and p-bromonitrobenzene. Three different TLCs were performed, one for each reference. A solvent system of Hexane:EtOAc (9:1) was used. This ratio for the eluant was predetermined as indicated in the lab manual and was chosen because it yielded the optimal

separation of spots on the TLC and had the optimal polarity as a solvent system (“Experiment 1 : Thin Layer Chromatography,” 2014). This is most likely because the compounds used in this TLC were aromatics. These types of compounds are nonpolar and so would move faster along the TLC plate (“Experiment 1 : Thin Layer Chromatography,” 2014). So, a more nonpolar solvent was used to counter this and ultimately yield a better separation. Like the first part of the lab, if a TLC had 3 spots with the same R_f value this would mean that the compound found in the sample is the same as the reference compound. This is because the R_f value of the sample would definitely be shared by the co-spot as it was also spotted with the sample and the only way that the R_f value of the reference could be the same as the sample is if the reference was the same as the sample. This provides a row of 3 spots to look out for when identifying the components of an unknown compound. In this case, it was determined that the unknown compound E contained m-bromonitrobenzene and p-bromonitrobenzene. This is because in figures 3.2 and 3.3, a row of 3 spots is seen. In figure 3.1; however, this is not seen, therefore demonstrating that the sample has nothing in common with the reference. This diagram in particular has 3 spots in the co-spot lane which is a representation of the separation of the reference and the other 2 compounds that the sample is composed of. One error that can be seen in the figures 3.2 and 3.3 is that the co-spot lane only has 1 spot when in reality it should have had 2 spots. 1 spot would be an overlap of the reference and one of the components of the unknown and the other would be the second component of the unknown sample. This was not the case and is further discussed as a source of error.

Sources of Error

A few sources of error may have hindered the success of this experiment. The TLC plate preparing process is vastly delicate, and if rushed will not yield perfect results.

An easy way to damage an already prepared TLC plate is to accidentally touch the silica side of the plate with one's fingers or palm. The skin is oily, coated in organic compounds, touching the silica will cloud the results of the molecules under analysis.

There was an experimentation error. The entire lab bench utilized the same capillaries for ortho, meta and para bromonitrobenzene. There were 3 capillaries, 1 for each solvent. It would only take one person in the lab to misplace the capillaries to damage all the TLC plates that were prepared using those capillaries. This would explain outliers seen in quantitative observations part C and other. The error can be avoided by being more careful and perhaps labeling the capillaries with some sort of marker to colour coordinate it to each solvent.

For part C, of the lab the co-spot in figures 3.2 and 3.3 are missing an extra spot. This could be because not enough of the sample (E) was loaded onto the co-spot. While visualizing the TLC plates for part C under the UV light, the spots were the faintest of all the previous TLCs.

Another human made error is due to not being able to see the solvent on the TLC plate with the human eye. As mentioned in the qualitative observations, all the solutions used were transparent and would dry out on the TLC plate within one second. If one was not careful it would be very easy to overload a solvent, or not put any at all. This can be avoided by being extra careful and recording everything that was done step by step into the lab notebook.

Conclusion

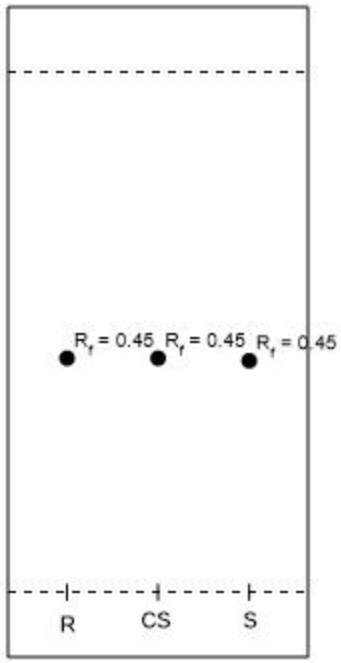
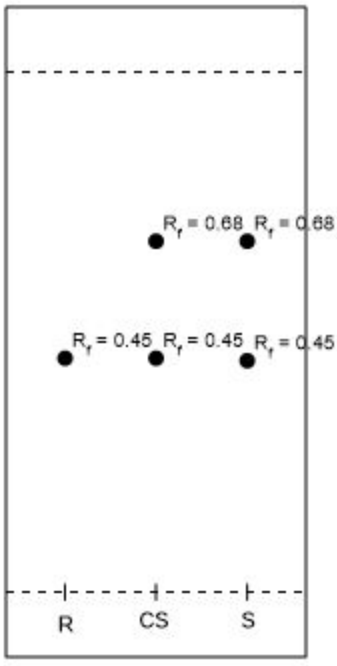
Part A: The unknown #43 sample was found to be biphenyl.
Part B : The solvent ethyl acetate is the more polar than hexane because the R_f values on the plates eluted in EtOAc are larger, in comparison.
Part C: The unknown E was a compound that consisted of meta and para bromonitrobenzene .

