Developing Reusable Microfluidic Hormone Test Chip

A Major Qualifying Project
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by

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Professor Andrew Teixeira, Advisor

Abstract

The goal of this project is to create an opportunity for chemical engineering students at Worcester Polytechnic Institute (WPI) to gain experience using microfluidic reactor systems. A procedure by Professor Andrew Teixiera was tested for feasibility as an experiment in the Unit Operations curriculum. Microfluidics applications were explored in lab-on-a-chip devices. To make these tests more sustainable, chemical solvents and chromatography materials were tested for reversibility and building of chip prototypes to make hormone testing reusable and therefore more accessible.

Acknowledgements

There were several professors at WPI without whom this project would not be possible. Firstly, I would like to thank Professor Susan Roberts for being so receptive to my ideas about working alone and creating a project of my own. I would also like to thank Professor Stephen Kmiotek for helping me to find an advisor to take me on in short notice when my previous plans fell through.

I would like to thank Professor William Clark for introducing me to microfluidics and taking me on to advise initially. His input was valuable at the beginning of my project as I was testing the microfluidics experiment for integration into the Unit Operations curriculum and as I explored the applications of microfluidics in hopes of creating a new objective of my own.

Above all, I want to thank Professor Andrew Teixeira for advising my project. His guidance, understanding, and willingness to listen helped me to grow as an engineer and find love for my major. I want to thank him for being so receptive to my ideas and giving me the opportunity to take this project in a direction that interested me. He never once made me feel like I wasn't doing or understanding enough, but was patient in explaining to me what I needed to learn or do. This meant the world to me and allowed me to feel strong, justified, and inquisitive regarding my ideas and questions.

Finally, I want to thank Tiffany Royal from the Chemical Engineering Department for ordering the materials I needed and making me my key to the lab, my best friend Chloe Sairs for explaining to me how antibodies and hormones work, and my boyfriend Zach Sharp for moving in with me so I could afford to stay in Worcester while I finished my degree and bringing home a myriad of plastic pieces from the craft store we worked at to choose from in making my test chip prototypes.

Executive Summary

The chemical engineering curriculum at WPI allows the opportunity for students to work hands-on with large scale chemical processing equipment, gathering data and analyzing results for fluid flow and heat transfer phenomena. Microfluidics is a fairly new development in the chemical industry, utilizing reactors 10-100 μL wide. The small size allows an entire lab bench to be condensed onto a single chip, or to use limited sample sizes. To familiarize students with this up and coming field, Professor Teixeira wrote an experiment for the Unit Operations course in which students construct a circuit with interchangeable microreactors, inject a tracer, and analyze data for residence time and dispersion. I tested the protocol using a Stamixco static mixer, a straight union, and a straight tube bypass.

Microfluidics opens a myriad of possibilities for applications, such as the at-home pregnancy test. These tests are microreactors which function using capillary action to move a sample through several points on a test strip to detect the pregnancy hormone HCG. The sample is first mixed with antibody-dye units The HCG will bind to these if it is present. This mixture continues along the test strip to two sets of immoble antibodies. One line of immobilized antibodies binds to HCG, forming the positive line. The other binds to free antibodies showing a control line which appears whether the test is positive or negative. In order to make these tests more accessible and sustainable, I thought it would be beneficial to make them reusable or reversible.

One study by Luo et al. found that exposure to salt solution could cause reversible self association in HCG antibodies, where the antibodies are unbound from the result sites, possibly freeing them up for reuse. To test this theory, I investigated three salt types at various concentrations for their ability to induce reversibility.

Professor Teixeira and I adapted the project to focus on reusability and allow for at home progress. I constructed a reusable prototype casing for interchangeable test strips and tested three different test strip materials with tracer dye. I photographed the process over time and evaluated the data by grayscale in ImageJ to obtain reactor characterization such as concentration over time, the velocity of the flow and the change in broadness of the peaks over time.

In conclusion, it was determined that the microfluidics is a worthwhile addition to the chemical engineering curriculum, providing students the opportunity to construct a process of their own as well as exposure to micromixing, residence time, and dispersion. The tests regarding salt inducing reversible self association showed some promise, showing better reversibility with greater salt concentrations. In the future, they should be tested at varying temperatures as well as with different solvents, such as

toluene, acetone, or alcohol. The prototypes were successful in demonstrating the velocity and mixing abilities of several different test strip materials.

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Introduction and Background

1.1. Importance of Microfluidics

In the chemical engineering industry, it is common for processes to include unit operations such as batch or continuous mixing tanks, gas and liquid extraction columns, vessels and reactor tanks. To optimize the performance and safety of this machinery, it is important to know the residence time distribution of the equipment. Residence time distribution, or RTD, is an indicator of the macro-mixing behavior of two reactants or phases in the reactor. It can measure bulk flow patterns and characteristics which gives engineers the opportunity to adapt the reactor's design to meet process requirements [1].

An up and coming development in the chemical industry is the use of micro-scale reaction systems, referred to as microfluidics, micromixers, or microreactors. Typically, these microreactors have inner diameters less than one millimeter, making it possible to fit an entire lab bench onto a single chip, allowing for construction of handheld analytics tools useful for limited samples or reactant conservation. The size of the reaction systems also allows for fluid phenomena not normally observed in conventionally sized equipment. With this field rising in popularity, Professor Teixeira thought it would be appropriate to introduce chemical engineering students to the topic with an additional experiment in the Unit Operations coursework which would present students with the opportunity to construct and run their own microfluidic system and test different mixers for residence time, dispersion, and other measurements pertaining to reactor efficiency.

In the WPI Chemical Engineering degree, one requirement includes two laboratory courses in Unit Operations in which students are able to experiment on larger scaled chemical processing equipment. The experiments allow students to work hands on with equipment such as plate and pipe heat exchangers, membrane reactors, packed bed towers, and fluid circuits. The students then work in teams to evaluate the data collected, analyze the results, and write a report or memo to be presented to their professors. Students are able to practice laboratory safety protocol, and foster group collaboration skills.

Microfluidics is an up and coming field in the chemical engineering industry, with development of micromixing applications in synthesis, crystallization, polymerization, and extraction, as well as biological analysis and detection technologies [2]. The concept of microfluidics enabling a lab bench to be scaled down into something that could fit in a pocket creates the potential to make these processes more accessible for personal use. The minute reactant requirements make these systems affordable, and

many home test chips do exist, such as hormone tests. Hormone testing is a necessary indicator of the health of people worldwide. For women who are trying to reproduce or, conversely, do not have access to birth control, pregnancy testing is crucial to allow them to make decisions about their body or life as soon as this change takes place. In some places these tests can be inaccessible to the common person. In the developing world, most women do not have the basic right to seek medical attention. Around 1,000 women globally die daily from pregnancy or childbirth related complications according to the World Health Organization; 99% of these deaths occur in Africa and Asia. For every woman who dies in childbirth, at least 10 more suffer serious injuries. These deaths are considered preventable as the solutions for treating these problems are common in developed parts of the world-- just inaccessible to women in remote or oppressive regions. Adequate care before, during, and after childbirth can mean the difference between life or death for a woman [3]. Therefore it is important to provide even the most basic necessities of reproductive health to those who need it in order to prepare pregnant women and prevent these tragedies from occurring.

Another issue regarding hormone testing is any user would be using it repeatedly, not just once, making this a recurring expense. These tests are single use, and commonly made of non-biodegradable materials such as plastic. To make these tests more accessible and sustainable, this paper will explore the options for creating a reusable microfluidic hormone testing system, which could be used repeatedly and regularly to check the health of its user.

1.2. Microfluidics

Microfluidics involves the flow of liquids through channels in the range of 10-100 μL. Microfluidic processes offer new levels of precision and micromixing, as well as offering availability and applications unachievable by conventionally sized reaction operations. The small channels allow for unique fluid flow phenomena which would not occur on a regular scale. They provide the option to reproduce the same reactions simultaneously in very repeatable manners, and only small volumes are needed, providing an excellent method for analysis of limited samples. This makes microfluidics chips especially useful for analysis of DNA, RNA, protein and cell samples. Since there is a small reaction space and a high surface to volume ratio, the greatest benefit of microfluidic systems is their fast mixing and accurate thermal control [4]. Microfluidic systems can take on many forms, from a miniature reaction circuit using tiny tubes in place of pipes or microfluidic chips which use photolithography to carve flat channels and reservoirs into a plastic or glass surface, allowing an entire lab bench to be consolidated onto a single small chip. Detection methods such as fluorescent markers or electrodes can monitor the outcomes of microfluidic reactions [5]. A microreactor or

micromixer can take the form of T-, Y-, or cross- mixers, spiral static mixers, or packed beds.

Figure 1. Stamixco helical polypropylene static mixer within ⅛" PFA tubing, used to enhance axial dispersion and micromixing.

