Blood Fractionation Chromatography

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Abstract

Fractionation is important to understand someone's health. How we can simulate blood fractionation through a simple, inexpensive ink spread experiment more about blood fractionation and liquid chromatography. Cotton will spread on ink the most because it has the most pores. Pilot ink spreads fast because it is water-based (low viscosity). Higher temperatures should result in more spread because of lower viscosity. We measured the ink spread by taking the average between high and low ink mark points. We check our measurement repeatability and determined our sample size. The results repeatedly showed that nylon was the material with the least ink spread, with water not spreading at all, pilot ink barely spreading, and India ink spreading very little. For polyester, water spread much better than nylon. It was clearly shown that higher temperatures meant more spread. We heated the cotton cloth only since using heat transfer from the water to the ink is too long and the water vapor would block the pores from absorbing the ink. We learned more about using statistics graphs and tables. We also learned how to control the experiment and determine the sample size through teamwork building. We would thank our teachers and parents for advising.

Keywords
Chromatography, Viscosity, Pilot Ink, Statistics

1. Introduction of Chromatography Science and Ink Spread Experiment

In medical Science, separation of blood helps understand the health condition. Red blood cells can carry oxygen to organs and remove carbon dioxide. White blood cells are essential to the immune system. Platelets are needed to clot your blood when you are bleeding. Though, the process of blood fractionation is costly and complex. The objective of this STEM project is to design a simplified, inexpensive ink spread experiment to study Chromatography Science and Applications, and Simulate Blood Fractionation. Chromatography is method for separating the parts of a mixture of either a gas or liquid solution containing different chemicals [1,2,3,4]. The mechanism of ink or blood spread is by a process called calligraphic motion. There is research on how to purify the blood by modern techniques [5,6,7]. In this STEM project, authors use liquid chromatography over gas chromatography due to budget constraint. During ink chromatography, mobile phase is water or ink, stationary phase is the different types of cloth. The solvent is the water. The solute is the ink pigments (blood cells). When ink/blood spreads, it is going through the pores in the medium (cloth). The affinity between the solute and the pores determines chromatography. The spreading difference among water mark, ink mark, and base mark can simulate the blood fractionation.

2. Define Project Scope

In order to define project scope, team has conducted (1) 5 Whys Analysis, and (2) SIPOC Analysis.

2.1 5 Whys Analysis

- **1st Why did the Pilot ink spread faster than Water?**
- **Answer=** Due to lower viscosity of the Pilot Ink.

- **2nd How to adjust the viscosity of the Pilot Ink?**
- **Answer=** Viscosity is lower at higher temperature.
- **3rd Why we care about lower viscosity?**
- **Answer=** Lower viscosity can help Chromatography separation (Blood Fractionation).
- **4th How does the viscosity impact chromatography?**
- **Answer=** The pores in the cloth will affect the ink spread.

Based on 5 Whys Analysis, the ink viscosity and media pore density are the most critical variables for simulating Blood Fractionation.

2.2 SIPOC Analysis

SIPOC Analysis is another powerful tool to help define the project scope. SIPOC starts with C-Customer Driven. In this STEM project, science teachers and Science Faire judges are the key customers to evaluate the project success. The project output deliverables are Project Management Trilogy: (1) cost: control budget within $200, (2) schedule: submit STEM paper by May 15, and (3) quality: faster ink spread (better blood fractionation). The process is Ink Spread Chromatography Science [8,9]. The input variables are media cloth (Polyester, Nylon, and Cotton) [10-18] , Ink (India Ink, Pilot Ink) [19] , Equipment Set-up. The supplier is Stores (Target, Walmart, and Online). Conducting SIPOC analysis has helped team lay out a thorough plan for next phases.

Table 1. SIPOC Analysis

<table>
<tr><th colspan="5">SIPOC Analysis</th></tr>
<tr><th colspan="2">X's</th><th>F's</th><th colspan="2">Y's</th></tr>
<tr><th>Supplier</th><th>Input</th><th>Process</th><th>Output</th><th>Customer</th></tr>
<tr><td rowspan="5">Stores</td><td>Cloth (Polyester, Nylon, Cotton)</td><td rowspan="5">Ink Spread Chromatography Process</td><td>Budget < $200</td><td rowspan="3">Science Teacher, Parents</td></tr>
<tr><td>Equipment and Method</td><td rowspan="2">Complete Project by mid May</td></tr>
<tr><td>India Ink , Pilot Ink</td></tr>
<tr><td>Ink Temperature</td><td rowspan="2">Faster Ink Spread</td><td rowspan="2">IEOM Judges</td></tr>
<tr><td>Microwave Temp and Duration</td></tr>
</table>

3. Experimental Setup and Materials Selection

In this section, team would design the experimental setup to measure the ink spread performance [4] and select the materials for studying the ink chromatography science.