Questions

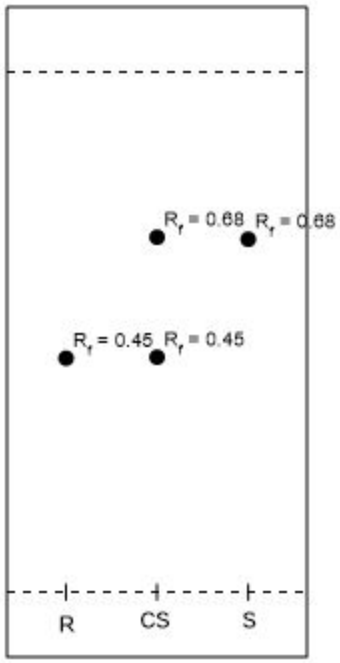
1. It is important that any sample or reference is applied to the co-spot last in order to prevent cross contamination because the same capillary tube will be used to spot each specific substance and the co-spot is an area where 2 substances, the reference and the sample, are applied. In the experiment, the first substance applied to the silica was the sample to the right and middle lanes (“Experiment 1 : Thin Layer Chromatography,” 2014). Next, the reference needed to be applied, first on the left lane and then lastly on the middle lane (co-spot) (“Experiment 1 : Thin Layer Chromatography,” 2014). If the reference was applied to the co-spot first and then the reference lane. The sample that was already applied on the co-spot might enter the capillary tube and the reference lane would be contaminated with the sample as well.

2. Increasing the polarity of the solvent would affect the distance that the sample spot is able to travel up the TLC. The more polar the solvent is the more it breaks down the intermolecular forces holding the compounds to the silica. Since the compounds are more free flowing they are more mobile and are able to move farther up the TLC plate. Since, the compound has been able to cover a larger distance the R_f value of the compound also changes and becomes larger as a result.

3.

a) **Figure 4.1.** TLC at the beginning of the reaction using a reference of compound A.	b) **Figure 4.2.** TLC after 50% completion of the reaction using a reference of compound A.
 Reference(R) = A CS = Co-Spot Sample (S)* = A [reaction mixture] *At the beginning of the reaction there will be only reactants.	 Reference(R) = A CS = Co-Spot Sample (S)* = A (50%) & B (50%) [reaction mixture] *At halfway through the reaction there will be 50% of reactants and 50% of products.

c) **Figure 4.3.** TLC at the end of the reaction using a reference of compound A.

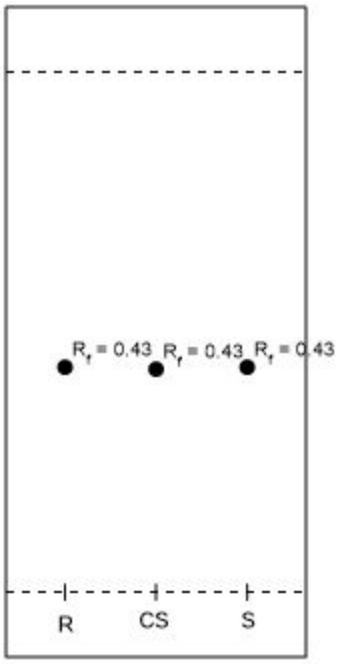
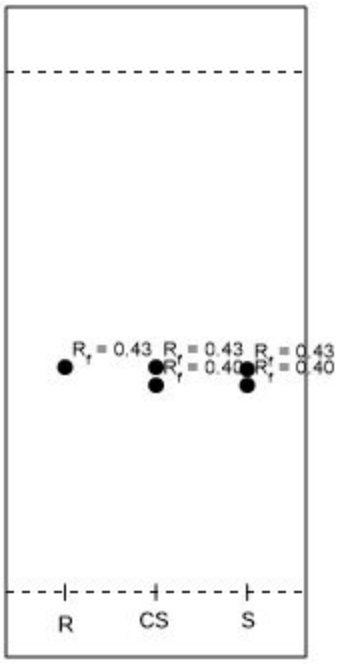


