Experiment 1:

Thin Layer Chromatography

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CHM 1321 Section C6

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Introduction:

Thin layer Chromatography is technique used to separate non-volatile mixtures. It is performed on a plate made of glass or aluminum with an adsorbent, silica gel. Because of the polar properties of silica gel, the more polar compounds will be more attracted to the gel, thus, it will move slower down the plate. Vice versa, the compounds less polar will move faster than the more polar compound. The solvent used will also affect the distance that the compounds move. With a more polar solvent, the distance moved will be greater, because the solvent will disrupt the intermolecular forces between the silica gel and the compounds. R_F (Retention Factor) is the distance traveled by the solute over the distance traveled by the solvent.

Procedure and Observation:

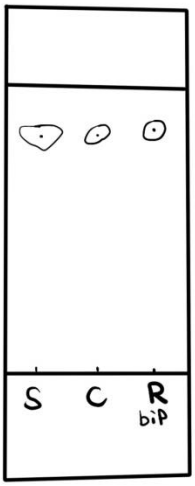
Part A:

1. Add 8ml of 2:8 mixture of ethyl acetate (EtOAc) and hexanes to the developing jar and close the lid.
2. Using a pencil, draw one line on two TLC plates, at least 1cm away from the edge. Then draw three equally spaced tics on the line you drew. The tics should be at least 5mm away from the edge of the TLC plate.
3. Obtain unknown compound from demonstrator. (Unknown sample 44 was obtained)
4. Dissolve 10 mg of the sample in to 2ml of dichloromethane. Label test tube.
5. Dip a capillary into the solution, and then use the end of the capillary to touch the TLC plate 2 times. Apply the solution on the appropriate lane. One should be the sample lane, and the other should be the co-spot lane.
6. Obtain 2ml of Benzophenone, to one of the TLC plates spot the reference lane first, then the co-spot lane.
7. Obtain 2ml of Biphenyl, to the other TLC plate spot the reference lane first, then the co-spot lane.
8. Carefully put one of the TLC plates into the developing jar, close the lid, and make sure the silica gel faces up, and the jar does not shake. Wait until the solvent reaches approximately 1cm from the top of the TLC plate, remove the plate and mark the finish line with a pencil.
9. Do the same with the other TLC plate.
10. Wait for the plates to dry. Then visualize the TLC plates under a UV light, gently mark the visible spots and measure the R_F values.

Table 1. Reagent table for Part A.

Compound:	Molecular Weight:	Amount:	Density:
2:8 EtOAc and Hexanes		8 mL	
Biphenyl	182.217 g/mol	≤ 2 mL	1.110 g/mL
Benzophenone	154.210 g/mol	≤ 2 mL	1.041 g/mL

Figure 1. Unknown sample 44 and Biphenyl, using solvent 2:8 mixture of EtOAc and hexanes.



Finish Line: 4.15 cm

Biphenyl: 3.50 cm

Sample: 3.40 cm

R_F Value: 0.819

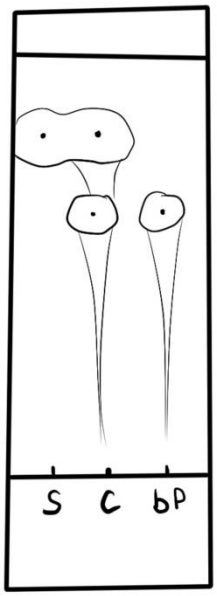
S: Sample 44

C: Co-spot

R: Reference

Bip: Biphenyl

Figure 2. Unknown sample 44 and Benzophenone, using solvent 2:8 mixture of EtOAc and hexanes.



Finish Line: 5.00 cm

Benzophenone: 3.10 cm

Sample: 4.20 cm

R_F Value: 0.840

S: Sample 44

C: Co-spot

Bp: Benzophenone

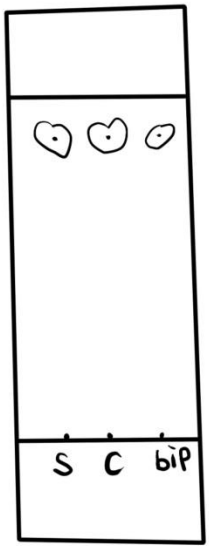
Part B:

1. Obtain 8 ml of ethyl acetate (EtOAc) in your developing jar, place lid on jar.
2. Prepare two new TLC plates. Repeat steps 2-10 from part A.
3. Clean the developing jar, and obtain 8ml of hexanes in the jar.
4. Prepare two new TLC plates. Repeat steps 2-10 from part A.
5. Draw a replica of the TLC plates in the lab report.

