uOttawa

CHM 1321 Organic Chemistry 1 Laboratory

Thin Layer Chromatography

Experiment 1: ***Thin Layer Chromatography***

Part A: Identifying the Components of an Unknown Mixture using TLC.

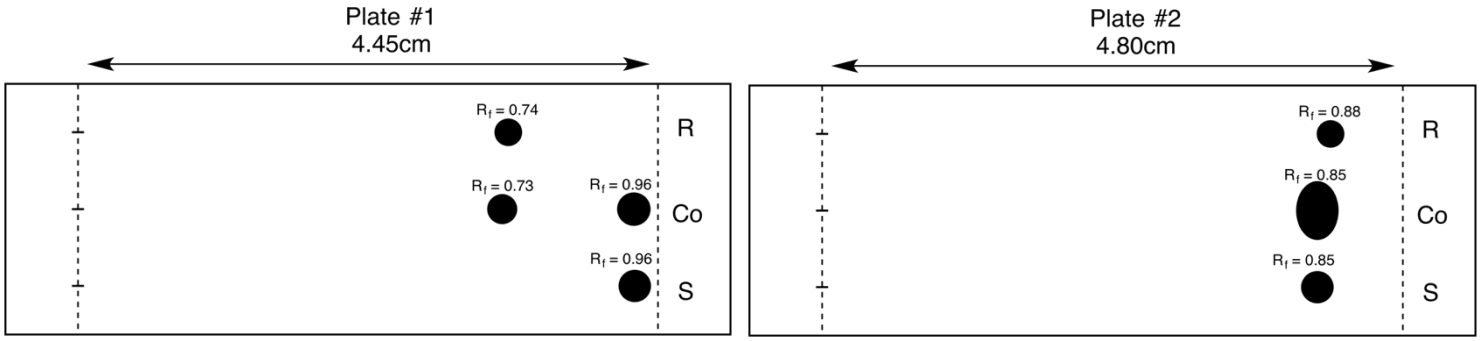
Experimental Procedure and Observations.

1. Two TLC plates were prepared with three lanes each. (Lanes labeled from left to right: Reference, Co-Spot and Sample respectively)
2. Unknown compound **#14** was obtained from Chris Demone.
3. Approximately 10mg of the unknown compound (a white reflective crystalline powder) was weighed using a centigram resolution scale. It was then mixed into approximately 2mL of Dichloromethane making sure to thoroughly agitate in order to obtain acceptable dissolution. A rubber stopper was used to seal the test tube during the agitation and light gas pressure formed inside as a result. The pressure was released upon removal of the stopper.
4. The solution prepared in the previous step was spotted using glass capillary tubes onto the Sample and Co-Spot lanes of the two plates prepared (Plates #1 and 2).
5. The **Benzophenone** reference solution was spotted onto the lanes labeled Reference and Co-Spot on **Plate #1.** The Reference lane was spotted first in order to avoid carrying minute amounts of the unknown sample already applied to the Co-Spot lane over into the Reference lane in order to avoid contaminating the Reference lane.
6. The **Biphenyl** reference solution was spotted onto **Plate #2** following the procedure described in the previous step (5.).
7. Both plates were inserted into the developing jar with the silica coated side facing upward. The level of the 2:8 mixture of EtOAc : Hexanes was measured in order to verify that the solvent level was below the spot height on the pencil line drawn on the bottom of the plates. This was done to avoid dissolving the spotted material into the surrounding solvent and losing all data in consequence.
8. The procedure had to be restarted three times due to the plates falling over and sticking face to face (silica to silica) in the developing jar when moving the developing jar around too quickly. This was done due to the fact that some of the spotted compound might have been transferred from one plate to another during the time they were in contact which would have skewed the data.
9. The solvent was allowed to elute for approximately 6 minutes until the solvent line reached approximately 1cm from the top of the 2 plates.
10. The plates were then removed and the solvent line was marked immediately in order to make sure the solvent line was recorded before the solvent had

evaporated. The solvent was allowed to evaporate for approximately 1 minute until the plates were visibly dry.

11. The plates were then observed under UV light and the spots were circled lightly with a pencil.

Results



Legend: R = Reference, Co = Co-Spot, S = Sample

Discussion

The results obtained indicate quite clearly that the unknown compound being assessed must be Biphenyl.
From Plate #1 it is possible to observe that the compound in the Reference lane has a significantly different polarity from the compound that was spotted in the Sample lane. The fact that the spot in the Reference lane has the same R_f (=0.74) as only one spot in the Co-Spot lane and that the spot in the Sample lane has only one spot with a matching R_f (=0.96) further reinforces this conclusion.