Reference (R) = A
CS = Co-Spot
Sample (S)* = B
[reaction mixture]

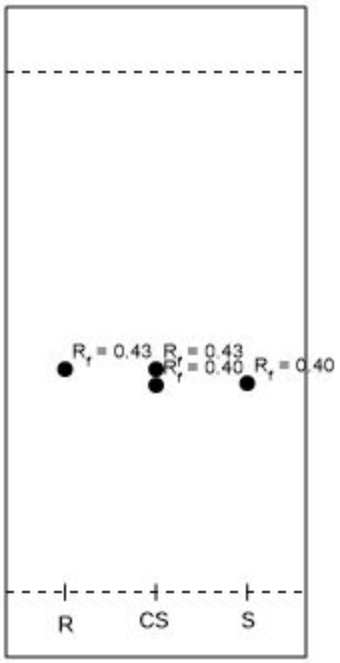
*All of the A molecules have been converted to B (product).

d) It is better to use a sample of molecule A than molecule B to follow the reaction because A is the starting material of the reaction. This allows us to monitor the reaction to its completion. This is possible because if the starting material is used as a reference it will be known that the reaction was completed when the dots in the sample lane do not match with those of the starting material because the starting materials molecules should not be present in the product of the reaction. This would tell that all of the starting material, the reactants, have turned into products. Molecule B or any product is not a good reference because firstly, the reaction has to be complete to acquire the products and secondly, it makes the reaction harder to track. Instead of looking for a point that should eventually disappear, one would be looking for a point to become fixed on the TLC to match with the reference. This is counterintuitive and there is a potential for error. Furthermore, if the products were used instead of the reactants, then it would be harder to know that the reaction taking place is the correct reaction.

4.

a) **Figure 5.1.** TLC at the beginning of the reaction using a reference of compound C.	b) **Figure 5.2.** TLC after 50% completion of the reaction using a reference of compound C.
 Reference (R) = C CS = Co-Spot Sample (S)* = C [reaction mixture] *At the beginning of the reaction there will be only reactants.	 Reference(R) = C CS = Co-Spot Sample (S)* = C (50%) & D (50%) [reaction mixture] *At halfway through the reaction there will be 50% of reactants and 50% of products.

c) **Figure 5.3.** TLC at the end of the reaction using a reference of compound C.

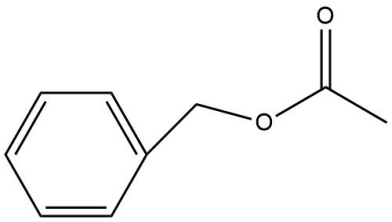
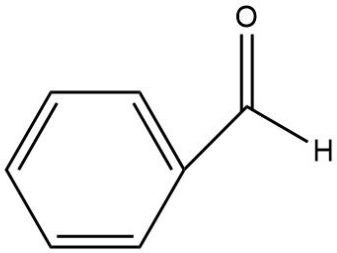
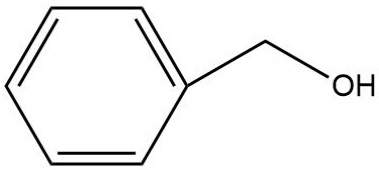


Reference (R) = C
CS = Co-Spot
Sample (S)* = D
[reaction mixture]

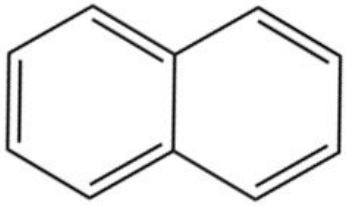
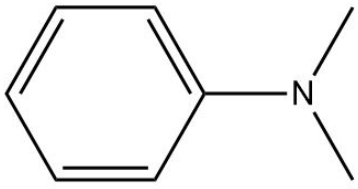
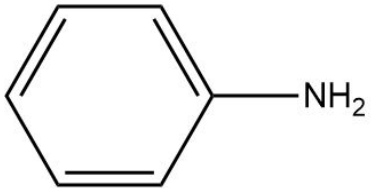
*All of the C molecules have been converted to D (product).