Table 2. Reagent table for Part B.

Compound:	Molecular Weight:	Amount:	Density:
EtOAc	88.105 g/mol	8 mL	0.902g/mL
Hexane	86.180 g/mol	8 mL	0.692 g/mL
Biphenyl	182.217 g/mol	< 2 mL	1.110 g/mL
Benzophenone	154.210 g/mol	< 2 mL	1.041 g/mL

Figure 3. Unknown sample 44 and Biphenyl, using solvent EtOAc.



Finish Line: 4.20 cm

Benzophenone: 3.80 cm

Sample: 3.80 cm

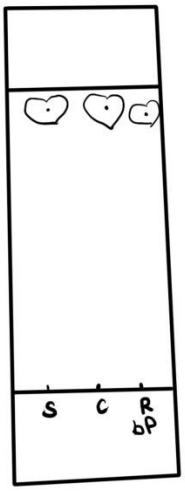
R_F Value: 0.905

S: Sample 44

C: Co-spot

Bip: Biphenyl

Figure 4. Unknown sample 44 and Benzophenone, using solvent EtOAc.



Finish Line: 4.20 cm

Benzophenone: 3.85 cm

Sample: 3.70 cm

R_F Value: 0.881

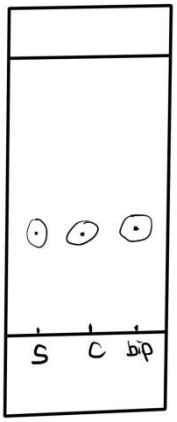
S: Sample 44

C: Co-spot

R: Reference

Bp: Benzophenone

Figure 5. Unknown sample 44 and Biphenyl, using solvent Hexane.



Finish Line: 4.90 cm

Biphenyl: 1.70 cm

Sample: 1.80 cm

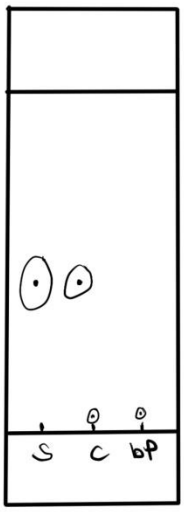
R_F Value: 0.367

S: Sample 44

C: Co-spot

Bip: Biphenyl

Figure 6. Unknown sample 44 and Benzophenone, using solvent Hexane.



Finish Line: 4.40 cm

Benzophenone: 0.20 cm

Sample: 2.00 cm

R_F Value: 0.455

S: Sample 44

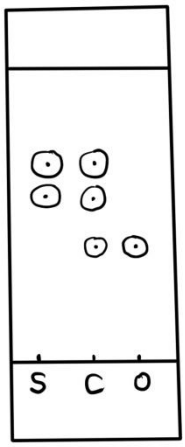
C: Co-spot

Bp: Benzophenone

Part C:

1. Obtain 2ml of unknown mixture of compound from demonstrator (unknown YY) in a 50 ml beaker.
2. Obtain 8ml of 9:1 Hexanes : Ethyl Acetate in developing jar.
3. Prepare three new TLC plates using same technique in part A and B, but instead of using Benzophenone and Biphenyl, use *o*-bromonitrobenzene, *m*-bromonitrobenzene, and *p*-bromonitrobenzene as reference.
4. Set one of the TLC plates into the developing jar, wait until it reaches to approximately 1cm to the top of the TLC plate, then take the TLC plate out and mark the finish line. Repeat this step for the other two TLC plates.
5. Once all of the TLC plates dry, put them under the UV lights, DO NOT mark them yet, take a picture with camera directly facing the TLC plate (one at a time, and be sure there are no angles).
6. After taking all the pictures, lightly mark the spots with a pencil.
7. Use ImageJ and the appropriate calibration curves to determine the ratio of compounds in your mixture.

Figure 7. Unknown sample YY and *o*-bromonitrobenzene, using solvent 9:1 mixture of Hexane and EtOAc.



Finish Line: 4.50 cm

o-bromonitrobenzene: 1.75 cm

$Sample_1$: 3.00 cm

$Sample_2$: 2.50cm

R_{F1} Value: 0.667

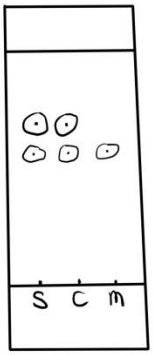
R_{F2} Value: 0.556

S: SampleYY

C: Co-spot

O: *o*-bromonitrobenzene

Figure 8. Unknown sample YY and *m*-bromonitrobenzene, using solvent 9:1 mixture of Hexane and EtOAc.