In Plate #2, the two compounds analyzed, the Biphenyl reference and the unknown compound #14, have similar polarities. In the Co-Spot lane on Plate #2, the two compounds appear to be overlapping and slightly out of line (horizontally) with each other which perfectly explains the slightly oval shaped spot. The R_f difference between the spot in the Reference lane and the one in the Sample lane being under 0.5 (0.88-0.85=0.3) indicates that the two compounds, the Biphenyl contained in the reference solutions and unknown #13, are one and the same.

The difference in polarities, assuming the unknown #14 is biphenyl, appropriately justifies the fact that the sample of the unknown solution eluted farther because it has no polar functional groups. Benzophenone is slightly polar due to it having a carbonyl group on it and this explains the fact that it has a smaller R_f value than the unknown #14 in Plate #1. ($0.74 < 0.96$)

Conclusion

It is safe to conclude that, within the limitations of the experiment, the unknown compound #14 must be Biphenyl. This is due to the fact that in a TLC analysis against a known reference solution of Biphenyl it compared favorably, whereas when compared to a reference solution of a slightly more polar compound, Benzophenone, it eluted much farther (Plate #1), further reinforcing the original conclusion.

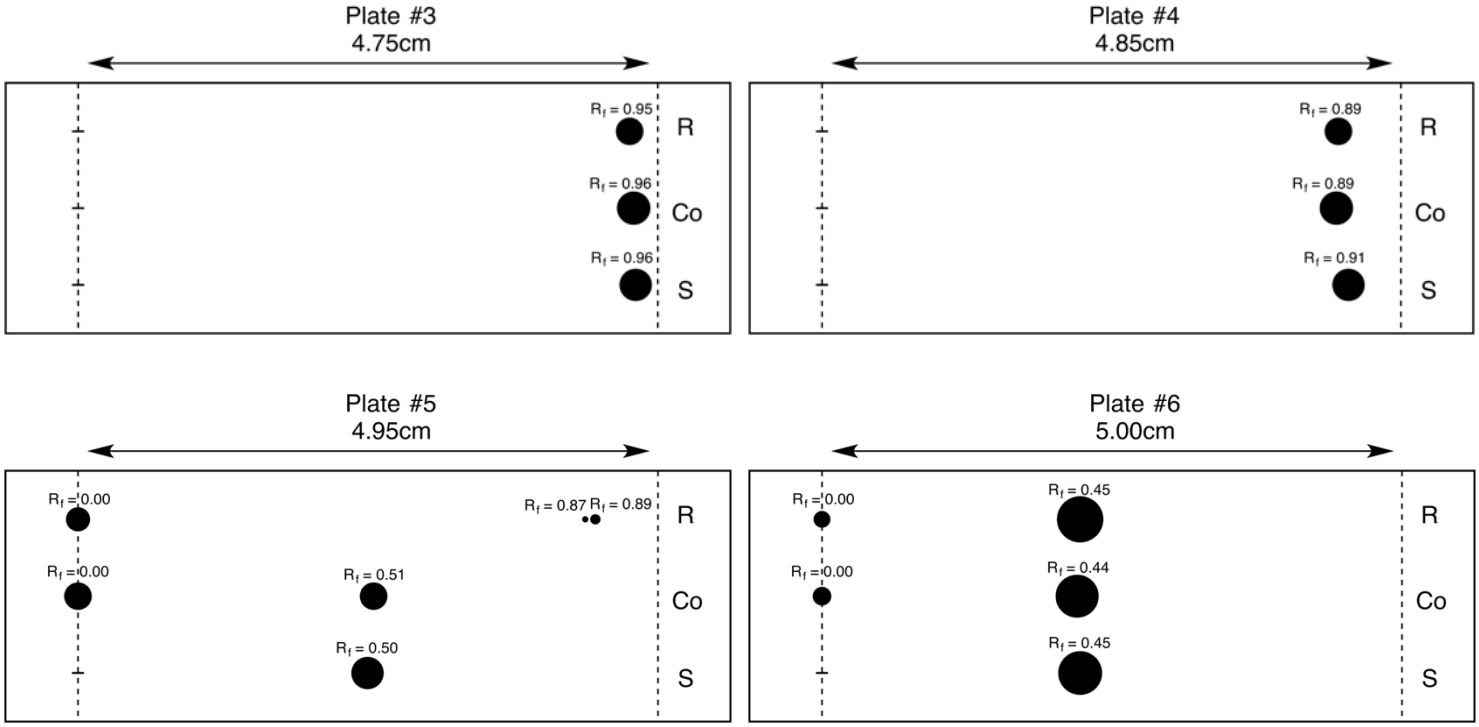
Part B: Effect of Solvent on TLC

Experimental Procedure and Observations

** Much of the procedure of part B is homologous to part A. In consequence, only differing steps will be mentioned in an effort to avoid redundancy in this report. **

