Experiment 1: Thin Layer Paper Chromatography

Date: January 10, 2017

Protocol

Part A

- Refer to steps 1-9 on pages 17-18 from the Laboratory Manual 2017(Introduction to Organic Chemistry; CHM1321 by Prof. William Ogilvie and Prof. Tony Durst.)
- Sample number 83 was used as the Unknown Sample.

Part B

- Refer to steps 1-7 on page 18 under *'Part B: Effect of solvent on TLC,'* in the Lab Manual.
- Sample number 83 was used as the Unknown Sample.

Part C

- Refer to steps 1-3 on page 18 under *'Part C: Ratio of compounds,'* in the Lab Manual.
- Unknown Sample '*ZZ*' was used.

Observation

- The chemical compounds used in this experiment were colourless and visible only under Ultraviolet (UV) lighting.
- Silica gel was semi-liquid allowing it to easily trap the oily residues on external objects such as the rulers and hands, which could interfere with the development of the TLC.
- Sample 83 was white crystals in its dry form, but when reacted with *dichloromethane*, it became a clear, versatile liquid.

Thin Layer Chromatography (TLC)

Legend

R= Reference Compound
CO= Co-spot, mixture of the known (reference) compound and the Unknown Sample.
US= Unknown Sample.
$\mathbf{D_{R/CO/US/SF}}$ = the distance travelled by reference Compound/Co-spot/Unknown sample, /Solvent Front, Respectively

Part A

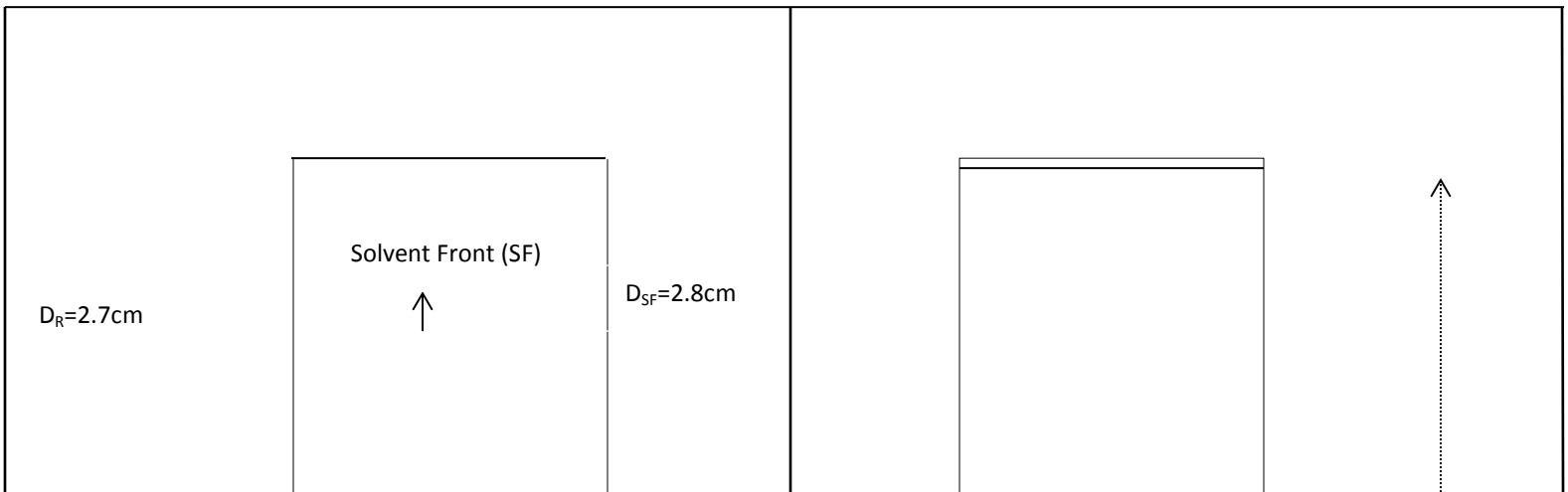
Solvent system: 2:8, Ethyl Acetate: Hexanes
Unknown Sample: #83 (10mg + 2ml of dichloromethane.)