Finish Line: 4.50 cm

m-bromonitrobenzene: 2.30 cm

$Sample_1$: 2.85 cm

$Sample_2$: 2.30cm

R_{F1} Value: 0.633

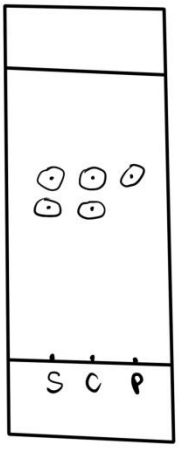
R_{F2} Value: 0.511

S: SampleYY

C: Co-spot

M: *m*-bromonitrobenzene

Figure 9. Unknown sample YY and *p*-bromonitrobenzene, using solvent 9:1 mixture of Hexane and EtOAc.



Finish Line: 4.00 cm

p-bromonitrobenzene: 2.80 cm

$Sample_1$: 2.80 cm

$Sample_2$: 2.30cm

R_{F1} Value: 0.700

R_{F2} Value: 0.575

S: SampleYY

C: Co-spot

P: *p*-bromonitrobenzene

Sample Calculations:

Part A. Figure1.

R_F Value= (Distant solute moved)/(Distant solvent moved)

= 3.40cm / 4.15cm

=0.819

Part C. Percentages of Peaks:

Table 3. Area of peaks in *m*-bromonitrobenzene TLC plate of Part C:

Peak:	Area:
1	39197.8
2	40381.8
3	36023.11
4	45464.24
5	51317.69

Percent Peak of *m*-bromonitrobenzene:

Percent peak 1= (area of peak 1)/(area of peak 1 + area of peak 2)x100%

= (39197.8/39197.8 + 40381.8) x 100%

=49.26%

Percent peak 2= 50.74%

Percent peak 3= 44.21%

Percent peak 4= 55.79%

Table 4. Area of peaks in *o*-bromonitrobenzene TLC plate of Part C:

Peak:	Area:
1	38558.48
2	39103.23
3	34664.04
4	35694.56
5	15612.62
6	32352.45

Percent Peak of *o*-bromonitrobenzene:

Percent Peak 1= 49.65%

Percent Peak 2= 50.35%

Percent Peak 3= 40.32%

Percent Peak 4= 41.52%

Percent Peak 5= 18.16%

Table 5. Area of peaks in *p*-bromonitrobenzene TLC plate of Part C:

Peak:	Area:
1	31378.14
2	32999.92
3	29585.19
4	26157.9
5	27260.04

Percent Peak of *p*-bromonitrobenzene:

Percent Peak 1= 48.74%

Percent Peak 2= 51.26%

Percent Peak 3= 53.07%

Percent Peak 4= 46.93%

Mole Percent:

Y= 0.976x + 0.804

$x_p = (y_p - 0.804)/0.976$

$x_p = (49.26 - 0.804)/0.976$

x_p= 49.65 mole%

x_m= 51.16 mole%

Discussion:

Part A: In this section, we found out that the R_F Values for our unknown sample 44 is 0.819 when compared with biphenyl, and it is 0.840 when compared with benzophenone. In the TLC plate with the benzophenone as reference, we can clearly see that the co-spot have two spots. This means that one compound is more polar than the other one, thus, it will stick to the silica gel more and move a relatively less distance than the other compound. And on the TLC plate with the biphenyl as reference, we can see that there is only one spot on the co-spot lane. This means that the sample and the biphenyl have the same polarity, and from this we can conclude that our unknown sample 44 is biphenyl.

Part B: In this part we were able to compare the difference of the results with different solvents used. The solvent's polarity will greatly affect the distance that the compound will move on the TLC plate, because the more polar the solvent, the more likely it is to disrupt the intermolecular forces between the silica gel and the compound. With a more polar solvent, the compound will be more attracted to the solvent then to the silica gel; hence, the solvent would drag the compound further on the TLC plate. Ethyl acetate is much more polar than hexane, this explains why on the plates using ethyl acetate as the solvent have all the compounds are dragged all the way to the finish line, and why the plates using hexane as the solvent have all the compounds in the middle of the start and the finish line. Even though on the hexane plate we can see that the compounds separated, but it is a really small distance, and thus we can conclude that with too polar or not polar solvents, we will not get a very accurate result. In conclusion, the distance of which the compound moves is affected by the polarity of the solvent.

