MICROFABRICATION OF BIO-ANALYTICAL DEVICES: MICROELECTRODE ARRAY AND TRAVELING-WAVE ELECTROPHORESIS

by

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ABSTRACT

Microfabrication of Bio-analytical Devices: Microelectrode Array and Traveling Wave Electrophoresis

by

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This thesis offers an in-depth look into the fabrication processes used to create two distinct analytical tools capable of examining biological components at a microscopic level. The microelectrode array is an electrochemical device that was designed to be capable of testing up to six components in a biological sample simultaneously inside of a microfluidic system. The array offers a quick analytical tool to examine electrochemical components within any given aqueous sample.

Through the use of techniques learned during the fabrication of the microelectrode array, electrodes for the traveling wave electrophoresis system were created and incorporated into the analytical system. Traveling wave electrophoresis is the process by which particles can be transported and separated inside a microfluidic system. The microelectrodes created were aligned along the top and bottom of a microfluidic channel in order to simulate a voltage wave capable of propelling charged
particles down the channel at a programmable wave speed. The fabrication process to create such a device is outlined in detail.

(119 pages)
PUBLIC ABSTRACT

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Neil Draper

The need for potable water is increasing with the ever-increasing world population. Further development of fast, portable, and cost effective analytical tools is necessary in order to create diagnostic techniques capable of supporting the water needs of the world’s population. Within the last decade microfluidics and Lab-on-a-Chip (LOC) technologies have increased the portability and speed of detection for aqueous samples. Photolithography techniques serve as a cost effective fabrication tool to create LOC electrodes on the micron scale.

An in-depth look at the fabrication process is undertaken in this paper in order to further the development of micro-scale detection techniques. An electrode array capable of detecting multiple targets within one aqueous sample was designed and fabricated. The electrode array was assessed for performance characteristics to determine if reproducibility is possible. The fabrication process was also detailed for a new chemical separation technique, traveling-wave electrophoresis (TWE). TWE could serve as a separation tool capable of separating out specific charged molecules for biological and chemical samples. The TWE device was assessed on the capabilities to move charged molecules.
ACKNOWLEDGMENTS

I would like to thank Dr. Anhong Zhou for his mentorship, leadership, and continued support throughout the many stages of my educational career. He has been both a motivator and a comforter during my time working on my thesis. I would also like to thank Dr. Boyd Edwards for giving me a wonderful opportunity to expand my horizons in Champaign Illinois and for serving as my mentor during my time under his direction. The understanding and advice from Dr. Charles Miller concerning the graduation process was also beneficial to me.

I am grateful for the financial support from the Utah Water Research Laboratory for the projects that were completed under the direction of Dr. Anhong Zhou. I also want to express my gratitude for the financial support of the National Science Foundation during my work in Illinois under the direction of Boyd Edwards.

I would like to thank my colleagues who worked by my side in the Molecular and Cellular Sensing and Imaging Lab at Utah State University and Brian Baker for his help training me in the microfabrication process. I would also like to thank Aaron Timperman for his help and direction at the Construction Engineering Laboratory in Champaign Illinois as I worked in conjunction with the Army Corps of Engineers.

Lastly I would like to make a special acknowledgment to my family for all the support and patience they have shown me during the difficult times I have had in completing my projects. They always gave me the emotional support and confidence that I needed to press forward and encouraged me to be the best version of myself.

-Neil Draper
FOREWORD

My thesis research offered me an opportunity to work in several different locations with a variety of organizations, and in an assortment of laboratories. The foundation and basis of my research was performed under the direction of those associated with Utah State University and all of my research opportunities outside of Utah State University tie back to professional connections associated with the university. I have had the opportunity to work in two different labs during my undergraduate career at Utah State University, but for the purposes of my graduate research I performed all of that work in the Molecular and Cellular Sensing and Imaging Lab under the direction of Dr. Anhong Zhou.

