Experiment 1:
Thin Layer Chromatography

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CHM1321 Section C1

Demonstrators:
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January 19, 2016

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Lab #1
Thin Layer Chromatograhy

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Procedure and Observations:

Part A: Identifying the component of an unknown mixture using TLC.

- 10 mL of 1:9 EtOAc:hexanes was poured into the developing jar and the lid was screwed on.
- Prepare 2 TLC plates were by using a pencil on the white silica face
- A starting line was drawn as a 1 cm horizontal line from the bottom of the plate.
- Then draw 3 equally-spaced tic marks on the line and labelled: r indicated reference, c indicated co-spot, and s indicated sample.
- Another line is drawn 1 cm below the top of the TLC plate.
- 10 mg of unknown sample 91 was dissolved in 1 mL of dichloromethane to make the sample solution.
- The sample solution was spotted a couple of times on c and s spots of each plate using a capillary.
- Different capillaries are used for different substances.
- 1 mL of acquired benzophenone was spotted onto r and c spots on one of the two plates.
- 1 mL of acquired biphenyl was spotted onto r and c spots of the second plate.
- After one another, TLC plates were placed into the developing jar in a way that the silica face faced upward. Solvent level was below the horizontal line. The lid was closed.
- The plates were removed by a tongs when the solvent rose up to the pencil mark 1 cm away from the top of the TLC plates.
- With the silica face upward, the plates were placed on paper towel to dry.
- After drying, the plates were brought to the UV lamp. The plates were visualized using the lamp. The dark spots were circled lightly with a pencil.
- The spots were measured and R_f values were calculated.
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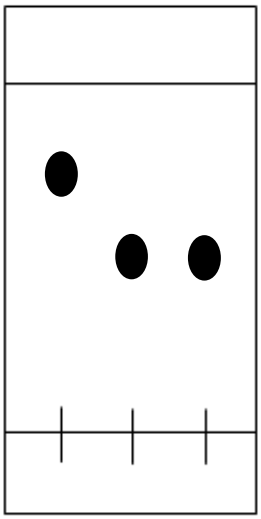
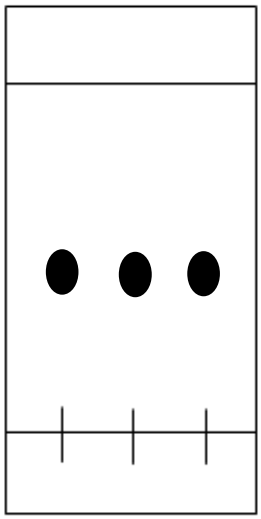