While the Unit Operations experiments examine large scale phenomena in heat transfer and fluid flow, microfluidics is a technique that is overlooked in the curriculum. There are also very few experiments in which a reaction is actually executed, few applications in which the students design and build a reaction system, and reactor phenomena such as mixing, dispersion, and residence time are not covered. Since the dynamics of a microfluidics system are so unique, and there are few other opportunities for students to work with these systems, it is important to give students exposure to this field. Because of this up and coming field, the Chemical Engineering Department wants to add an experiment to the Unit Operations course to familiarize students with microfluidic systems as they become more commonplace in the chemical engineering world. The students will also have the opportunity to set up their own reaction process using the pumps, tubing, reactors or mixers, valves, and analysis tools. We plan to create an experiment in which students set up an apparatus from the microfluidics materials, collect data on residence time using a spectrometer, and analyze the data for mixing performance from a reaction in which two reactions between ions occur at two different speeds. By differentiating between how much of the fast product formed rather than the slow, we can tell how well mixed the reactants are before leaving the reactor using a Villermaux-Dushman analysis [6].

One of the more commonplace applications of microfluidics is the at home-pregnancy test. Most home pregnancy tests use a sample of the subject's urine, which is introduced to a test strip by dropping or soaking. The sample is mixed with an antibody bound to a dye indicator. If the sample contains HCG, it will bind to these indicators. Via capillary action, the sample mixed with the dye markers is wicked along the test strip. It first reaches a strip of antibodies which are fixed in position and will bind to antibodies that are not already bound to HCG, giving the negative or control result. The mixture then crosses a strip of different antibodies that will only bind to an antibody bound to an HCG hormone. This gives the pregnant result.

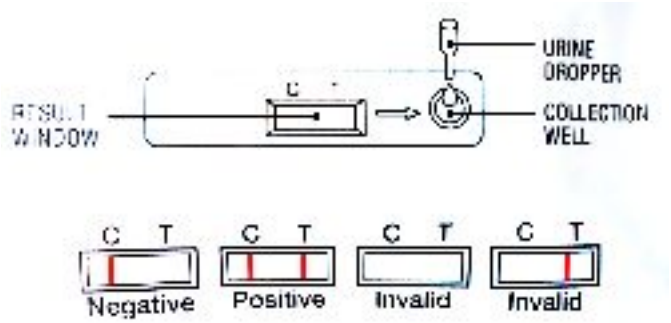


Figure 2. Diagram of a VeriQuick pregnancy test showing where the sample is dropped, and where the result is shown.

Reversible Self Association

In a study by Medimmune, it was found that, when treated with salt, these human gonadotropic antibodies will demonstrate levels of reversible self association (RSA) in which the binding of the hormone to the markers on a HCG test can be reversed. They found high concentrations of NaCl to be the most effective, with $CaCl_2$ inducing less RSA. They also found that higher pH environments can increase RSA. It was found that RSA is more likely at high concentrations due to enhanced attractive intermolecular interactions. They tested seven salts and found that all induced RSA to some degree, with different salts resulting in different levels of RSA [7].

Residence Time Distribution

In a reaction system of any size, the residence time distribution of a mixer gives characterization of the axial dispersion in the system. Axial dispersion is the mixing in a direction perpendicular to the flow. A perfect system would have no axial dispersion, only mixing in the direction of flow. A good indicator of this can be the residence time of a fluid in the mixer, which can be demonstrated by injecting a sample of dye and observing the concentration curve as the tracer leaves the reactor. When plotted as concentration versus time, a perfect plug flow reactor would have a sharp drop in concentration after the initial injection, while a more poorly mixed reactor would have a curve which trails off in concentration as particles remain trapped for longer inside the reactor [8]. More axial mixing and dispersion creates a broader peak in concentration after the sample is injected.

The residence time distribution also gives mean residence time, the length of time each molecule remains in the mixer. This is critical knowledge for optimization of the reactor, adjusting size to account for maximum mixing and favorability of the preferred products.

Beer's Law

In order to collect data for the calibration curve, a sample should be chosen and then tested over a UV spectra to determine the optimal wavelength at which to take absorbance readings. The sample, in my case a red dye called Intracid Rhodamine WT or Rhodium B, is then tested using the spectrometer to obtain a plot of absorbance vs wavelength. The wavelength at which the sample has the highest absorbance can be taken as the peak wavelength. With these parameters concentration can be calculated using the Beer's Law equation below

$$A = \varepsilon c l$$

Equation 1. Beer's Law. The measured absorbance A equals the molar absorption coefficient ε times the molar concentration c times the length of the path traveled by the light l.

Calibration Curve

The next step in obtaining the Beer's Law correlation for the chosen sample is to dilute the original stock sample to several known, lower concentrations. Fixing the spectrometer to the optimal wavelength, the absorbance of the sample is read for each of the dilutions. The results of this experiment can then be plotted as concentration versus absorbance. The results should be linear, and the equation of the line gives the Beer's Law correlation for the substance tested. From this, any measurement of absorbance can now be converted easily to a value of concentration through the linear relationship demonstrated in the equation below [9].

$$A=mc+b$$

Equation 2. Linear Equation For Calibration Curve. A is absorbance, related to concentration c through the slope m and y intercept, which should be close to zero.

Processing of RTD Data

After obtaining a calibration for Beer's Law, the system must be set up to perform the residence time distribution experiment. The Elvenflow six way valve should be set up with a 100 µL loop to allow for injection of the tracer dye into the system. When the system is ready, water begins to pump, passes through the valve, then the reactor or mixer, and then the absorbance is read at the spectrometer at the end. At t=0, the valve is toggled to the injection position, and a known volume of the red tracer dye passes through the system. Once the dye reaches the mixer, it is mixed and dispersed with the

water flowing previously before and after it. In ideal flow the front of the dye to the water should remain distinct. With axial mixing, the concentration of dye bleeds into the water before and after the majority passes into the mixer. The greater the deviation from plug flow, the broader and more gradual the concentration gradient as the concentration of the liquid leaving the mixer gradually returns to zero.

The data collected from this experiment will be in the format of transmittance versus time. Transmittance is converted to absorbance using the equation below.

$$Absorbance = log\left(\frac{1}{Transmittance}\right)$$

Equation 3. Conversion between absorbance and transmittance

Then, the absorbance can be converted directly to concentration using the Beer's Law correlation. This gives a plot of concentration versus time. The breadth of the peak is indicative of the residence time, and the sharpness of the decreasing side of the peak shows how the sample was affected by axial dispersion, keeping the reactant inside the reactor for longer than if it were perfect plug flow.

Time Distribution Function

In order to normalize the data obtained from this experiment, the concentration must be divided by the area under the curve [10].

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt}$$

Equation 4. Residence Time Distribution Function

The mean residence time can then be calculated using the equation below and a processing software.

$$\bar{t} = \frac{\int_0^\infty t*C(t)dt}{\int_0^\infty C(t)dt} = \frac{V}{Q}$$

Equation 5. Mean residence time equation where V is the volume of the mixer and Q is the flow rate [11].

In the situation where a mixer is being compared to a system without a mixer, called a bypass, the following equation should be used for deconvoluting the measurements. By doing this, it can be ensured that only the residence time of the mixer itself is being analyzed, rather than the residence time of the entire system [12].

$$E(t) = E_{bypass}(t) * E_{mixer}$$

Equation 6. Convolution of bypass from mixer.

Dispersion Model

The standard plug flow reactor model ideally has no axial mixing. The term $\frac{D}{\mu L}$ was added for axial dispersion to quantify reactor mechanics in imperfect mixing and flow conditions. The result was the dispersion model in equation 7, to describe when dispersion reaches conditions of advection, transfer of the fluid due to bulk motion, where D is about equal to μL thus $\frac{D}{\mu L}$ equals about 1.

$$E(\theta) = \frac{1}{\sqrt{4\Pi(\frac{D}{uL})}} e^{\left[-\frac{(1-\theta)^2}{4\theta(\frac{D}{uL})}\right]}$$

Equation 7

$$E(\theta) = \bar{t}E(t)$$

Equation 8

$$\theta = \frac{t}{\bar{t}}$$

Equation 9

For these equations, E(θ) is the residence time distribution function, θ is the dimensionless time variable equal to time over mean residence time, D is the dispersion coefficient of the mixer, u is the mean linear velocity of the liquid, and L is the mixers axial characteristic length [13].

Villermaux-Dushman Analysis

In order to better characterize the microscopic or fast initial mixing that occurs in microfluidic systems, a Villermaux-Dushman reaction can be run. In the Villermaux-Dushman reaction, a series of reactions from a set of reactants take place at slower and faster rates. In a mixer which has good mixing and little dispersion, the fastest reaction can be neutralized by the slower reaction, causing only the product of the slower reaction to be produced. However if the mixing is poor, unreacted reactants from the slower reaction do have time to react and form a secondary product which can be detected and differentiated from the first.