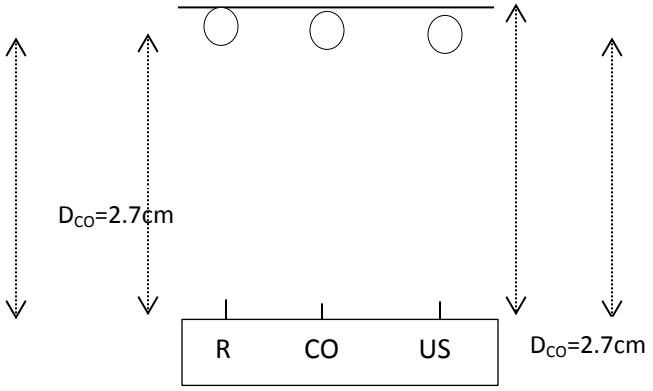
TLC1

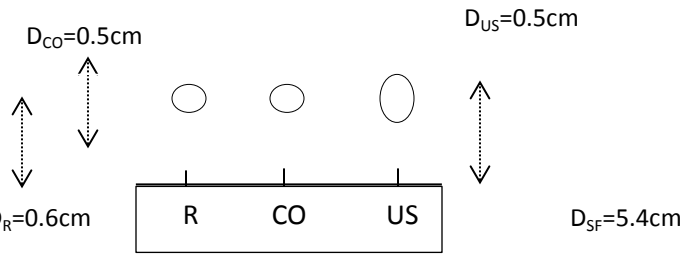
Reference Compound: Benzophenone

TLC2

Reference Compound: Biphenyl







Rf_R	Rf_{Co}	Rf_{US}
0.9	0.9	0.9

Rf_R	Rf_{CO}	Rf_{US}
0.1	0.1	0.1

Part Bi

Solvent System: Ethyl Acetate

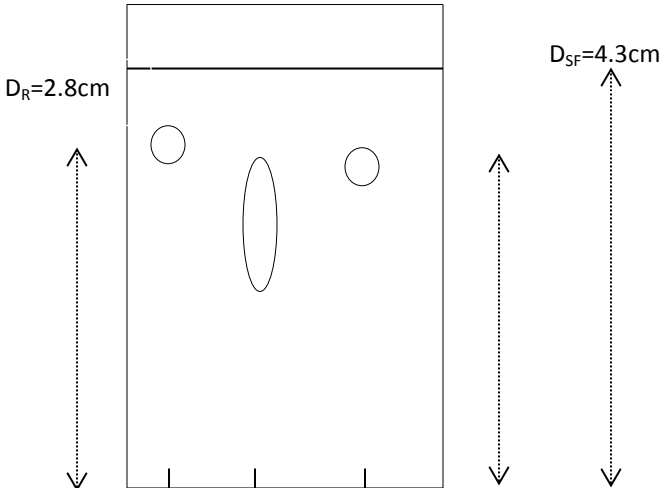
TLC3

Reference Compound: Benzophenone

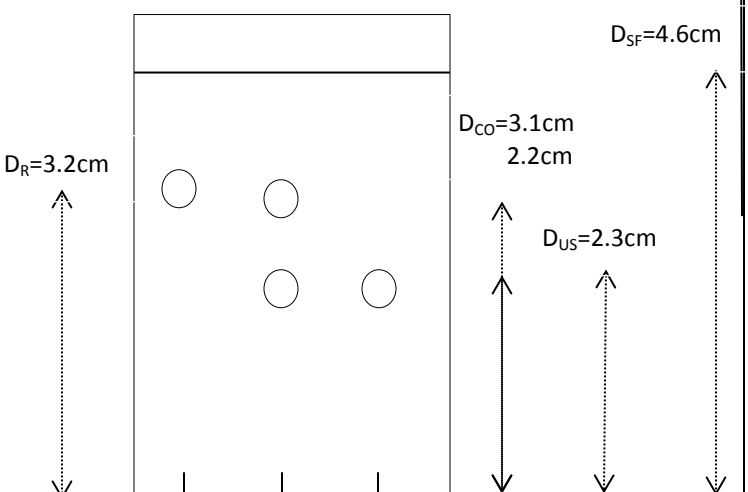
TLC4

Reference Compound: Biphenyl

Rf_R	Rf_{Co}	Rf_{US}
0.65	0.63	0.67