d) It is important to use a co-spot because it allows to get a clearer differentiation for compounds that have very similar R_f values. As in the above TLC's, compound C and D share very similar R_f values. This difference is only made visual by using a lane with a co-spot. This allows us to observe both the spots in the same lane and makes the differences in R_f values much clearer. If the co-spot was not made, then there is a high chance that it would not be known that the R_f values were different and it would seem as though the reaction is not occurring. This would be a major error, but is easily preventable by the co-spot.

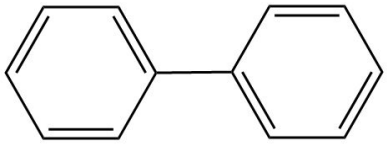
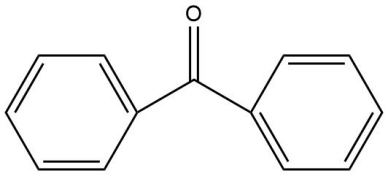
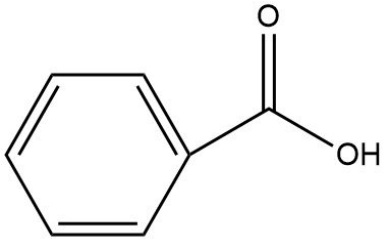
5.

i) Least Polar → Most Polar Highest Rf → Lowest Rf		
benzyl acetate	benzaldehyde	benzyl alcohol
		

- ➢ all three molecules are polar, some more than others
- ➢ benzyl alcohol can accept and donate hydrogen bonds therefore benzyl alcohol will have the lowest Rf value
- ➢ between benzyl acetate and benzaldehyde, benzaldehyde is more polar. The molecule is smaller, and has more hydrogen bonding potential with silica than benzyl acetate

ii) Least Polar → Most Polar Highest Rf → Lowest Rf		
naphthalene	N,N-dimethylaniline	aniline
		

- ➢ Naphthalene is a symmetrical molecule; therefore it is non-polar (least polar)
  - ○ Won't bind to silica, will have the highest Rf value
- ➢ N,N-dimethylaniline and aniline are both polar, both have a nitrogen
  - ○ The nitrogen on N,N-dimethylaniline is attached to two non polar methyl groups
  - ○ The nitrogen on aniline is bonded with two hydrogens, creating an amino functional group which is polar. More hydrogen bonding potential
  - ○ N,N-dimethylaniline has more atoms than aniline and is more heavy, this is also a reason for it to be less polar-
- ➢ Therefore aniline is more polar than N,N-dimethylaniline and will have the lowest Rf value

iii) Least Polar → Most Polar Highest Rf → Lowest Rf		
biphenyl	benzophenone	benzoic acid
		
➢ biphenyl is a symmetrical molecule; therefore it is non-polar (least polar) ○ Won't bind to silica, will have the highest Rf value ➢ benzophenone and benzoic acid are both polar ➢ benzoic acid is more polar due to having two electronegative oxygen atoms, while benzophenone only has one ○ the acid has a hydroxyl group and therefore will have potential to create hydrogen bonding with silica ○ benzoic acid would bind to silica faster than benzophenone resulting in a smaller Rf value and therefore will show to be most polar.		

References

Durst, B. T., Scaiano, T., Flynn, A., & Focsaneanu, K. (2019). CHM 1321 Organic Chemistry Laboratory Manual 2019, 1–18.

Experiment 1 : Thin Layer Chromatography. (2014), (September), 1–6.

