Experiment 17: Chromatography

Nicholas Egan

With Partners Corbin Edmondson and Ashley Faddis

March 26, 2015

Chem 113, Section 003, TA Elizabeth Crowe

I. Introduction

Chromatography is defined to be a chemical method of component separation where two phases – the mobile phase and the stationary phase – are used to dissolve and divide parts of a liquid. In its oldest and first uses, chromatography was used in the 1800s to separate chlorophyll components.[1] However, paper chromatography was not invented or widely-used until years later when scientists discovered the method while separating parts of a protein. These two English scientists, Martin and Synge, were awarded the 1952 Nobel Prize in chemistry, and their contributions and methods are still practiced today.[2]

Speaking on the method used for paper chromatography, the visual aspect is not very hard to understand: a solvent moves up the paper, pulling with it colored components of the separable liquid. However, the chemistry behind the method is a bit more complex. Capillary action drags the mobile phase – the components and solvent that move across the paper – up the stationary phase – the porous paper itself.[1] However, the distance the components travel is determined by their polarity and attraction to both the paper and the mobile phase. In most cases, chromatography paper is polar and laden with OH^- molecules. As the solvent travels across the stationary phase, the different components will begin to move across the paper and appear colorful due to the differences in polarity.[2] By this method then, the polarity of each individual component will force it to separate onto the stationary phase at a certain distance. Non-polar components carried by a non-polar solvent (like the proponol mixture used in this lab) will travel far up the paper, while polar components will be the first to separate out.[3] This makes sense, as the non-polar solvent will always carry similar molecules of the non-polar form, but will not have as strong of an attraction to polar components. Another important characteristic of paper chromatography is the retention factor, commonly denoted as the R_f value. This equates to

the approximate ratio of molecules visible on the paper (the mobile phase), and is a relatively constant and reliable comparison method.[1]

Of course, paper chromatography is not the only method used to identify substances, specifically ink. In investigative businesses such as the FBI, there are several identification methods used, such as mass spectrometry, liquid chromatography, and infrared sensing that can be used to determine the identity of an unknown ink. Additionally, there are methods that are less-destructive – paper chromatography essentially destroys the ink sample – which are beneficial if evidence needs to be saved.[4] Liquid chromatography is an interesting method, as there are many different variations of it. The process could range from having several high-tech pumps and equipment to simply running liquids through a filter, as was accomplished in part of this laboratory exercise.[1]

Chromatography is also useful in purifying liquids or solutions in the manufacturing industry. Since the method is a relatively confined – meaning that it can be done entirely in a column filter – and easy one, it is widely-used and appreciated in large-scale filtration. While this process of liquids and vapors rising up a tube may be different from paper chromatography, both methods serve the same purpose: allowing different components to separate from a liquid.[5] Another use for chromatography is separating mixtures, such as sugar in foods, to show the differences in coating colors and dyes.[6]

In Experiment 17, the objective is this: to get components to separate out of ink to properly identify unknown pen samples based on trial runs. Students are supposed to use prior knowledge – coupled with results from early parts of the lab – to construct chromatograms that will enable the student to determine what inks were spotted on the paper.[1] This being said,

application, trials, and problem-solving are important concepts in this laboratory exercise, as they should be in any experiment. For this lab, I developed a hypothesis regarding the solutions that would best identify my unknown ink samples:

> "Based on observations of the base paper chromatogram [the fact that the some of the pen components did not travel very far up the paper] (the 2:1 isopoponol:water), [I] decided that in order to investigate and determine which pens are which, a solution with a higher polarity would be needed. This is because, since the base was of non-polar solution, some polar pens (the black Pilot G-2 07) did not separate out into components. With a more polar solution, this pen and others will be able to move up the solvent front further because of like polarities.[7]"

II. Procedure

The entire procedure for this laboratory exercise was taken from PSU Chemtrek 2014-2015 *and is cited as such.*

I began the experiment by creating several chromatograms. One of these was similar to the one created in Section A of the experiment where making chromatograms was first explained, in that it included all the colors and models of pens to serve as a base reading. The creation of a chromatogram is obviously an important procedure for this lab, so it should be explained. First, I obtained a piece of chromatography paper, all fifteen styles of pens, and the 2:1 proponol:water mixture provided for this lab. Using a pencil and a ruler, I drew a line across the length the paper 0.5 cm from the bottom and made small tick marks on that line every 1 cm. On each of these tick marks, I made a small ink dot with each pen, noting the placement of the pens:

Position on Chromatogram	Pen Color & Style
1	Blue InkJoy 300 RT
2	Blue Pilot G-2 07
3	Blue Pilot Easy-Touch
4	Blue Pilot V-Ball BG
5	Blue BIC Ultra-Round
6	Black BIC Ultra-Round
7	Black Pilot V-Ball
8	Black Pilot Easy-Touch
9	Black Pilot InkJoy 300 RT
10	Black Pilot G-2 07
11	Red Pilot Easy-Touch
12	Red BIC Ultra-Round
13	Red InkJoy 300 RT
14	Red Pilot G-2 07
15	Red Pilot V-Ball

I proceeded by pouring enough of the 2:1 mixture into the top-half of a Petri dish so it would cover the base. I stapled the chromatogram together as described on page 17-12 in *PSU Chemtrek* and placed the part of the paper cylinder with the ink dots down in the Petri dish. I covered it with a plastic cup to prevent evaporation and waited approximately twenty minutes until the solvent front was 0.5 cm from the top of the chromatography paper. After letting the chromatogram dry, I measured the distance the middle of each component traveled, calculated the R_f (retention factor) values for each pen type, and recorded them in *Table 1*, noting the color differences as well.

Next came the part of the experiment that involved application of knowledge to determine identities of unknown pen samples provided by the TA. Though each of us was provided with different unknown samples to test, we were allowed to work in a small group (consisting of Corbin Edmondson, Ashley Faddis, and myself) to hypothesize and come up with a method for successful identification (see Introduction). As stated in my Introduction, we decided that a solution of higher polarity would likely be needed to bring out some components

of specific pens. Therefore, I decided to use distilled water as a solvent since, according to the Snyder Polarity Index, it was the most polar liquid we had[8] (refer to *Figure 2* in the Results section). We briefly used the Snyder Index to not only compare some liquids to run chromatograms with, but also to brainstorm mixtures that might drag out more components. The second solution I decided to test was a 1:1 water:methanol mixture, because we hoped that the polar water mixed with the alcohol would both drive some of the components out to measurable range, while also conserving some of the data found from the base 2:1 test. Corbin tested an ethyl alcohol solution and 100% methanol, but they did not prove to be helpful in further identification of black pens.[9] Ashley ran 100% isoproponol, but this solution was much more non-polar than the base 2:1 and did not produce much more useful data.[10]

For identification of my unknown pens, I decided to first use the 1:1 water:methanol mixture, since it looked to be helpful in determining some of the pen styles on my unknown chromatogram (see *Figure 7* in Results). While it produced similar results to some of the pens in the trial, I was only able to confidently identify one pen style with this method. Therefore, I used the second chromatogram to run the 2:1 proponol:water solution, with hopes that I could use the base trial to help identify the unknown pens (refer to Results, *Figure 8*). This proved successful, as both the R_f values and the color patterns matched some of the pens on the base trial, which allowed me to identify the rest of the unknown pens.

III. Results

Figure 1: 2:1 Proponol:Water Base Chromatogram[7]

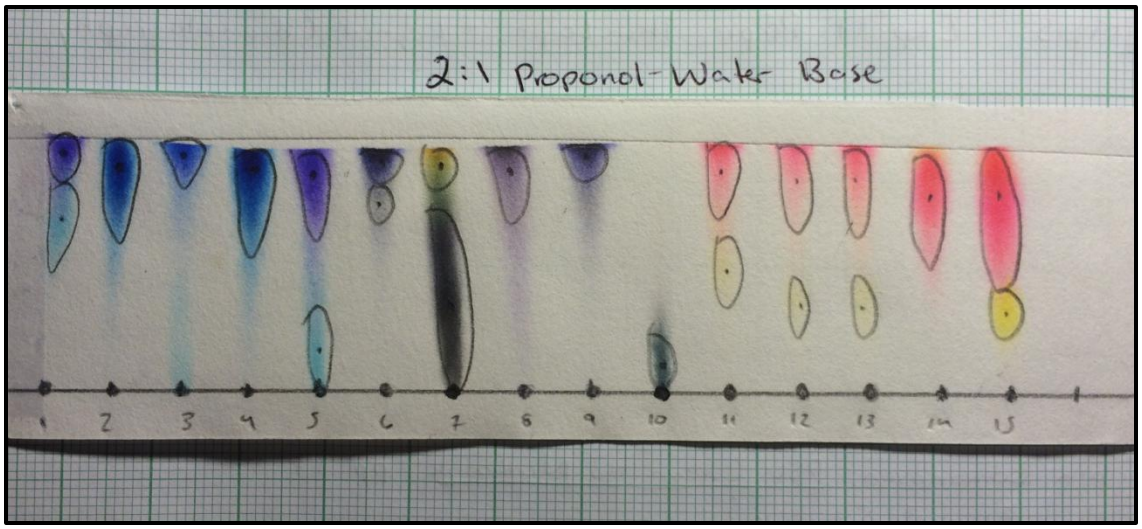

Table 1: R_f Values of 2:1 Proponol-Water Base[7]

Position Number*	Color of Component	R_f Value	Color of Component	R_f Value
1	Light blue	0.694	Purple	0.792
2	Blue	0.917		
3	Blue	0.972		
4	Blue	0.889		
5	Light blue	0.167	Purple	0.861
6	Yellow	0.778	Black	0.945
7	Black	0.361	Yellow	0.917
8	Purple	0.917		
9	Purple	0.972		
10	Black	0.111		
11	Yellow	0.500	Pink	0.889
12	Yellow	0.361	Pink	0.861
13	Yellow	0.333	Pink	0.889
14	Dark pink	0.806		
15	Yellow	0.333	Dark pink	0.806

***Note:** The "Position Number" corresponds to the table of positions and pen styles in the Introduction.

Sample R_f Value Calculation[7]

$$R_f = \frac{distance\ component\ moved}{distance\ solvent\ front\ moved}$$

$$R_f = \frac{2.5\ cm}{3.6\ cm} = 0.694$$

Figure 2: Distilled Water Chromatogram[7]

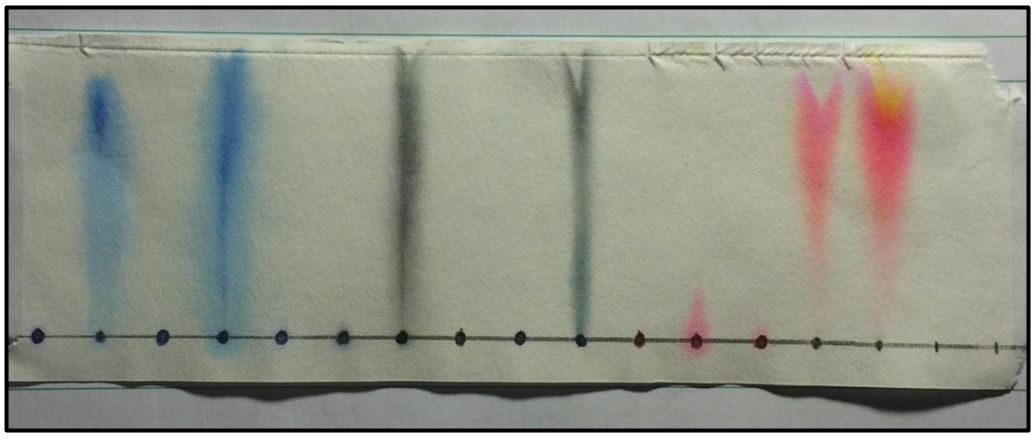

Figure 3: 1:1 Methanol:Water Chromatogram[7]

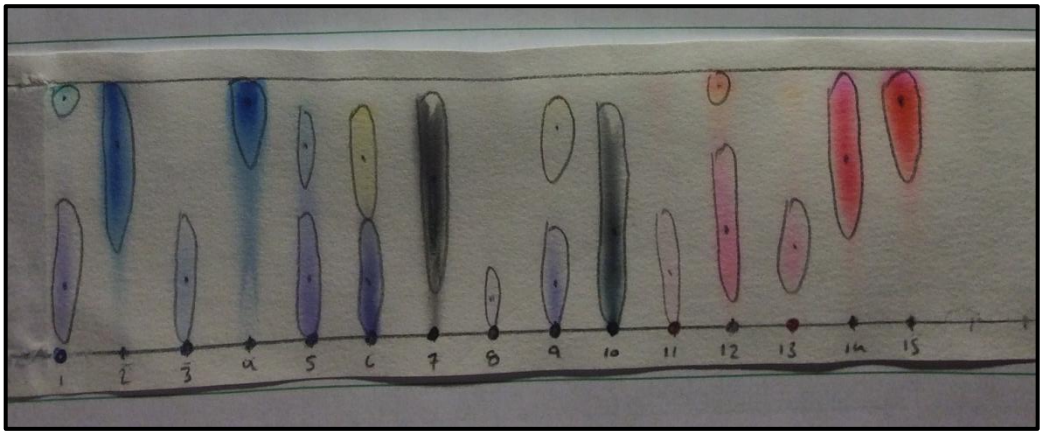

Table 3: R_f Values of 1:1 Methanol:Water[7]

Position Number	Color of Component	R_f Value	Color of Component	R_f Value
1	Purple	0.256	Blue	0.949
2	Blue	0.769		
3	Light blue	0.256		
4	Blue	0.897		
5	Purple	0.230	Blue	0.744
6	Purple	0.205	Yellow	0.692
7	Black	0.598		
8	Light purple	0.128		
9	Purple	0.197	Yellow	0.769
10	Black	0.359		
11	Light pink	0.205		
12	Pink	0.385	Orange	0.923
13	Pink	0.308		
14	Dark pink	0.641		
15	Dark pink	0.684		

Figure 4: 100% Methanol Chromatogram[9]

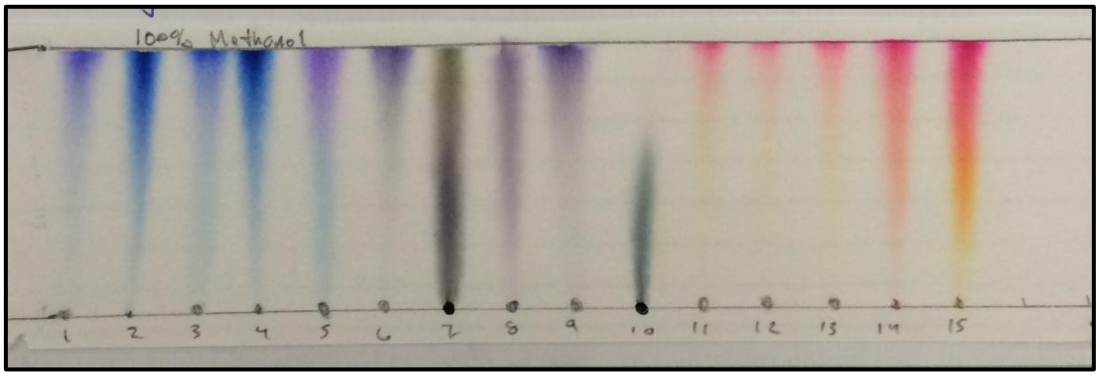

Figure 5: Ethyl Alcohol Solution Chromatogram[9]

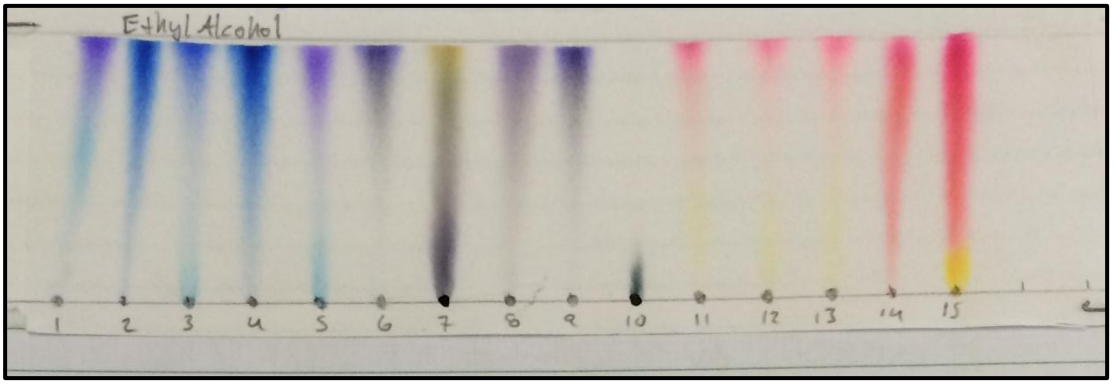

Figure 6: Isoproponol Solution Chromatogram[10]

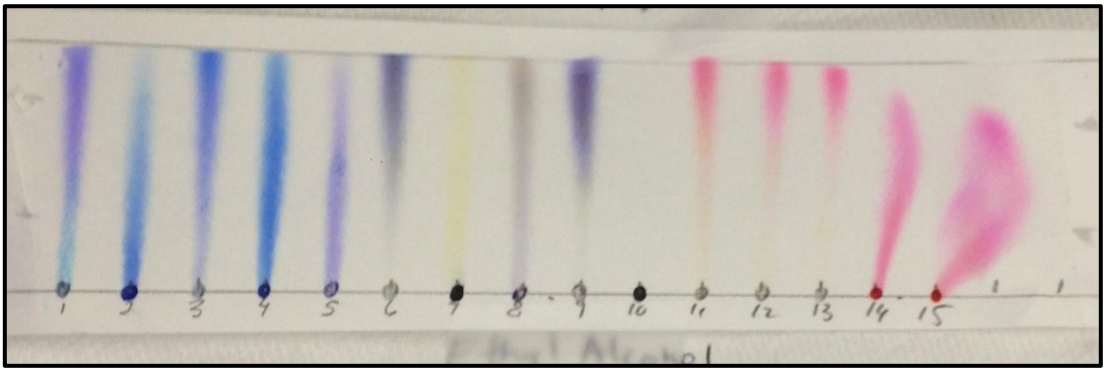

Figure 7: Chromatogram of Unknown Inks, Test 1 – 1:1 Methanol:Water[7]

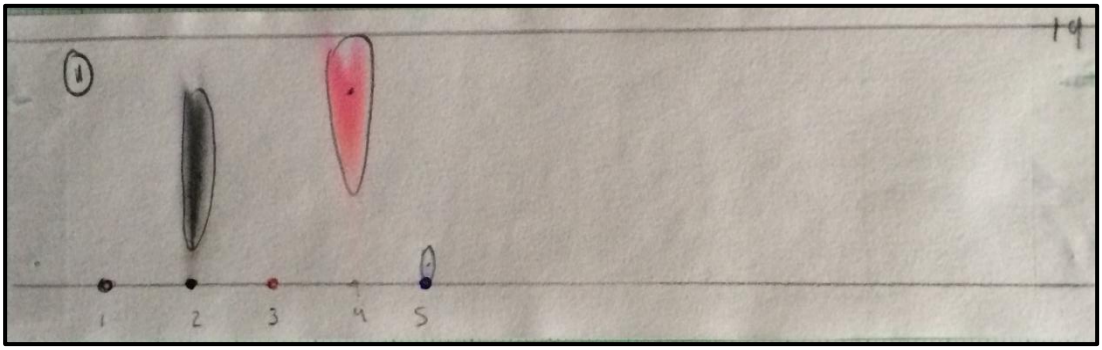

Table 7: R_f Values of Unknown Inks, Test 1 – 1:1 Methanol:Water[7]

Position Number	Color of Component	R_f Value
1	Unidentifiable	0.000
2	Black	0.459
3	Unidentifiable	0.000
4	Pink/Red	0.784
5	Purple	0.081

Figure 8: Chromatogram of Unknown Inks, Test 2 – 2:1 Proponol:Water[7]

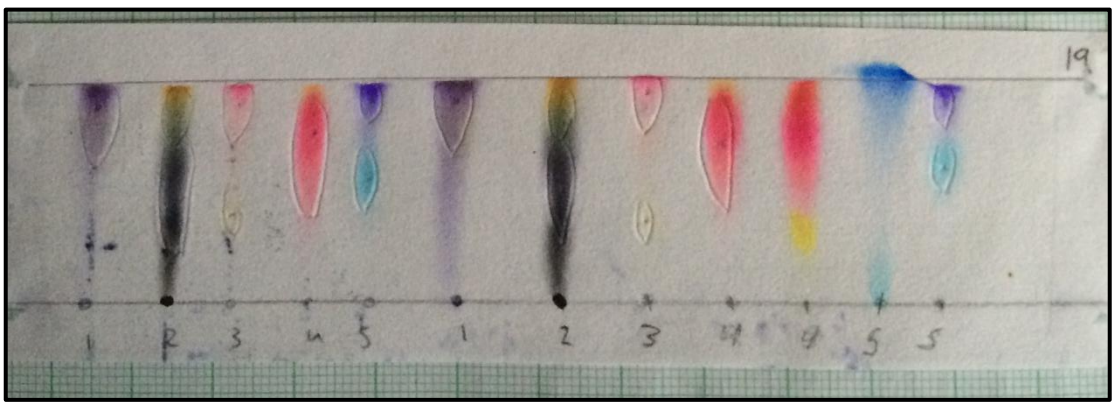

Table 8: R_f Values of Unknown Inks, Test 2 – 2:1 Proponol:Water[7]

Position Number	Color of Component	R_f Value
1	Purple	0.882
2	Black	0.500
3	Pink	0.912
4	Dark pink	0.765
5	Light blue	0.559

Table 9: Identification of Unknown Ink Samples[7]

Ink Sample Position Number	Pen Number (from 2:1 base)	Ink Pen Style
1	8	Black Pilot Easy-Touch
2	7	Black Pilot V-Ball
3	11	Red Pilot Easy-Touch
4	14	Red Pilot G-2 07
5	1	Blue InkJoy 300 RT

As one can see, the amount of data collected for this lab was extensive; however, all the data was used to ultimately make educated guesses about the identities of my unknown ink samples. *Figure 1* and *Table 1* show the chromatogram and collected data from the 2:1 proponol:water base test, which was somewhat of an introductory exercise. However, after running some more solutions and creating numerous chromatograms, it proved to be the most effective method for identifying my inks. One can argue that, because of the balance of polarity between the alcohol and water in the mixture, the components were able to separate in a balanced manner (i.e. the solution was not too polar or non-polar, and thus pulled the colors out at a fair distance). *Figure 2* and *Table 2* show chromatograms that were placed in water; however, the 1:1 methanol:water mixture appeared to give a good set of component data and R_f values. Therefore, I originally decided to use this solution to run my first set of unknown samples (*Figure 7*). Again though, the dominance of water did not produce good readings, and much of that data was not helpful in identification.

As stated in the Introduction and Procedure, the mindset my group and I used to experiment with new solvents was to increase the polarity of the solutions, in hopes that the components' colors and R_f values would be more unique and distinguishable. While the six chromatograms we created (see Figures 1-6) produced helpful data, there was still a guess factor that needed to be considered when identifying our inks. However, these guesses were not blind; by comparing R_f values from corresponding tables and doing color analysis, a sound determination could be achieved (see *Table 9*). One of these comparisons was to known pens that I spotted on my second unknown chromatogram:

Table 10: Self-Predicted Ink Samples on *Figure* 8[7]

Position (past the unknowns) of Pens	Pen Color & Style
1	Black Pilot Easy-Touch
2	Black Pilot V-Ball
3	Red Pilot Easy-Touch
4	Red Pilot G-2 07
4	Red Pilot V-Ball
5	Blue BIC Ultra-Round
5	Blue InkJoy 300 RT

For positions 4 and 5 on *Figure* 8, I was unsure, and decided to spot two different styles of red and blue pens, respectively (I was rather sure about the identities of unknowns 1, 2, and 3). Using R_f and color comparison, I was able to make the distinction that appears in *Table 9.* To conclude, I will add that all five of my identified ink samples matched with what they were supposed to be.

IV. Discussion

Due to the nature of this laboratory exercise, the procedure that I adopted for identification of my unknowns did not change much from what was listed in *PSU Chemtrek*. In Section E of the procedure, there is a lot of flexibility for the student to choose his/her own method for unknown ink identification; additionally, creating a hypothesis is at the student's discretion and is reliant on prior knowledge.[1] Therefore, the procedure that I adopted for this lab is very close to what is stated in the Procedure section of this report.

There are one or two items that I changed, however. *PSU Chemtrek* suggested that students may want to use wider chromatography paper to create their chromatograms (with hopes that the longer solvent-front distance could drag out more components).[1] However, wider paper was not available for this lab, and therefore I relied on changing the solutions in which to run the chromatograms. Additionally, the instructions suggested we use the Snyder Index, which is a sheet listing properties (including polarities) of different solvents available for use in this experiment.[1] I initially decided not to use the index of values, but while forming my report I felt it necessary and appropriate to reference it. Doing so helped in finding some error and reasons why the 1:1 methanol:water chromatogram test did not work as well. While we knew water was a very polar solvent (index of 9), we thought methanol was much more non-polar than it actually is (index of 6.6).[8] While we wanted to make sure the solutions were more polar, as per the hypothesis, we believed it was important to make sure the components would not turn out as horizontally spread as the water test. Therefore, we mixed methanol and water, but in hindsight we most likely should have used something with a slightly lower and more non-polar index.

Speaking to the results, I feel that my chromatogram tests and methods proved to be successful, seeing that I was able to successfully identify all five of the ink samples. As explained above, while I probably chose the incorrect solvent in which to run the first chromatogram, I quickly realized my mistake of using a solvent composed of too much water and polar molecules. Because water is a very polar molecule, it is more likely to diffuse and spread horizontally across the polar paper, as seen in *Figure 2.* Since the ratio of methanol-to-water was only one-to-one, there was not enough polar substance to drag out most of the components, like inks 1, 3, and 5 in *Figure 7.* It is possible that the solution was too polar to show some components, which made it difficult to confidently identify a majority of the unknowns. Conversely, the 2:1 proponol:water solution had a much higher non-polar concentration as compared to water. This allowed the components of my second unknown ink test to successfully appear, and created R_f values that were similar to those of the original base test (*Table 1* and *Table 8*).

After running the second unknown chromatogram in the 2:1 proponol:water solution, it was relatively easy to choose what inks matched certain pens. I started by spotting certain pens on *Figure* 8 that I thought matched the unknown ink spots. Then, the first step was to compare the components' colors to the 2:1 base test, *Figure 1.* I compared the calculated R_f values from *Table 1* and *Table 8* to make sure the similarities made sense both visually and mathematically. From these two comparisons, I was able to confidently identify unknown ink positions 1, 2, and 3. However, I was still unsure about 4 and 5, which I anticipated when initially guessing what pens I should spot on my unknown chromatogram. Therefore, I decided to spot two similarly-looking pens for both positions. This was beneficial and incredibly helpful, as I was able to correctly compare and identify inks 4 and 5.

The results showed too that the different color pen inks acted differently based on what solution the chromatogram was placed in. For example, the first test of my unknowns – seen in *Figure 7* – was able to drag out the black component of the pen in position 2, and the pink component in the red pen which was in position 4. However, the yellow and blue components were not apparent in the chromatogram, which speaks to the polarity issue. Blue and yellow hues have more polar groups than a red dye, as evidenced with Section A of the experiment with the food dyes.[1] While this may not be true and synonymous for all dyes of these colors (such as these pens), my experimental data strongly validates this claim. It makes sense that the blue and yellow dyes would not migrate as far on a chromatogram which has a large amount of polar molecules, such as the 1:1 methanol:water solution. However, it is evident in the 2:1 proponol:water solution that the blue and yellow components were dragged out more than in the 1:1 chromatogram. This shows the trend (at least for these ink samples) that a non-polar-laden solution is needed for a successful comparison.

Noticeably, this does not go along with my hypothesis that was developed in the Introduction. Possible error in judgment or experimentation could be due to the solvents we used. While it may be true (and probably correct) that polar molecules would bring out more components, the 1:1 methanol:water solution contained too much of a percentage of water. It probably would have been more successful if there were more parts methanol, as the water would not diffuse horizontally as much. Another source for error could be human experimental error. While my group and I tried to work diligently and precisely to gain the outcomes we hoped for, there could have possibly been some error with mixing solutions, testing chromatograms, or measuring R_f values. For example, a retention factor is not something that is set in stone. It requires precisely identifying the center of the visible component and then

creating a ratio based on the distance the solvent front moved; it is quite possible that we measured the center incorrectly, which would make our ratio incorrect and vary the comparison. Speaking to mixing solutions properly, there is a fine line to walk when dealing with polarity. If we added a few extra drops of methanol to our methanol:water mixture, it likely would have messed up the polarity we expected, and thus the results and R_f values would be incomparable.

V. Conclusion

The goal of this lab was "to create an experiment and process to better-separate colors of ink and correctly identify them.[7]" I would suggest that my data, results, and findings proved that there are different (but not necessarily better) methods to test ink samples and identify unknowns. Corbin Edmondson, Ashley Faddis, and I worked together to develop a hypothesis, stating that in order to better-identify unknown pen ink samples, we would need to develop and mix a more-polar solution than the 2:1 proponol:water one used in Section A of the lab.[7] We proceeded and followed that hypothesis, but the resultant chromatograms did not exactly match what we anticipated. Instead, the original base solution – the 2:1 mixture – gave me the best mix of R_f value comparison and color identification. While this disproved my hypothesis, it did show that the laboratory exercise was successful, as I was able to correctly identify all five ink samples. If I were to conduct this lab again, I would be sure to use solutions of higher polarity using the Snyder Index as a reference, and ones that were not necessarily water. Regardless, I would be interested to see how a variety of solutions could be used to potentially identify unknown ink pen samples, especially if the method used was not paper chromatography but something like mass spectrometry or infrared analysis.

Works Cited

1. Thompson, Stephen. *PSU Chemtrek: Small-Scale Experiments for General Chemistry;* Keiser, Joseph T., Ed.; Hayden-McNeil: Plymouth, MI, 2014; pp. 17-1 – 17-21.

2. Florida Institute for Human & Machine Cognition. Paper Chromatography Science Background. http://www.ihmc.us/groups/voluntology/wiki/291f0/Paper_Chromatography_Science_Background.html (accessed Mar 13, 2015).

3. Chemguide. Paper Chromatography: Paper Chromatography Using a Non-Polar Solvent. http://www.chemguide.co.uk/analysis/chromatography/paper.html (accessed Mar 13, 2015).

4. Brewer, Jason D.; Egan, James M.; and Hagan, Kristen A. Forensic Analysis of Black Ballpoint Pen Inks Using Capillary Electrophoresis. *Forensic Science Communications:* FBI [Online] July 2005, Vol. 7, No. 3. http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2005/research/2005_07_research01.htm (accessed Mar 14, 2015).

5. Bordonaro, Julie and Carrier, Rebecca. Rensselaer Polytechnic Institute. Intro to Chromatography: Chromatography. http://www.rpi.edu/dept/chem-eng/Biotech-Environ/CHROMO/chromequip.html (accessed Mar 16, 2015).

6. Burdge, Julia and Overbay, Jason. *Chemistry: Atoms First;* McGraw-Hill: New York, NY, 2012; pp.7-8.

7. Egan, Nicholas. *Chemistry 111/113 Notebook: Penn State University;* Hayden-McNeil: Plymouth, MI, 2009; pp. 15-23.

8. Kok ChemWare. HPLC: Solvent Properties. http://www.sanderkok.com/techniques/hplc/eluotropic_series_extended.html (accessed Mar 25, 2015).

9. Edmondson, Corbin. *Chemistry 111/113 Notebook: Penn State University;* Hayden-McNeil: Plymouth, MI, 2009; p. 19.

10. Faddis, Ashley. *Chemistry 111/113 Notebook: Penn State University;* Hayden-McNeil: Plymouth, MI, 2009; pp. 23-24.