1. Part A is repeated twice in Part B with two different eluants in order to observe how different solvents (with different polarities) will influence the R_f of each compound.
2. The first reiteration (Part A, steps 1 and 4–11) was accomplished using **Ethyl Acetate alone (EtOAc**) as an eluant. **(Plates #3 and 4)**
3. The second reiteration (Part A, steps 1 and 4–11) was done using **Hexanes** as the eluant. **(Plates #5 and 6)**

Results



Legend: R = Reference, Co = Co-Spot, S = Sample

Discussion

In this part of the experiment it is possible to observe how different solvents affected the R_f values obtained in part A.

In the first half of part B, the solvent used was EtOAc which is significantly polar when compared to the polarity of the ratio of eluants used in part A of the experiment (2:8 EtOAc to Hexane). This is because in part A, the EtOAc was diluted with an essentially non polar solvent, Hexane, which was done in order to have observable and appreciable R_f value differences between the two compounds used. As it is possible to observe in Plates #3 and 4, without this "polar fine tuning", the data becomes completely inconclusive. This is due to the fact that, although there are two different compounds on the same TLC plate, they both appear to be the same when using a

very polar eluant such as pure EtOAc because they all migrate as far as possible making discrimination impossible.

Adding Hexanes to an eluant after observing results such as those seen in Plates #3 and 4 is akin to "magnifying in" on the data in order to change the relative scale of R_f values and create an appreciable and quantifiable difference in R_f between the two compounds.

In Plates #5 and 6 there appears to be some form of contamination. For the purposes of this discussion, the 2 small spots on Plate #5 in the Reference lane (R_f =0.88) will be ignored. The spot with R_f of 0.45 in the Reference lane in Plate #6 will also be voided. The reason these points will not be considered is that in all other plates no separation was observed in the Reference lane alone. The Reference lane in Plate #6 must have been contaminated with some of the unknown sample (assumedly, biphenyl).

Upon further observation of Plates #5 and 6, it is possible to observe that the Sample lane's R_f is consistent throughout the two plates (approximately 0.5) which indicates that, even though the eluant used was pure Hexanes, it appears to be of useable polarity in order to discriminate biphenyl from other compounds. The fact that the main spot in the Reference lane on Plate #6 has the same R_f value as the spots in the Co-Spot lanes (0.45) indicates that the unknown compound #14 must indeed be biphenyl.

In Plate #5, the fact that the reference compound appears to not have moved at all is appropriate when considering the fact that Benzophenone, which is a slightly polar compound, will not be eluted by an essentially non polar solvent. It will bind to the Silica coating and not have an R_f value at all. This can be seen by observing the two spots with R_f of 0 in the Reference lane and the Co-Spot lane on Plate #5.

Conclusion

This further set of testing has reinforced the conclusion drawn in part A of the experiment: the unknown compound must be biphenyl.
This is due to the fact that it eluted an appreciable distance in an essentially non polar solvent (pure Hexanes). If it was Benzophenone, it would not have moved, as can be seen by the Reference lane of Benzophenone in Plate #5