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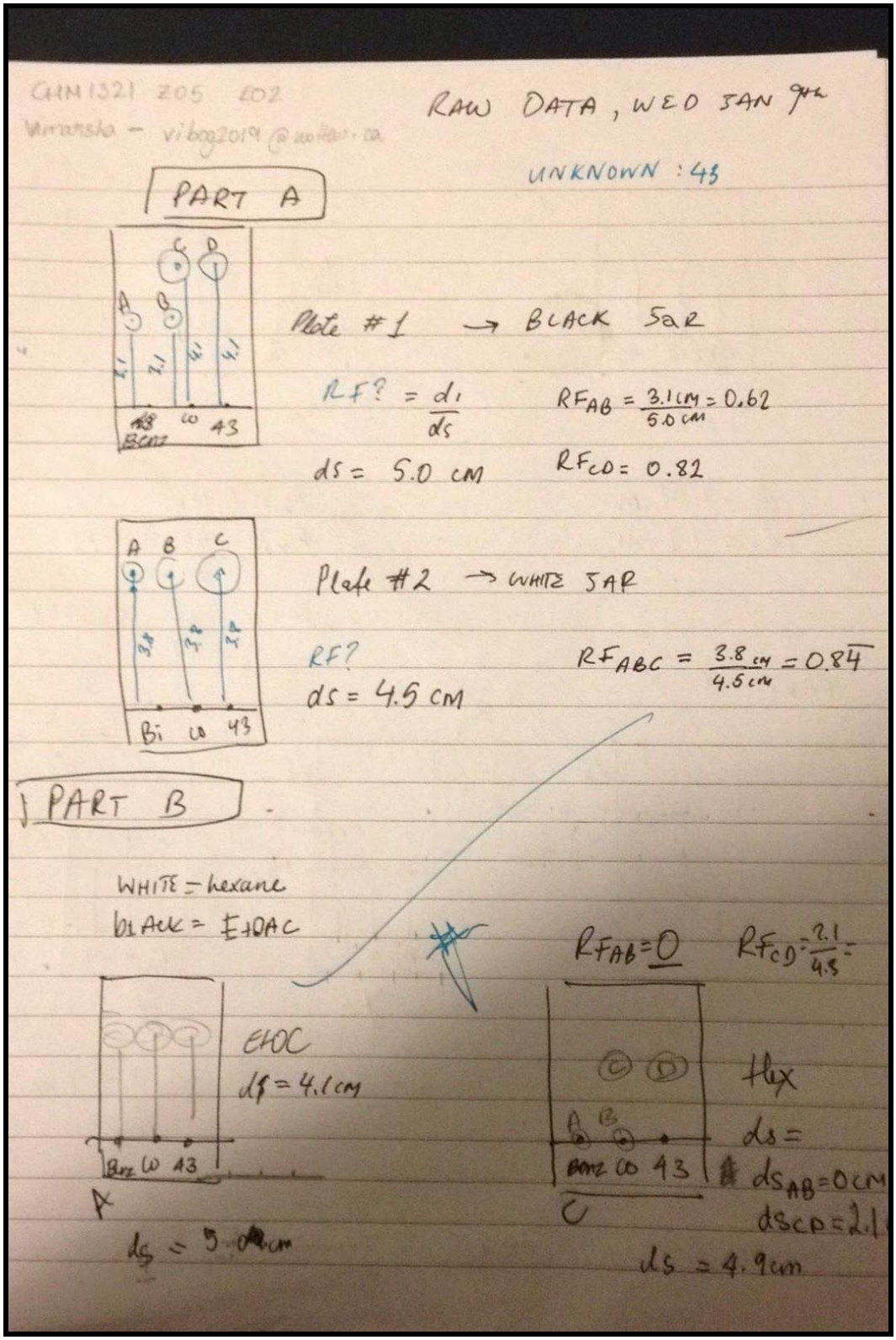
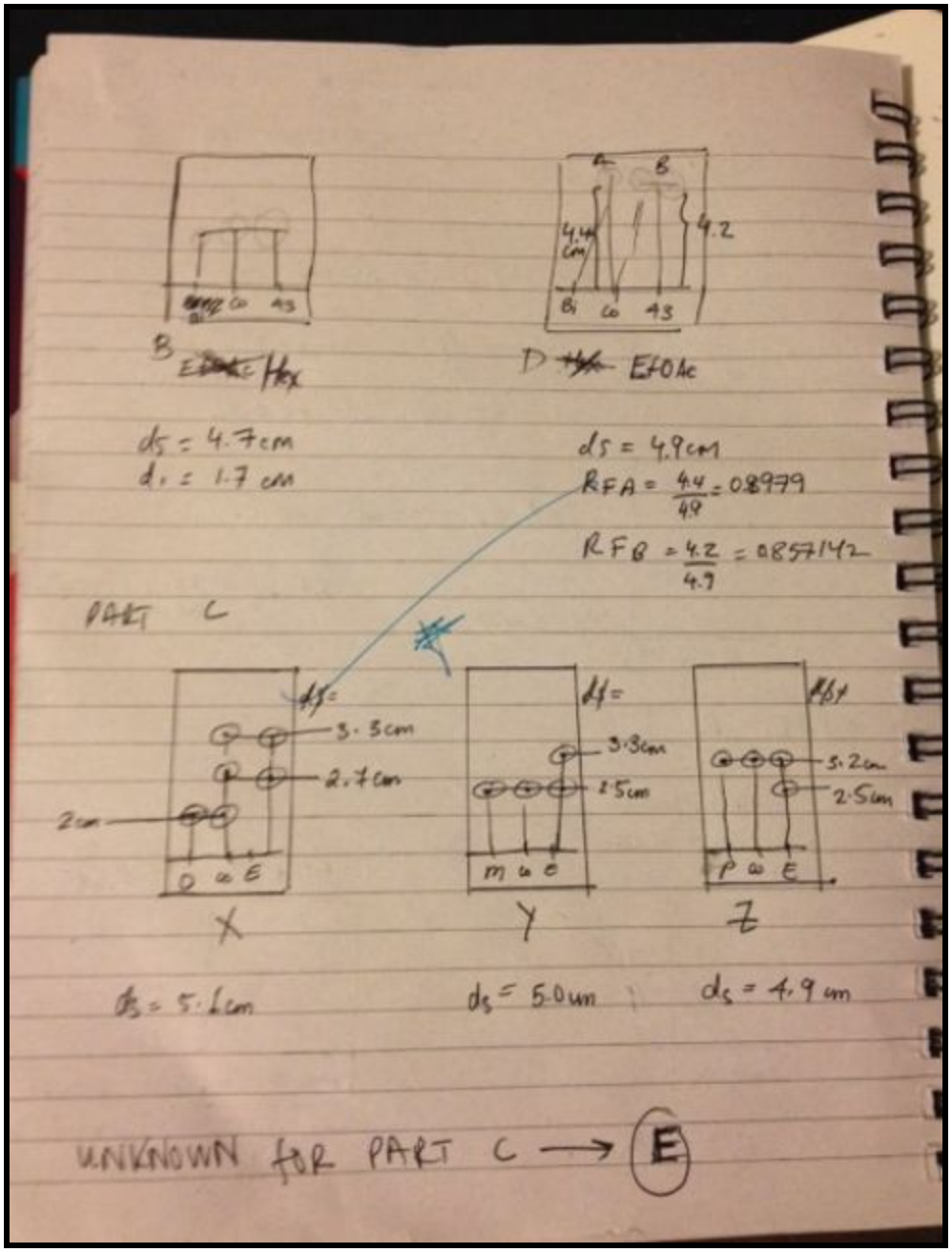
Raw Data


CHM 1321 Z05 Z02
RAW DATA, WED JAN 9th
UNKNOWN: 43
PART A
Plate #1 → BLACK SAR
RF? = d1/ds
ds = 5.0 cm
RFAB = 3.1 cm / 5.0 cm = 0.62
RFCD = 0.82
Plate #2 → WHITE SAR
RF?
ds = 4.5 cm
RFABC = 3.8 cm / 4.5 cm = 0.84
PART B
WHITE = hexane
BLACK = EtOAc
EtOC
ds = 4.1 cm
RFAB = 0
RFCD = 2.1 / 4.5 =
Hex
ds =
dsAB = 0 cm
dsCD = 2.1
ds = 4.9 cm


B
D
4.4 cm
4.2
4.9cm
RFA = 4.4/4.9 = 0.8979
RFB = 4.2/4.9 = 0.857142
PART C
3.3cm
2.7cm
2cm
3.3cm
2.5cm
5.2cm
2.5cm
X
Y
Z
5.1cm
5.0cm
4.9cm
UNKNOWN FOR PART C → E