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Microchip Capillary Electrophoresis

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Date: June 3, 2014

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED

Microchip Capillary Electrophoresis

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE IN BIOENGINEERING

THESIS ADVISOR

DEPARTMENT CHAIR

MICROCHIP CAPILLARY ELECTROPHORESIS

by

Daniel Shull, Mark Vinopal, and Scott Hardy

SENIOR DESIGN PROJECT REPORT

Submitted in partial fulfillment of the requirements
for the degree of
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School of Engineering
Santa Clara University

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Abstract

For the study of neuromodulation in *Cancer borealis* we have designed a microfluidic device to separate and detect bioamine concentrations with a high temporal resolution. Our goal is to use this device to measure the concentration of continuous bioamine microdialysis samples directly from the pericardial cavity (the area surrounding the heart) of *Cancer borealis*. The microfluidic device that we designed is made from polydimethylsiloxane (PDMS) and exhibits an off-channel configuration of capillary electrophoresis (CE) by incorporating micellar electrokinetic chromatography (MEKC). CE is used to separate bioamines based on charge and size due to the applied electrical potential. In the off-channel configuration, the potential is applied across the separation channel and grounded by the palladium decoupler, which lies just before the detector. Microchip CE is advantageous because it uses small amounts of analyte and completes fast run times. We will use MEKC to separate dopamine and octopamine, since they are structural isomers, by their difference in affinity to sodium dodecyl sulfate (SDS) micelles. This results in different elution times for dopamine and octopamine. We were able to drive the fluid in the correct direction. The creation of this device has valuable implications, allowing for baseline concentrations of neuromodulators with the *Cancer borealis* to be established. The effect of different stimuli on these crabs can then be more accurately determined.

Acknowledgements

We would like to thank our Bioengineering advisor Dr. Ashley Kim for her guidance throughout the project. We would also like to thank our collaborating professors Dr. Steven Suljak (Chemistry), and Dr. John Birmingham (Physics) for their assistance. Additionally, we would like to thank Dr. Paul Abbyad (Chemistry) for allowing us to use his facilities and equipment.

We are very grateful for our funding from the Santa Clara School of Engineering and the use of some funding from Dr. Suljak's grant: "Investigating the Role of Neurohemal Biogenic Amines in Shaping Stomatogastric Motor Programs of the Crab *Cancer Borealis,*" Multiple Investigator Cottrell College Science Award [co-PI: John T. Birmingham, SCU Physics], Research Corporation for Science Advancement, July 2011 – June 2013, $75,000.

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1 Introduction

This project involves an interdepartmental effort between Dr. Ashley Kim's lab (bioengineering), Dr. Steven Suljak's lab (chemistry), and Dr. John Birmingham's lab (physics) to study the effects of neuromodulators (chemical signals released by neurons used to control the muscles of the organism) common to the crab *Cancer borealis*. The goal is to study how different stimuli affect the neuromodulators of the crab. In order to do this, it is necessary to create a device that has a high temporal resolution to measure an accurate baseline.

Research done by an earlier group has focused on the concentrations of neuropeptides and their correlation with physiological changes in *Cancer borealis*[1]. This background provided us with a foundation for our proposed work, so that we could focus on the analysis and measurement of these neuromodulators and more precisely quantify the reactions of *Cancer borealis* in different environments. This particular crab species serves as a simple model organism, allowing us to study the effects of these neurohormones using only a small range of stimuli.

In order to obtain our sample data, we designed and manufactured a microfluidic device that can detect the presence of very small concentrations of dopamine, norepinephrine, octopamine, serotonin, tyramine, and gama-aminobutyric acid (GABA). We can then use this device to obtain baseline concentrations from our target organisms, the crabs themselves. Once we have a baseline, we can apply different stimuli to the crabs and measure the real-time changes in concentrations of these neuromodulators from hemolymph samples taken from the crabs using microdialysis.

In summary, we have made a microfluidic device to detect concentrations of six specific neuromodulators that requires small volumes of sample, yields high sensitivity, achieves fast detection, and is inexpensive.[2,3]

2 System

2.1 System Overview

Below in Figure 2.1 is an illustration of the various components of our microfluidic device and how they come together.

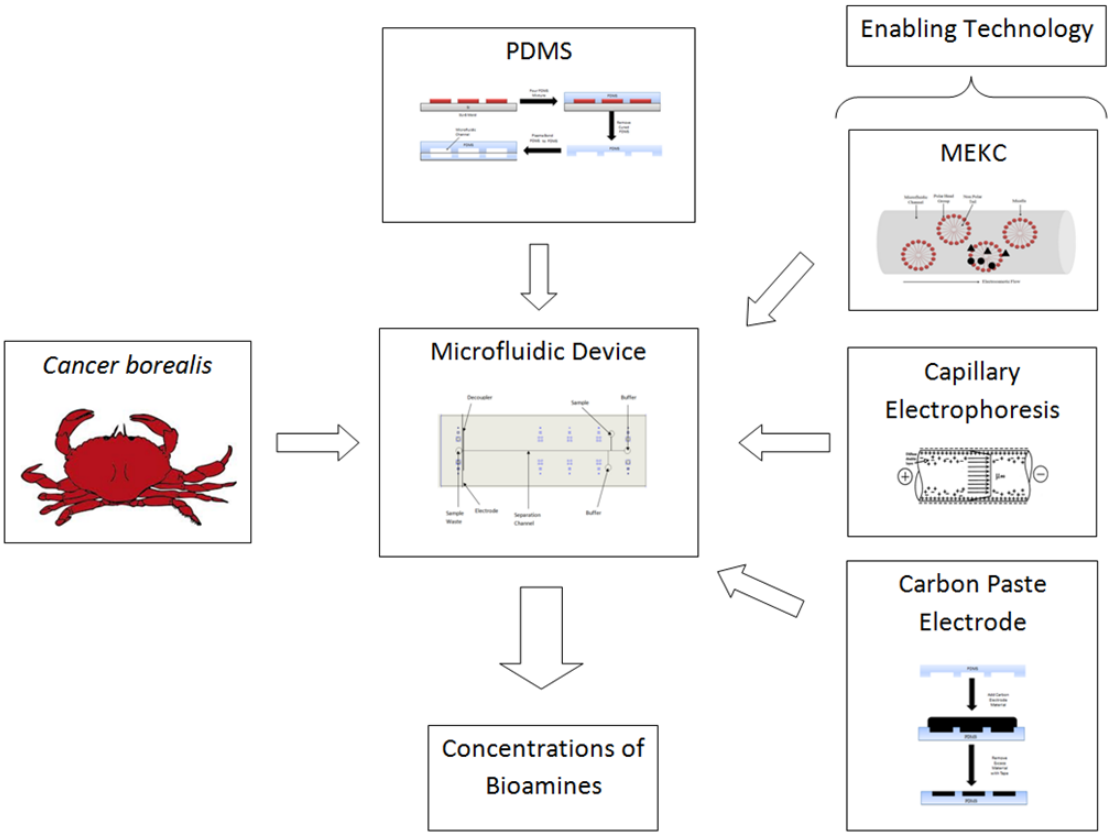

Figure 2.1: *An overview of the microfluidic device project.*

We used these enabling technologies and materials in the design, fabrication, and experimentation of our device. The PDMS (polydimethylsiloxane) forms the device itself, while the MEKC (micellar electrokinetic chromatography), capillary electrophoresis, and the carbon paste electrode technologies allow us to separate and detect our analytes. See Table 2.1 below for more details.

Our device uses the sampling technique microdialysis. Microdialysis is important to the use of our device because it allows for continuous measurements, resulting in a higher time resolution. While microdialysis can have slow sampling times, since it relies on diffusion, the use of microchip capillary electrophoresis will decrease the sampling time. Microdialysis will also allow us to filter out larger proteins and lipid chains in the hemolymph sample. We will go into more detail on that later.[1,4]

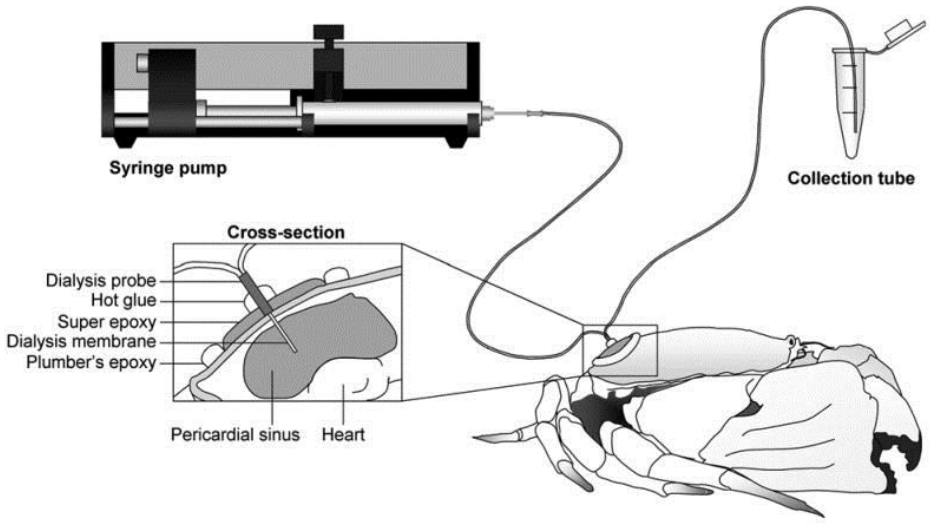

Figure 2.2: *The sampling technique microdialysis is placed in the pericardial sinus of the crab. The fluid diffuses through a semi-permeable filter into a collection tube. In our case, the fluid will flow into our device directly.*

Table 2.1: *Enabling technologies used in our design, along with brief descriptions and summaries of how they will be used in our design.*

Name	Description	Use
Capillary electrophoresis (CE)[5,6]	Electric field separation of compounds with different electrokinetic properties	Separate neuromodulators from a *Cancer borealis* hemolymph sample for detection
Amperometric detection[5,6]	Detection of chemicals due to changes in electrical current	Detection of the neuromodulators after they have been separated by CE
Micellar electrokinetic chromatography (MEKC)[7,8]	A technique to separate compounds in CE with similar electrokinetic properties	Separation between dopamine and octopamine (structural isomers). Will use sodium dodecyl sulfate micelles

2.2 Device Overview

Below is a simplified schematic of our device's final design, as well as a picture of our device as set up for experimentation.