While working under the direction of Dr. Anhong Zhou, I had the opportunity to study, develop, and use a multitude of biological sensors and technologies including surface plasmon resonance, electrochemistry, fluorescence microscopy, fluorescence spectroscopy, flow cytometry, and atomic force microscopy. In an effort to improve and develop better biological sensors I was able to work at the University of Utah learning nano- and microfabrication techniques in the Nanofabrication Lab under the direction and guidance of Brian Baker. Working at the University of Utah in the nanofabrication lab allowed me to learn photolithography techniques that were essential to all of the projects that I worked on during my graduate research.

During the course of my research creating and fabricating a microelectrode array at Utah State University and the University of Utah, I was brought into contact with Dr. Boyd Edwards who had interest in collaborating with my major advisor, Dr. Anhong
Zhou. The project that Dr. Edwards was working on had need of someone with knowledge and experience with photolithography to perform the experiments the project required. Dr. Edwards offered me the opportunity to go live in Champaign Illinois and work with a former colleague and collaborator of his, Dr. Aaron Timperman.

In Champaign Illinois I worked under the direction of Aaron Timperman who was working for the Army Corps of Engineers. I got the opportunity to work inside the Construction Engineering Research Lab, which is one of seven national labs associated with the Army Corps of Engineers. The facilities at the Construction Engineering Research Lab didn’t allow for the photolithography that needed to take place for the success of the project, so photolithography work in Illinois was performed at the University of Illinois in the Micro and Nano Technology Lab. Also to further my education and complete the requirements for a master’s degree, I took one class at the University of Illinois.

I feel incredibly privileged to have been presented with all of these opportunities. I feel as though these varied experiences will allow me to bring a unique perspective to all of my future endeavors. I hope that those who follow after me may also have the chance to work in a variety of locations with several different institutions as I have learned a lot from my experiences.
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CHAPTER 1

PROJECT INTRODUCTION

1.1 Motivation

There is a persistent need for potable water with the ever increasing world population. Given this driving force for potable water, fast, cheap, effective, and portable methods for the detection of contaminants in an aqueous sample are continually being developed [1]. Many diseases found in the world today are often caused by bacteria, whose DNA are specific to the given species to be infected. For this reason, fast and inexpensive ways to detect specific sequences of bacterial DNA can lead to a reduction of illness by identifying which strains are harmful to which specie of animal. Electrochemical techniques have proven to be effective in the differentiation of DNA strands down to the single base pair mismatch level [2-4]. Paired with an ability to give an almost instantaneous response, electrochemistry proves to be a significant target in the development of DNA detection systems as well as other potential water contaminants [5-7]. Using electrochemistry and microfabrication techniques, disposable microelectrode arrays can be designed and tested for accuracy and repeatability in order to prove the viability of electrochemical systems to analyze contaminants such as DNA or heavy metals. The integration of these electrode arrays into a microfluidic system would allow for sample manipulation and increase the portability of a detection method [8].
1.2 Overview and Solution

1.2.1 Traveling-Wave Electrophoresis

Traveling-wave electrophoresis, TWE, is an interesting analytical chemical separation technique for microfluidic device fabrication purposes. TWE utilizes a tunable potential wave to propagate charged particles down a microfluidic channel [9]. As the frequency of the potential wave is tunable, TWE allows for specificity within any given separation. TWE separates charged particles based on the inherent electrophoretic mobility of the charged molecules within the system.

When two charged particles each have a different electrophoretic mobility, a potential wave can be generated that has a wave speed flanked by the electrophoretic mobility of each of the charged particles. When the wave travels faster than the electrophoretic mobility of a charged particle, the particle will lag behind the wave. When the wave travels slower than the electrophoretic mobility of a charged particle, the charged particle will travel at the wave speed. The difference in the traveling speeds of the charged particles creates the separation of the two particles as they travel down a microfluidic separation channel.

When compared to capillary electrophoresis TWE performs admirably [10-12]. Capillary electrophoresis has long been the standard for high efficiency analytical chemical separations [13-16]. The reason that TWE performs well when compared to capillary electrophoresis is due to the potential energy wells that are created by the voltage waves. The resolution of samples inside of a capillary electrophoretic system is
limited by diffusion. The length of the capillary determines the length of the separation that can be achieved, but as you increase the length of the capillary the concentration of the ions is reduced. During TWE the ions travel within a potential energy well as seen in Figure 1.1 and this limits the diffusion along the travelled path.