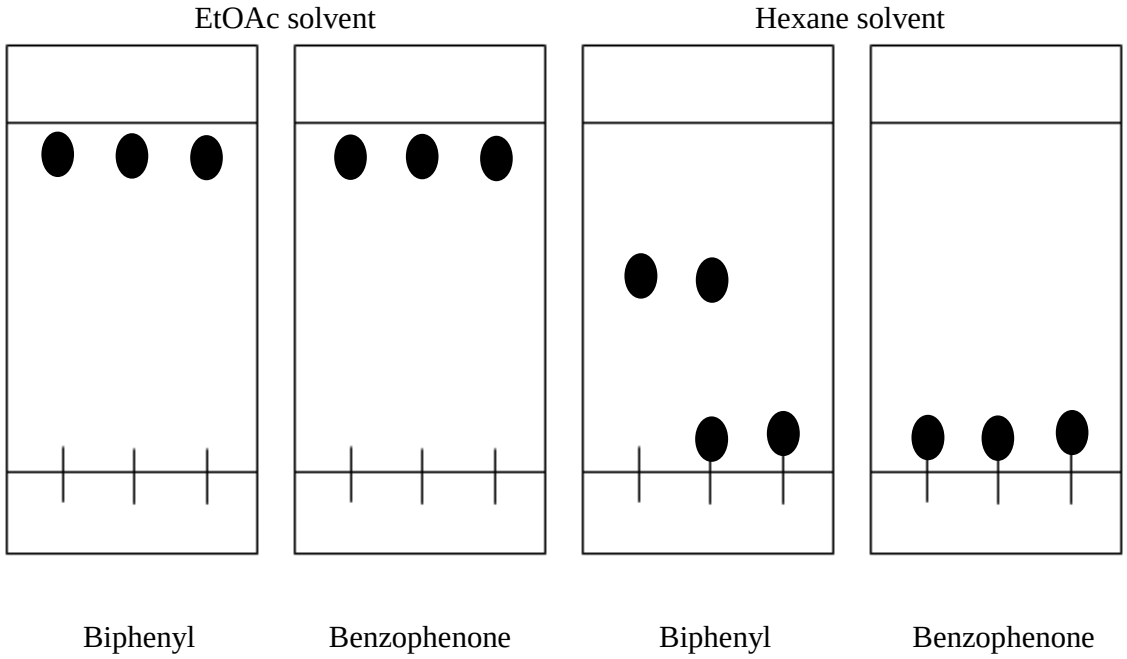
Biphenyl



Benzophenone

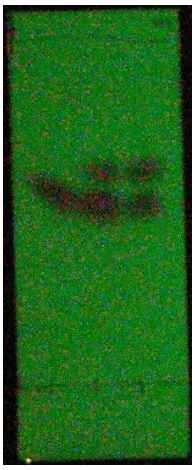
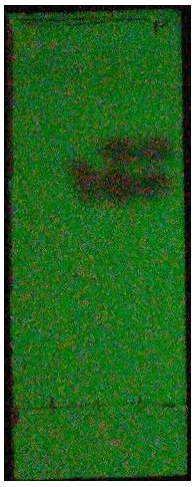
Part B: Effect of solvent on TLC.

- 10 mL of EtOAc was poured into another developing jar and the lid was screwed on.
- 2 new TLC plates were prepared as described in Part A. All drawn lines and spotted solutions are the same.
- After the plates were developed in EtOAc, they were left to evaporate and visualized under the UV light just like the procedure in Part A.
- The developing jar was cleaned with acetone and water and was then filled with 10 mL of hexanes.
- 2 new TLC plates were made in the same way as for Part A.
- After the plates were developed in hexanes, they were left to evaporate and visualized under the UV light just like the procedure in Part A.
- R_f values were calculated for all 4 plates.



Part C: Ratio of compounds.

- 10 mL of 1:9 EtOAc:hexanes was placed in the jar and the lid was placed on.
- Solutions were spotted in the same way as for Part A, but this time YY is the sample solution and there are 3 different reference solutions.
- Unknown compound mixture YY was acquired and spotted onto 3 different TLC plates.
- *o*-bromonitrobenzene was spotted onto the first TLC plate. Beside the r, o- was indicated for *o*-bromonitrobenzene.
- The same was done for the *meta* (m-) and *para* (p-) isomers on the second and third plates.
- The plates were all developed, dried, and visualized like the procedure in Part A. However, in part 3, the dots were not circled.
- Instead, pictures of the plates were taken under UV light with the phone.
- ImageJ software was used to determine the ratio of compounds in the YY mixture.

 *ortho* isomer  *meta* isomer  *para* isomer

Discussion:

In experiment 1, the same procedure is used throughout for preparing and analyzing TLC plates. The only difference is the solvents and the sample compounds. The starting line has to be above the solvent level in the jar so that the compounds in the spots are not diffused into the solvent. The lanes had to have enough space between them to see the differences.

Lane r, stands for reference, is where the known compounds were spotted on. Lane s, stands for sample, is where the sample compounds were spotted on. Lane c, stands for co-spot, is where both unknown and known samples were spotted on for differentiating compounds with similar R_f values. Different capillaries were used when putting different compounds on the plates to avoid cross contamination between compounds. Also, the compounds must be spotted in lane r or lane s before lane c to avoid contamination.

While the solvent is eluting, lid must remain on the jar to prevent any evaporation from the plate. The pencil mark at the top of the TLC was used as a stopping point instead of marking the stopping point after the solvent has eluted because the solvent may evaporate too quickly before we could get a chance to mark down the stopping point. In this way, the R_f values are more accurated.