The mixing performance can be quantified using the segregation index. In an example, the reactions are as follows:

$H_2BO_3^- + H^+ \leftrightarrow H_3BO_3$ quasi-instantaneous

$$5I^- + IO_3^- + 6H^+ \leftrightarrow 3I_2 + 3H_2O$$ very fast

$$I_2 + I^- \leftrightarrow I_3^-$$ quasi-instantaneous, secondary after formation of I_2

The segregation index X_s is given by

$$X_s = \frac{Y}{Y_{ST}}$$

Equation 10. segregation index

Y is the ratio of the acid consumed by the fast reaction and the total acid injected. Y_{ST} is the value of Y in total segregation where acid is consumed in proportion to the concentrations of $H_2BO_3^-$ and IO_3^-.

$$Y = \frac{2(n_{I_2} + n_{I_3^-})}{n_{H^+}}$$

Equation 11. ratio of acid consumed in fast reaction to total acid injected

$$Y_{ST} = \frac{6n_{IO_3^-}}{6n_{IO_3^-} + n_{H_3BO_3^-}}$$

Equation 12. value of Y in total segregation

Using these equations, the segregation index X_s goes to zero in perfect micromixing and 1 in total segregation [14]. As in the procedure for determination of residence time, the concentration values can be obtained via spectroscopy.

Methods

Microfluidics Experiments

With the help of Professor Andrew Teixeira, I tested a microfluidics experimental procedure for the Unit Operations senior year laboratory course at WPI. Professor Teixeira ordered the components necessary to construct various microfluidics circuits, including Harvard Apparatus and Vici M6 pumps, a six port two position ElvenFlow valve, an Ocean Optics Spectrometer, and several micromixing reactors. For my experiments I used the Stamixco Static Mixer inside a piece of 1/8” PFA tubing (both 200 mm long), a piece of 1/8" tubing without the static mixer (200 mm long), and a straight union junction. The tubing size used for the entire rest of the circuit was 1/16” PFA (0.031” ID).

Table 1: Types of micromixing components (obtained from "Experiment No. X Micromixing in Flow" (Teixeira).

Mixers	Packed Beds ID (mm) x L (mm)	Packing (um)
Stamixco	2.1x200	75-90
Straight Union	2.1x250	63-75
Straight Tube	3.2x200	
	4.6x200	

The protocol was written by Professor Teixeira and I tested it for feasibility as a Unit Operations experiment. I used Rhodium B as a dye, creating a stock solution of a known concentration and diluting it down to several known dilutions. The dilutions were run through the spectrometer to determine peak wavelength and Beer's Law for conversion between absorbance and concentration.

To set up the microfluidics circuit, I cut a loop of 1/16" tubing with an inner diameter of 0.031" to 20 cm to create a volume capacity of 100 uL. The circuit was then set up to include two Vici M6 pumps, one connected to a reservoir of tracer and the other to a reservoir of tap water.

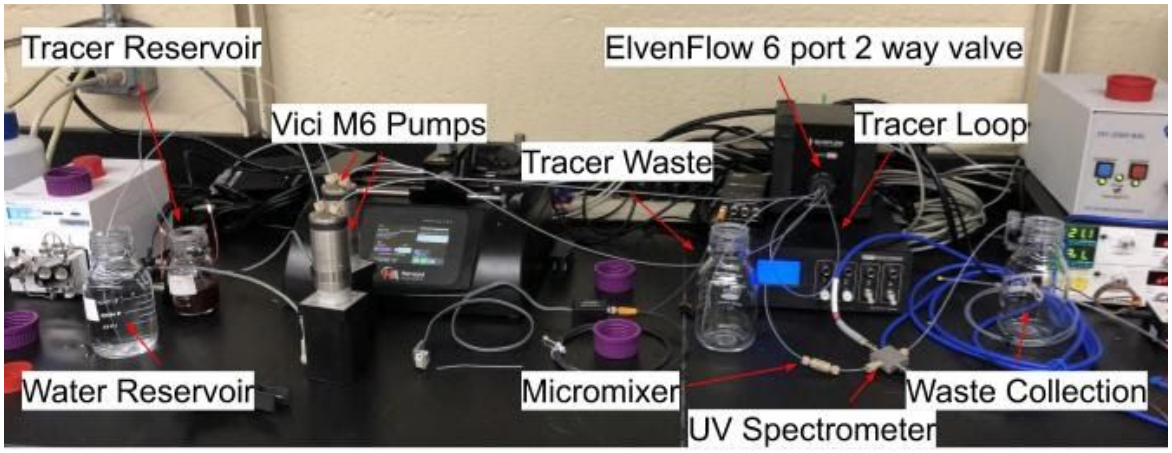


Figure 3. Microfluidics circuit configuration used in data collection.

After assembling the microfluidics circuit, the tracer was loaded and flow initiated by setting a flow rate of 1 ml/min using Labview to automate the Vici pumps. After hydrodynamic steady state was established through observation of stable UV readings, the six position valve was toggled to position 2 (depicted in part C of Figure 4), and the tracer in the loop was thus injected into the circuit. The UV spectrometer recorded

absorbance over the elution of the tracer through the mixer, and the concentration allowed to return to steady state before turning off the pumps and reconfiguring the circuit with the next mixer.

I demonstrated this experiment using a Stamixco Static Mixer, a straight union, and a straight tube the length of the static mixer to create a bypass. The configuration of the circuit relative to the ElvenFlow 6 port 2 way valve is depicted below.

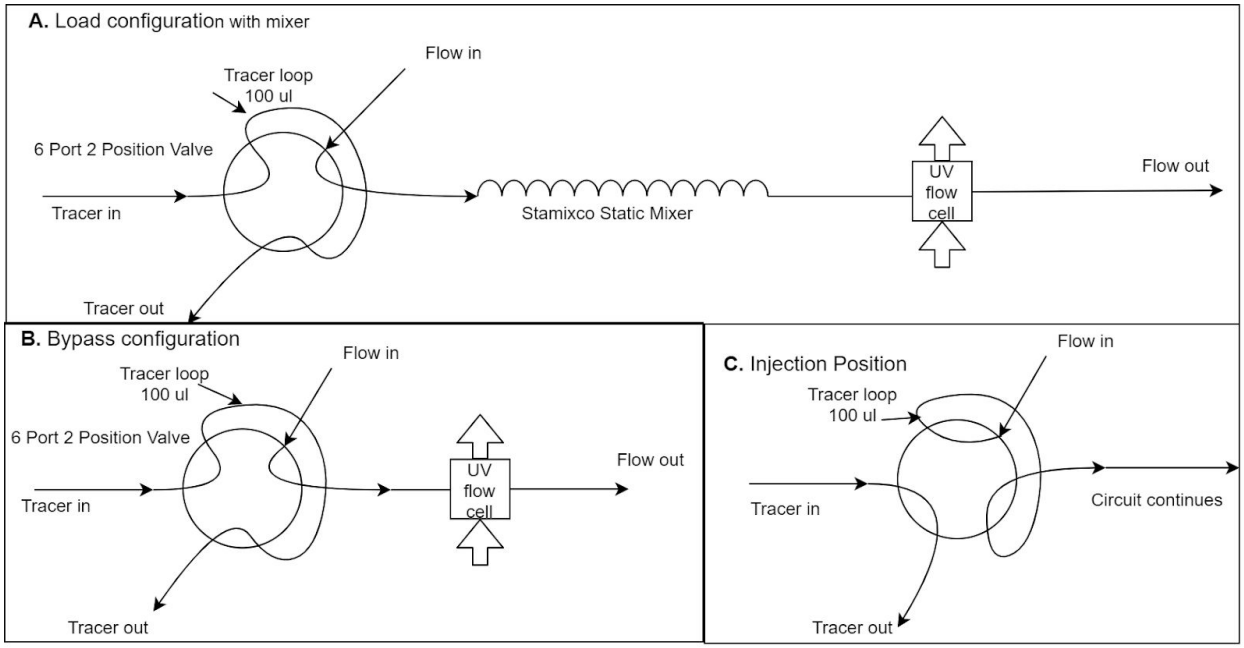


Figure 4. Diagram of schematic of microfluidics circuit showing a mixer with 6 port 2 position valve in tracer load position (A), load position with bypass (B), and tracer injection position (C).