This type of separation is of interest in multiple fields including proteomics, cell biology, and genomics to name a few [11]. TWE can be used inside a microfluidic channel allowing for the integration of the technique into lab-on-a-chip devices. This could be used as an upstream process before the microelectrode array performs any electrochemical detection.

1.2.2 Microelectrode Array

Drinking water shortage as well as the degradation of water quality will always remain as important factors contributing to the global sustainability in the foreseeable future. Although most of the sources for water in the United States are already monitored very closely, many developing countries still have major problems with potable water due to pollutants such as pesticides, heavy metals, and saturation of nutrients such as phosphorous [17]. Electrochemical techniques have been derived to detect many of these pollutants, and these techniques could be tested using a microelectrode arrays inside a microfluidic device [18].
Figure 1.1 - A schematic illustrating the potential energy wells in TWE and movement of positive (blue), negative (orange,) and neutral (black) ions.
One example of a biological contaminant that could be detected using a microelectrode array is Cryptosporidium parvum, a potentially fatal protozoan parasite [19, 20]. C. Parvum infects specific species of animals based on a short DNA sequence that has been identified and added to the Genbank database [21-23]. Although several methods such as flow cytometry, laser scanning, immunomagnetic separation, and PCR have been utilized for successful detection, electrochemistry offers unique benefits to many of these systems [24-26]. Electrochemistry offers a detection system that is fast, reliable, inexpensive, and portable.

Electrochemical detection of single nucleotide polymorphisms (SNPs) involves transduction of a molecular genotyping reaction into a measurable signal process. Direct electric detection-based electrochemical genotyping and genosensing assay systems are attractive as a potentially robust and reliable approach to detection of SNPs. SNPs are common DNA sequence variations and strongly correlated with the individuals and hence can be used as a “bio-signature” (high density marker) of the genes [27]. Electrochemical techniques can discriminate single base mismatch mutations [28, 29].

Compared with other biosensing techniques, the electrochemical biosensors have gained great attention in molecular diagnostics due to their high sensitivity, small dimension, low cost, ease-of-use and compatibility with microfabrication and integrated array technology [30, 31]. Given these advantages, a detection system for any specific DNA sequence can be designed and integrated into a microfluidic system that can be produced quickly and inexpensively.
There are essentially two different ideas on this type of electrochemical detection. The first employs the use of an intercalating agent to detect the hybridization of two DNA strands [32]. The second uses a hairpin DNA structure to produce an electrical response when no hybridization has occurred, and no electrical response once DNA hybridization happens [33]. While both techniques seem to be viable options the hairpin DNA structure seems to be the most attractive as no extra reagents need to be integrated into this type of process. This will increase the ease of use and make the detection system simpler for all who use it. An example of this type of detection system can be reviewed in an article published by Ferguson et al. [33].

Given the ability of electrochemistry to detect and evaluate multiple pollutant factors, an electrochemical system that can be incorporated into a microfluidic device will create a cost effective, fast, portable, and near comprehensive detection system. Any electrodes that are fabricated must be easily reproduced and provide consistent and accurate results. The performance of the individual electrodes in the microelectrode array need to be tested using standard electrochemical techniques to test the reproducibility and accuracy of the sensors.

1.2.3 Electrochemistry Techniques

Two electrochemical tests, cyclic voltammetry and differential pulse voltammetry, will be performed to analyze the performance of the microelectrode arrays. Cyclic voltammetry will be useful in the analysis of each electrode to perform both oxidation and reduction reactions. Differential pulse voltammetry is useful to
analyze the performance of each electrode across a concentration gradient and to
determine a detection limit. Both of the techniques utilize a three electrode detection
system similar to what is seen in Figure 1.2. The electrochemical setup consists of a
working electrode, a counter electrode, and a reference electrode. The reactions inside
the system happen between the working electrode and the counter electrode that
function as the anode and cathode respectively. The reference electrode serves as
reference by which a voltage differential can be measure.