In part C, the spots on the plates were not circled by pencil because the Image J program would not be able to analyze the plates. However, in part A and B, pencils were used to outline spots for the measurements to calculate the R_f values.

To measure the R_f value of a compound, one simply take the distance traveled by the spot and divide it by the distance traveled by the solvent. A smaller R_f value indicates a more polar compound and a larger R_f values indicates a more nonpolar compound. The silica has a strong pull on polar compounds so polar compounds have less displacement. On the other hand, silica has a weak pull on nonpolar compounds so nonpolar compounds have larger displacement.

Part A:

- R_f benzophenone in 1:9 EtOAc:hexanes = $2.1/4.7 \approx 0.45$
- R_f biphenyl in 1:9 EtOAc:hexanes = $3.2/4.3 \approx 0.74$
- R_f sample 91 in 1:9 EtOAc:hexanes = $(1.9/4.3 + 2.1/4.7)/2 \approx 0.44$

The compound in the unknown mixture 91 is likely to be benzophenone, since they have more similar R_f values 0.44 and 0.45.

Part B:

- R_f benzophenone in EtOAc = 4.5/4.6 ≈ 0.98
- R_f biphenyl in EtOAc = 4.5/4.6 = 0.98
- R_f sample 91 in EtOAc = (4.5/4.6 + 4.5/4.6) ≈ 0.98

With a pure EtOAc solvent, all of the spots are pushed up to the top of the plate. R_f values are difficult to measure and compare because they are all the same. As we can see, EtOAc is very polar and will pull all of the spots upwards at the same rate, making them seem like they have very similar R_f values. Therefore, the pure EtOAc solvent is too polar for the spotted compounds.

- R_f benzophenone in hexanes = 0.1/4.9 ≈ 0.02
- R_f biphenyl in hexanes = 2.1/4.7 ≈ 0.37
- R_f sample 91 in hexanes = (0.3/4.7 + 0.3/4.9)/2 ≈ 0.06

With a pure hexanes solvent, the polar compound benzophenone does not move much away from the starting point because there is not enough force to pull it up. The unknown compound also experiences only a little bit of movement. Biphenyl experiences a pull up to the middle of the plate. Therefore, the unknown substance is the compound benzophenone. The pure hexanes solvent is completely nonpolar and has almost no pull on polar compounds, so the polar compounds' R_f values are very small and insignificant. Biphenyl is nonpolar so it experiences a pull and has a measureable R_f value.

Part C:

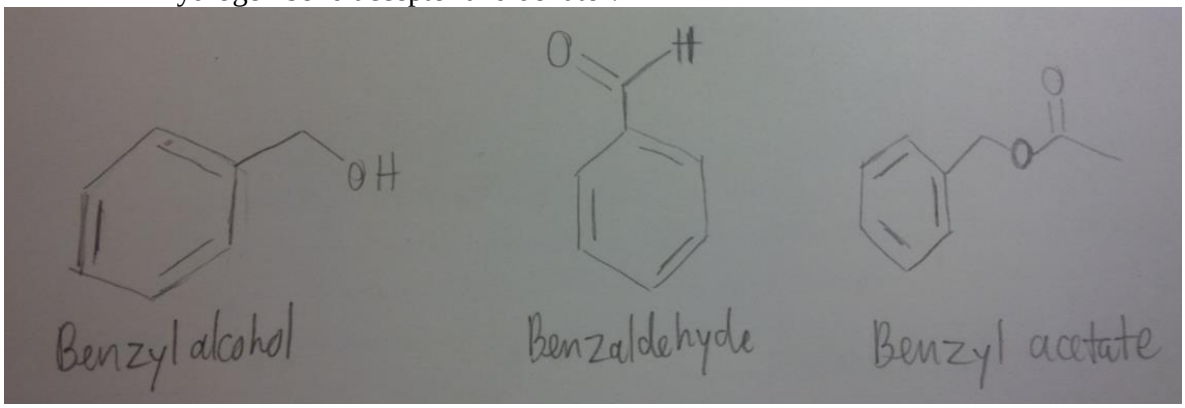
YY is a meta-para mixture of bromonitrobenzene because YY spots were corresponded with the *m*- and *p*-bromonitrobenzene, but not those of *o*-bromonitrobenzene.
In the ImageJ program, lane profile plots were analyzed using the areas at the peak of the sample. If the peak of the unknown isomer lines up with the peak of the known isomer, they are the same compound. After that, the percentage of the peaks on the same graph was calculated by using the area of both peaks as below. Appendix III of the CHM 1321 lab manual and calibration curves were consulted.