Reusable Hormone Test Chips

Creating a Negative Result

As a secondary objective, Professor Teixeira urged me to research fields in which microfluidics could be applied. I came to him with several ideas we might be able to replicate in the lab. We decided to study hormone testing, and found that similar studies had tested different salt solutions for their ability to unbind the pregnancy hormone HCG from the antibodies fixed at the result window [7]. In bulk we ordered VeriQuick Early Pregnancy Tests from Live Action Safety, which are microfluidics chips in that they are miniature analysis reactors. The plastic casing surrounding the test strip was pried open using a screwdriver. A pipet was used to distribute 3 drops of tap water from the Goddard Labs basement onto the collection side of the test strip. The

antibodies, marked with pink dye, were observed as they were wicked by capillary action to the bond site, ensuring that the antibodies bound to display the negative result. A full procedure can be found in Appendix A.

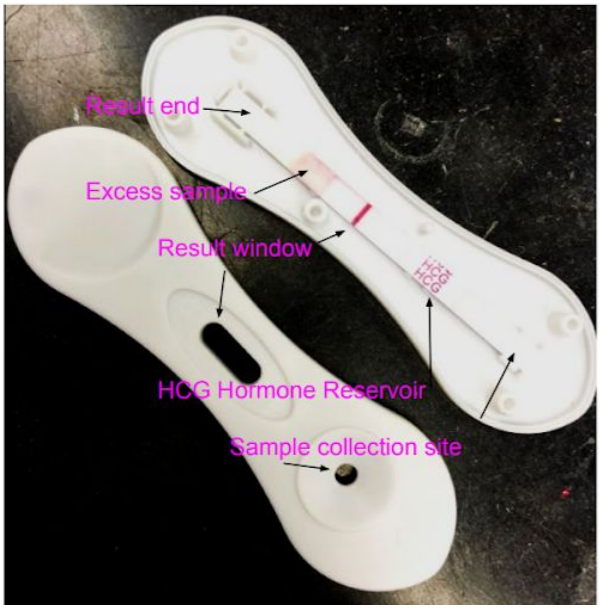


Figure 5. Negative result on opened HCG test, where sample has been allowed to flow into the end site.

Testing for Reversibility with Various Salts

Solutions of potassium chloride, sodium chloride, and calcium chloride were created at concentrations of 1, 2, and 3 M for each. Several pregnancy tests were opened and activated using the procedure for creating a negative result. The salt solutions were then pipetted onto the result end of the test strip after the antibodies eluded past the result window, but before reaching the sponge-like area at the result end. A visible change in direction of the antibodies and tracer, moving back towards the collection area, would be a sign of a successful test. A procedure can be found in Appendix B.

Testing Effect of Drying Prior to Salt Addition

Three test kits were opened, activated, and allowed to dry for 20 minutes. Solutions of KCl, NaCl, and $CaCl_2$ were created at concentrations of 3 M. Three drops of salt solution were added to the end site of the test strip, and observed for signs of antibody unbinding. The procedure is included as Appendix C.

Soak in 4 Molar Salt Solutions

Three pregnancy tests were opened, activated, and allowed to dry. Solutions of 4 Molar KCl, NaCl, and $CaCl_2$ were prepared and placed in small beakers. The test strips were placed collection site down in the beakers with the result site submerged and the end site exposed to the air. The experiments were left overnight and observed the next day for movement of the bound antibodies from the result site. A full procedure can be found in Appendix D.

Reusable Test Chip Prototype

Next I built a prototype for a reusable HCG testing chip. I obtained several pieces of folded plastic chips from the scrapbook section of AC Moore, 5 cm wide by 16 cm long, folded in half along the short side (32 cm long when unfolded). A 1 cm channel was marked longways in the center. Four layers of packing tape were placed on top of one another on either side of the channel on the inside of the fold, and smoothed to remove any bubbles. Three test strip materials would be varied to study their velocity and elution, tissue, toilet paper, and paper towel. The test strips were constructed by separating the material into plys and cutting a 1 cm wide strip. The strips are laid in the channel with excess trailing out of the bottom, and a line of dye placed a few centimeters from the entrance to the channel on the test strip. The chip is folded closed and held tight with bulldog clips. The outer top can be marked with centimeters along the visible strip to allow for ease in measurement and data analysis. A protocol can be found in Appendix E.

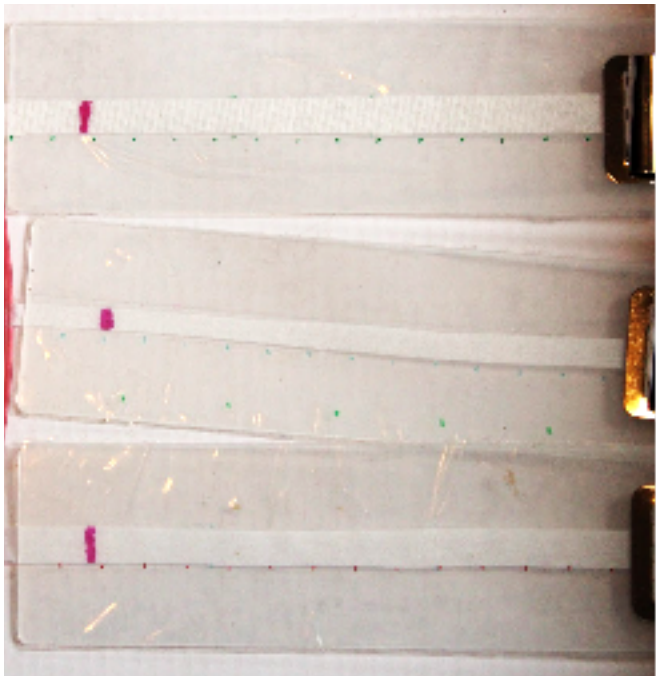


Figure 6. Three prepared test chip prototypes, with varying test strip materials from top to bottom as follows: Paper Towel, Tissue, and Toilet paper

Prototype Testing

The three prototypes were tested simultaneously. A plate of water was set up and the three chips placed beside it, with the tail of the test strip adjacent to the water reservoir. A tripod was set up directly above it to take photos of the test chips every 10 seconds. At t=0, all three tails of the test strips were inserted into the reservoir, allowing capillary action to wick the water into the test chips. The photos were analyzed for saturation over distance and time, where saturation of the dye in the photo is analogous to concentration of dye in the chips.

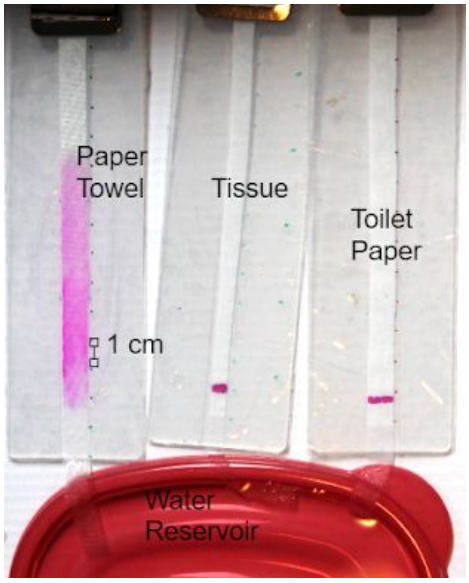


Figure 7. Prototype test, where paper towel has already wicked considerable amounts of dye up the chip.

Results and Discussion

Microfluidics Experiment Results

The absorbance vs time data was transferred from LabView to Excel and processed, calibrating t=0 to the instant where the valve was switched. Concentration was plotted as a function of time for the Stamixco Static Mixer, straight union, and straight tube bypass configurations.