3.1 Ink Spread Experimental Methods

There are some challenges as following when designing the experimental setup:

- Control Initial Cloth-Ink Contact Area by designing a special fixture (shown in Figure 1)

- Control Heating Process (Container Size, Water Volume, Heating Power…)
- Control Solvent Volume (30 mls)
- Control Ink Spreading Duration (5mins)
- Control Ink Drying Time (10mins)
- Control Heat Transfer Experiment is critical to verify temperature effect.

Figure 1. Design Ink Spread Fixture

Team has spent significant time to brainstorm what could be the measurement variations in order to control and minimize any experimental variations (Noise). Control each vital experimental factor is critical to ensure reliable Ink Spread. Team has created a Fishbone Cause-Effect diagram as shown in Figure 2.

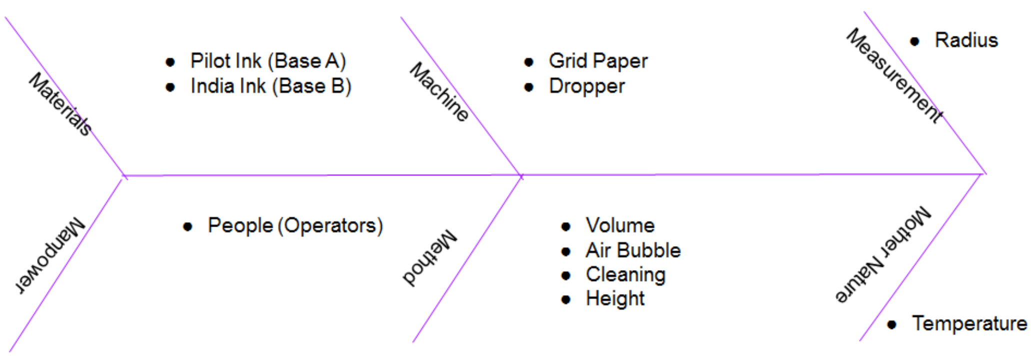

Figure 2. Cause and Effect Diagram of Experimental Control

3.2 Select Materials

Cloth pore affinity is critical. Three types of Cloth were chosen: (1) Cotton: easily absorbs 27% of the water, (2) Polyester: not as absorbent as cotton. Polyester dries fast and is water- repellent, and (3) Nylon: similar properties as polyester (synthetic fabrics). Nylon is more water-resistant compared to Polyester [10-18].

Ink viscosity is critical. Two types of Ink were chosen: (1) Pilot Ink has lower viscosity, and (2) India Ink has high viscosity. Materials are shown in Figure 3.

Figure 3. Materials: Cloth and Ink

3.3. Measure Spreading Rate

We will put in a paper in the ink tank, and take it out instantly to know the length of paper that is in the water/ ink. Therefore, we will minus the length of paper in the water from the length of spread. Baseline Level: 1.1 cm (B in the formula below). In Section 3.1, team has tried very hard to control this baseline level at 1.1cm+/-0.1cm in order to control the initial ink volume and paper-ink contact area which is critical to impact the Ink spreading property.

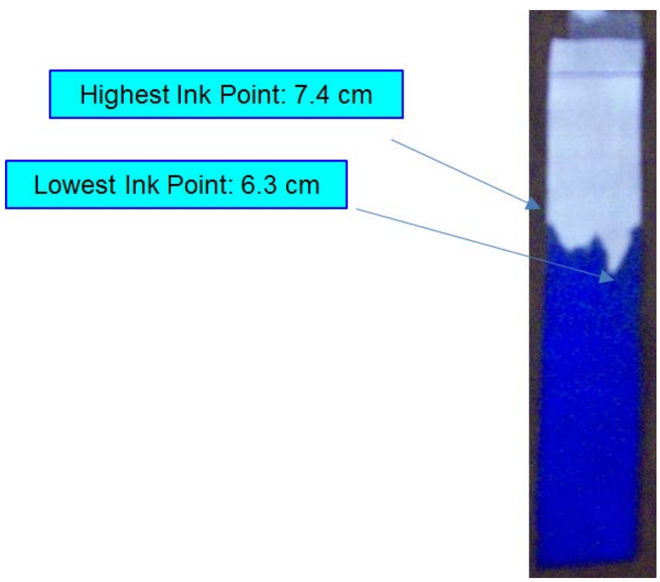

Figure 4. Ink Spreading Marks

To quantify the Rate of Ink/water Spread: authors have recorded the Highest Point (Hpi) and the Lowest Point (Lpi) as shown in Figure 4 to determine the spread distance.