Part C: In this section we used the same way we found sample 44 to find out that YY is a mixture of *m*-bromonitrobenzene and *p*-bromonitrobenzene. By using the program, ImageJ, we found the percent area, and by using the percent area we calculated the mole percent with the calibration curve, Y= 0.976x + 0.804.

Sources of error: At first, we tried to put two TLC plates into one developing jar, but that would mess up the results since they may interfere with each other. Other sources of error may be that the UV light wasn't as bright, showing an unclear result. When using ImageJ the results may vary according to the photo quality.

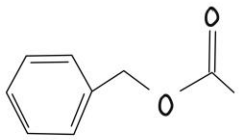
Questions:

1. Like said before, when the polarity increases in the solvent used, the distance that the compounds move increase significantly, this may give an inaccurate result because when the solvent is too polar, all the compounds will be dragged to the finish line. When the solvent is less polar, the compounds may not move or just move a little. This can

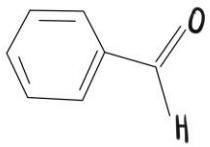
also give bad results, because the compounds may not be separated enough to see significant difference in the R_F values.

2.

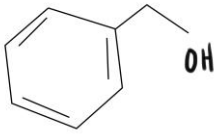
a.



Benzyl acetate



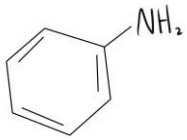
Benzaldehyde



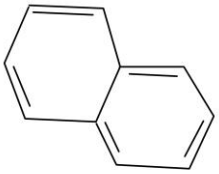
Benzyl alcohol

Benzyl alcohol would have the smallest R_F value, because only benzyl alcohol has an OH group attached to it, making it the most polar out of the three. The more polar it is, the more it is attracted to the silica gel, and thus, it shall have the smallest R_F value.

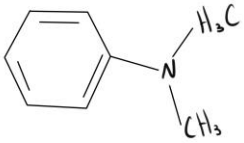
b.



Aniline



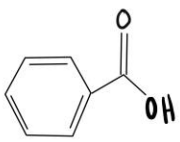
Naphthalene



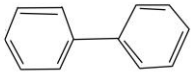
N,N-dimethylaniline

In this group, aniline is the most polar. First of all, out of the three, the structure of aniline is relatively not as symmetrical as the others. And in N,N-dimethylaniline, even though there are two carbon atoms contributing to the polarity, but the nitrogen atom in aniline has a larger electronegativity than the carbon atoms, thus aniline is the most polar, and will have the smallest R_F value.

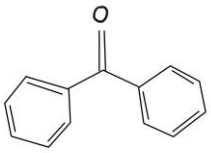
c.