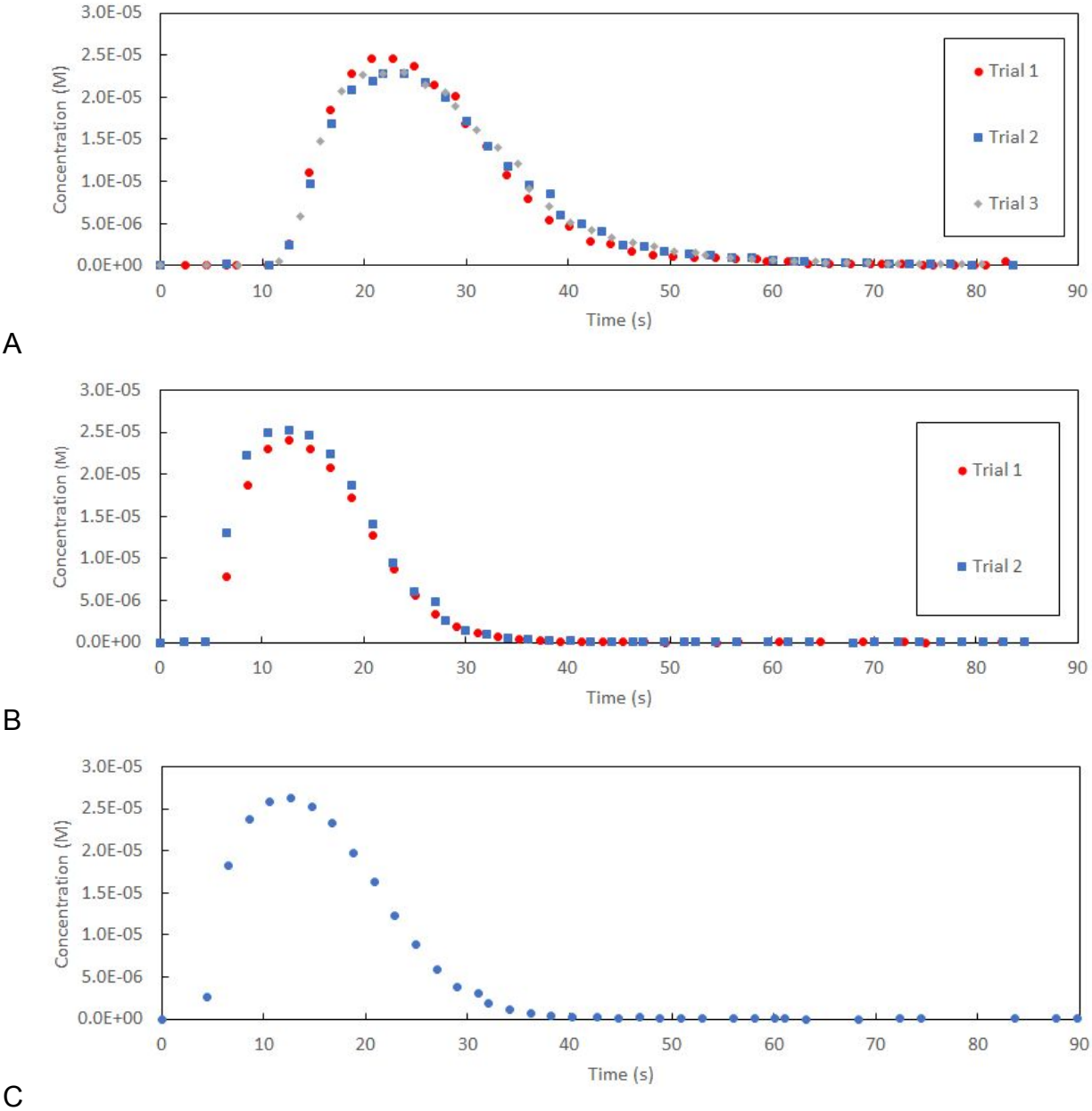


Figure 8. Tracer Injection Plots. Plots A-C depict the concentration over time. Panel A is the Stamixco static mixer. Panel B is the straight union. Panel C is the straight tube bypass. The flow rates were 1 ml/min. The concentration of the stock solution was 3.88×10^{-5}M.

The concentration peak in panel A when the static mixer is applied is considerably broader than the straight union or bypass. The average maximum concentration occurs at 22.8 seconds after the tracer enters the mixer, at a concentration of 2.256×10^{-5} M. The broader peak occurs due to the mixing of the tracer with the water, creating greater dispersion perpendicular to flow and a longer residence

time for the dye to remain inside the mixer. The average peak occurs for the straight union in panel B at 12.7 seconds and 2.46×10^{-5} M. For the straight tube bypass in panel C, the peak occurs at 12.6 seconds and 2.632×10^{-5} M. The static mixer retains the tracer almost twice as long as the other tested components, making it advantageous for micromixing and reactions.

The broadness of the peaks associated with the straight tube and straight union are about equally as broad, demonstrating that the union does not hold the molecules any longer than a straight tube plug flow reactor would. The most concentrated stream is caused by the straight tube, showing the least mixing is induced. The lesser maximum concentration from the straight union shows that some mixing and axial dispersion does occur, most likely caused by disturbance to the flow, breaking the stream line as it passes through. The union is not meant to mix but to serve as a junction between two tubes, and the bypass is the same length and width of tubing inserted the same way as the static mixer, but with no actual mixer. I hypothesize that the bypass tube creates more predictable, smoother flow, whereas the straight union causes disturbances in flow patterns and thus should be avoided by using a longer tubing line rather than connecting two. Photos of the static mixer and straight union are demonstrated below. The straight tube was the same setup as the static mixer but without the helical structure inside (plain 1/8" tubing of the same length, 200mm).

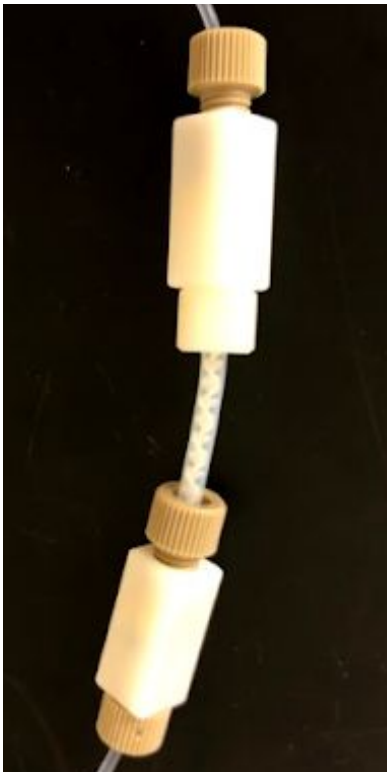
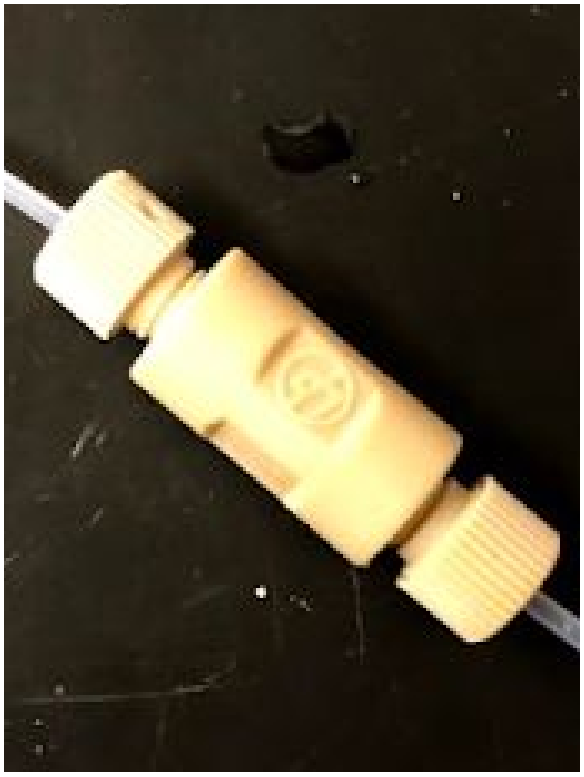
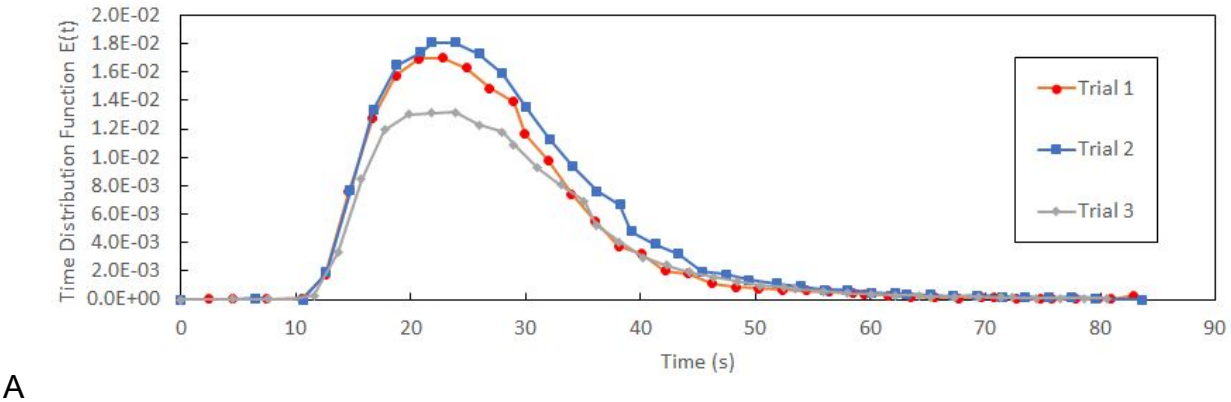
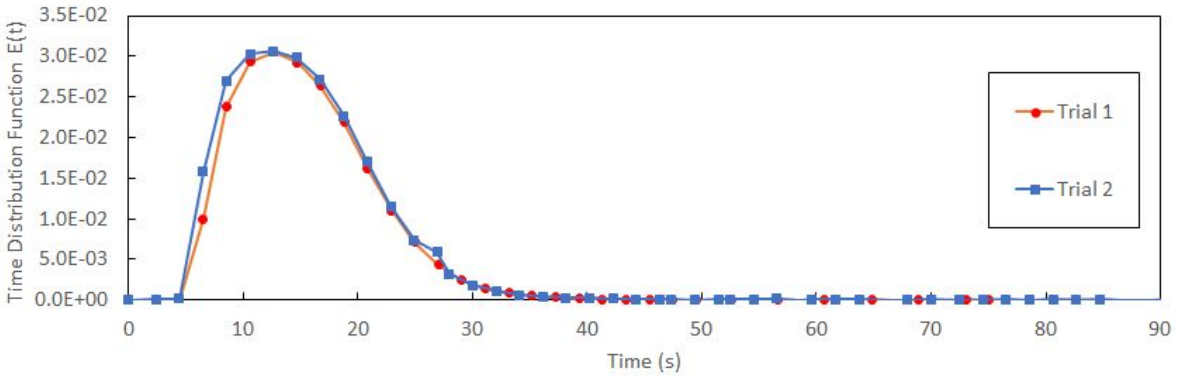
A  B 