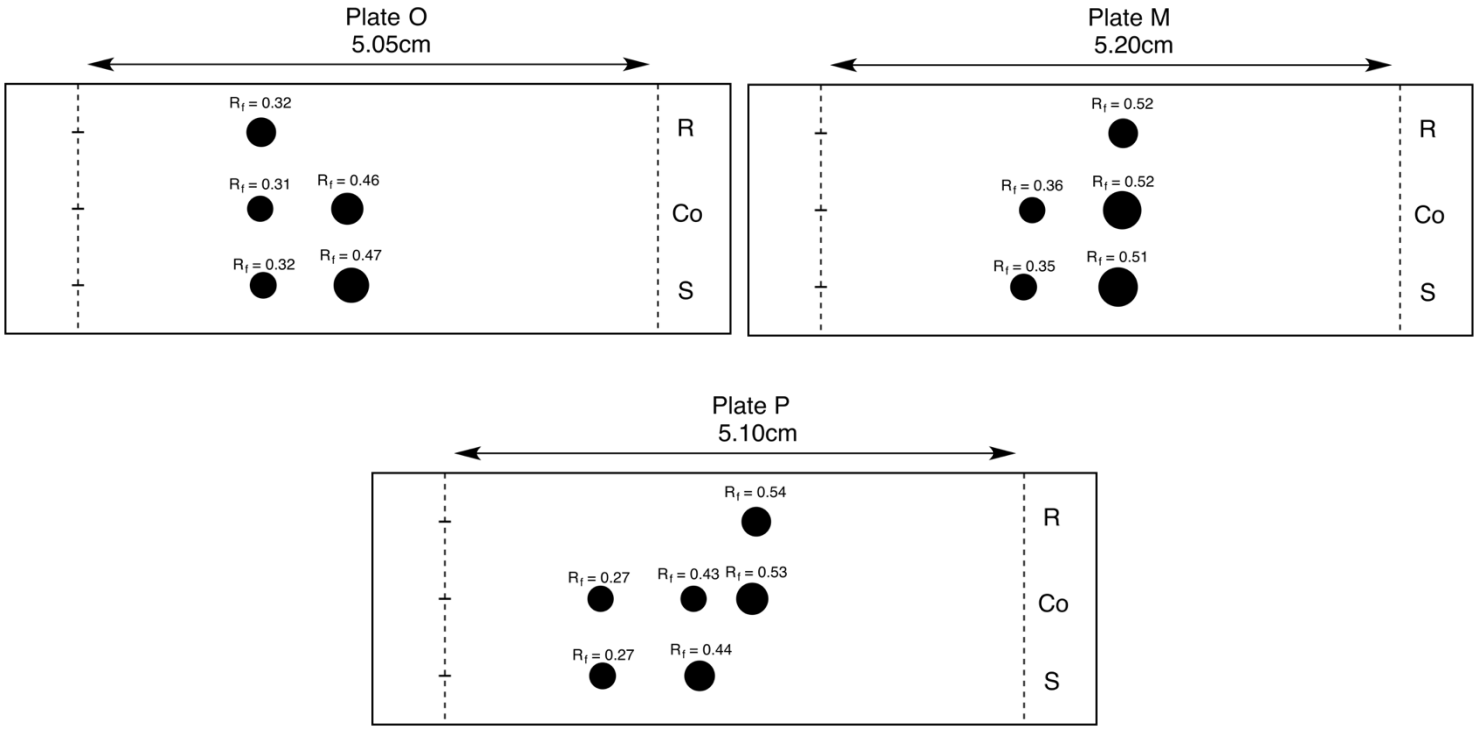
Part C: Ratio of Compounds

Experimental Procedure and Observations

** Much of the procedure of part C is homologous to part A. In consequence, only differing steps will be mentioned in an effort to avoid redundancy in this report. **

1. 2mL of the unknown mixed solution ***ZZ*** was obtained. (Translucent, slightly yellow tint)
2. Using a 9:1 ratio of Hexanes:Ethyl Acetate as the eluant, three TLC plates were prepared (Part A, steps 1 and 7–11) spotting the Reference and Co-Spot lanes with:
   O-Bromonitrobenzene (Plate O),
   M-Bromonitrobenzene (Plate M) and
   P-Bromonitrobenzene (Plate P)

Results



Legend: R = Reference, Co = Co-Spot, S = Sample

Discussion

In part C of the experiment, the unknown solution chosen was ***ZZ***. Upon immediate visual inspection of the three reference solutions, it was observed that only one of the three reference solutions was yellowish in color and so was our unknown which immediately indicated that the unknown must contain a certain amount of it.

Through the analysis of TLC Plates O, M and P, it becomes obvious that the unknown solution ***ZZ*** must be composed of both **O-Bromonitrobenzene and M-Bromonitrobenzene**. This can be seen by observing that there are spots in the Sample and Co-Spot lanes that match the R_f value of the only spot in the Reference lane of Plates O and M.

It is safe to assume that the unknown solution ***ZZ*** did not contain P-Bromonitrobenzene for several reasons. Firstly, there are seemingly only two compounds in our unknown solution ***ZZ*** because there are only two individual spots in the Sample lane of all three plates with an appreciable difference in R_f. Secondly, the two spots in the Sample lane of Plate P do not have comparable R_f values to the spot in the Reference lane of Plate P.

Conclusion

From the data obtained through TLC analysis of the unknown solution ***ZZ*** it is possible to determine that it must contain both O-Bromonitrobenzene and M-Bromonitrobenzene. The data also indicates that it must not contain P-Bromonitrobenzene.