Cyclic voltammetry, CV, produces a potential waveform as seen in Figure 1.3.
The cathodic potential will first increase over a given potential range and then once it
reaches the maximum it will decrease until it cycles back to the minimum. Current is
recorded as a function of the voltage potential range that cycled. If the oxidation and
reduction potentials fall within the voltage range specified for the experiment, peaks
can be observed in the current verse potential curve as seen in Figure 1.4. For all of the
tests recorded the parameters can be seen in Figure 1.5.

Differential pulse voltammetry, DPV, is different from cyclic voltammetry in that
only the oxidation or the reduction reaction is measured. The potential waveform for
DPV can be seen in Figure 1.6 and is produced as a series of voltage pulses that are
produced over a linear voltage range. Similarly to cyclic voltammetry current is
measured as a function of potential producing a current verse potential graph. An
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Figure 1.7 - A diagram showing the general shape and significant parts of a DPV voltagram.
1.2.4 Hypothesis and Specific Aims

The hypothesis that this thesis addresses is if it is possible to develop two microfluidic devices that could be used in tandem to separate and analyze chemical ions within a solution. Specifically it is the aim to be able to develop a microelectrode array capable of typical electrochemistry techniques. If the microelectrode array is capable of electrochemistry then we can prove the capability for chemical diagnostics and analysis within a microfluidic system. The other specific aim is to be able to charged molecules within an open microfluidic channel. If open channel microfluidic separations are possible, a large area or diagnostics and research could be opened as the separations could be used in conjunction with other microfluidic techniques for diagnostic purposes.
CHAPTER 2

FABRICATION AND CHARACTERIZATION OF MICROELECTRODE ARRAY

2.1 Fabrication

2.1.1 Microelectrode Array Design

The purpose of this project is to create an electrochemical detection system to analyze multiple targets that could be incorporated inside a small microfluidic chamber. An eight electrode system was developed allowing for six working electrodes capable of independent testing with one reference electrode and one counter electrode that act as the counter and reference electrodes for each of the working electrode sensors. This will allow for the detection of multiple samples within one microfluidic system. The design of the microelectrode arrays can be seen in Figure 2.1.

2.1.2 Photomask Fabrication

The photomask is what creates the design in the photolithography process and was created using the Heidelberg MicroPG 101. A glass plate deposited with chrome and photoresist serves as the photomask during fabrication. The glass plate is placed photoresist up inside the Heidelberg and the design, Figure 2.1, is programmed into the machine. Using a laser, the photoresist is exposed inside the areas of the design. AZ300 MIF developer is used to remove the design areas of the photoresist after exposure. The areas of the photoresist that are removed open a clean layer of chrome where the design will be. Finally the photomask is placed inside chrome etch to remove the
exposed chrome where the design area is located. A completed photomask for the microelectrode array can be seen in Figure 2.2.

![Figure 2.1 - The design for the fabrication of six working electrodes (WE, middle) combined with one counter electrode (CE, top rectangle) and one reference electrode (RE, bottom rectangle).](image)

2.1.3 Glass Substrate Preparation

B270 glass plates were ordered from Valley Design with dimensions of 125x125x2mm. The dimensions were chosen to comply with the microfluidic system developed in the lab that requires a 2mm thick glass chip to fit inside the microfluidic chamber. By using a glass sheet that is 125mmx125mm we can create up to twelve chips containing microelectrode arrays during one full completion of the photolithography. Glass sheets were cleaned using a piranha solution bath for 5 minutes followed by an oxygen plasma cleaning before photoresist deposition.
2.1.4 Photoresist deposition

AZ9260 photoresist was spin coated onto the glass sheets at 2500rpm for 30 seconds. After spin coating the sheets were prebaked on a hot plate at 95°C for 1 minute. Using the photomask that was created for the microelectrode array, see Figure 2.2, the glass sheets were exposed to UV light for 45 seconds using the Suss MA1006. The substrates were subsequently developed using AZ 300 MIF developer by dipping them in a bath of this developer for 45 seconds until the array designs were exposed within the photoresist.
2.1.5 Metal Deposition and Lift-Off

Plasma deposition was used to deposit the metals that are needed for the microelectrode array. The TMV super SS-40C-IV Multi Cathode Sputtering system was used to complete the metal deposition process. Two metals were deposited onto the substrates including chromium as an adhesion layer and a gold layer that will act as the electrode surface for testing. The chromium layer was deposited under a vacuum and a flow 150ccm of Argon gas at a power of 45 Watts for 3 minutes allowing for approximately 10nm of chromium to be deposited onto the glass surface. The gold layer was also deposited under vacuum and a flow of 150ccm of Argon gas, but the gold was deposited at a power of 45 Watts for 10 minutes to allow for a thicker electrode surface layer of 100nm.

Lift-off is the final step in the microelectrode array fabrication process. Once metal has been deposited and covers the entire glass sheet the area of the substrate the still contains photoresist must be removed leaving the microelectrode arrays on the glass. The photoresist is dissolved and removed from the glass substrate using an acetone bath for 4 hours. After a 4 hour acetone bath, the photoresist and gold will peel away from the glass substrate and a quick rinse in deionized water allows for results seen in Figure 2.3. In order to create individualized chips, the Disco DAD641 dicing saw was used cut the glass into the specific size for the microfluidic chamber design.
2.2 Microelectrode Characterization

2.2.1 Cyclic Voltammetry

To verify the functionality of the electrodes that were fabricated two common electrochemical measurements, cyclic voltammetry and differential pulse voltammetry, were performed using the new fabricated arrays. Cyclic voltammetry measures the oxidation and reduction reaction that occurs within a reactive species. The oxidation and reduction reactions can be observed as a spike in current seen by graphing voltage versus current for a given system.

For the cyclic voltammetry tests a solution of phosphate buffered saline at a concentration of 0.01mM was used to dilute potassium ferricyanide. Measurements were taken at potassium ferricyanide concentrations of 0.1mM, 0.2mM, 0.5mM,
0.8mM, 1mM, 2mM, and 5mM. All six working electrodes were used with a platinum counter electrode and a silver/silver chloride reference electrode to measure all 7 concentration levels. The six locations of the working electrodes can be seen in Figure 2.4. The results from these tests can be seen in Figure 2.5 and A.1-5.

Figure 2.4 - A schematic of the six working electrodes that were tested.
Figure 2.5 - Cyclic voltammetry curves measured varying the molarity of potassium ferricyanide and working electrode number six.

Looking at the shape of all the cyclic voltammetry curves we can see that all six of the electrodes are able to record both an oxidation and a reduction peak. Analyzing the differences in the amplitudes of the oxidation and reduction peaks we are able to determine that all six of the electrodes that were fabricated produce a similar consistent result. By looking more precisely at the reduction peaks that were measured for the solution of potassium ferricyanide at molarity 5mM, we can quantitatively determine the accuracy between the six working electrodes that were fabricated. The average amplitude for the six reduction peaks measured for the 5mM concentration of
potassium ferricyanide was measured to be -11.7 microamps with a standard deviation of 0.9 µA for the samples in Figure 2.5. As the standard deviation across the six electrodes that were measured is 7.7% of the average peak voltage, it is reasonable to conclude that all six electrodes produce similar results using cyclic voltammetry. The amplitudes of the oxidation and reduction peaks have a direct relationship with the electrode surface area. Since we can see that all six electrodes produce similar amplitudes, we can conclude that the fabrication process produced electrodes of similar size capable of making separate distinct but similar measurements on any electrochemical sample. The reproducibility of the CV tests can be seen in Figure 2.6 and Figures A.6-11 in the Appendix. Graphs showing the average voltagram across the six electrodes with a standard error calculation for CV tests at each concentration can be seen in Figures A.12-18.

2.2.2 Differential Pulse Voltammetry

Differential pulse voltammetry is another electrochemical technique by which current differential is measured before and after a pulse of voltage is introduced into an electrolytic solution. Varying pulses of voltage are introduced to the system and the current is plotted as a function of voltage. This technique is useful to determine the detection limit of the electrodes that were fabricated as well as analyze the ability of the electrodes to perform across a concentration gradient.
Figure 2.6 - An overlay of the CV measurement 5mM ferricyanide solution of all six working electrodes.

Once again the electrolyte solution of phosphate buffered saline at 0.01M was used to dilute potassium ferricyanide into varying concentrations of 0.1mM, 0.2mM, 0.5mM, 0.8mM, 1mM, 2mM, and 5mM. The six working electrodes and the same counter and reference electrodes as the previous tests were used to run all of the differential pulse voltammetry tests. The results from the tests can be seen in Figure 2.7 and Figures A.19-23 in the Appendix.
Figure 2.7 - Differential pulse voltammetry results for the working electrode at position six. The potassium ferricyanide concentration of 5mM was removed from the results to more clearly see the concentration gradient in the graph.

Through qualitative analysis it is observed that all six of the working electrodes produced similar results. This is consistent with the results observed in the cyclic voltammetry curves. Quantitatively we can analyze the peaks in the current to determine the consistency throughout the six working electrodes. The peak currents for all six electrodes measured at a concentration of 2mM potassium ferricyanide solution produce an average current of 1.30 µA after adjusting for the baseline. The standard
deviation for the peak current in the six electrodes is 0.08 µA or 6.6% of the average peak current. Once again these results are consistent with the cyclic voltammetry results and confirm that the electrode size and shape are consistent throughout the array. The reproducibility of the DPV tests can be seen in Figure 2.8 and Figures A.24-29. Graphs showing the average voltagram across the six electrodes with a standard error calculation for DPV tests at each concentration can be seen in Figures A.30-36.

Figure 2.8 - An overlay of the DPV measurement 5mM ferricyanide solution of all six working electrodes.
The limit of detection is determined as the lowest concentration of the sample that can be consistently and accurately measured. To determine this limit of detection one can analyze the peak current values of the lowest concentrations and determine if the results are consistent and accurate. The average peak current values for the concentration of 0.1mM ferricyanide solution is 6.06 nA with a standard deviation of 3.1 nA. The standard deviation at the concentration is 51% of the average peak current and shows the inconsistency of the results. These results show that the sensors cannot accurately detect the ferricyanide solution at a concentration of 0.1mM.

At a concentration 0.2mM the average peak current is 252 nA with a standard deviation of 22 nA. This standard deviation is 9% of the average peak current. As the standard deviation is less than 10% of the average peak current, one can conclude that the sensors are providing a consistent result. Since all of the sensors produce similar and consistent results, the detection limit is determined to be at 0.2mM for this ferricyanide solution.

To measure the consistency of the sensors across a range of concentrations, a calibration curve is calculated from the results seen in Figure 2.8. The calibration curve can be seen in Figure 2.9. The consistency of the electrodes across the concentration gradient can then be determine using the R² value once a regression line is tabulated. For the regression curve, the result from the concentration of 5mM is added and the result of the concentration of 0.1mM is subtracted as we have determined that result to not be accurate.
Figure 2.9 - The calibration curve for electrode number four using varying concentrations of potassium ferricyanide solution.

The calibration curve produced an $R^2$ of 0.9941. That means that using the regression line on the calibration curve one can predict the peak current at any concentration along that curve with 99.41% accuracy according to the results calculated in Figure 2.9. Calibration curves for the other five working electrodes are found in the Appendix under Figures A.37-41. The electrodes not only perform consistently when compared to the other electrodes in the same array, but they also perform very consistently across a variety of concentrations.
CHAPTER 3

FABRICATION OF A TRAVELING-WAVE ELECTROPHORESIS DEVICE

3.1 Electrode Fabrication

3.1.1 Substrate Preparation

Glass coverslips, 24mmx50mm, and 3”x1” glass microscope slides (Fischer) were sonicated in acetone and isopropyl alcohol (Fischer) for 5 minutes with each sonication step followed by a water rinse to remove the chemicals used in the sonication step. Each slide and coverslip was individually dipped into Nanostrip (Cyantek) for 30 seconds and rinsed in running water to finish cleaning.