Figure 9. Static Mixer and Straight Union. The static mixer is a helical structure placed inside of a piece of 1/8" tubing with two unions on either side depicted in panel A. The straight union I tested is depicted in panel B; the two 1/16” tubes are inserted into the ridged portion then screwed into the central union piece, which connects them with a pinhole in the center. The straight tube bypass was constructed with a 1/8" piece of tubing with the same configuration as panel A, excluding the helical structure that is the static mixer. Both the static mixer and the bypass tube were 200 mm long.

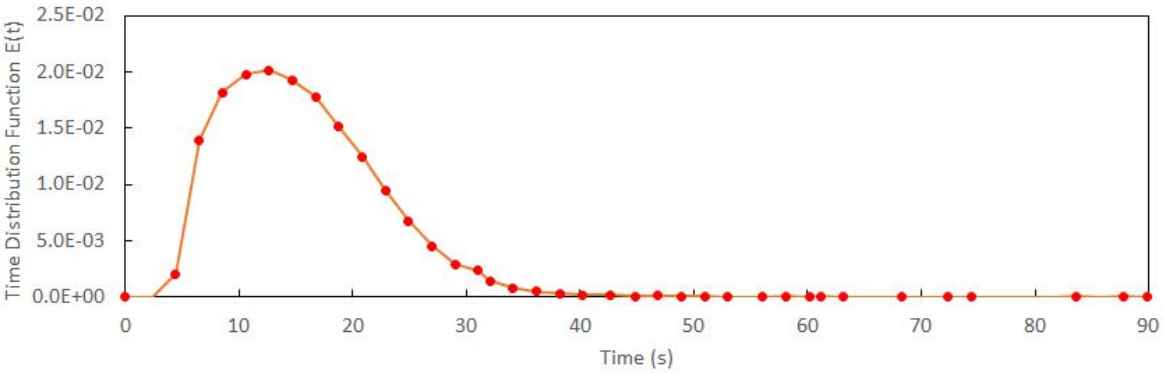
The data was analyzed with the residence time distribution equation (equation 4) to create the residence time distribution plots in figure 10. The x -axis is indicative of time and the y-axis is a dimensionless tracer concentration. The area beneath the curve is indicative of the fraction of molecules which have spent x amount of time in the reactor.



A



B



C

Figure 10. Residence Time Distribution Function for Stamixco Static Mixer (Panel A), Straight Union (Panel B), and Straight Tube Bypass (Panel C). The residence time curves are obtained using equation 4, where E(t) equals concentration divided by the area under the concentration curve.

With an average peak at 22.8 seconds and a nondimensional concentration of 1.6×10^{-2}, the static mixer retains far more molecules for a longer amount of time than in the straight union or bypass. This makes collisions more likely to occur thus encouraging mixing and reacting. The straight union's average peak occurs at 12.7 seconds at a concentration of 3.1×10^{-2}. The peak for the bypass occurs at 12.6 seconds

and a concentration of $2.0x10^{-2}$. The concentration produced by the straight union is about twice as much as that of the static mixer, showing the static mixer is more effective at combining the injected tracer with the water stream. For the bypass, the greatest number of tracer molecules remained within the tube for only 12.6 seconds. The bypass is the same length and width as the static mixer, for which nearly the same proportion of molecules remain mixing in the reactor for 22.8 seconds, 1.8 times as long, making it nearly twice as likely for collision to occur between molecules of the 100 µL tracer and the water stream.

The breadth of the residence time peak is indicative of the axial dispersion. While miniscule amounts of particles remain in the mixer for 90 seconds, bulk amounts of tracer remain in the mixer for 60 to 70 seconds for the static mixer and 30 to 40 seconds for the straight union and the straight tube. This reinforces the analysis that particles spend nearly twice as long in the static mixer than a bypass or union, confirming that the static mixer induces axial dispersion to double mixing and collision effectiveness. The peak is sharper and taller on both the straight union and straight tube bypass plots than the static mixer peak, like the straight union residence time peak, making the static mixer the best option for a reactor.

To better process this data, the static mixer could be deconvoluted by the bypass using Matlab and equation 4 to remove the effects of simply going through the 200 mm length of tubing and show only the mixing effects caused by the helical mixer. Dispersion can also be quantified by using equations 5-7. A Villermaux-Dushman analysis can also be performed using reactants to show whether the fast or slow reaction will persist in the mixer.

Reversible Hormone Test Chip Results

By dropping water into the collection site of the VeriQuick pregnancy test strips, it was confirmed that tap water could wick the marker antibodies to bind at the result window, producing a negative test result.

Table 2. Tap water was confirmed to activate a negative test result on the HCG tests.

Test	Result
Will water create a negative result?	Yes

In testing reversibility with KCl, NaCl, and $CaCl_2$, my results were contrary to those observed in the Medimmune study. NaCl did not display any reversibility in my tests, and $CaCl_2$ produced reversibility more readily whereas it had shown to produce

only a small amount of reversibility in the Medimmune study. In congruence with the Medimmune tests, higher concentrations were more likely to result in unbinding of the two antibodies [7].

Table 3. Results of application of counter flow of three different salt types at 1, 2, 3, and 4 M.

Salt	Dye Flow 1 M	Dye Flow 2 M	Dye Flow 3 M	Dye Flow 4 M
KCl	No	No	Yes	Yes
NaCl	No	No	No	No
$CaCl_2$	No	Yes	Yes	Yes

Drying of the activated test before addition of salt did not increase reversibility, but in fact none of the salt solutions were able to reverse the binding once dried.

Table 4. Results from application of 2 M salts after drying the activated strip.

Salt (2 M)	Reversibility Upon Drying First
KCl	No
NaCl	No
$CaCl_2$	No

Soaking the activated test strips overnight did not cause observable unbinding via capillary action or dissolution into the solvent.

Table 5. Activated test strips were soaked in 4M salt solution overnight. At the end of the test it was not apparent that any hormone teg dye indicator had been removed from the negative point by capillary movement up the strip or dissolution into the salt baths.

Salt (4 M)	Dye Movement when Soaked Overnight
Potassium Chloride	No
Sodium Chloride	No
Calcium Chloride	No

Reusable Test Chip Prototypes

Over time, the marker eluded through the test strips, as can be seen in the photos below, which stack the photo taken at each time frame in order for the three test strip materials. The photos of the experiment were taken every 10 seconds, stacked and evaluated using ImageJ to map the saturation value over the distance, which was marked in green every centimeter. The test strips visually demonstrate the concentration peaks similarly to the concentration graphs from the micromixing experiments. The most concentrated or saturated areas contain the most tracer molecules, whereas the outer edges of the tracer are mixed with water. A test strip with a broad peak or tracer stain will induce the best mixing as it retains the molecules for the longest amount of time.

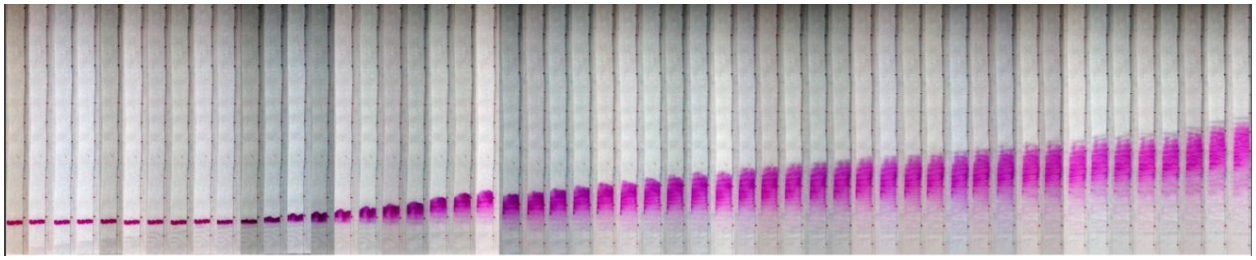


Figure 11. Toilet Paper Velocity and Concentration Test With Ink. Taken in ten second intervals with one ply of toilet paper used as the prototype test strip material, centimeters are marked in green.