Rf_R	Rf_{CO}	Rf_{US}
0.70	0.70 & 0.48	0.50



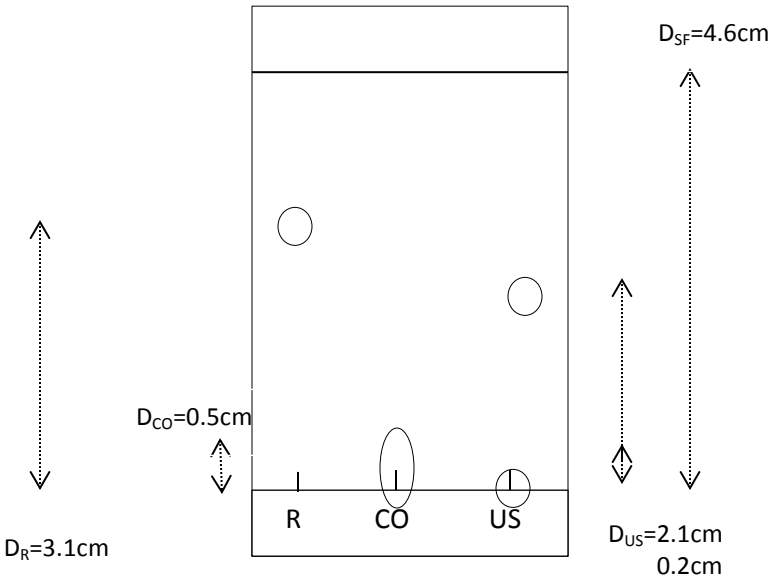
D_{CO}=2.7cm

Part Bii

Solvent System: Hexanes

TLC5:

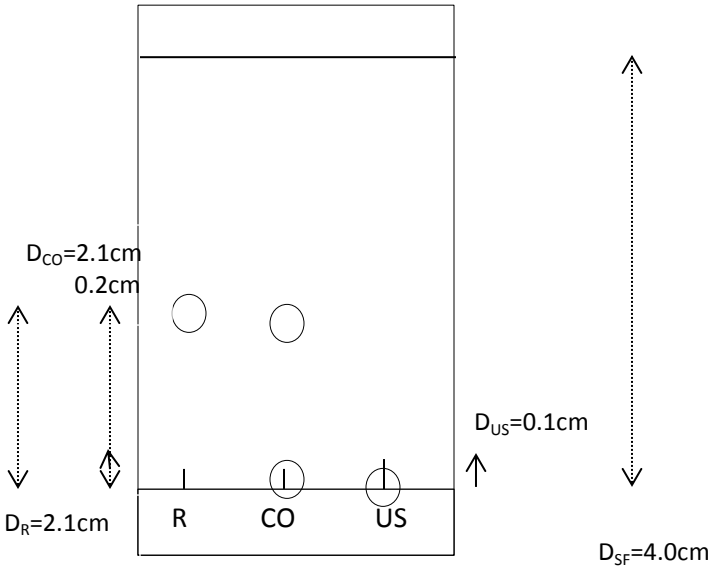
Reference Compound: Benzophenone



Rf_R	Rf_{Co}	Rf_{US}
0.67	0.11	0.46 & 0.04

TLC6:

Reference Compound: Biphenyl



Rf_R	Rf_{CO}	Rf_{US}
0.53	0.50 & 0.05	0.03

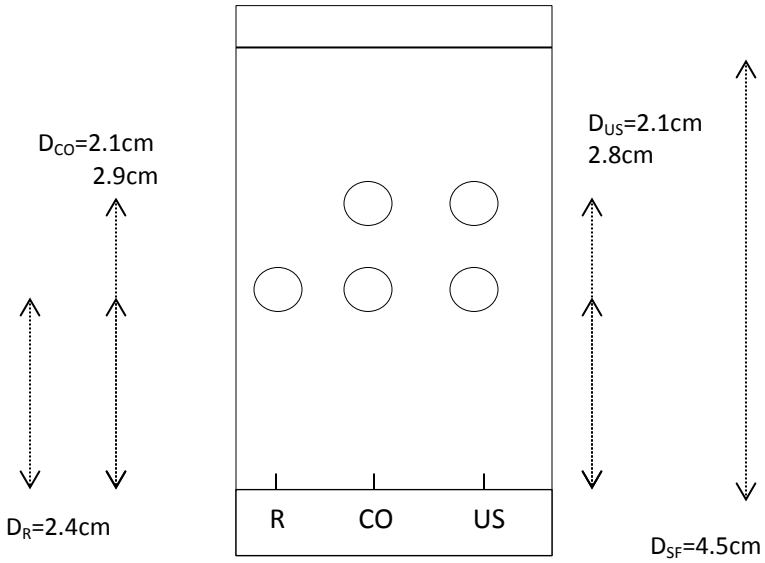
Part C

Solvent system: 9:1, Hexanes: Ethyl acetate

Unknown Sample: ZZ

TLC7:

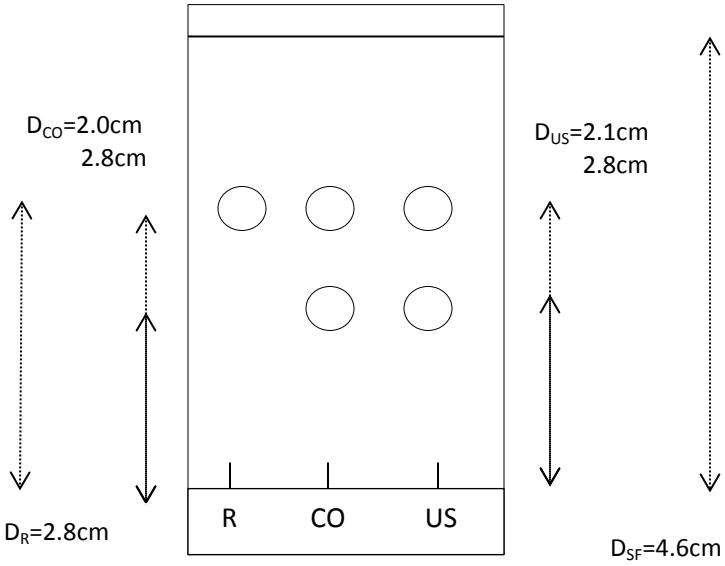
Reference Compound;
o-bromonitrobenzene



Rf_R	Rf_{Co}	Rf_{US}
0.53	0.47 & 0.64	0.64 & 0.47

TLC8:

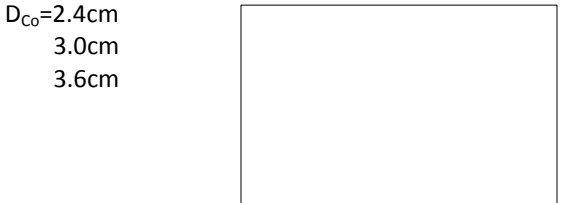
Reference Compound;
m-bromonitrobenzene

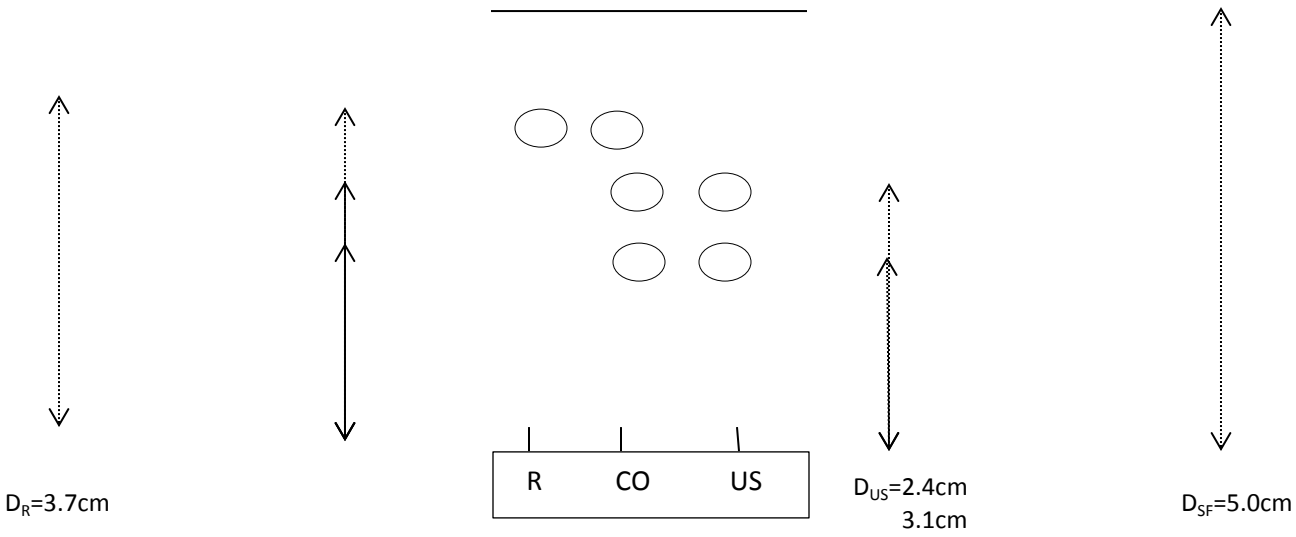


Rf_R	Rf_{CO}	Rf_{US}
0.61	0.43 & 0.61	0.46 & 0.61

TLC9

Reference Compound: *p-bromonitrobenzene*





Rf_R	Rf_{Co}	Rf_{US}
0.74	0.48 & 0.60 &0.72	0.48 & 0.62

Calculations

Retention Factor (Rf)

Rf value = $\boldsymbol{Distance\ travelled\ by\ compound \div Distance\ travelled\ by\ Solvent}$

Example: Part A, TLC 1,
$D_{uS} \cong$ 2.8cm
$D_{SF} \cong$ 3.0cm
Rf = $D_{US} \div D_{SF}$
= 2.8cm ÷ 3.0cm
= 0.9

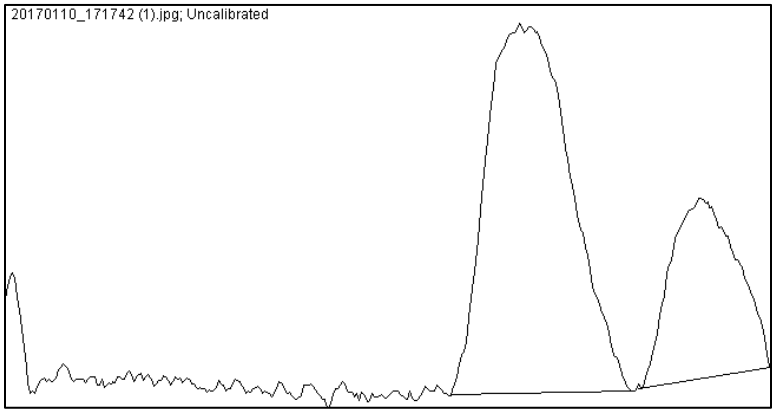
Percentage Absorbance

Absorbance of meta =26084

Absorbance of ortho = 10108

% Absorbance (meta) = (26084/ *26084 + 10108) × 100

= **72%**



% Absorbance (ortho) = 100% - 72 %= **28%**

(TLC 8)

Calculate the mole percentage

y = % Absorbance (meta) = 72 %
y = 1.7373x + 0.31
(y – 0.31) / 1.7373 = x
72-0.31/1.7373 = x
41.2% = x

x = mole % (meta) = **41.2 %**
mole % (ortho) = **58.8 %**

Discussion

1. Thin Layer paper chromatography has two phases; the mobile and stationary phase. The mobile phase is the solvent placed in the developing jar, this solvent moves up the TLC via capillary action. The stationary phase is the silica gel, or other absorbent and polar semi liquid chemicals. Silica gel is a very polar compound while the solvent can be polar or nonpolar or a mixture of chemicals with both properties.

    - There are 3 types of compounds spotted on the TLC; one with known structure and polarity, other with an unknown polarity and structure, the third compound is a mixture of both known and unknown.

    - As the solvent moves up the TLC, it binds to the chemical(s) with similar polarity and moves it along the TLC. The silica gel will bind to the polar compound(s) and prevent them from moving up the TLC. The result is a range of distances travelled by the different compounds on the TLC. This

process can be quantified with the Retention factor (Rf). *(Calculations explained above.)*

- Compounds with the same (± 0.1) Rf values are considered to have the same polarity as they formed bonds with either of the phases in a very exact manner and so must be composed of the similar chemical elements in similar sequence. In this way Rf values can be compared to detect whether the Unknown sample is or contains the known sample.

2. In <u>part A</u>, the unknown sample used was #83.
   - Unknown sample #83 contains benzophenone. The Co-spot, Reference Sample and Unknown sample travel the same distance up the TLC 1, concluding that Sample #83 contains benzophenone. This relation can be proved using the Retention Factor (Rf) value, which is 0.9 for all three substances.

   - Unknown Sample #83 also contains Biphenyl based on the *qualitative* observations. In TLC2 all three of the spotted compounds travel the same distance from the base line. The Rf value cannot be used as the qualitative proof of the observations because the solvent was allowed to rise up and beyond the TLC. Hence the Solvent front is inaccurate, resulting in inaccurate Rf values.

   - The recognition of sample #83 from TLC 1 and 2 is inconclusive *from the results in part A of the experiment.* The result for TLC 2 was corrupted therefore so was the comparison between the two TLC plates.

   - ***In Part B I & ii, it can be observed that the Rf value of benzophenone and the Unknown sample #83 are closer together (TLC 3 &5) when compared with the Rf values of Biphenyl & unknown sample # 83(TLC 4 &6.) Therefore the unknown sample contained benzophenone***.

3. In <u>Part B i) and ii),</u> the effect of different solvents was observed
   - Excluding experimental error there is a pattern developing with the change of the solvent systems. In TLC 2 & 3 EtoAc is used as the solvent and the Rf values are higher. This was the result of all three of the compounds travelling higher up the TLC when compared to TLC 4 & 5, when Hexanes was used as the solvent.

   - Ethyl acetate is very polar compound and it disrupts the Intermolecular forces between silica gel and the polar compounds by binding to the silica gel and the compounds. Since the solvent is moving up via Capillary action, it will keep moving up and take the polar compounds along to a longer distance. Ethyl acetate is not however polar enough to bind with silica gel and dissolve it, so the stationary phase is intact.

- Hexanes are nonpolar. Only the non-polar ends of the compounds move along the TLC. Most compounds involved in this experiment had a polar dominance except for biphenyl, resulting in lower Rf values when a nonpolar mobile phase was used.

4. In Part C, TLC were used to determine composition of Unknown sample 'zz'

    - TLC 7 results in similar Rf values of o-bromonitrobenzene and 'zz'. TLC 8 results in similar Rf values for m-bromonitrobenzene and 'zz'. Hence, the unknown sample 'zz' contains ortho and meta bromonitrobenzene.

    - In TLC 9, the unknown sample 'zz' has Rf factors of 0.48 and 0.62, which are close to the Rf factors for ortho-bromonitrobenzene in TLC 7 and meta-bromonitrobenzene in TLC 8 respectively, reconfirming their presence in the sample. The Rf for para-bromonitrobenzene is relatively different, so it is not a constituent of the sample, 'zz.'

    - TLC 8's co-spot is composed of m-bromonitrobenzene and 'zz'. Since Unknown sample is composed of m-bromonitrobenzene and o-bromonitrobenzene (as discussed above.) The Rf value of the co-spot is of m-bromonitrobenzene and o-bromonitrobenzene and can be used to extract the mole percentage of both compounds in the sample 'zz.' Being **41.2 % and 58.8 %** respectively.

5. The sources of error in this lab may include the following

    - For Part A of this experiment, both TLC were placed in the same developing jar, such that TLC 2 was on top of TLC 1. This blocked TLC 2 from clear sight of vision resulting in the solvent going beyond the TLC plate, hence corrupting the value for the distance travelled by the solvent front; Rf value. This can be corrected by placing only one TLC plate per developing jar, such that the movement of the solvent can be seen clearly, so that the TLC can be removed from the jar for a clear solvent front.
    - The placement of TLC plates on top of each other may have allowed the silica gel to absorb extra chemical compounds from the aluminum background of the TLC in front. These components would be seen under the UV light and disrupt the results as they do not belong to any criteria of the chemicals used. This can be corrected by placing only one TLC per developing jar with the silica gel facing upward.
    - The developing jars were opened during the developing process of the TLC in order to correct the placement of the plate inside the gel. This 'disturbance' may have allowed the versatile molecules of the solvent to become unsettled and so evaporate from the TLC plates. This unauthenticated the mobile phase. This can be corrected by placing the

TLC and fixing it's position before closing the developing jar, and then not disturbing it.

- When preparing the TLC plates, hands often touched the silica plate, allowing it to absorb the oil residue on the latter's surface or displacing the silica gel from the plate of TLC. This added unwanted compounds on the plate that would be developed and observed. This issue can be solved by wearing gloves when handling the TLC plates or placing the TLC on a high rise flat surface with frictional support, so that the TLC does not glide when preparing them.
- It was difficult to spot smaller dots of compounds on TLC. Different sized spots have different centres, affecting the measured distance travelled by each one. The large size of the spots makes results less efficient as there is a wider range of values to be considered. This can be corrected by placing the spots at a 90° angle with the spotter for smaller and similar spot sizes placed on the TLC.
- No physical markings were made to label the lanes. Only a template TLC was used for comparison (left to right in order of R, Co, US.) This may have resulted in some confusion on the confirmation of the specific lanes' recognition. This issue can be avoided by labelling the lanes at either the very top of the TLC, where solvent front is not to reach. Or at the bottom, below the base line. This way the probability of doubt is decreased.

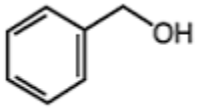
Questions

1. The results of the TLC depend on the distance travelled by the spotted compounds. These distances are a direct result of the different polarities of the solvent system and the stationary phase. When the polarity of the solvent system is increased the following occur:
    i. The mobile phase binds with the silica gel, therefore decreasing the possible binding sites between silica gel and the polar compounds.
    ii. With the weakened intermolecular forces between the spotted compounds and silica gel, the more polar solvent will form more bonds with the polar compounds. Resulting in a higher attraction between the polar compounds and the mobile phase instead of the stationary phase.
    iii. Capillary action moves the solvent up and so its intermolecular forces with silica gel do not inhibit its movement up the TLC. Therefore, the polar compounds move up with the solvent up the TLC.

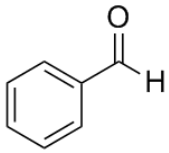
2. Smallest Rf value occurs when the distance travelled by the spotted compounds is short, due to their attraction to the polar stationary phase (silica gel.)

    a. Most polar to least: Benzyl alcohol > Benzaldehyde > Benzyl acetate

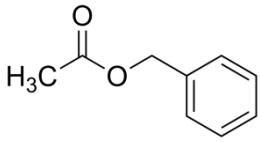
Benzyl alcohol will have the smallest Rf value as it is the most polar of the three compounds and so will bind more to the stationary phase. The hydroxyl group in alcohol allows for hydrogen to be donated and create a hydrogen bond. Benzaldehyde cannot hydrogen bond amongst them; they can however accept hydrogen bonds from other compounds



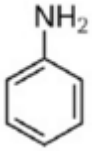
Benzyl Alcohol



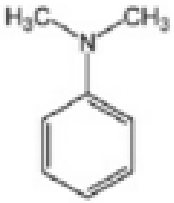
benzaldehyde



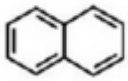
b.



(Aniline)



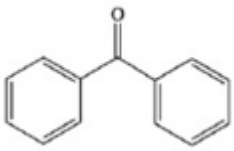
(N,N-dimethylaniline)


Naphthalene

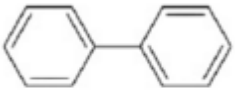
Most polar to least polar: Aniline > N,N dimethylaniline>Naphthalene

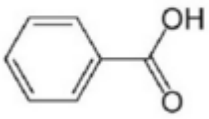
Aniline will have the smallest Rf value as it is the most polar of the three compounds. The difference in electronegativity between the Nitrogen and Hydrogen allow there to be a dipole charge. This charge allows the Aniline to form hydrogen bonds with polar molecules. The Other two compounds are non-polar, they are symmetrical and do not have an effective uneven share of charge.

c.



(Benzophenone)


(Biphenyl)



(Benzoic acid)

Most polar to least polar: Benzoic acid >Benzophenone> Biphenyl

Benzoic acid will have the least Rf value as it is most polar chemical and will be highly attracted to the stationary phase. The Carbonyl group allows the compound to accept hydrogens from other compounds while the Hydroxyl group allows it to share the hydrogens, and form hydrogen bonds. The presence of these functional groups makes benzoic acid very polar. Benzophenone carbonyl group will allow the acceptance of hydrogens but, it is largely unipolar due to the benzene rings. Biphenyl is a symmetrical non-polar molecule, so will have a high Rf value not low.