Spread Distance= [(Hpi - B) + (Lpi- B)] ÷2

3.4. Measure Repeatability and Sample Size

Authors would verify the measurement repeatability by using water solvent on cotton media. Water-Cotton combination can travel more than other combinations to enlarge the separation signal. Water is cheaper and we need to save ink materials within the budget within $200. Repeat the same spread measurements twice and the repeatability (noise range) should be smaller than 10% of the separation signal.

Repeatability Criteria: [(Test #1+ Test #2)/2] (0.1) ≥ range of test #1 & test #2
Test #1= 6.7, Test #2= 6.9, Average= 6.8, Range= 0.2 < 10% of Average= 0.68
Conclusion: Pass Measurement Repeatability Test.

Based on the measurement repeatability data, authors have furthered calculated the Power Test and determine the sample size by Minitab Statistics Software for conducting the Hypotheses Tests. Authors set Alpha Risk at 5% and Beta Risk= 10% (Power= 0.9) which is typical risk levels for Biomedical Industry. The Minitab result summary is shown in Table 2 below. The calculated sample size=4 is within the project budget.

Table 2. Power Test and Sample Size.

Difference	Sample Size	Target Power	Actual Power
0.2	4	0.9	0.915181

4 Analyze and Verify Hypotheses

There are three major hypotheses: (1) which cloth type can facilitate spreading, (2) which ink type can move faster, and (3) is ink/cloth temperature critical on spreading property?

4.1 Cloth Material Test

As shown in Figure 5, the ink spreading (Y Axis) is highly dependent on the cloth type (X Axis). Different cloth materials have different pore density which would significantly impact the affinity with water. As expected, cotton can absorb water and facilitate Ink Spreading better.

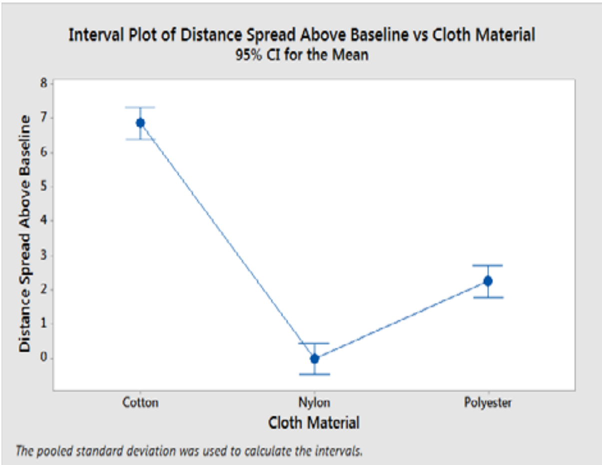

Figure 5. The confidence interval plot of spread distance among cloth types.

4.2 Ink Compare and Contrast

Also as shown in Figure 6, Ink is spreading much slower than water (less blood fractionation). Pilot Ink spreads faster than India Ink. This is because Pilot Ink is has lower viscosity, and India Ink has high viscosity [21,22] . A faster spreading rate of Pilot Ink can simulate better Blood Fractionation.

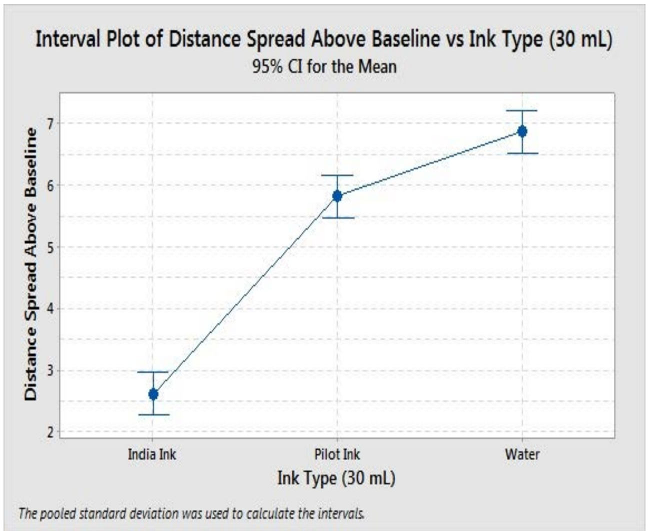

Figure 6. Spread comparison among water and two different inks.

4.3 Temperature Effect

As the temperature rises, the kinetic viscosity should decrease as well as shown in Table 3 [20]. The higher the temperature, the farther the ink should spread.

Table 3. Viscosity Property vs. Temperature

Temp. [°C]	Dyn. Viscosity [mPa.s]	Kin. Viscosity [mm²/s]	Density [g/cm³]
20	5.751	5.35	1.0743
25	4.875	4.55	1.0719
30	4.177	3.91	1.0695
35	3.615	3.39	1.0669
40	3.156	2.97	1.0642
45	2.776	2.62	1.0614
50	2.460	2.324	1.0585

Instead of heating ink (much harder to control the temp), authors have used Microwave to heat cloth. When the cotton cloth was heated, ink spread faster than cotton cloth not heated as shown in Figure 7.

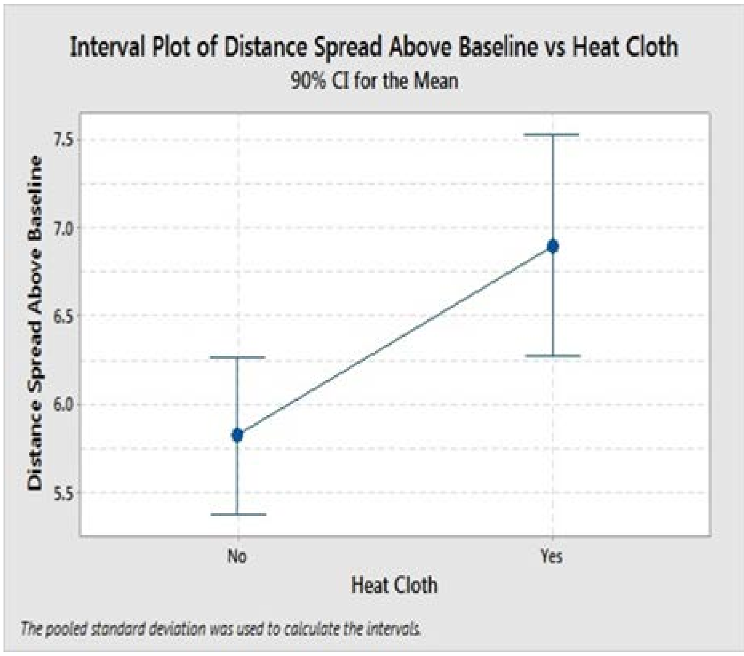

Figure 7. Ink spread vs. Heat Cloth.

5 Conclusions

This project has designed ink spreading experimental and demonstrated the ink spread property to simulate the blood fractionation. It's very critical to control the measurement repeatability and quantify the spread distance. The cotton cloth has a better spread behavior over Polyester and Nylon due to its better media pore affinity. Pilot Ink is also over India Ink due to its lower viscosity. Heating the cloth over Ink can better control on temp factor and improve the ink spreading. Confidence interval statistics and Power Test sample size are applied in order to meet the project budget limit.

Acknowledgements
Special thanks to Chia Lin and Chao-Yuan Chen for supporting our research. Thanks to my classmate Amrit and Science Teacher Mr. Richards for Science assistance.

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Biography

Mason Chen is student in Stanford On-Line High School Program. Mason has certified Lean Six Sigma Black Belt through IASSC (International Associate of Six Sigma Certification), and also certified IBM SPSS/Modeler Statistics and Data Mining Certificates. Mr. Chen has been invited to several conferences like IEOM, ASQ, AQI, ASA, JMP/SAS and local ASQ SV statistics group to present his STEM Projects. His STEM projects have drawn interest in Robotics/EV3, JAVA Science, Poker Probability, Powerball Lottery, Sports Analytics, Biostatistics and Healthcare Statistics… Mason is familiar with Lean Six Sigma DMAIC, DFSS, and Minitab 17, JMP 13, SPSS 24, and Modeler 18 Statistics Software. Mason has also been learning Data Mining and Big Data Analytics through several STEM Projects. As a Stanford High School Student, he has published several Conference Papers in IEOM, IWSM, FSDM conferences.