- % isomer = $\frac{area\ of\ isomer\ peak\ in\ sample}{total\ area\ of\ peaks\ in\ sample} \times 100\%$. Mole % depends on calibration curve.
- % *meta* isomer = $\frac{6391.740}{6391.740 + 4724.426} \times 100\% \approx 57.5\%$. Mole % ≈ 58%
- The remaining percentage must be of the *para* isomer. 100% - 58% ≈ 42%

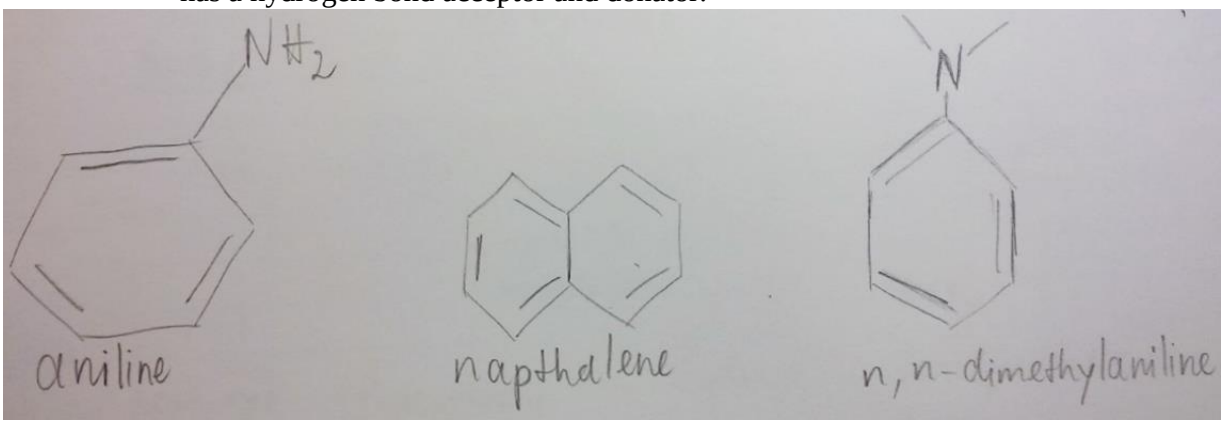
Questions:

1. Increasing the polarity of the solvent system will move all of the spots up higher on the plate. It would be beneficial only if the unknown compound is very polar. Since silica is a very polar substance, polar samples will tend to attach to it more. A more polar solvent system will displace the spot. On the other hand, a more nonpolar solvent system will not be able to move the samples at all. However, a solvent system that is too polar compared to the samples will pull all of the samples up to the top of the plate and R_f values will be difficult calculated and could be useless just as in part B of the experiment where EtOAC is used as the solvent.

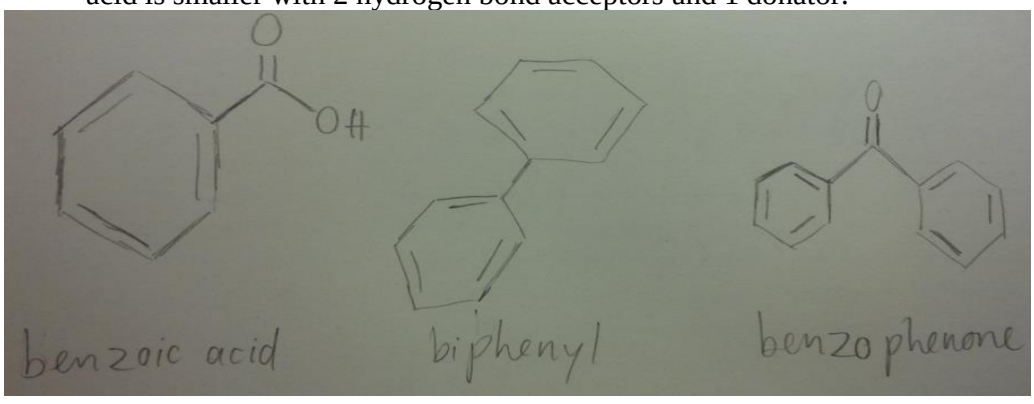
2. If a compound has a small R_f, it means that that compound is more attracted to the silica plate than the solvent. Since polar compounds have stronger pulls on other polar compounds, the smallest R_f samples must be the most polar.
    a. Benzyl alcohol would have the smallest R_f value. Benzaldehyde only has one hydrogen bond acceptor. Benzyl acetate has 2 hydrogen bond acceptors; however, it is a larger molecule than benzyl alcohol. Benzyl alcohol is a smaller molecule with a hydrogen bond acceptor and donator.