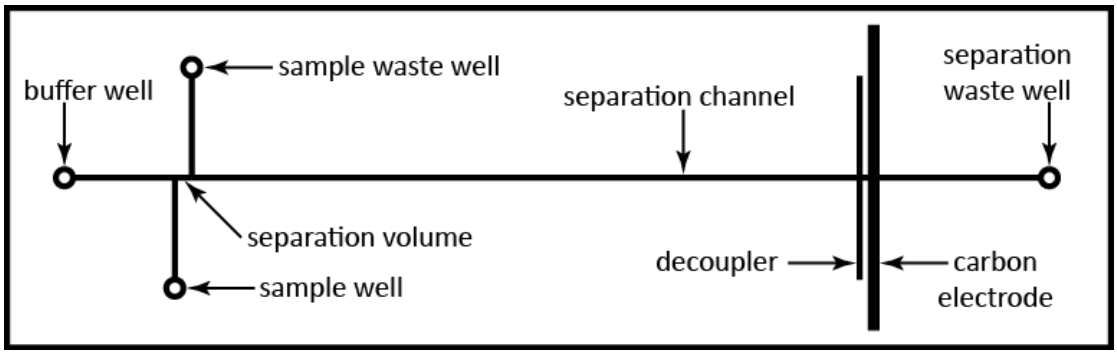

Figure 2.3: *Schematic of microfluidic device, features not drawn to scale. For approximate scale, the entire device is about 5.5cm long.*

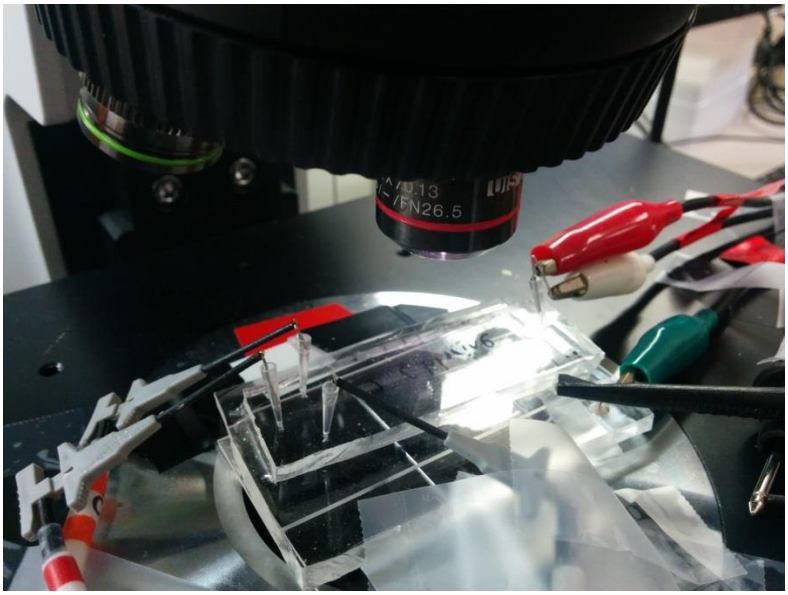

Figure 2.4: *Picture of our device in an experimental setup. The three microclips on the left and the red/white alligator clips on the right are connected to platinum probes inserted into the wells. The microclip on the right is connected to the palladium decoupler, and the green alligator clip is connected to a copper lead wire for the carbon paste electrode.*

We first fill our device with 1M NaOH for 20 minutes to precondition out device. Our device is then filled with our 20mM TES[a] buffer before our sample is inserted into the sample well. Our fluid flows through our device in two steps. In the load step, we set our sample well to 600 V and the sample waste well to 0 V and pinching voltages of 570 V and 550 V for the buffer well and decoupler. The fluid flows down the voltage

[a] (2-[1,3-dihydroxy-2-(hydroxymethyl)propan-2)yl]amino]ethanesulfonic acid). TES is a buffer with a pH range from around 6.8 to 8.2

gradient from the sample well to the sample waste well. We then apply 600 V at the buffer well and 0 V at the decoupler while having a pinching voltage of 454 V and 444 V for the sample well and sample waste well. This results in the fluid flow from the buffer well to the decoupler taking the separation volume that has our sample, down the separation channel to flow over the carbon electrode.

2.3 Customer Needs and System-Level Requirements

The customer needs a higher temporal resolution than already existing technologies. Our device uses a smaller sample volume along with having a higher sensitivity than other existing technologies. As a result, we are able to satisfy the needs of the customer.

2.4 Benchmarking Results

Current mechanisms for studying the relationship of neuromodulators in *Cancer borealis* and humans include capillary electrophoresis and liquid chromatography– mass spectrometry. These technologies detect amine concentrations, but suffer from poor sensitivity, poor time resolution, and/or high cost. The device we have designed provides low cost fabrication, high time resolution, high sensitivity, and multi-analyte detection of bioamines in *Cancer borealis.*

Table 2.2: *Comparison of existing technologies and our device.*

Technology	Approx. Sensitivity	Sample Size	Speed	Cost
Capillary Electrophoresis[9]	nanomolar (10^{-9})	around 100µL	Fast	Low
Liquid Chromatography-Mass Spectroscopy[10]	micromolar (10^{-6})	around 1mL	Slow	Very high
Microchip Capillary Electrophoresis with Amperometry (our device)	picomolar (10^{-12})	around 10-30nL	Very fast	Very low

2.5 Functional Analysis

There are three sequential functions that our device was designed to perform:

Table 2.3: *Description of three main functions of our microfluidic device. These functions happen separately and sequentially in practice.*

Function	Description
Sample loading	The sample well is filled with the sample to be analyzed. Current flows from the sample well to the sample waste well to electroosmotically fill the separation volume with sample.
Analyte separation	Current flows from the buffer well to the decoupler, which leads to electroosmotic flow and electrophoretic separation of the analytes in solution. Even though current stops at the decoupler, the fluid continues moving.
Analyte detection	Each volume of separated analyte flows past the carbon paste electrode, where it is detected using amperometry.

2.5.1 Sample Loading

During the sample loading phase, our device is filled with buffer, and then sample is injected into the sample well. We then apply an electric field across our device, and our analyte is moved from the sample well to the sample waste well through a process called electrophoresis. In the presence of an electric field, the charged ions present in the buffer move towards the well set at the lower voltage. This causes bulk flow of the sample, and the solution flows through the "T" segment in our device. Figure 2.4 shows a simple schematic of this.

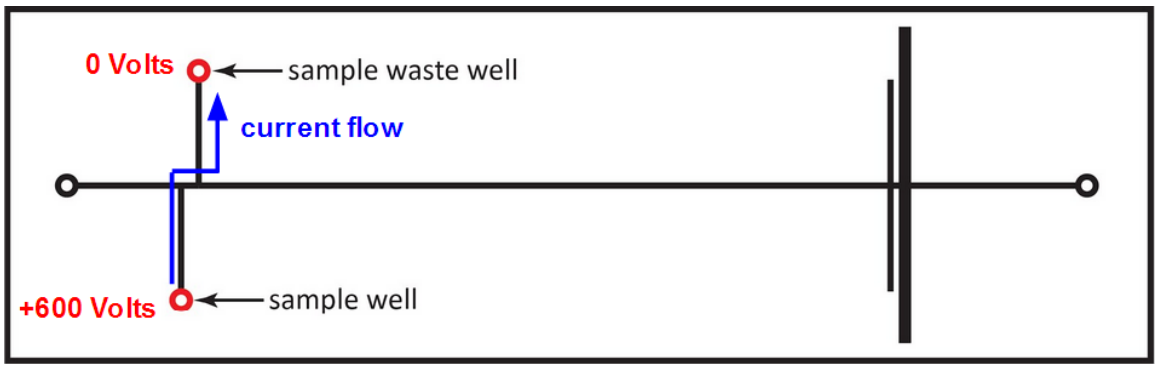

Figure 2.5: *Our sample fluid flows from the sample well to the sample waste well as a result of the voltage gradient that we create.*

2.5.2 Analyte Separation

Once the "T" segment of the device has sample in it, we change the direction of the electric field, as shown in Figure 2.5, setting the higher voltage at the buffer well, and ground at our decoupler. Through electrophoresis, the sample that was present in the

"separation volume" area of the T, is moved down the channel toward the electrode. Separation occurs due to the differing electrophoretic mobilities, or mobility of an analyte in an electric field, of the analytes in solution. This variable is affected by the molecular size and charge of each particle. Equations 1 and 2 are used to calculate electrophoretic mobility (μ_p), using electrophoretic migration velocity (u_p), electric field strength (E), length from inlet to detection point (L), migration time (t_r), length of capillary (L_t), and the voltage applied (V).

