

SEPARATION TECHNIQUES

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Chemistry

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Every honest researcher I know admits he's just a professional amateur. He's doing whatever he's doing for the first time. That makes him an amateur. He has sense enough to know that he's going to have a lot of trouble, so that makes him a professional.

— Charles F. Kettering (1876-1958) (Holder of 186 patents)

ACKNOWLEDGEMENTS

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I also thank Prashansa Gupta and Srijit Mukherjee for their contribution to this report as my lab-partners, who made the task of performing experiments immensely comfortable and productive at the same time.

CONTENTS

1	THIN LAYER CHROMATOGRAPHY (INTRODUCTORY)	1
1.1	Aim	1
1.2	Chemicals Required	1
1.3	Theory	1
1.4	Procedure	2
1.5	Observations and Results	3
1.6	Precaution	3
1.7	Acknowledgements	4
2	THIN LAYER CHROMATOGRAPHY (CONSTITUENT DETECTION)	5
2.1	Aim	5
2.2	Chemicals Required	5
2.3	Theory	5
2.4	Procedure	6
2.5	Observations and Results	7
2.6	Precaution	7
2.7	Acknowledgements	8
3	COLUMN CHROMATOGRAPHY (INTRODUCTION)	11
3.1	Aim	11
3.2	Chemicals Required	11
3.3	Theory	11
3.4	Procedure	12
3.5	Observations and Results	13
3.6	Precaution	14
3.7	Acknowledgements	15

LIST OF FIGURES

Figure 1	TLC plates after Iodine visibility treatment	3
Figure 2	Diagrammatic representation of the test setup	6
Figure 3	TLC plates for Mixture 1	8
Figure 4	TLC plates for Mixture 2	9
Figure 5	p-Nitroanline	11
Figure 6	TLC plates for the Initial Run	13
Figure 7	TLC plates for various test tubes	14
Figure 8	TLC plates for the rest of the test tubes	15

LIST OF TABLES

LISTINGS

ACRONYMS

THIN LAYER CHROMATOGRAPHY (INTRODUCTORY)

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1.1 AIM

TO find the R_f values of the following compounds:

1. Naphthalene (least polar)
2. Benzophenone (partially polar)
3. Aniline (polar)

1.2 CHEMICALS REQUIRED

1. Naphthalene
2. Benzophenone
3. Analine
4. Hexane
5. Ethyl Acetate
6. Iodine
7. Silica

1.3 THEORY

Thin Layer Chromatography is a separation technique that involves the use a rigid porous structure (like that of a silica layer on a glass slide). The mixture is dissolved in a suitable solvent (the precise meaning of suitable will be made clear in a specific example) and a spot of this mixture is made near one end of the structure. This end is now dipped in a suitable solution (again suitable will be defined later), and due to capillary action, this solution begins to move up. When it comes in contact with the spot, the constituents of the spot, differentially move up the structure, allowing us to separate them.

For viewing the different components, some of the techniques used are as follows:

1. UV

2. Iodine Staining
3. KMnO₄ Staining

We now define an R_f value for a given concentration of the solution

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solution}} \quad (1)$$

1.4 PROCEDURE

1. Preparing the TLC plates
 - a) Prepared a silica slurry, using silica and ethyl acetate
 - b) Dipped two glass slides, held together, into the solution, to coat about eighty percent of it with the slurry.
 - c) Allowed them to dry (could blow air on it to accelerate the process) and then separated them.
2. Visibility Chamber
 - a) Added a few granules of Iodine with silica granules dominant in number, in a beaker, covered with a watch glass.
3. 10% Ethyl Acetate Soln. in Hexane
 - a) Using a measuring cylinder, measured 1 mL Ethyl Acetate and then made the volume 10 mL using Hexane. Transferred the contents in a suitable beaker and covered it with a watch glass.
4. Prepare a solution of the given compounds in Ethyl acetate and using a capillary tube, put a spot on the TLC plate, near the silica coated edge. Also, marked physically, by a method suitable, the position of the spot.
5. Placed the TLC plate, carefully (it's fragile) inside the the 10% Ethyl Acetate Soln., such that the spot is above the level of the solution initially and covered it again, with the watch glass.
6. Kept a watch on the TLC and removed it as soon as the solvent crossed about 90% of the height of the silica coating and placed it cautiously in the Visibility Chamber, until the spots became visible.
7. Now marked the lower portion of the visible spots (should ideally be only one, excluding the Ethyl Acetate Solution) and the Ethyl Acetate solution and measured their distances from the initial position of the spot marked earlier.



In our experiment, we scratched off some silica to mark, using the capillary tube



We actually had to repeat the experiment as we broke the silica coating!

1.5 OBSERVATIONS AND RESULTS

1. Naphthalene: $\frac{4.2}{4.4} = 0.954$
2. Benzophenone: $\frac{3.3}{4.2} = 0.785$
3. Aniline: $\frac{0.7}{4.4} = 0.159$

For details, please refer to

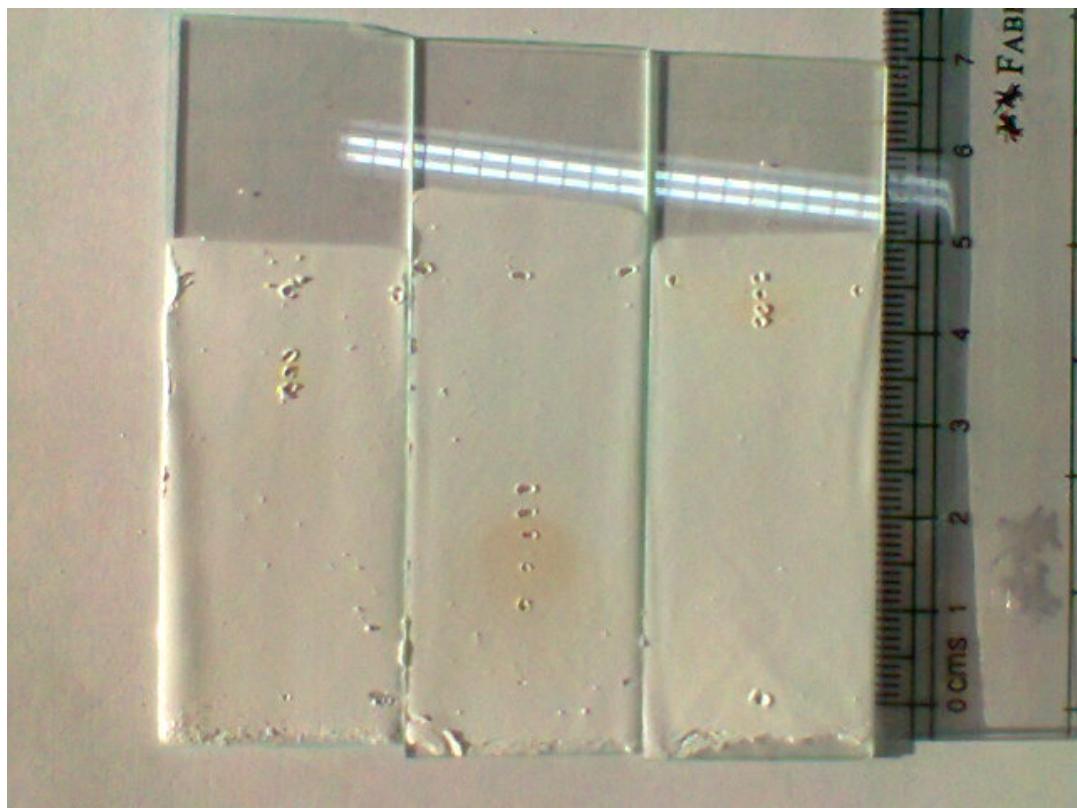


Figure 1

The R_f value decreases with increase in polarity of the compound being analysed.

1.6 PRECAUTION

1. The slurry shouldn't be very thick
2. Cover the beakers with a watch glass to ensure there's no loss of volatile substances (minimal that is)
3. The coating is very fragile, thus the TLC plates must be handled with caution

1.7 ACKNOWLEDGEMENTS

I thank Dr. R Vijaya Anand for his guidance during the experiment. I also acknowledge the contribution of my lab partners, Prashansa and Srijit for performance of the same.

2

THIN LAYER CHROMATOGRAPHY (CONSTITUENT DETECTION)

January 21, 2013

2.1 AIM

To find the constituents of the mixture samples given, which contain two of the following each:

- A) Resorcinol
- B) p-Hydroxybenzaldehyde
- C) 2,4 - Dinitrophenylhydrazine
- D) p-Nitro Aniline

and to find the corresponding R_f values, using suitable solvent systems.

2.2 CHEMICALS REQUIRED

- 1. Resorcinol
- 2. p-Hydroxybenzaldehyde
- 3. 2,4 - Dinitrophenylhydrazine
- 4. p-Nitro Aniline

2.3 THEORY

The theory here is rather straight forward. We already know that the sample contains two components. We, on a TLC, make three spots, the extreme right comprising of only, say A, the middle comprising of A and the mixture, and the extreme left spot, comprising of only the mixture. Now as shown in the diagram, we'll obtain three spots in the middle (called the co-spot), if A is not already present in the compound. If A is present, then only two spots will appear in the middle.

It is the same as saying that the extreme left should have fewer spots than the co-spot if A is not present, in general, and the 'extra spot' in the co-spot, must match with the spot of A on the extreme right.

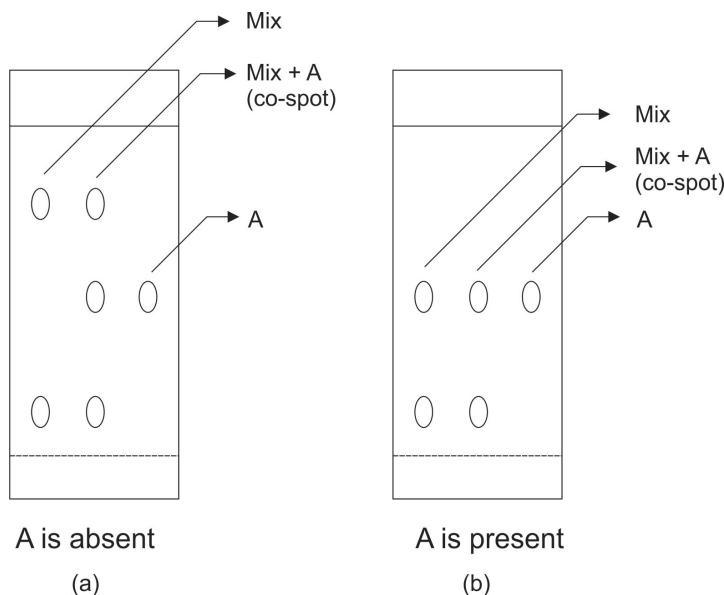


Figure 2

2.4 PROCEDURE

1. Preparing the TLC plates (same as the previous experiment, repeated here for convenience)
 - a) Prepared a silica slurry, using silica and ethyl acetate
 - b) Dipped two glass slides, held together, into the solution, to coat about eighty percent of it with the slurry.
 - c) Allowed them to dry (could blow air on it to accelerate the process) and then separated them.
2. Visibility Chamber (again, same)
 - a) Added a few granules of Iodine with silica granules dominant in number, in a beaker, covered with a watch glass.
3. Various concentrations (5%, 10% and 20%) Ethyl Acetate Soln. in Hexane
 - a) Using a measuring cylinder, measured the required volume of Ethyl Acetate and the made the volume 10 mL using Hexane. Transferred the contents in a suitable beaker and covered it with a watch glass.
4. Diluted the given solutions of A, B, C, D, Mixture 1 and Mixture 2 appropriately and using a capillary tube, put the spots as described in the theory, using compounds A, B, C and D, one after the other, on the TLC plate, near the silica coated edge. Also, marked physically, by a method suitable, the position of the spot.



Our
dilutions were not
sufficient the first
time we attempted
this and we had to
redo the entire
procedure

5. Placed the TLC plate, carefully (it's fragile) inside the Ethyl Acetate Soln. (its concentration was changed progressively in accordance with displacement of the spots), such that the spot is above the level of the solution initially and covered it again, with the watch glass.
6. Kept a watch on the TLC and removed it as soon as the solvent crossed about 90% of the height of the silica coating and placed it cautiously in the Visibility Chamber, until the spots became visible. This is the same as before, except that we setup two chambers simultaneously to speeden up the process.
7. Now marked the positions of all the spots, deduced presence of the substance taken and also calculated the R_f value for A, B, C and D.



We once ended up dipping the spots in the solution itself, which lead to trouble later

2.5 OBSERVATIONS AND RESULTS

Mixture 1 was found to be constituted of compounds A and B, viz. Resorcinol and p-Hydroxybenzaldehyde. In a 20% system, we observed:

1. Resorcinol : $R_f = 0.129$
2. p-Hydroxybenzaldehyde : $R_f = 0.477$
3. 2,4 - Dinitrophenylhydrazine : $R_f = 0.028$
4. p-Nitro Aniline : $R_f = 0.202$

Mixture 2 was found to be constituted of compounds C and D, viz. 2,4-Dinitrophenylhydrazine and p-Nitro Aniline. In a 20% system, we observed:

1. Resorcinol : $R_f = 0.129$
2. p-Hydroxybenzaldehyde : $R_f = 0.422$
3. 2,4 - Dinitrophenylhydrazine : $R_f = 0.085$
4. p-Nitro Aniline : $R_f = 0.259$

For details, please refer to [Figure 3](#) and [Figure 4](#)

2.6 PRECAUTION

Precautions are same as those in the previous experiment, viz.

1. The slurry shouldn't be very thick
2. Cover the beakers with a watch glass to ensure there's no loss of volatile substances (minimal that is)
3. The coating is very fragile, thus the TLC plates must be handled with caution

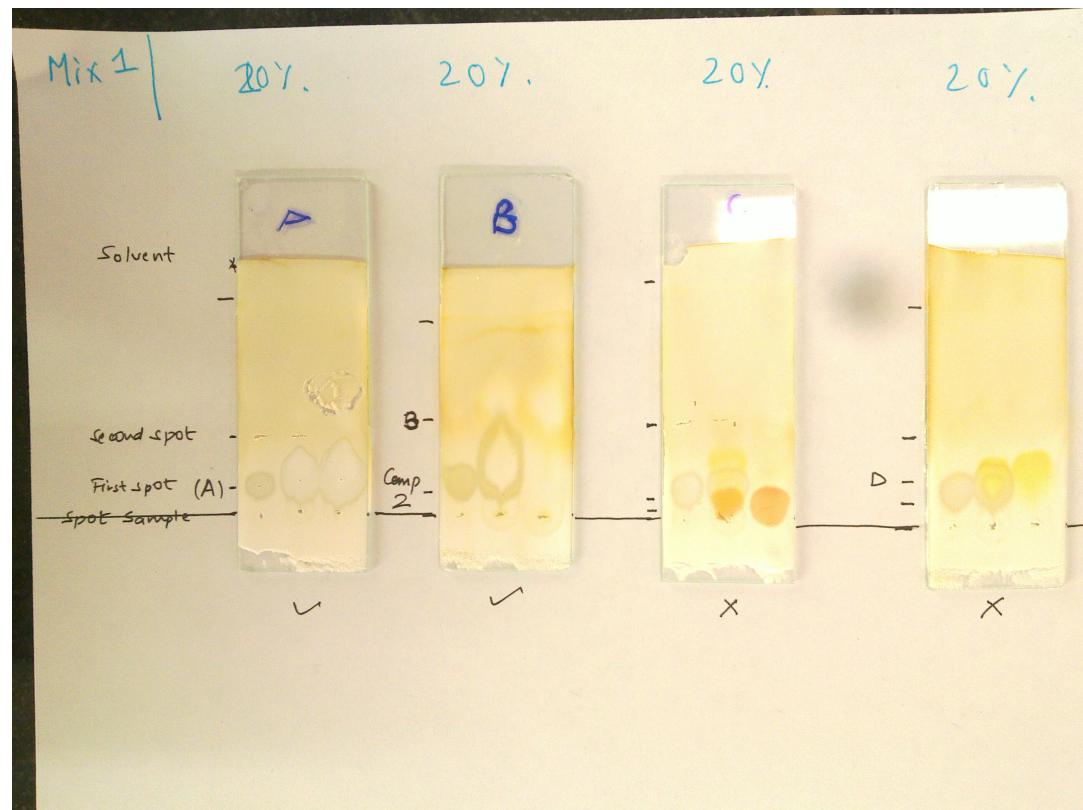


Figure 3

2.7 ACKNOWLEDGEMENTS

I thank Dr. R Vijaya Anand for his guidance during the experiment. I also acknowledge the contribution of my lab partners, Prashansa and Srijit for performance of the same. I especially thank them for maintaining their calm while we were forced to repeat the experiment numerous times.

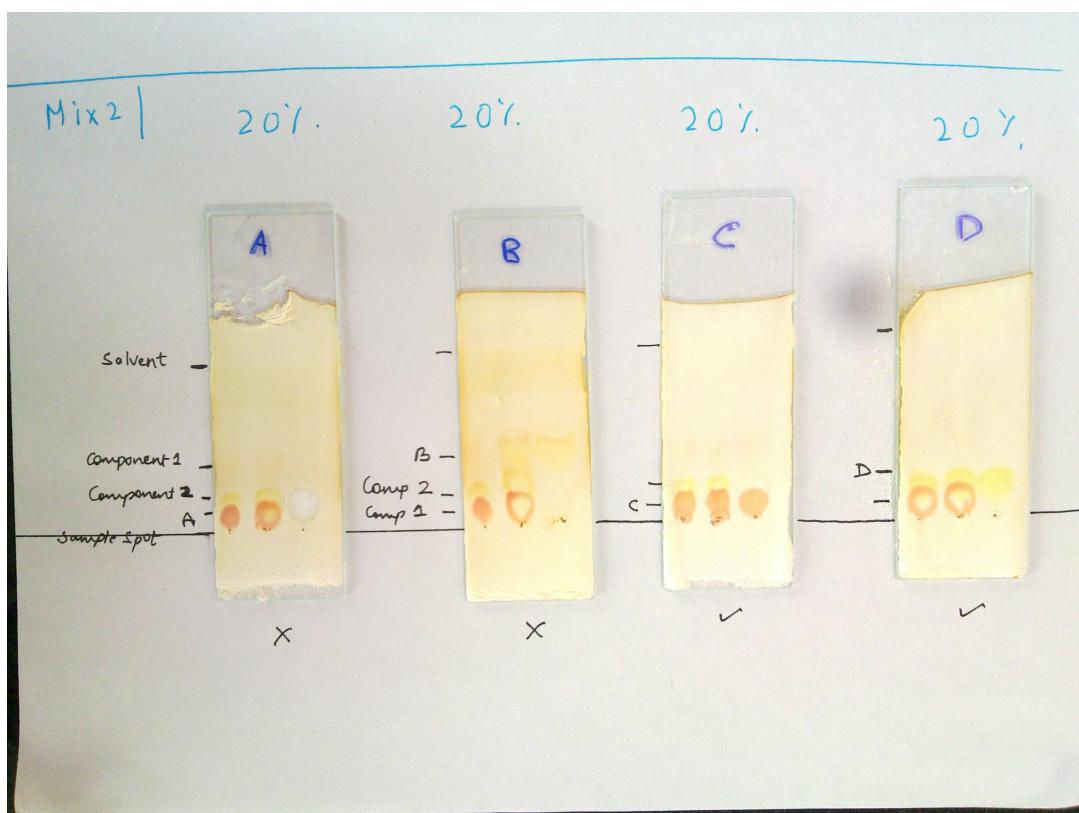


Figure 4

3

COLUMN CHROMATOGRAPHY (INTRODUCTION)

January 21, 2013

3.1 AIM

To get a hands on experience with the Column Chromatography technique, using a single compound, viz. p-Nitro Aniline.

3.2 CHEMICALS REQUIRED

1. Silica
2. Iodine
3. Ethyl Acetate
4. Benzene
5. p-Nitro Aniline (given compound, refer to ??)



Figure 5

3.3 THEORY

TLC is good for detecting what constitutes a mixture. However its yield is very little. To overcome this difficulty, we use a technique known as "Column Chromatography". The principle of differential binding of compounds with the solvent is still harnessed. However, instead of relying on the capillary action, we now rely on gravity. The setup consists of a vertical wet (hexane) silica column, contained in a suitable glass container with a flow control apparatus and a nozzle at the bottom. The compound (if solid, then first dissolved in an appropriate solvent) is added on top of the silica column and on top of it the eluent (a suitably polar solution) is added. This moves down through

the silica column, differentially moving the constituents of the mixture (which is just a compound in our case). Then if the compound is visible, we can easily use this property and collect the constituents in different test tubes. However, if the compounds are not visible, we may need to run a TLC for each small volume collected.

Wikipedia has a very concise and accurate description of the same, which can also be referred to.

3.4 PROCEDURE

1. Determining the concentration of Elluent to use
 - a) Prepared the TLC plates as in the previous experiments
 - b) Setup the Visibility Chamber, again as before
 - c) Various concentrations (50%, 30% and 20%) Ethyl Acetate Soln. in Hexane were created
 - d) The given compound was diluted in Ethyl Acetate.
 - e) TLC was run using the given compound for the various concentrations, till the R_f value was found to be less than $\frac{1}{2}$, just as described in the previous experiments.
 - f) Use the concentration value for which the TLC is roughly less than half, as the Elluent's concentration. This was found to be 20% for our case as is given in the next section.
2. Preparing the Wet Column
 - a) While the TLCs run, a slurry of silica was created in hexane (with about 20-30 spatula of silica) and it was poured in glass column, as described in the theory.
 - b) To this, hexane was added, and it was shaken until all air bubbles disappeared. Further the volume of hexane was reduced to about 1 cm above the silica gel alongside, by allowing hexane to flow through the nozzle.
 - c) The compound, along with silica, were mixed with ethyl acetate to form a thick slurry.
 - d) This mixture was transferred on top of the silica column.
 - e) After it settled reasonably, cotton was added to further level and to ensure that addition of elluent doesn't disturb the mixture (in this case the compound). This ensures that when the separation process proceeds, it doesn't happen out of plane (with respect to the cross section of the container)
3. Running the Column
 - a) Got a set of ordered test tubes in a suitable holder.



This step helps determine the kind of solution we use for the elluent as it determines the speed at which the separation process will take place.

- b) The Elluent was poured into the glass column cautiously and sealed from the top.
- c) The liquid was allowed to flow through the nozzle of the glass column, into the test tubes, progressively, as they filled.
- d) For various test tubes, TLC was run again to find its composition. However, since the compound was coloured, this process was initiated only when the test tubes were known to visibly contain the compound (as could be readily seen from the column as time progressed).

3.5 OBSERVATIONS AND RESULTS

To find the concentration of Elluent to use, please refer to [Figure 6](#). We obtained the following results

1. With 50% $R_f = 0.85$
2. With 30% $R_f = 0.62$
3. With 20% $R_f = 0.25$



Figure 6

To find the constituents of the test tubes, we run TLC with a 20% system, on each of them and the results are as follows. Refer to [Figure 7](#) and [Figure 8](#) for details.

1. Plate 1, $R_f=0.17$
2. Plate 2, $R_f=0.18$

3. Plate 3, $R_f=0.21$
4. *Plate 4, $R_f=0.27$
5. Plate 5, $R_f=0.18$
6. *Plate 8, $R_f=0.10$
7. Plate 10, $R_f=0.17$
8. Plate 11, $R_f=0.15$

* appear to be outliers Since the mixture consists of only one compound, we expect to get the same R_f values for all coloured test tubes, which seems to hold good for the first place of decimal.

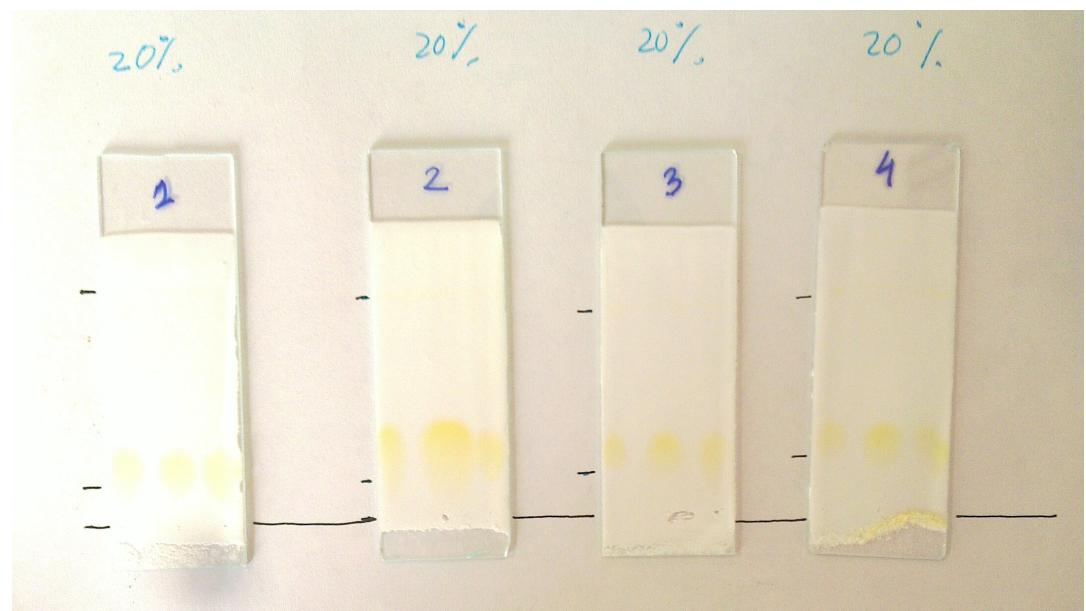


Figure 7

3.6 PRECAUTION

Precautions are same as those in the previous experiment, viz.

1. The slurry shouldn't be very thick
2. Cover the beakers with a watch glass to ensure there's no loss of volatile substances (minimal that is)
3. The coating is very fragile, thus the TLC plates must be handled with caution

Further,

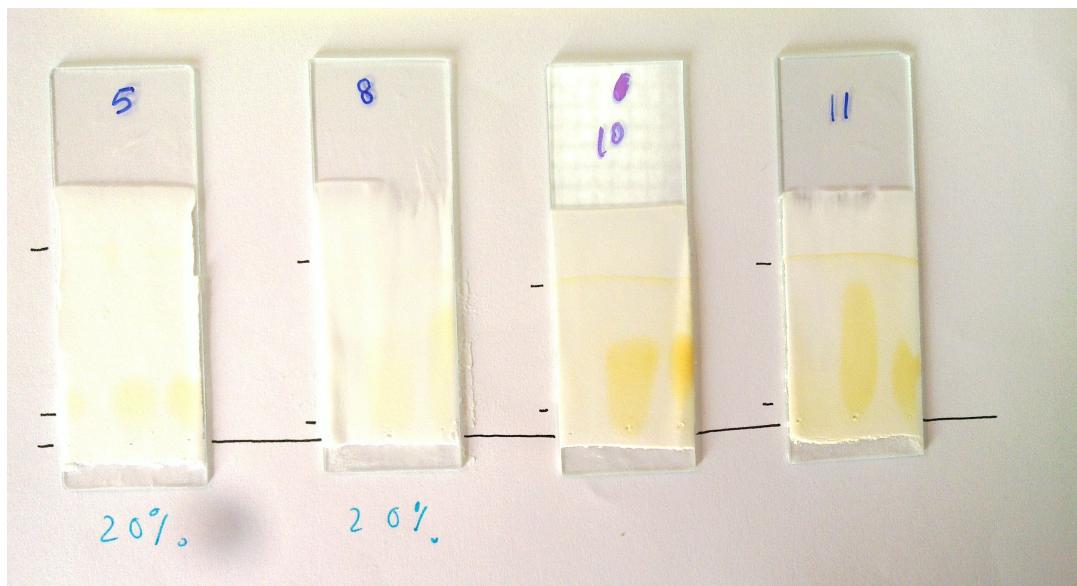


Figure 8

1. We've to ensure there aren't any air bubbles in the column created, by tapping it enough.
2. The hexane level shouldn't drop below the silica column's top, else cracks would begin to appear.
3. Ensure the test tubes aren't cracked.

3.7 ACKNOWLEDGEMENTS

I thank Dr. R Vijaya Anand for his guidance during the experiment. I also acknowledge the contribution of my lab partners, Saumya, Vivek, Prashansa and Srijit for performance of the same, especially for being able to work in harmony, despite being a large group of five. I also thank our PhD guide for demonstrating the experiment and her assistance in general, with performance of the same.



One of our test tubes was in fact cracked from the bottom and we lost that volume of the recovered compound.

COLOPHON

This document was typeset using the typographical look-and-feel `classicthesis` developed by André Miede, for L^AT_EX.
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