    b. Aniline would have the smallest R_f value. Naphthalene is a nonpolar compound. N,N-dimethylaniline only has a hydrogen bond acceptor and it is a large molecule. Aniline has a hydrogen bond acceptor and donator.



    c. Benzoic acid would have the smallest R_f value. Biphenyl is a nonpolar compound. Benzophenone only has a hydrogen bond acceptor and it is a large molecule. Benzoic acid is smaller with 2 hydrogen bond acceptors and 1 donator.



Conclusion:

Through this first lab in learning how to use TLC to separate and identify the unknown compounds and the effect of different solvents on the spotted compounds, it is very clear that more polar compounds will be more attracted to the silica gel and do not move as far as the more nonpolar compound. Therefore the R_f values of the more polar compounds are smaller because they are more attracted to the silica, and the R_f values of the more nonpolar compounds are bigger because they are easier to be pulled up by the solvent. Moving over to the solvent, if the solvent is too polar, it might pull all spots up at the same rate. Consequently, R_f values of all the compounds would look all the same and there is no way to identify which one is which. However, if the solvent is not polar enough, the polar compound would be stuck at the bottom of the starting point, which means their R_f values are all the same and they cannot be differentiate.

Throughout the lab, everything went well. The only problem I encountered is when I took pictures of the TLC plate for part 3 of the lab. My pictures were not defined enough and very blurry to get a good view of the dots. The ImageJ software could not read my pictures. So I have to borrow the pictures from another person to be able to do part 3 of the lab. The TLC pictures I used in part 3 are from Rami Hamoudeh, and his student number is 8134985.The positions of the dots on his TLC plates are corresponded to the ones on my TLC plates.

Reference:

Department of Chemistry. (2013). *CHM 1321 Introductory Organic Chemistry*. Ottawa, ON: University of Ottawa.

The TLC plates for part 3 of the lab are borrowed from Rami Hamoudeh, 8134985.

Raw Data

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Part A:

Identification #91

Solvent: 4.3 cm
Sample: 1.9 cm

Biphenyl: 3.2 cm

Solvent$_2$: 4.7 cm
Sample: 2.1

Benzophenone: 2.1 cm

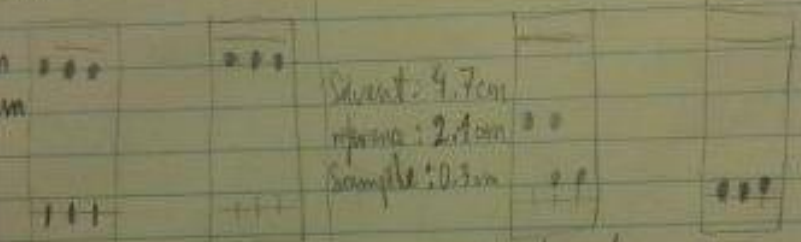
Biphenyl Benzophenone

Part B:

Solvent: 4.6cm
All spots: 4.5cm

Solvent: 4.7cm
reference: 2.1cm
Sample: 0.3cm

Solvent: 4.9cm
Sample: 0.3 cm
reference: 0.1 cm



Biphenyl Benzophenone Biphenyl Benzophenone

Solvent: EtOAc Solvent: hexane

Part C: