# IDENTIFICATION OF UNKNOWN PEN INKS BY PAPER CHROMATOGRAPHY

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Introduction

Chromatography has thousands of applications from forensics to identification of chemicals. It has been used to identify anything from strands of the polio virus[11] to types of steroids.[10] Chromatography has allowed for so many advancements in science and it would not have been possible without Mikhail Tsvett. Chromatography was first discovered by Mikhail Tsvett, a Russian Scientist, who was trying to separate the hidden dyes within leaves. He tried to separate them by mixing a crushed solution of leaves with a specific powder. The hidden colors separated in different areas depending on how easily they were absorbed by the powder.[3] After Tsvett's discovery of chromatography the field was left relatively unexplored until the early 1900s. Two other scientists, Martin and Synge, were fascinated by the possibilities of chromatography and refined Tsvett's procedure; developing paper chromatography. Using their refined version of chromatography Martin and Synge were able to separate the amino acids in a protein and were awarded the Nobel Peace Prize in 1952 for their work. Many more scientist have come after Tsvett, Martin, and Synge and have refined chromatography even farther and now there are several different variations of it including the use of liquid chromatography to identify DNA.[4] Paper chromatography is also used within forensic labs. It can be used to identify a mixture of a substance found at a crime scene by comparing it to known control samples of a similar substance.[2]

Paper chromatography was the method of chromatography used in this lab. There are two phases in paper chromatography; stationary and mobile. The stationary phase is the solvent that is used to draw the mobile phase up the paper, it is what allows the dyes to separate. If a solvent is too polar or too nonpolar (too different) than the mobile phase will not adhere to the paper correctly and will not separate quickly enough or will not move at all. The mobile phase is what will separate and travel along the paper following the stationary phase. The objective of chromatography is to completely separate the components of the substance that is being tested. In order to completely separate the components the

component spreading should be minimized and the migration differences should be maximized.[9] This information was the basis of this lab. The goal was to use paper chromatography to identify unknown pen inks based upon chromatograms of known inks. Even though paper chromatography is used in this lab there are other techniques available to identify inks such as electrophoresis.[12] Based upon the data gathered from sections A-D of this lab the hypothesis solution that is a ratio of a polar and nonpolar solvent would be best to separate the dyes into their separate components.

Procedure:

The procedure was developed based on knowledge gained from sections A-D of the experiment completed week before. The first steps of this experiment was to determine if the inks were polar or nonpolar and from previous knowledge it was determined that they are mostly polar. The next step was to create a solution that would separate the components of the dyes. Each of the members of the team were assigned a solution to test. The procedure for testing the solvents was to use assign each group member different ratios of solvents with a total volume of 15 mL each time. This allowed for simple ratio calculations. The 15 mL were measured in 10 mL graduated cylinders and then poured into a petri dish, both of these materials were thoroughly cleaned after each solvent. Then the chromatograms with the inks drawn on them were stapled and placed on the petri dish containing the solvent. Once the chromatogram was placed on the petri dish it was covered with a plastic cup creating a “tank.” The chromatograms were then observed until the solvent reached ~1 cm from the edge of the paper. Once the solvent reached that distance the chromatograms were removed and set aside to dry.

Based upon the results of the separation of the inks the ratios were discarded or slightly changed. Once the final ratio of 2:1 methanol to propanol was decided upon that chromatogram was set aside and the unknown inks were tested. In order to identify the unknowns a direct comparison was used. The unknown was placed directly beside the known chromatogram and depending on the

separation the unknowns were identified. Another test was used for further comparison of the unknown inks. Both the known and unknowns were placed under a UV light which caused a component of the black ink to glow. This was used to further differentiate between the brands of the color of inks.

The key for the chromatograms from left to right for the color is black, blue, red, and then the pen brands follow the key below.

Left Bic Pilot G-2 Pilot Easy Touch Pilot V-Ball Papermate Right

Results

The results from each of the chromatograms were compared to each other to determine the best solvent for the separation of the pen inks.

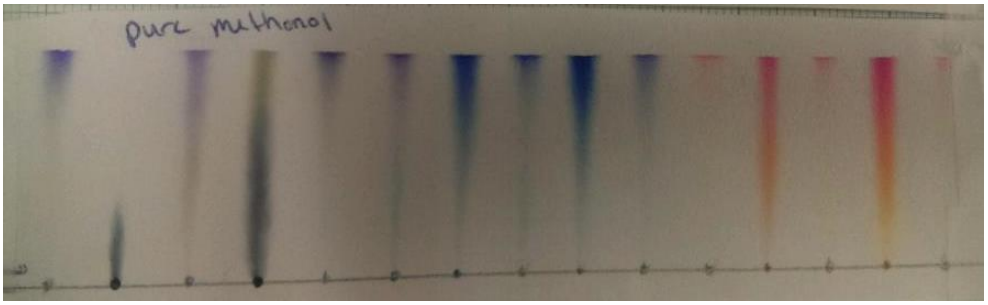


Figure 1: Pure Methanol[8]

Pure methanol was determined to be too polar as the components did not have a clear separation.

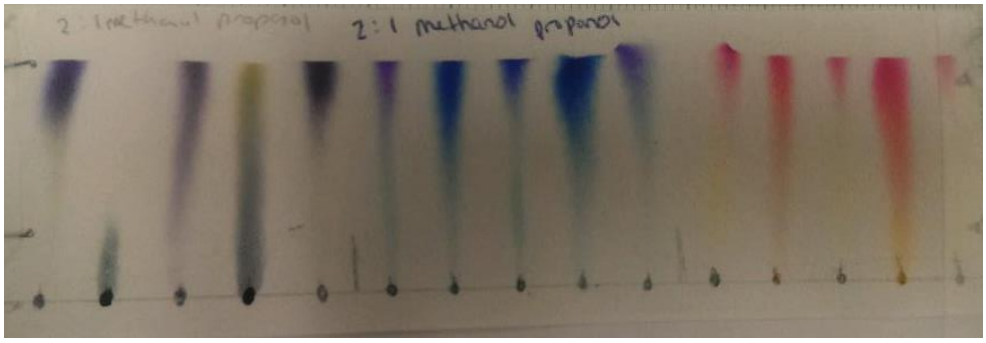

Figure 2: 2:1 Methanol Propanol[8]

2:1 Methanol propanol was determined to be the ideal solvent as the components had the clearest separation in the shortest travel distance.

Samantha's Chromatograms[7]

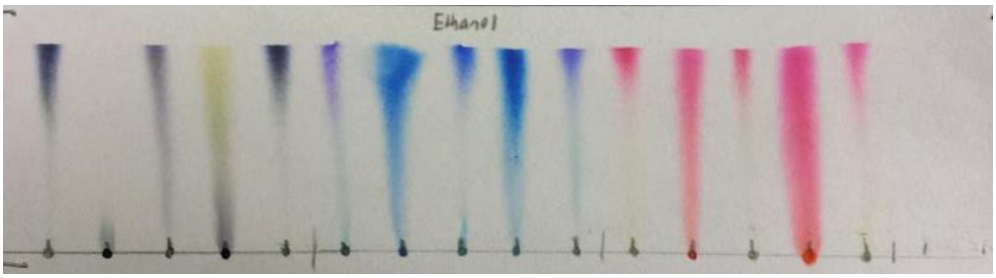


Figure 4: Ethanol

Pure ethanol was still too polar because the inks did not separate, instead they spread apart as they were too attracted to the water molecules.

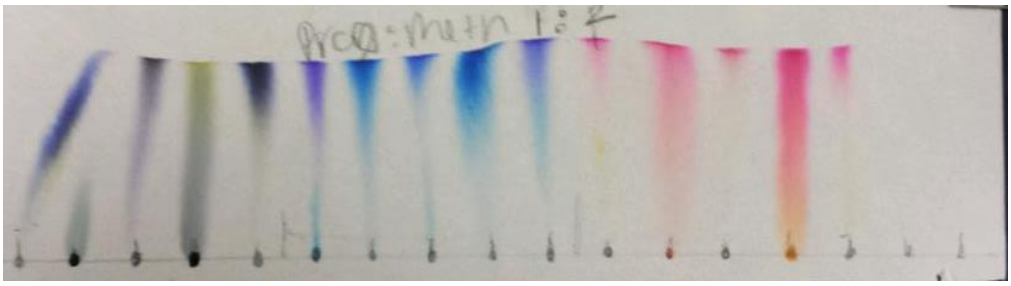

Figure 5: 1:1 Methanol Propanol

1:1 ethanol propanol was too non polar because the components were separating but were taking too long to separate.

Taylor's Chromatograms[5]

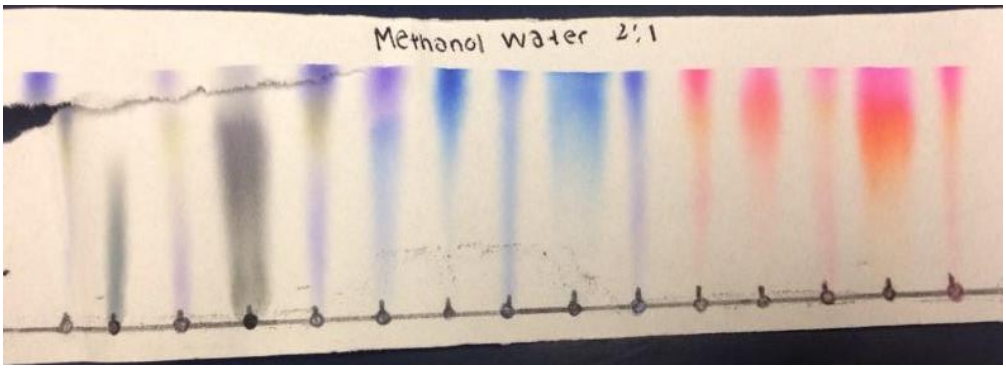


Figure 6: 2:1 Methanol Water

2:1 methanol water was far too polar, the ink spread too far across the page.

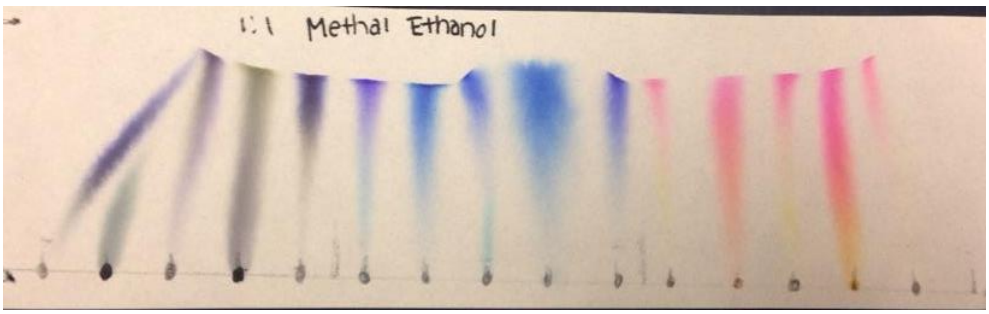


Figure 7: 1:1 Methanol Ethanol

1:1 Methanol ethanol was too polar, the components did not separate well.

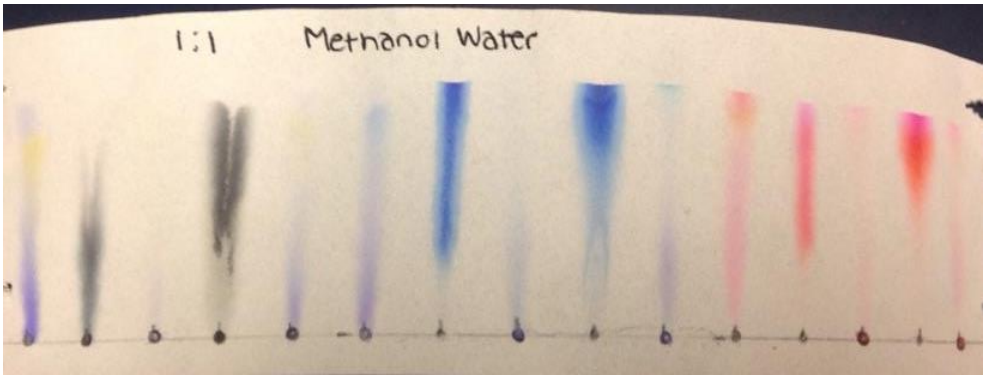


Figure 8: 1:1 Methanol Water

1:1 methanol water was too polar, the components visible blurred across the page.

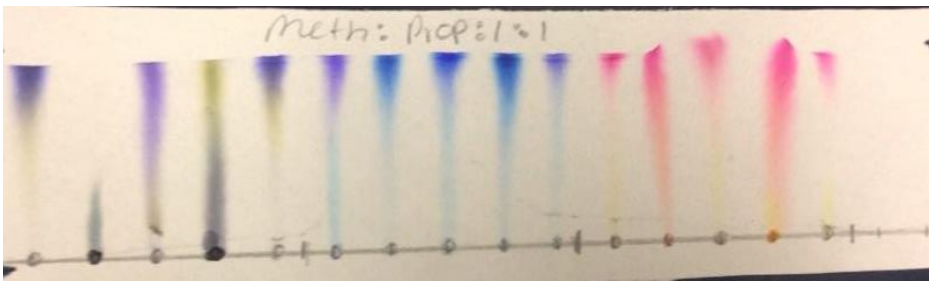


Figure 9: 1:1 Methanol Propanol

1:1 Methanol propanol was determined that it was still too polar as the components were still too attracted to the stationary phase and did not separate.

Alex's Chromatogram's[6]

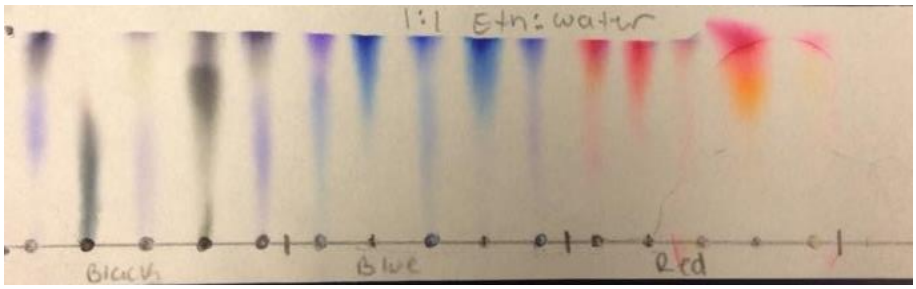


Figure 10: 1:1 Ethanol Water

1:1 Ethanol water was too polar, the components did not separate.

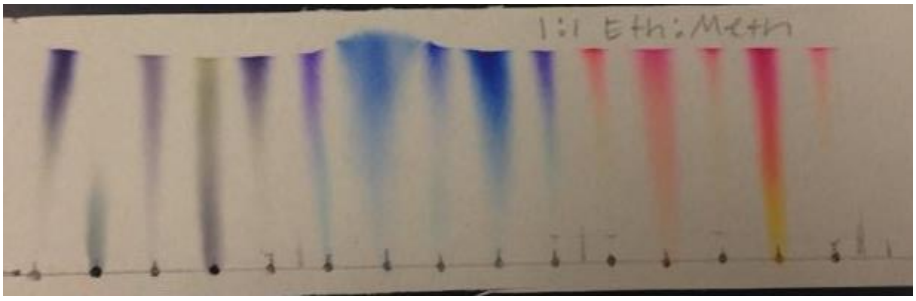


Figure 11: 1:1 Ethanol Methanol

1:1 ethanol methanol was still too polar, while the components did separate the migration distance was too close and it was inconclusive.

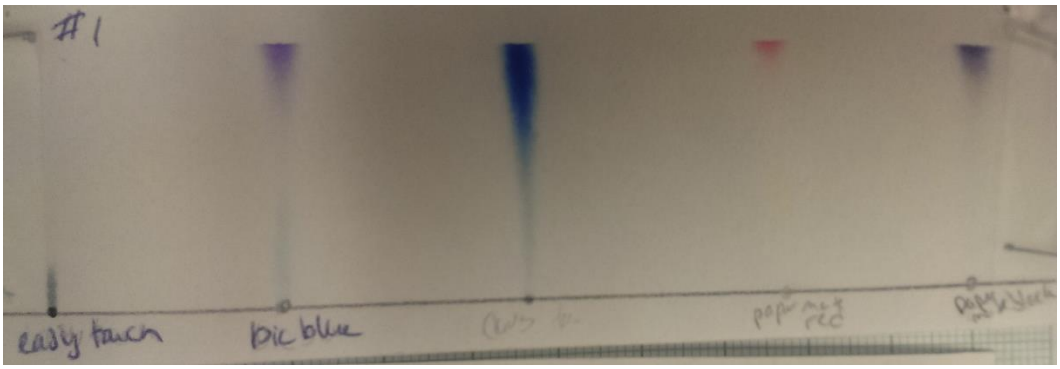


Figure 12: Unknown #1 chromatogram[8]

The ideal chromatogram was chosen by comparison of each team members chromatograms. The ones that did not show any component separation were discarded. The chromatograms that were left were compared to each other and the one that showed the best separation was chosen, 2:1 methanol propanol. This solvent was polar enough that the components were attracted to it but nonpolar enough that the component migration distance was distinguishable. 15 mL of 2:1 methanol propanol was then measured out and poured into a petri dish to test the unknowns. The unknown chromatogram was stapled, placed in the petri dish and covered.

Once the components separated the unknowns were compared to the knowns using both direct comparison and the UV light. The first unknown on the chromatogram easily matched the G-2 black ink pen because it was the only one that had a black component that did not move. The second unknown was a little harder but was ultimately identified by the same fluorescence as the Bic blue ink pen. The third unknown had the same component migration and colors as the V-ball blue. The fourth was a match for fluorescence and the same yellow and pink components as the Papermate red ink pen. The fifth and final unknown was difficult to determine by the naked eye but it had the same fluorescence has the Bic black ink pen under the UV light. Again the identities were determined and are as follows from right to left; G-2 black, Bic blue, V-ball blue, Papermate red, and Bic black.

## Discussion

In order to identify the unknown inks, the correct solvent had to be used. Choosing the correct solvent was tricky and the first step was to determine if pen ink is polar, so the inks were tested using water as the solvent. The results proved that the ink was polar because it was too attracted to the solvent and spread out all over the chromatogram and did not separate. Based upon this knowledge the solvent was changed to be more polar than the previously attempted solvent from the previous week (methane). Since the solvent had to be more polar than just methane but less polar than water, it was decided that a mixture of a nonpolar and polar solvent would be used. The given solvents that could be used (methanol, propanol, ethanol, and water) were ordered from most polar to least polar. From this information different ratios of solvents were decided on and were tested between group members. After all of the solvents were tested they were compared and the one with the best component separation and migration distance was chosen. The ideal solvent turned out to be a 2:1 ratio of methanol to propanol. This supported the hypothesis that the pen inks required a mixture of a polar and nonpolar solvent. The changes made did support the hypothesis as different combinations of polar and nonpolar solvents were tried.

The final procedure that was selected was to use 15 mL 2:1 ratio of methanol to propane and to use a comparison of this solvent with the unknowns vs. the solvent with the known inks. Both of the chromatograms were placed side by side and the best fit was chosen based upon their similarities to the fluorescence and the separation of dyes. The final procedure allowed for successful identification of the unknowns. The 2:1 ratio of methanol to propanol allowed for the identification of the unknown chromatogram #1.

## Conclusion:

Paper chromatography requires both a mobile and a stationary phase. The stationary phase's intermolecular forces have to attract those of the mobile phase but they cannot be too attractive. This was the problem that was set forth at the beginning of this lab. A ratio of solvents had to be found that allowed for the movement of the pen inks but not enough to allow the inks to run or not separate. Experimentation led to the conclusion that an ideal solvent for pen inks is a 2:1 ratio of methanol to propanol. This was determined by the separation of the components and the approximate distance between the separation of the components. The use of this solvent allowed for the correct identification of all the unknowns by comparison to the knowns. The unknowns were as follows from right to left; G-2 black, Bic blue, V-ball blue, Papermate red, and Bic black. This solvent supported the hypothesis that a mixture of a polar and nonpolar solvent would produce the best results. Further research could be conducted based upon the concepts learned in this lab. An unknown ratio of chemicals could be analyzed with a given solvent and compared to controlled known samples and the ratio could be identified. Other concepts that could be further explored could be changed the height of the paper or examining the use of the UV light for identification even further; the possibilities are endless.

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