(1) $$u_p = \mu_p E$$

(2) $$\mu_p = \left(\frac{L}{t_r}\right)\left(\frac{L_t}{V}\right)$$

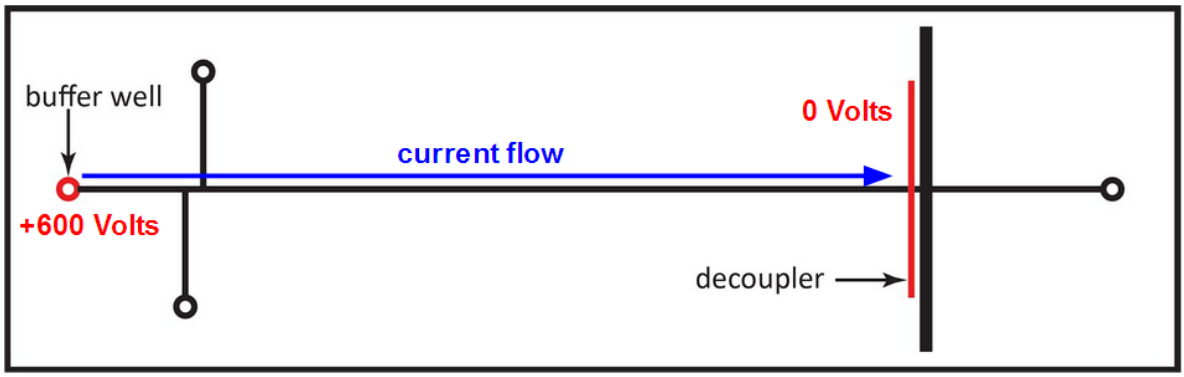

Figure 2.6: *An electrode is connected to the buffer well and the decoupler so that we can create a voltage gradient to cause the sample to flow down the separation channel.*

2.5.3 Analyte Detection

Detection of our analytes is achieved by oxidizing the OH groups present on our neuromodulators. This is done by holding our carbon paste electrode at a constant voltage and measuring the current across it. When a molecule of analyte comes in contact with our electrode, the oxygen-hydrogen bond is broken, causing the analyte to rearrange its structure to accommodate the new carbon-oxygen double bond, which in turn releases a free electron into the system. Since our analyte molecules have two OH groups, two electrons are released per molecule. These electrons causes the current through our electrode to increase slightly; the more analyte in contact with the electrode, the larger the increase in current. As the sample moves past the electrode and there are no more OH bonds to break to free up more electrons, the current dips back to the normal level, giving us a spike. Since these neuromodulators move at different speeds, the current spikes for the different analytes will take place at different times, helping us distinguish how much of each neuromodulator is present in the sample.

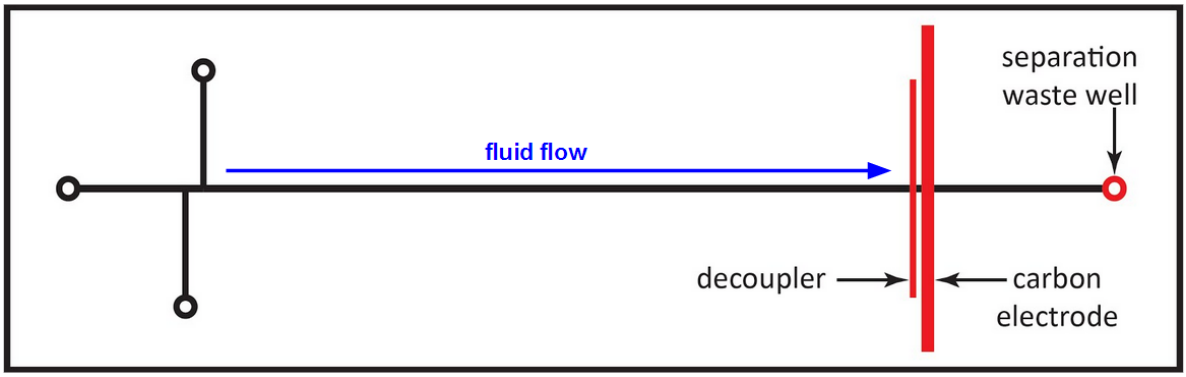

Figure 2.7: *The fluid flows past the decoupler and carbon paste electrode into the separation waste well. As the bioanalytes flow over the carbon paste electrode, a change in current is detected.*

2.6 Technical Challenges

There were some technical challenges that we had to solve in order for our device to be successful. The challenges and solutions are described in table 2.4.

Table 2.4: *Technical challenges faced during the project. This may need to be turned back into paragraphs if we need more detail in the explanations behind the decisions.*

Challenge	**Solution**	**Explanation**
Separating structural isomers	MEKC[7,8]	Sodium dodecyl sulfate could be used in low concentrations to form micelles that have a different binding affinity to each of the structural isomers, meaning that one of our analytes will move more slowly down the channel.
Removing hemolymph matrix effects, extra proteins, and lipids	Use microdialysis with a semi-permeable filter	As the fluid is extracted from the crab, the fluid goes through a semi-permeable membrane that has a specific molecular weight cut-off to only allow the smaller molecules that we want through, while the larger proteins are filtered out.
Quantifying differences in signals	Conduct a large quantity of runs to develop current standards for set concentrations of analytes.	Once we start getting repeatable data, we can run solutions spiked with set concentrations of analyte and measure the signal current. When we have enough of this data, we can have an idea of what signal we can acquire for what concentration.
Electrode fouling	Run a cleaning step	By running a voltage sweep that goes

	before and after each run.[11,12]	outside of the normal range for oxidation and reduction of our analytes will serve to clear our electrode of any fouling.
Bubbles in the device	Ethanol runs	After each session with the device, we can clear the separation channel with ethanol to prevent the buildup of unoxidized analyte, which will cause bubbles the next time the channel is filled with buffer.

2.6.1 Separating Structural Isomers

The structural isomers (dopamine and octopamine in figure 2.7) will move down the separation channel at the exact same speed, and since during detection, we can only see free electrons, we are unable to tell the difference between them. In order to combat this, we inject our samples with SDS (sodium dodecyl sulfate). This forms little spheres in the solution (micelles) that the bioamines stick to. Dopamine has a higher affinity than octopamine which causes the dopamine molecules to be slowed down more than the octopamine molecules.

Figure 2.8: *The structural isomers octopamine and dopamine*

2.6.2 Hemolymph Filter

The hemolymph sample would not be able to be flowed through the device directly after it was obtained from the crab because there are protein and lipid chains that will clog the device. We will use microdialysis to solve this problem. The probe, which has a semi-permeable membrane with a specified molecular weight cut-off, is placed within the pericardial sinus. The smaller molecules diffuse through according to the concentration gradient while the larger proteins and lipid chains are filtered out.

2.6.3 Signal Differences

Different concentrations of bioanalytes will give different current values. In order to establish a current standard for different concentrations, multiple experiments for the same concentrations are run until repeatable data is obtained. This is done for multiple concentrations for different bioanalytes.

2.6.4 Electrode Fouling

After one or two experiments our carbon paste electrode will not be as sensitive at detecting the bioanalytes. As a result, we cleaned the electrode by running a voltage sweep that goes outside of the normal ranges for the oxidation and reduction of the analytes, returning the electrode to its original condition.

2.6.5 Bubbles in the Device

Bubbles in our device are detrimental to our success. One of the reasons that bubbles can form is a result of a buildup of analyte around the decoupler and electrode. We flowed ethanol through our device after each session of experiments to prevent this buildup from occurring.

2.7 Team and Project Management

The following are the steps we are taking to ensure fair treatment among team members:

- We scheduled meeting times in the lab when we do our research.
- We scheduled meetings with our professor as a team where we expressed our concerns and ideas about the project.
- Everyone is allowed to do everything and knows the procedure of how to do anything within the project, allowing for better conceptual understanding and problem solving within the group.

Communication is key when doing any sort of group project. We started by setting up a group text message thread where we could post problems, updates, and talk about meeting times, making sure that everyone was included and aware of what was going on in the lab. Since our lab space and instruments were being shared by several groups, we also decided to use a lab calendar to orchestrate when different groups could use the microscope, Chi instrument, etc. to maximize lab time and space. All of our research was also posted to a google document to allow multiple group members to edit and add to our thesis and lab materials at the same time. We feel that the steps that we have taken to ensure communication between team members are sufficient.

Refer to the Gantt chart in appendix A for more specifics on what each person contributed to the project throughout the year.

3 Subsystems

3.1 Wells

The wells, illustrated in figure 3.1, in our design were made out of approximately the last 5 mm of plastic pipette tips which we cut manually with a razor blade. The wells were pushed down through holes punched in the PDMS. Although all of the wells were constructed in the same way, their functions were different. Below is a figure showing the locations of the wells and a table describing their functions.

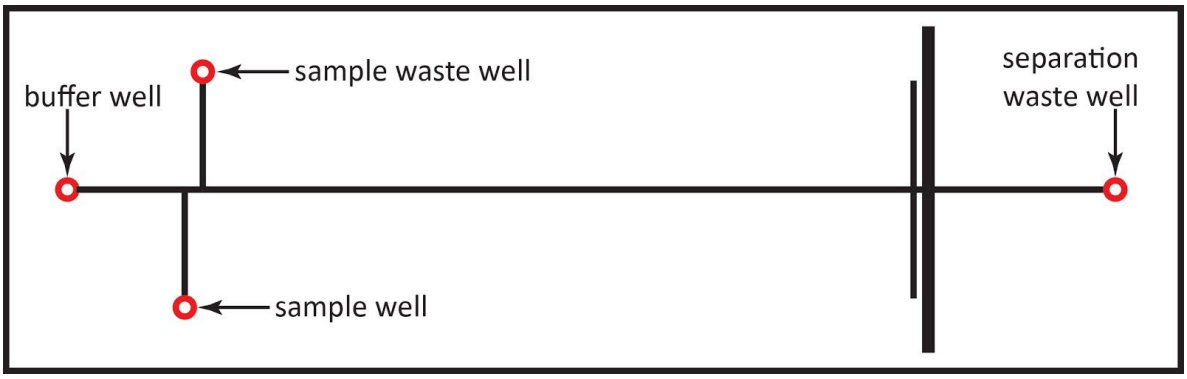

Figure 3.1: *Illustration showing the locations of the four wells in the device.*

Table 3.1: *Table describing functions of the four wells in the device.*

Well	Function
Buffer well	Held extra buffer solution
Sample well	Held the sample to be analyzed
Sample waste well	Collected excess sample after it had flowed from the sample well during the load step
Separation waste well	Collected waste sample after it had flowed down the separation channel and across the detection electrode during the separation step

3.2 Separation Channel

The separation channel, shown in figure 3.2, is approximately 55mm long, 50µm deep, and 50µm wide. This is the length of the channel past our sample well that will allow the different analytes to separate based on electrophoretic mobility, or how quickly they move in an electric field.

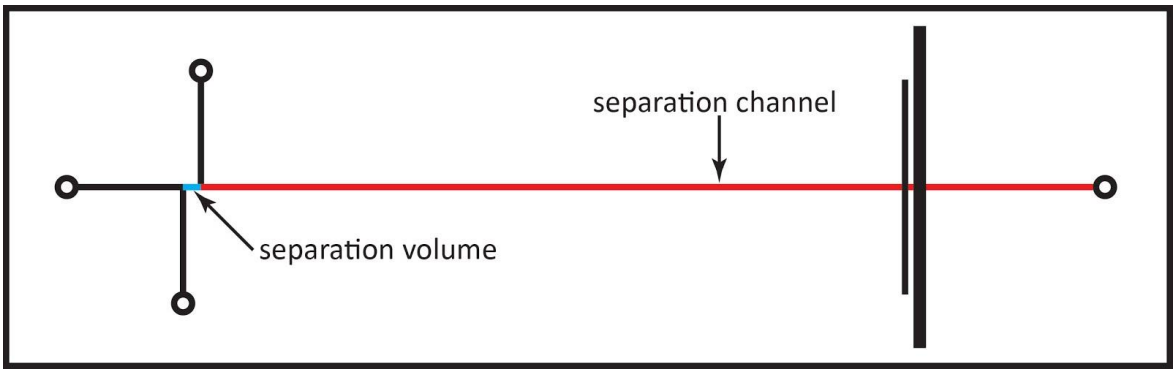

Figure 3.2: *Illustration showing the location of the separation channel and our sample volume.*

3.3 Decoupler

The decoupler, shown in Figure 3.3, was made of 0.025 mm diameter palladium wire. Palladium was chosen for its excellent electrical conductivity. We use a decoupler to electrically separate our grounding voltage from our carbon paste electrode. By placing the decoupler in front of the electrode, we make sure that any effect that the electric field used in electrophoresis could have on the electrode is mitigated. We did some tests to measure current with the decoupler placed behind the electrode, and the results prove that the decoupler placement is key. See figure in Appendix E.

Figure 3.3: *Illustration showing the location of the decoupler.*

3.4 Carbon Paste Electrode

The carbon paste electrode, shown in figure 3.4, was the detection mechanism for the analytes once they had electroosmotically separated during their flow down the separation channel. It was composed of graphite powder, multi-walled carbon nanotubes, mineral oil, and PDMS. See appendix D for specific composition.[1]

Figure 3.4: *Illustration showing the location of the carbon paste electrode.*

3.5 Micelles

A small concentration of sodium dodecyl sulfate (SDS) can be added to our sample to separate the two structural isomers that we are quantifying, octopamine and dopamine. SDS forms micelles, or small spherical aggregates of around 60 monomers, in polar solutions. Dopamine and octopamine have different polarities due to their chemical structure, which causes them to have different binding affinities to the SDS micelles. The analyte that has a stronger affinity to the micelles will move more slowly down the separation channel, and so will show up as a separate peak in our amperometric detection step at the electrode.[7,8]

3.6 Electrochemical Analyzer

The electrochemical analyzer was used as our detection monitoring system. The device we used was the CHI800D model. While the electrochemical analyzer can be used for multiple applications, we chose to use it for two main techniques: cyclic voltammetry and amperometric detection. Cyclic voltammetry was used to determine that our electrode was functional. The main technique used was amperometric detection. Amperometric detection measures the current versus the time. As previously stated, when neuromodulators flow past the electrode, there is a change in current that is detected, with a different current peak for each neuromodulator. We are then able to determine the concentrations of the different neuromodulators by measuring the current peaks. We calculated the motility of each neuromodulator to determine which current peak belonged to each neuromodulator. This technique is highly sensitive, which is what we were looking for in our device.

3.7 High Voltage Sequencer

We use a high voltage sequencer in order to move our sample through the device. We have four leads coming from the sequencer: one for the buffer well, sample well, sample waste well, and one connected to the decoupler. We used platinum wire for the electrodes that were in the wells while the lead for the decoupler was connected directly. Voltage was applied to the device which in turn created electroosmotic flow. The neuromodulators were separated based on their charge. Positive molecules travel the fastest followed by neutrally charged molecules and negatively charged

molecules. Electroosmotic flow is preferred to pressure-driven flow because the neuromodulators move in a “plug-like” profile while pressure driven flow moves the neuromodulators in a parabola like profile. The electroosmotic flow will give our device a higher sensitivity and better time resolution.

4 System Integration, Test

4.1 Fluorescent Test

Before we jumped straight into measuring bioanalytes through amperometric detection, we wanted to make sure that the fluid would flow in the correct direction. To determine this, we ran our experiment with fluorescein-dextran, figure 4.1, so that we could visually see the fluid flow through the channel.

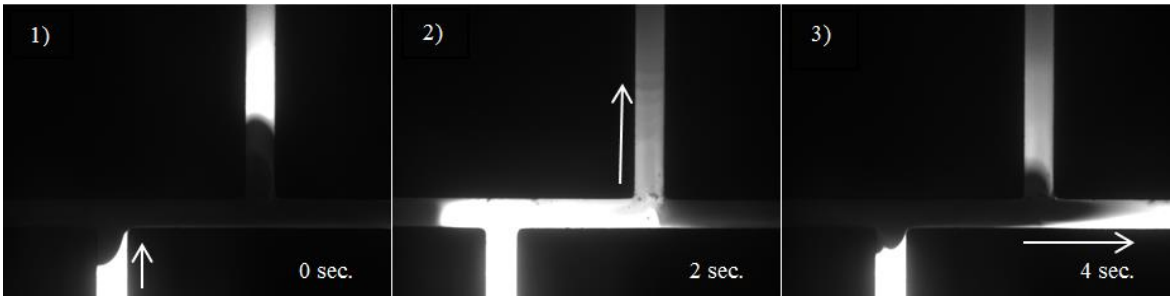

Figure 4.1: *Fluorescent 40x magnification images of double T sample injector filled with fluorescein-dextran by EOF. (1-3) Right leads to CE separation channel, top leads to sample waste reservoir, left leads to buffer reservoir, and down leads to sample reservoir.*

4.2 Cyclic Voltammetry

We also wanted to determine that our carbon paste electrode would be able to detect the bioanalytes through a change in current. We checked this first through cyclic voltammetry. The results of that experiment our illustrated in figure 4.2.

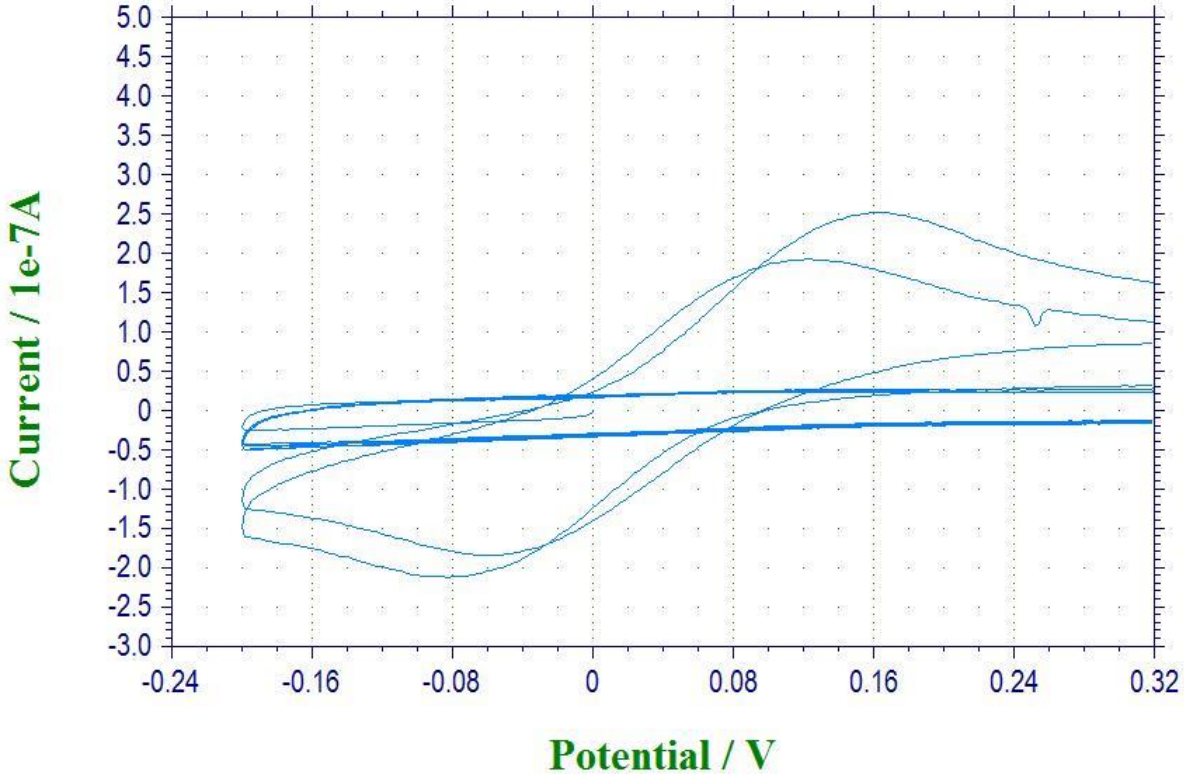

Figure 4.2: *CV conducted for our carbon paste electrode. Working electrode was the carbon paste, counter and reference electrode were the Pd wire decoupler. The straight lines around 0 microamps show when the solution only consisted of the buffer. When a drop of 1 mM dopamine was added to the solution, the current was able to fluctuate between around 0.2 and -0.3 microamps.*

4.3 Amperometric Detection

4.3.1 Pressure Driven Flow

We checked that our carbon paste electrode could also detect the bioanalytes as the fluid flowed through the channel. This was done through amperometric detection using pressure driven flow from a syringe at the buffer well. The results are shown in figure 4.3.

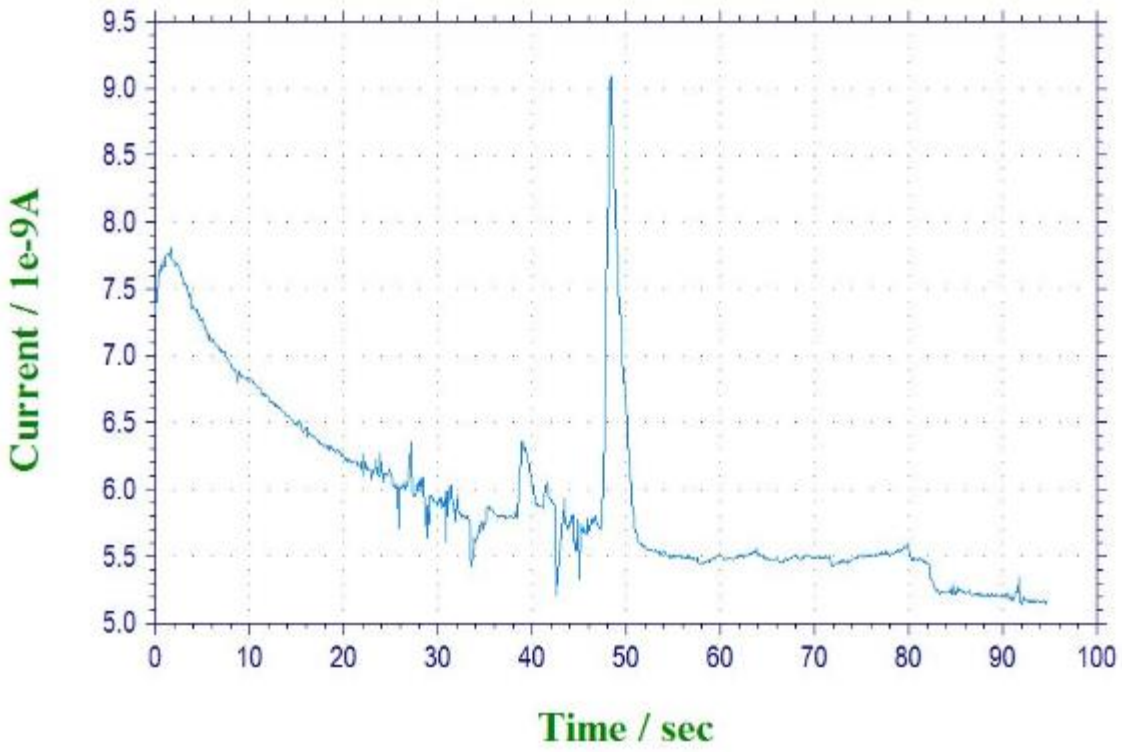

Figure 4.3: *Amperometric detection of 1mM dopamine solution using electroosmotic flow for the load step, and pressure driven flow for the separation step.*

4.3.2 Spiked Dopamine Runs

Now that we were able to confirm that our fluid was flowing in the correct direction and that our carbon paste electrode could detect these bioanalytes, we experimented using amperometric detection with electroosmotic flow. Figure 4.4 and figure 4.5 show our experiments using a 1 mM spiked dopamine solution.

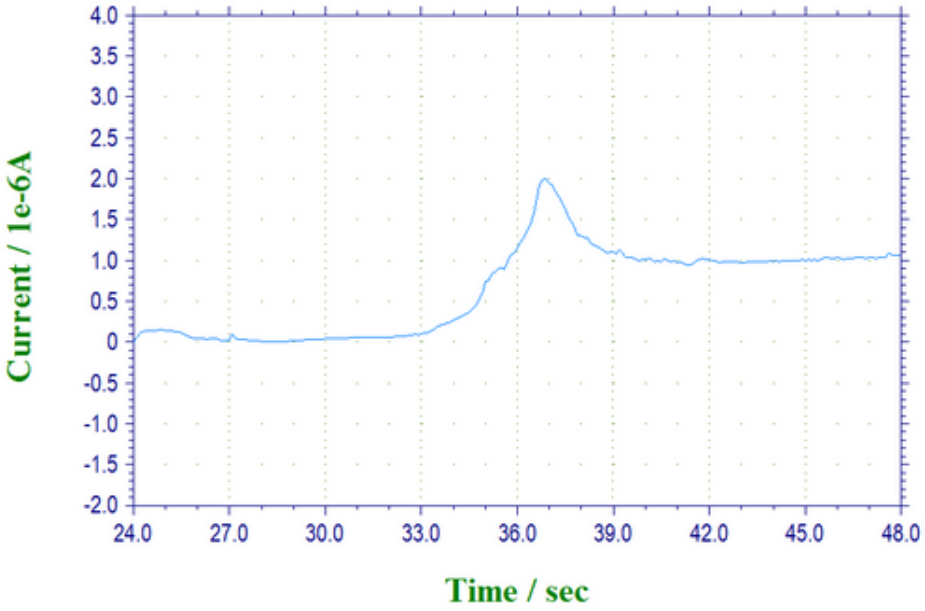

Figure 4.4: *Amperometric detection of 1mM dopamine solution using electroosmotic flow for the load and separation step. Note the wide sloping peak, and the uneven fall off in current after the analyte has passed. Most likely caused by current continuing to leak from the separation well, causing uneven flow of analyte down the channel.*

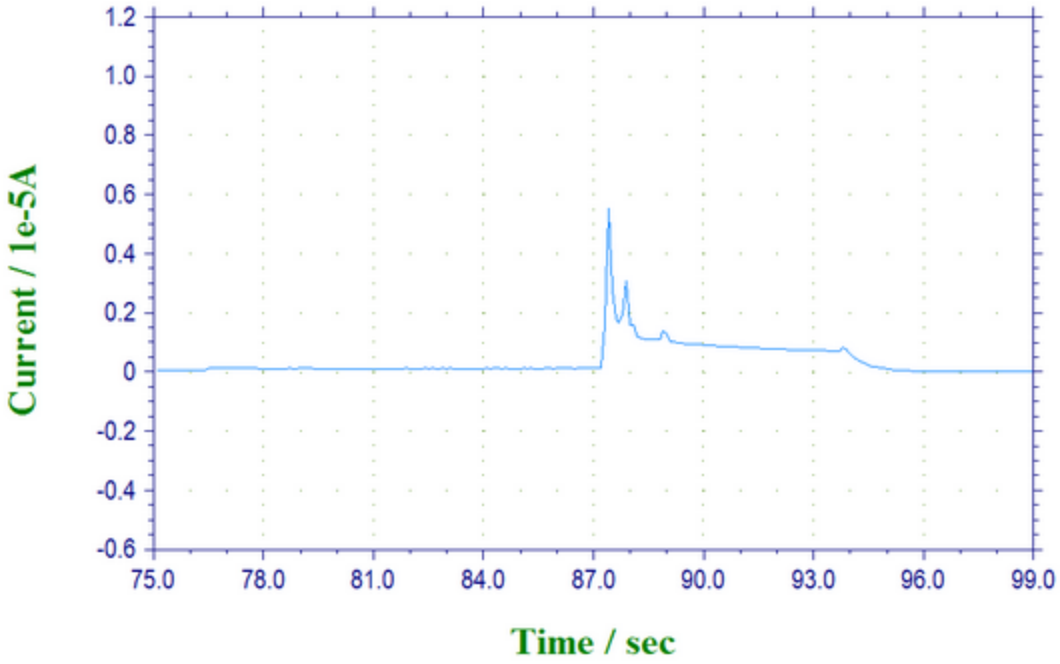

Figure 4.5: *Amperometric detection of 1mM dopamine solution using electroosmotic flow for the load and separation step. This steep peak shows the desired increase in current, but the other smaller spikes after the large one are also from dopamine (the only analyte in the solution), and are most likely caused by a faulty separation step.*

4.3.3 Catechol Run

We also tried our experiments with catechol instead of dopamine and got similar results.

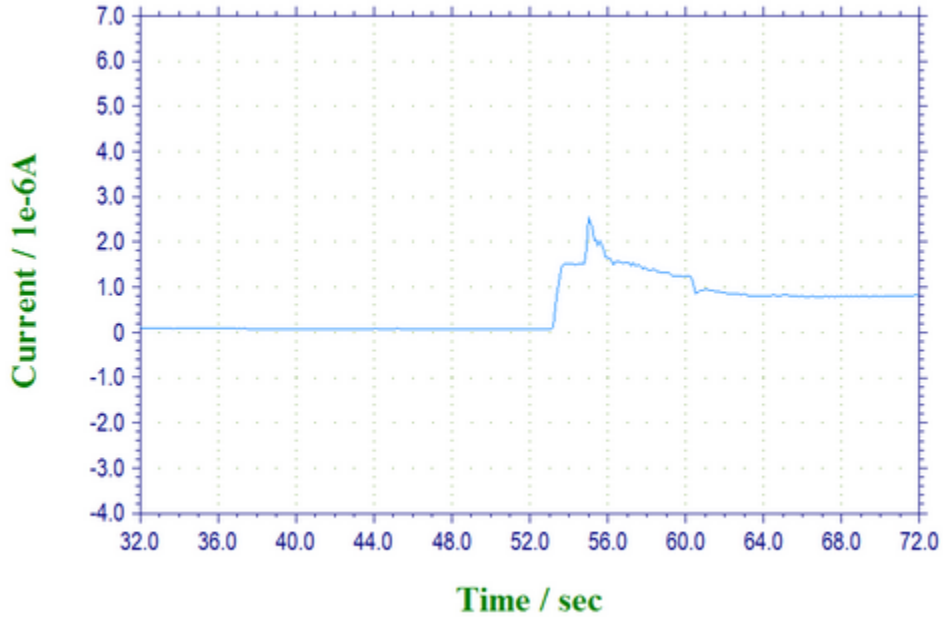

Figure 4.6: *Amperometric detection of 1mM catechol solution using electroosmotic flow for the load and separation step. The sharp increase in current shown is what we expect, however the slow fall off could indicate a faulty separation step as well. Note how the catechol peak occurs around 22 seconds after the separation step, while the dopamine runs took between 12 and 14 seconds. This is expected, because catechol is neutral at this pH, and therefore has a lower electrophoretic mobility.*

4.4 Platinum Node Test

In order to determine that our platinum nodes were all working the same, we ran some current tests through our device to measure the currents that each platinum node was giving off as well as receiving. Figure 4.7 illustrates the result of one of these tests. We did the same experiment on all the platinum nodes and got similar results.

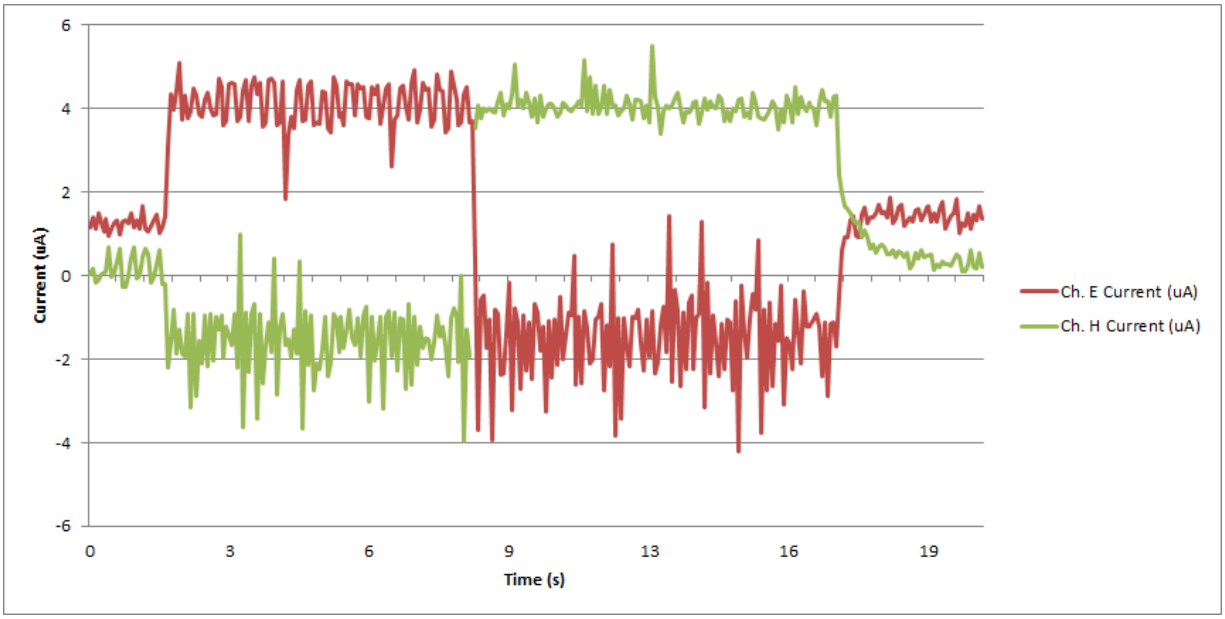

Figure 4.7: *We had one electrode in the sample well (ch. E) and another electrode in the sample waste well (ch. H). We applied 600 V to ch. E and 0 V at ch. H. We then reversed the voltages around 8 sec. We did this test multiple times and swapped different platinum nodes for each test. Each test gave similar results.*

4.5 Known Resistor Voltage Test

When checking our voltage sequencer to see if it was giving off the correct current, we connected our instrument to a known resistance of 30 MΩ. We ran multiple voltages through the circuit and measured the currents that the voltage sequencer instrument software gave us. We compared these to the theoretical values. The difference between the two values versus the voltage is shown in figure 4.8.

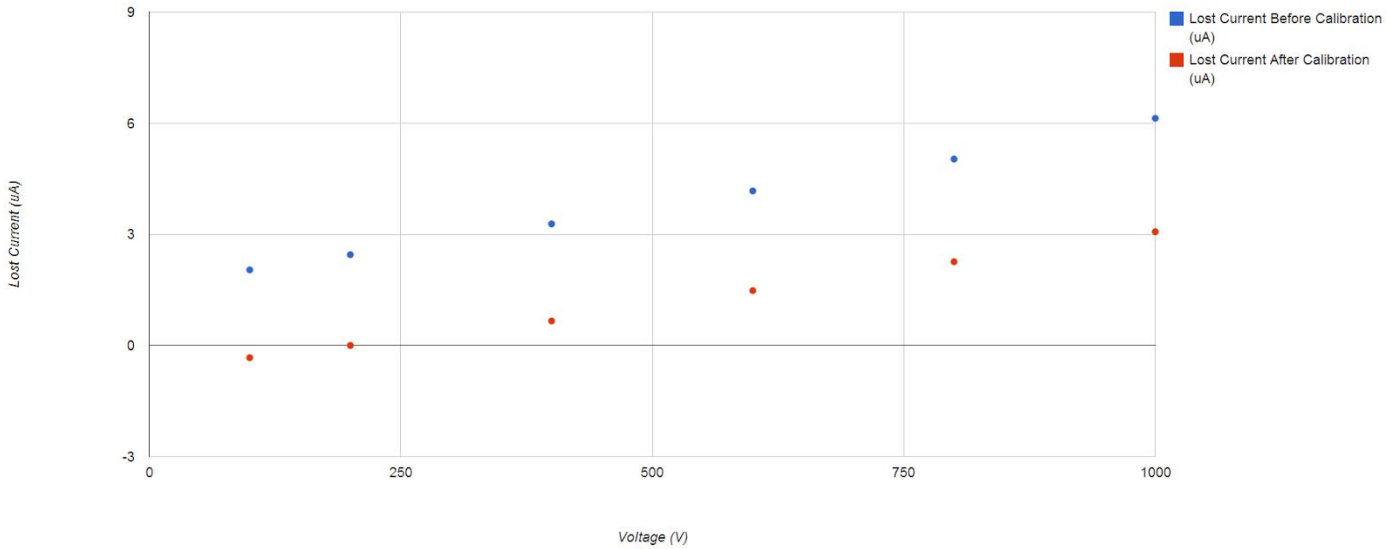

Figure 4.8: *We connected our high voltage sequencer to a known resistance of 30 MΩ and measured the current at different voltages. We compared that to the current that we expected.*

5 Discussion

The first experiment that we ran with our device was to use fluorescent dye instead of our crab sample to verify that our fluid was travelling in the correct direction. As shown in figure 4.1, we did get the fluorescent dye to flow in the correct directions for the load step and separation step. We then moved on to amperometric detection through electroosmotic flow of a stock solution of 1 mM dopamine. Unfortunately, we were not able to get very favorable results. We were able to get the load step to work almost every time, but the separation step was inconsistent. There were a few experiments which gave potential dopamine peaks, but there was either so much noise that we could not say with certainty that the peaks were a result of the dopamine being detected at the electrode, or the current would increase quickly and then decrease slowly, which was most likely a result of dopamine leaking into the separation channel from the other channels.

We hypothesized that the potential problems with our device could be with the carbon paste electrode, the platinum nodes, or the voltage sequencer. In order to determine that the carbon paste electrode could detect bioanalytes we first performed cyclic voltammetry (CV). Figure 4.2 shows that when dopamine was added to the solution during CV, the stereotypical "anode" and "cathode" peaks were present, showing at which current the dopamine molecules were getting oxidized and reduced respectively. This tells us that our electrode is capable of holding detecting the current change when these two phenomena occur.

We could then conclude that our carbon paste electrode was able to detect the dopamine. We also wanted to verify that the carbon paste electrode could detect dopamine as it was flowed over it. We tested this by doing a pressure driven flow test. We conducted the load step as we normally would using electroosmotic flow, but instead of using electroosmotic flow for the separation step, we used a syringe at the buffer well to push the sample down the separation channel and past the carbon paste electrode. Figure 4.3 shows the results of this test.

With the positive result we were able to conclude that carbon paste electrode was not the problem. We then tested the platinum nodes to see if those could be the problem. To do this we used sandpaper to rub off any residue that could be on the platinum wire and then immersed them in ethanol. The load step was performed with each of the platinum wires to determine if there was any difference between them. Figure 4.7 shows the current that was detected for one of the tests, and the other test give similar results. This led us to conclude that since all of the platinum nodes acted the same, they were not the problem. The last component we tested was the voltage sequencer. A known resistor of 30 MΩ was connected to the voltage sequencer and the current was measured at different voltages. Figure 4.8 shows that we determined a false positive current coming from the voltage sequencer. The voltage sequencer is part of the problem, and we will continue to test other components to determine if there are other problems.

Some improvements that we will incorporate to our project in the future is first to try our experiments with a different voltage sequencer to determine if that solves our problems. If that does solve our problems, we will move forward with detecting dopamine using amperometric detection and eventually use a hemolymph sample in our device that separates multiple bioanalytes. If it does not solve all of the problems, we will attempt to change the design of our device. There will be another group next year that will continue this project, and we intend to leave them with the knowledge necessary to address these issues.

6 Cost Analysis

A large budget is not needed to make our device (refer to appendix C). In actual practice, our device costs even less to make than what the budget illustrates because hundreds of devices can be made using our budget. This accomplishes our goal of making an inexpensive device. The only drawback is that in order to detect the neuromodulators using our device, a high voltage sequencer and an instrument that uses amperometric detection is needed. These two pieces of equipment can be very expensive with them costing over $10,000 each. However, while it may be a large investment at the beginning, we could amortize the cost of the equipment.

7 Engineering Standards and Realistic Constraints

7.1 Ethics

Ethics is an important topic to look at when creating an invention. Our team is acting ethically when it comes to our project because our project is for the purpose of making a better world and there are no materials or resulting devices that cause harm to anyone. The only ethical concern that people might have is that we use crabs for our experiment, and there have been some studies that show that crabs could feel pain.

One big issue with animal testing is whether or not the animals are sentient. Sentience is a difficult term to define, but it's generally understood to mean that the organism can distinguish itself from others. It is unknown as to when a nervous systems becomes complex enough to be known as 'self-aware'. Generally, most mammals and some birds are considered sentient, but the invertebrate world is not generally considered to possess this attribute. There have been some studies that allow for the possibility of cuttlefish and octopi being sentient, but given our current knowledge, we don't think that insects and worms, or the crabs that we use for our samples, are self-aware. Whether or not the crabs feel pain however, is another issue entirely.

According to a few studies (one most recently Dr. Elwood at the Queens University Belfast in the UK), crabs exhibit a type of avoidance behavior that has been associated with feeling pain.[13] During Dr. Elwood's study, crabs were given mild shocks when they entered one type of shelter over another, and after repeated trials, the crabs were far more likely to avoid the shelter types that had shocked them and would prefer to seek shelter elsewhere. A similar test was done on mice, and the evidence gleaned from these experiments supported the case that mice do feel pain, prompting many quality-of-life changes to allowed testing procedures. While not being completely harmless, our procedure is designed to be quick and effective, and gives the crabs time to get used to the dialysis probe before starting to take samples. We also attempt to numb the crab by placing them in cold water and ice for almost 20 minutes before performing our surgery. We do have to poke a small hole through the shell in order to insert the probe however, and this approach may cause trauma to the animal. If this procedure causes the crabs too much pain, we might need to change the way that we get samples, or chose another sample organism outright.

After we acquire enough data to establish what the baseline (normal state) concentrations of these neuromodulators are, then large changes in certain hormones that we are testing for might indicate that we are causing too much pain. Norepinephrine (NE) and dopamine (DA) for example, are two of our analytes that we could use to determine our impact on the crabs. Norepinephrine is a stress hormone that is responsible for the fight or flight response, as well as increasing heart rate and blood pressure. Dopamine has many tasks, but is the hormone that is most responsible for the sensation of pleasure. A steep increase

in NE, or a precipitous drop in DA concentrations could indicate that we are causing too much trauma to our subject.

If that is the case, we could attempt to further numb the crabs with general anesthesia before inserting the probe into their shells. This would be more expensive (on account of the need to purchase the drug), and would most likely ruin any data that we would attempt to acquire over the next few days, as the anesthetic would take time to break down inside the organism. If this doesn't work, we could always just take the frozen hemolymph samples from the physics department after they have already 'sacrificed' the crabs for their neurobiology research. Unfortunately this method would severely limit any post detection analysis that we could conduct because all of our data would be from dead crabs, preventing us from seeing any changes in neuromodulator concentrations due to altered environments.

7.2 Social

We have an ethical duty to our potential users to make the device safe to use. If we start to take advantage of our user's welfare then we have gone too far and need to rethink what we are doing. There is always some sort of ethical responsibility that a company has for using a product or service. Even though the customer is using it, there is still the responsibility of the company to not sell them an unethical device. Now if the user starts to change the manufactured settings, then it becomes primarily the user's responsibility. Our device does not have any unintended consequences. It detects concentrations of different bioamines. If someone decided to change it and detect concentrations of something else, we see no problem with that in itself.

7.3 Environmental

When creating an invention, it is important to consider if the device itself will be environmentally friendly. Our device is created out of PDMS which, while non-biodegradable, does not have any harmful effects towards organisms. In fact, it is in some fast food products.

7.4 Health & Safety

There are two main risks pertaining to our project: shocking ourselves with the voltage sequencer and accidentally sticking ourselves with a needle. In order to ensure safety while using the voltage sequencer, we announce to everyone around that we are going to turn on the sequencer so that everyone knows not to touch the leads. Also, we make sure to only touch one lead at a time so as not to accidentally complete the circuit if the sequencer happened to be on. With the needles we just make sure to never point them towards anybody, and to constantly keep them covered when not in use. We also use low

concentration sodium hydroxide to clean our device between runs, and 98% ethanol to remove dust particles from our working surfaces. Other than those risks, there are not any other concerns because aside from the sodium hydroxide, our materials are not bioactive.

7.5 Economic

When thinking about who the consumers of our product would be, we came to the conclusion that it would most likely be people in the developing world. As a result, we wanted to make our device affordable to that type of consumer. The materials that we chose were a big factor because we did not want them to be really expensive. We chose to use PDMS because it is affordable and has been known to be used for similar applications. We also had to consider the other instruments that would need to be used in addition to our device. Unfortunately, the voltage sequencer and electrochemical analyzer are not very affordable, but that is a challenge for all microfluidic systems.

7.6 Aesthetics

Our design is more for the functionality than the aesthetics, but our device design (Figure 2.2) is a very simple and symmetrical device. It is small and does not require very much skill to use. The look of the device is not the most attractive thing, but as we make progress we can make a shell for the device so as to make it more appealing and look simpler. But one thing that does make our device aesthetically pleasing is its simple, straightforward design in performing a complex task: the detection of bioamines. The approach we used is aesthetically impressive because it is an elegant and sensitive solution to a problem traditionally solved with larger, more expensive technologies.

8 Summary

We are involved in a joint project with the physics department and chemistry department that is studying the effects of neuromodulators common to the crab *Cancer borealis*. We aimed to create a device that had a temporal resolution, which in turn would provide an accurate baseline of neuromodulators in the crab. Our finished device seeks to have fast detection, be highly sensitive, require small sample volumes while maintaining affordability. The use of PDMS, microchip capillary electrophoresis, carbon paste electrode, and MEKC are all components that will help to achieve that goal. The new accomplishments that we achieved this year were the following:

- Established the PDMS-PDMS bonding procedure.
- Created multiple channel and electrode master molds using photolithography techniques, which was much cheaper than ordering them from Stanford.
- Attained successful fluorescent dye test showing the flow of the fluid in the correct direction.
- Successful pressure driven flow test, showing that we can detect bioanalytes using amperometric detection with small sample volumes.

These new accomplishments will help the next group who takes on this project to truly focus on figuring out the amperometric detection with electroosmotic flow with our device. Once that can be accomplished, more extensive tests will be feasible with multiple bioanalytes and eventually hemolymph sample from the crab *Cancer borealis*. This will allow for a baseline of neuromodulator levels to be established, allowing researchers to see how different stimuli affect these neuromodulator levels. Eventually, this device could potentially be used for similar tests in humans.

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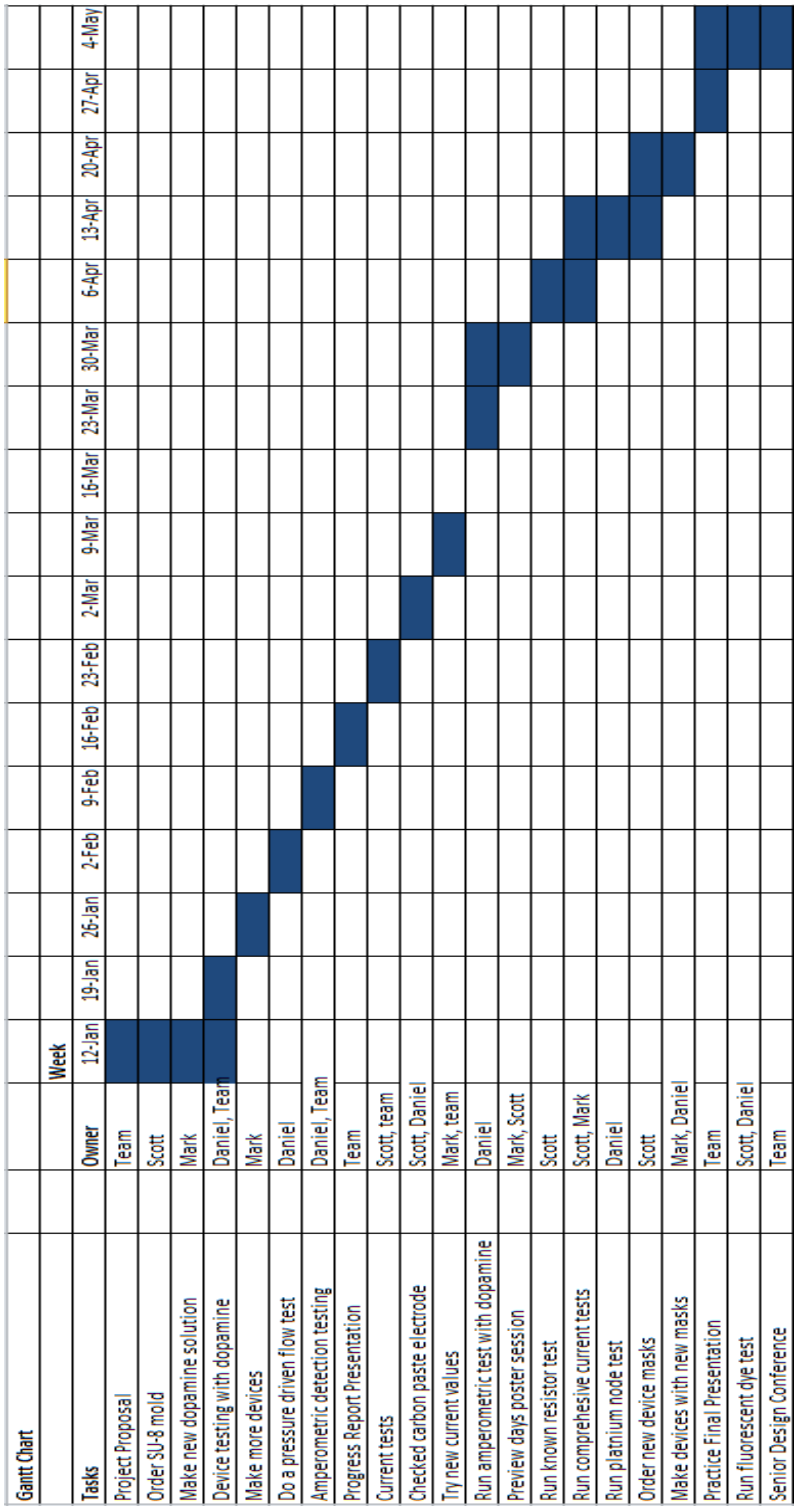
10 Appendices

Appendix A: Gantt Chart



Gantt Chart																								
			Week																					
Tasks		Owner	12-Jan	19-Jan	26-Jan	2-Feb	9-Feb	16-Feb	23-Feb	2-Mar	9-Mar	16-Mar	23-Mar	30-Mar	6-Apr	13-Apr	20-Apr	27-Apr	4-May
Project Proposal		Team																	
Order SU-8 mold		Scott																	
Make new dopamine solution		Mark																	
Device testing with dopamine		Daniel, Team																	
Make more devices		Mark																	
Do a pressure driven flow test		Daniel																	
Amperometric detection testing		Daniel, Team																	
Progress Report Presentation		Team																	
Current tests		Scott, team																	
Checked carbon paste electrode		Scott, Daniel																	
Try new current values		Mark, team																	
Run amperometric test with dopamine		Daniel																	
Preview days poster session		Mark, Scott																	
Run known resistor test		Scott																	
Run comprehesive current tests		Scott, Mark																	
Run platnium node test		Daniel																	
Order new device masks		Scott																	
Make devices with new masks		Mark, Daniel																	
Practice Final Presentation		Team																	
Run fluorescent dye test		Scott, Daniel																	
Senior Design Conference		Team																	

Appendix B: Risks and Mitigations

Table B.1: *Risks and Mitigations*

Risk Mitigation		
Risk	**Consequences**	**Solutions**
Shock by voltage sequencer	Neural injury and potential death	Never touch two leads at the same time
Sticking yourself with a needle	Infection	Be careful when working with needles
Working with NaOH	Exposure to strong base burn	Wear gloves when working with it

Appendix C: Budget

Table C.1: *Budget*

Item	Use	Amount	Amount	Actual
PDMS Mix	Forms Device	5 x 0.5 kg	$216.00	$327.00
Palladium decoupler (.05 mm)	Disrupts Applied Potential	2 m	$326.00	$271.00
Epoxy	Secures wires/insulation	8 pack	$52.00	$0.00
Scalpel	Cuts device to size	2 pack	$20.00	$8.00
Tubing	Used during testing	1 m	$8.00	$0.00
Pipet tips	Used as the well		$16.00	$34.00
Weigh boats	Used to mix chemicals	200 pack	$30.00	$0.00
Hole Punch	Forms well for injection site	2	$30.00	$0.00
Masks	Design mold	3	$100.00	$95.00
Tape	Removing excess carbon paste	12 rolls	$24.00	$0.00
Maintenance			$300.00	$0.00
Shipping			$50.00	$0.00
Stanford mold	Mold to make devices	1		$301.00
Cleanroom wipes	No lint wipes to wipe up liquid	300 pack		$16.00
TOTAL			$1,172	$1,052

Appendix D: Fabrication Procedure[14,15]

Device fabrication takes place in several steps.

Mold Fabrication

Blue film mold. For our electrode side slab, we used a mold that we created ourselves. By using the mask from Dr. Abbyad's mold, we were able to print 50μm blue film onto a glass slide, that we then developed using UV light using the following procedure:

1. Cover one side of a 3"x2" glass microscope slide with 50μm UV-sensitive blue film, making sure that there are no bubbles present.
2. Fold a piece of regular printer paper in half and place the glass slide inside the folded halves. Run the paper and slide through a heat laminator (speed 6 for our laminator).
3. Carefully align the mold mask on top of the blue film and expose with UV light for three seconds.
4. Develop in 1% potassium carbonate solution for about 5 minutes by submerging the mold in the solution and then vigorously squirting the the surface of the mold with a pipette. Pay close attention to the amount of undeveloped blue film on the surface, and stop developing immediately once it has all been dissolved.

For the other side of our device, the channel side, we used a mold that we bought from Stanford. The longer, thinner channel gave us much more trouble, and we would often over or under develop our mold, and be forced to start over. By ordering a SU-8 mold from Stanford, we added a little cost to our project, but removed a large source of error in the fabrication of our device.

PDMS Curing

After our molds are created, we measure out silicon base and binder in a ratio of 10:1, allowing for approximately 20g of PDMS per device we are trying to make. Before pouring the uncured PDMS onto the mold, we make sure to vigorously mix the base and binder together. Once the PDMS is poured onto the mold, we place the mold into a vacuum desiccator to pull out the air bubbles that are dissolved in the uncured PDMS before it hardens. After about an hour in the desiccator, we transfer the mold over to the 78º oven for 4 hours to completely cure it.

Device Prepping

After the PDMS has cured in the oven, we cut out the slabs from the extra PDMS and pull them off of the mold. We then make the carbon paste electrode by taking a drop of the uncured PDMS from the mixing bowl and an equal weight of mineral oil in a dish. Graphite powder and MWCNT (multi-walled carbon nanotubes) are then added in a 10:1 ratio to equal the mass of the mineral oil and uncured PDMS together. This is then mixed very well until it is of even consistency.

Then we screen print our electrode slot with the carbon paste mixture, ensuring that only the correct amount of paste is on our device by pulling excess layers off with strips of Scotch tape.
Whenever our slabs are not in use, we cover the printed side with a layer of scotch tape to prevent dust from getting caught in our channel.
After our electrode has been printed, we need to insert our palladium decoupler into the decoupler slot. To accomplish this, we carefully cut into the decoupler slot along the edge of the device with a razor blade, and then pull the wire into these slots and around the back of the device, taping it off at both ends.

Bonding[16]

After the decoupler has been secured inside its slot, we bring the two unbonded slabs to the plasma bonder. We punch 1mm holes through each of the wells to allow for connections to the outside of the device once the pieces are bonded together. We then remove the protective tape and place the slabs face up into the plasma bonder.
In order to secure a good vacuum and good plasma, we turn on both the pump and the plasma cleaner at the same time, and then do not turn the actual plasma on until the pressure inside the chamber is below 300 mtorr. When this threshold is passed, we turn off the vacuum and turn the plasma on high, allowing a small inlet to form, increasing the quality and volume of the plasma (displayed by a rosy pink color), which we maintain for 50 seconds. We then turn off the plasma and the plasma cleaner, and open the door after the vacuum has been released.
The two sides of the device are firmly pressed together and then this process is repeated to bond a glass slide to the back of the device, adding structural support.

Appendix E. Current without Decoupler

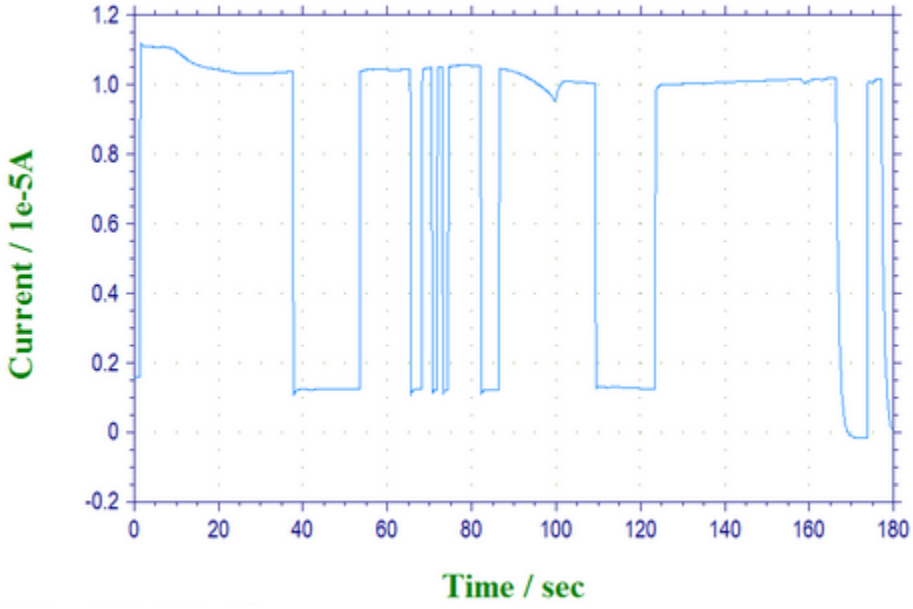


Figure E.1: *Amperometric detection step of purely buffer solution using a device without a decoupler. The two step level currents are purely generated by the field present in the load step (the lower currents) and the separation step (the higher currents). This shows the necessity of having a decoupler before the electrode.*

Appendix F: Material Sources

Table F.1: *List of the instruments and materials we used from other suppliers.*

Item	**Supplier**	**Item Number**
Electrochemical Analyzer	CH Instruments	CHI800D
Voltage Sequencer	LabSmith	HVS448-6000D
Palladium (decoupler)	Goodfellow	PD005113
Pipette tips (wells)	Fisher Scientific	02-707-447
Photolithography mask	CAD/Art Services	N/A