Benzoic Acid



Biphenyl



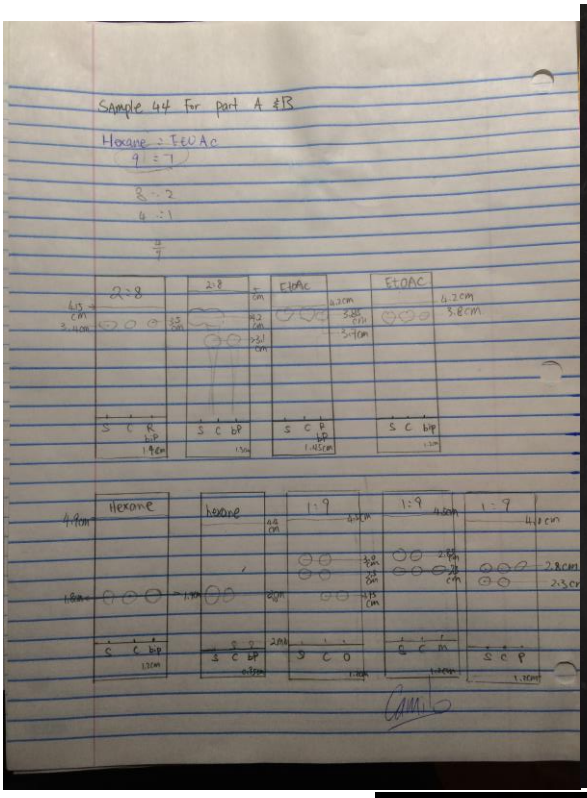
Benzophenone

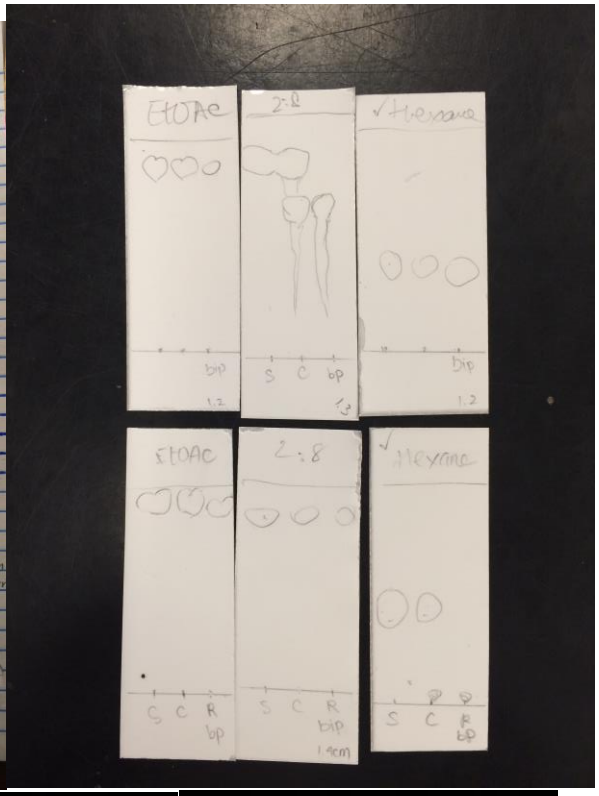
Benzoic acid is much more polar, and thus will have the smallest R_F value. Benzoic acid not only has an OH group, but also a double bonded oxygen atom, this all contributes the polarity of benzoic acid.

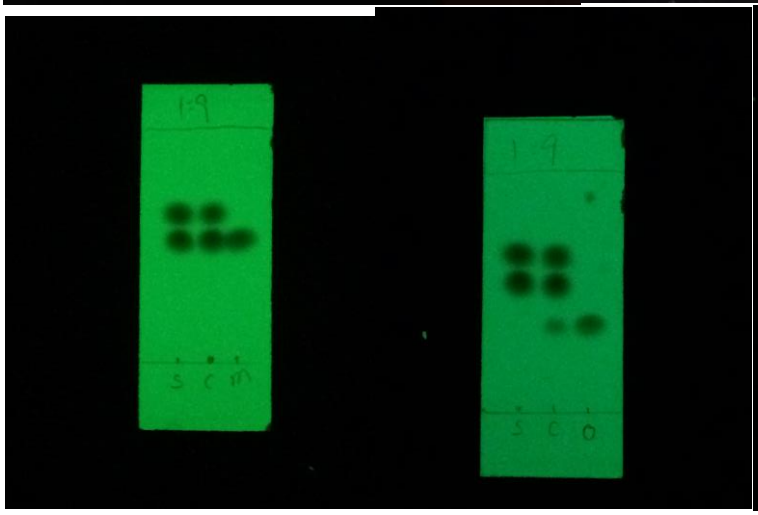
Conclusion:

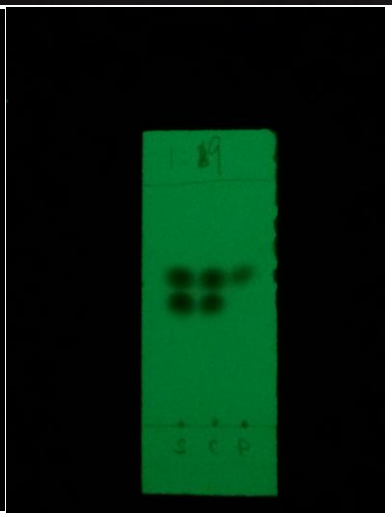
In conclusion, the unknown sample 44 is biphenyl. Unknown YY is *m*-bromonitrobenzene and *p*-bromonitrobenzene, each with a mole percent of: 49.65 and 51.16 accordingly. The solvent's polarity will affect the R_f value, the more polar the solvent, the bigger the R_F value. The less polar the solvent, the smaller the R_F value.

Raw Data:


Sample 44 for part A &B
Hexane : EtOAc
9 : 1
8 : 2
4 : 1
2:8
EtOAc
Hexane
1:9


EtOAc
2:8
Hexane
S C bp
S C R bp


1:9
S C m
S C O


1:9
S C P