It is also possible to determine that ***ZZ*** must contain a higher concentration of M-Bromonitrobenzene than O-Bromonitrobenzene due to the diameter of the M-Bromonitrobenzene spots in the Sample lane being consistently larger.

Questions from the Lab Manual

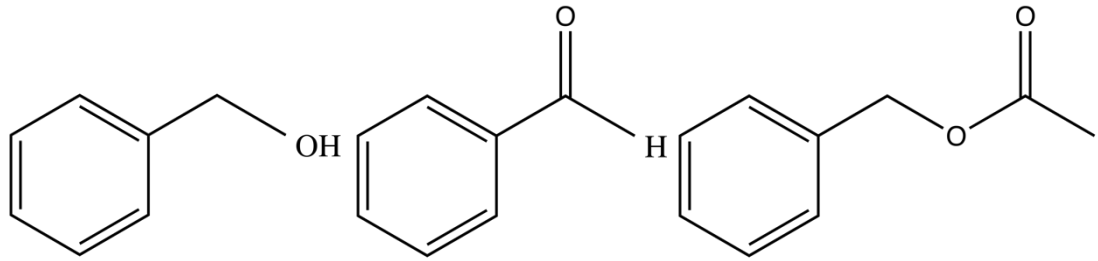
1. ***How does increasing the polarity of the solvent system affect the results of a TLC?***

Answer: Increasing the polarity of the eluant will effectively make the compound being analyzed run “faster” up the TLC plate. This is due to the fact the solvent is overcoming and balancing out the overwhelmingly polar nature of the Silica coating on the plate, therefore compounds are not bound as rigidly to the Silica, or rather are bound more equally between the eluant and the Silica coating.

2. ***In the following sets of compounds, which would have the smallest R_f on silica gel? Use a sentence to explain your reasoning. Draw line structures of each compound.***

a.

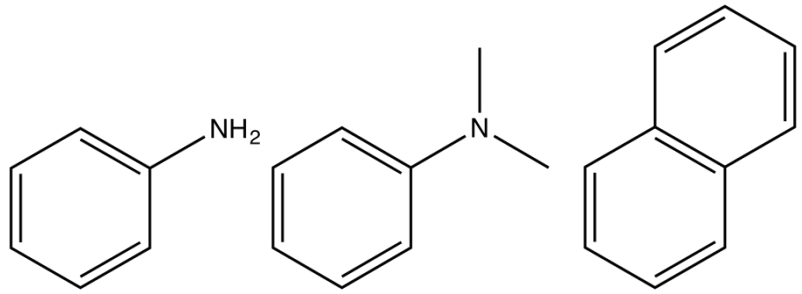
1. Benzyl Alcohol ***2. Benzaldehyde*** ***3. Benzyl Acetate***



I predict that Benzyl Acetate would be the most polar of the three and would therefore have the smallest R_f value. This is because it has 2 oxygen atoms and is therefore more polar than both the alcohol and aldehyde groups.

b.

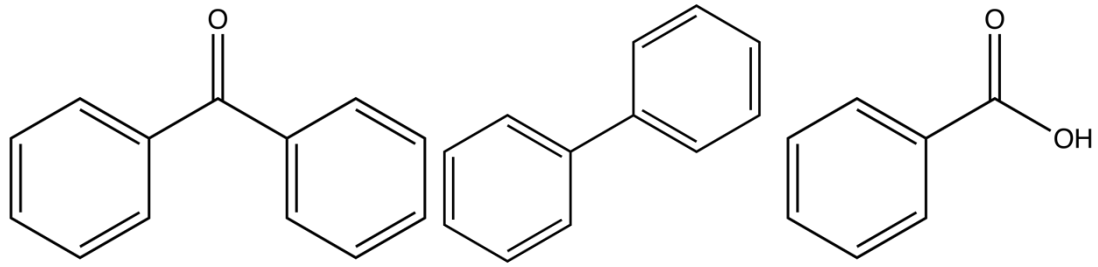
1. Aniline ***2. N,N-Dimethylaniline*** ***3.Naphthalene***



I predict that N,N-Dimethylaniline will be the most polar of the three and will therefore have the smallest R_f. This is because naphthalene is essentially non polar and N,N-Dimethylaniline has more electrons to pull in due to the two methyl groups than Aniline, because methyl groups can be electron donating and Nitrogen is fairly electronegative.

c.

1. Benzophenone ***2. Biphenyl*** ***3. Benzoic Acid***



I predict that Benzoic acid would most certainly have the smallest R_f due to it being the most polar of the three. Biphenyl is non polar and Benzophenone is less polar than Benzoic acid.