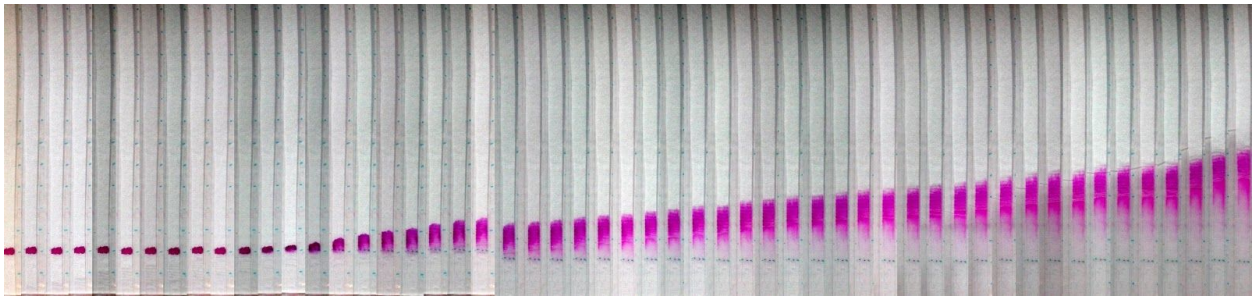


Figure 12. Tissue Velocity and Concentration Test With Ink. Taken in ten second intervals with one ply of a tissue used as the prototype test strip material, centimeters are marked in green.

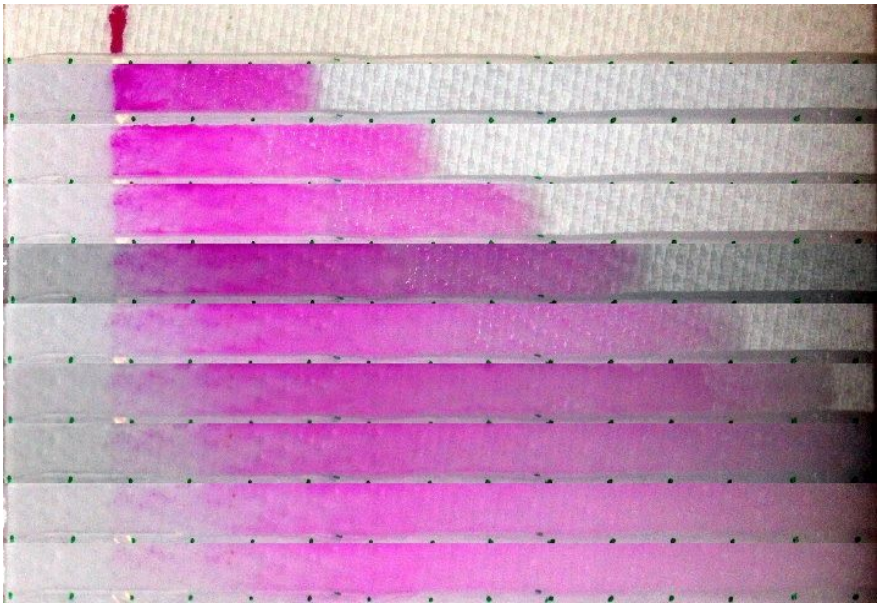


Figure 13. Paper Towel Velocity and Concentration Test With Ink. Taken in ten second intervals with one ply of a paper towel used as the prototype test strip material, centimeters are marked in green.

By plotting the distance and concentration of the peak saturation point for each time frame, figure 14 was created. This plot demonstrates that the peak concentration decreased as the tracer traveled up the test strip, and in turn the breadth of the peak increased as the concentration peak moved further from the origin.

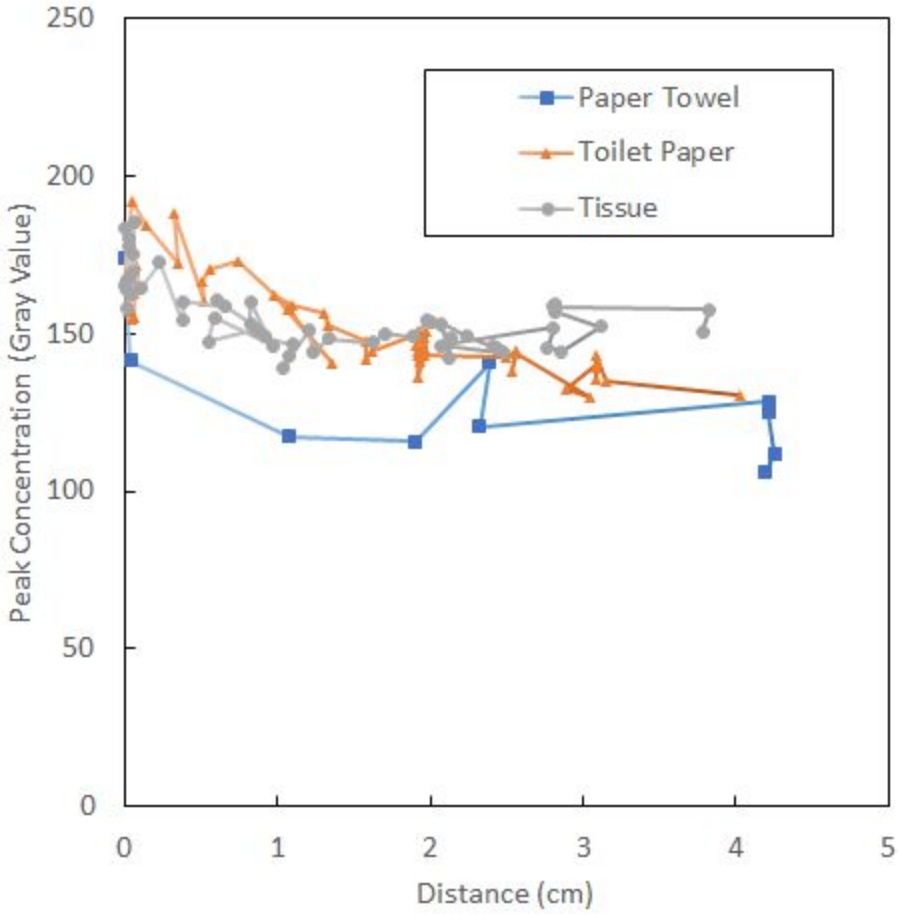


Figure 14. Peak Concentration vs Distance for Paper Towel, Toilet Paper, and Tissue

Several time frames of the tissue test strip were evaluated for the standard deviation of their respective concentration vs distance peaks. As time increased, the standard deviation of the peaks increased. This shows the peaks became broader and shorter. So, with time, concentration at any given point decreased and the length of the test strip which contained tracer elongated, showing more mixing of the tracer with the surrounding water.

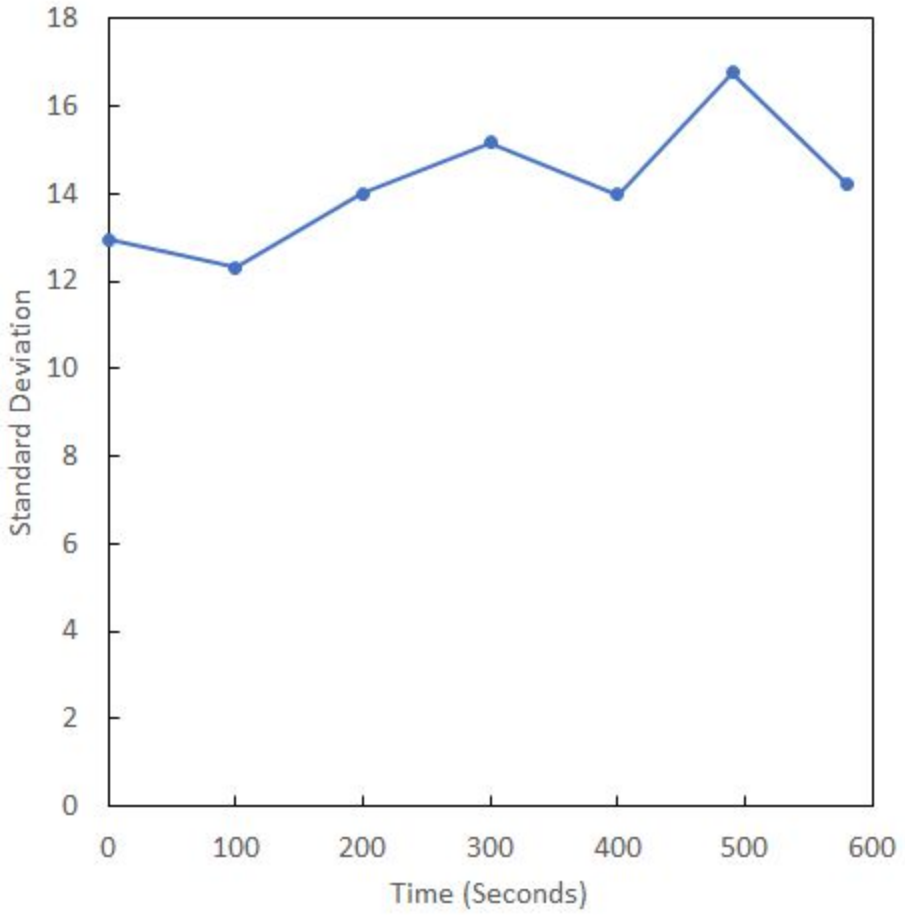


Figure 15. Tissue Standard Deviation Of Concentration Peak Over Time. This demonstrates the breadth of the peaks increasing over time.

The concentration peak gives the average distance which the molecules of tracer have traveled. The peak concentration location, average distance, was plotted for each time frame, demonstrated below in figure 16. The slope of each test strip trial helps to visualize the speed at which the tracer is eluding through the test strips. The slope of each test strip material calculated from the slope of these linear plots is tabulated in table 6.

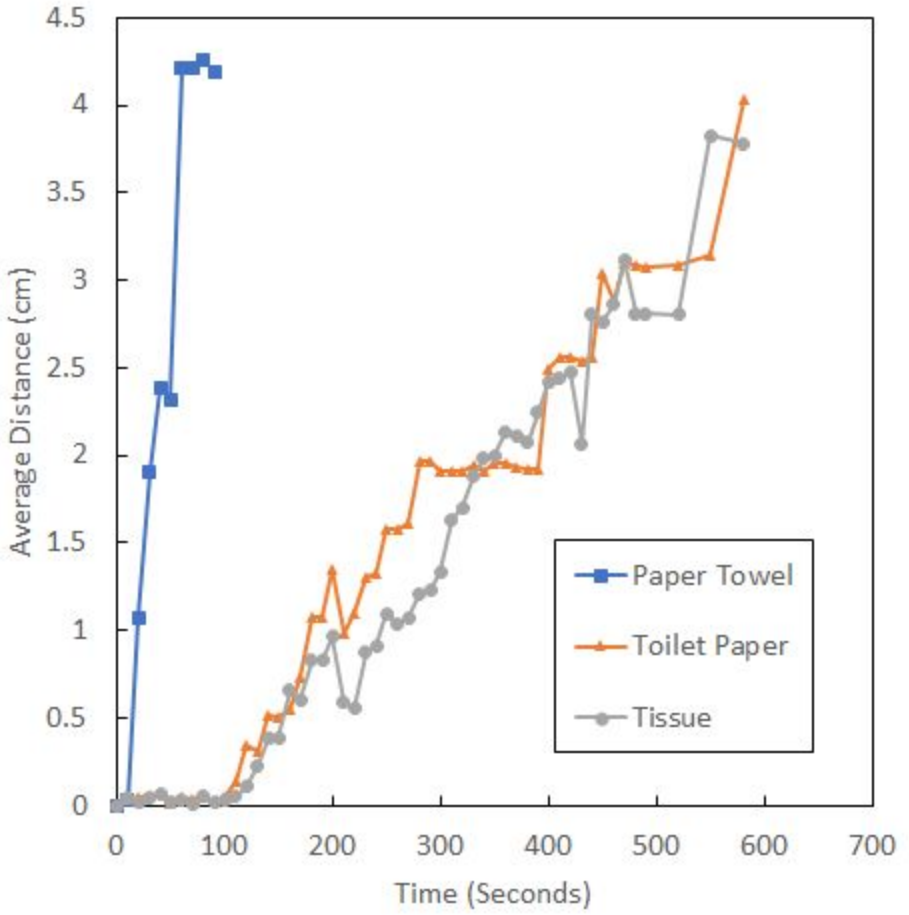


Figure 16. Average Distance vs Time

By finding the slope of each line in the average distance versus time, the average velocity can be determined. The calculated velocities are shown in Table 6 below.

Table 6. Velocities Associated with Three Test Strip Materials.

Test Strip Material	Velocity (cm/s)
Paper Towel	0.0544
Toilet Paper	0.0069
Tissue	0.0069

The differences in properties between the toilet paper and tissue test strips were slim. They had the same average velocities as one another, and similar peak concentrations. The paper towel wicked the tracer almost ten times as quickly, but the peaks were far broader. This implies that the tracer mixes better with water on the paper

towel test strip than the toilet paper or tissue test strips, in terms of speed and concentration.

Conclusion

In regards to the micromixing experimental procedure, I found the lab to be challenging but rewarding. It was very difficult to learn how to properly construct a microfluidics circuit and use LabView to automate it and collect data. The learning curve allowed me to observe dispersion and residence time phenomena very clearly between the different mixing apparatuses I inserted into the circuit. The results allow me to clearly differentiate between good and poor mixing units, and it would be a worthwhile addition to the Unit Operations curriculum.

I conclude that reversibility of the HCG antibody binding with salts requires more research so reusable pregnancy tests can replace single use tests to increase sustainability and accessibility. In my results it was shown that $CaCl_2$ at high concentrations was the most effective at inducing reversibility. Had I had more time, I would have liked to experiment with different solvents such as toluene or acetone or varying temperature.

In constructing reusable test chip prototypes, I think I was successful. The chips could be opened and the strip itself replaced with a new one. If the manufacturer wanted to optimize speed, paper towel would likely be the best choice for a test strip material. However, the narrower peaks of the toilet paper and tissue strips may be more beneficial for accuracy and maintaining high concentration if the time can be allowed. Research into which was cheaper would probably determine which should be used in manufacturing to increase profit margins. This could probably even be replicated with the HCG antibody attached.

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Appendices

Appendix A

Pregnancy tests with just water

1. Use a screwdriver to pry open the plastic casing of a VeriQuick Early Pregnancy Test, ordered from Live Action Safety
2. Using a dropper, drop 3 drops of water on the collection side of the test strip
3. Wait until the water crosses the result site, turning the control line pink

Appendix B

Pregnancy tests with KCl, NaCl, and $CaCl_2$

1. Prepare a solution of 1 M KCl, obtained from Sigma
2. Use a screwdriver to pry open the plastic casing of a VeriQuick Early Pregnancy Test, ordered from Live Action Safety
3. Using a dropper, drop 3 drops of water on the collection side of the test strip
4. Wait until the water crosses the result site, turning the control line pink
5. On the opposite side from the collection site, there is a spongy area we will call the result end. Just before the water reaches the end site, apply 3 drops of the solution to the result end
6. Repeat with 1 M solutions of NaCl and $CaCl_2$, both also obtained from Sigma
7. Repeat with 2 M and 3 M solutions of all three salts
8. Observe whether the antibody dye moves from the original line back towards the collection site

Appendix C

Testing Effect of Drying Prior to Salt Addition

1. Prepare a solution of 3 M KCl
2. Use a screwdriver to pry open the plastic casing of the test unit
3. Using a dropper, drop 3 drops of water on the collection side of the test strip
4. Wait until the water crosses the result site, turning the control line pink
5. Allow the test strip to dry out

6. Apply 3 drops of the solution to the end site
7. Repeat with 3 M solutions of NaCl and $CaCl_2$
8. Observe whether the antibody dye moves from the original line back towards the collection site

Appendix D

Soak in 4 Molar Salt Solutions

1. Prepare solutions of 4 M KCl, NaCl, and $CaCl_2$
2. Use a screwdriver to pry open the plastic casing of a VeriQuick Early Pregnancy Test, ordered from Live Action Safety
3. Using a dropper, deposit 3 drops of water on the collection side of the test strip
4. Wait until the water crosses the result site, turning the control line pink
5. Allow the test strip to dry out
6. Place the solutions in three separate 100 mL beakers
7. Place one test strip end site down in each beaker, submerging the control line but not the collection site
8. Allow to sit overnight
9. Observe whether the antibody dye moves from the original line back towards the collection site or has been removed from the control line into the solution

Appendix E

Reusable Test Chip Prototype.

1. Folding plastic piece (AC Moore scrapbooking) 1 ⅞ inx6.5in folds along short side so opens to just under 13 in
2. Mark center and edges of channel to create 1 cm channel
3. Lay one piece of packing tape on either side of the marked channel
4. Smooth with credit card
5. Build up layers of packing tape if desired, smoothing in between i did three
6. Tissue, toilet paper, and paper towel split into plys cut into 1 cm by 20 cm
7. Lay ply in channel allowing excess to hang off the end
8. About 1 inch from bottom place line of dye Nicole Premiere permanent marker
9. Mark centimeters on outside top layer of plastic to allow measurements