3.1.2 Photoresist Application and Development

AZ5214 photoresist was spun onto coverslips and slides at 4000rpm for 30 seconds to form an even thin layer for development. Slides were then heated on 95°C hot plate for 60 seconds before exposure to ultra-violet light at 271 watts. After UV exposure the slides rinsed in AZ 300 MIF developer for 30 seconds to remove the parts of the photoresist exposed to the UV light in the previous step. Drawings of the patterns used for the electrodes can be seen in Figure 3.1.
3.1.3 Metal Deposition

Metal deposition was performed using thermal evaporation on a Cooke Ebeam/Thermal Evaporator. The evaporation chamber was allowed to pump down to a pressure between $8 \times 10^{-7}$ and $1.5 \times 10^{-6}$ Torr before evaporation began. Approximately forty slides at a time were placed on a rotating platform to ensure uniform deposition throughout the evaporation process. Titanium, 20nm, was deposited onto the glass slides before gold was added, 100nm, as the top layer on the electrodes.

3.1.4 Lift-off Process

After metal deposition, slides were left to in an acetone bath overnight to remove the excess metal and photoresist. Any photoresist that did not peel off during the acetone bath was rinsed in more acetone using a pressurized spray bottle to remove
photoresist that was stuck firmly to the glass substrates. The completion of this process yielded results seen in Figure 3.2.

![Figure 3.2 - A CAD drawing of the thick and thin glass slides after deposition.](image)

### 3.2 Microfluidic Channel Fabrication

#### 3.2.1 SU8 Mold Fabrication

Single side polished silicon wafers (University Wafer) were used as a substrate to create an SU8 mold. SU8 was spun onto the silicon wafer at 1670 RPM for 30 seconds in order to produce a nominal height of 14µm. See Figure 3.3. On a hot plate set to 95°C the SU8 was prebaked for 3 minutes before being exposed to UV light for 45 seconds. Following exposure a post bake for 1 minute at 95°C was completed before a bath in SU8 Developer for 3 minutes removed the areas of the SU8 that hadn’t been exposed to the UV light from the surface of the silicon wafer. The SU8 mold was then rinsed of
developer solution using isopropyl alcohol, dried under nitrogen gas, and placed on a hot plate at 140°C for 30 minutes to finalize the curing process. After final curing the SU8 mold can now be cleaned and rinsed with all types of solvents with little to no damage done to the SU8 mold. Completion of the SU8 mold yielded a channel design that can be seen in Figure 3.4.

Figure 3.3 - The profile of the SU8 mold measured across the separation channel that shows an average height of 14.7 microns and a width of 55 microns.

Figure 3.4 - A drawing of the photomask used to create the microchannel in the SU8 mold.
3.2.2 Silanization of the SU8 Master Mold

In order to facilitate the removal of the microchannel from the SU8 mold, the mold must be silanized previous to the introduction of the PDMS polymer. To silanize the mold, the SU8 mold is placed inside of a plastic petri dish with 2-3 drops of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane. The plastic petri dish was placed under vacuum in a desiccator for an hour in order to allow the silane to coat the silicon and SU8 mold. The silanization process is completed by placing the master mold onto a hot plate at 120°C for 30 minutes.

3.2.3 PDMS Preparation and Application

Polydimethylsiloxane (PDMS) is used to form the microfluidic channels in the TWE device. Sylgard 184 Silicon Elastomer Base and Sylgard 184 Elastomer Curing Agent (Dow Corning) were thoroughly mixed together in a 10:1 mass ratio. The PDMS mixture is placed inside a dessicator under a weak vacuum to induce the removal of all of the air bubbles from the PDMS. After 20-30 minutes under a weak vacuum and with a fully silanized SU8 the PDMS mixture is carefully applied to the SU8 mold using a metal spatula assuring that no air bubbles are introduced into the PDMS mixture upon application. All parts of the SU8 must be covered with PDMS before the placement of the thin glass slide, Figure 3.2, on top of the mold.
3.2.4 Coverslip Placement and Electrode Alignment

Coverslips with gold electrodes previously deposited onto the glass surface are placed onto the master mold dispersing the PDMS across the entire surface of coverslip and electrode. This process must once again be accomplished without the introduction of air bubbles to the PDMS. Any and all air bubbles can distort the microchannel and can leave the channels unusable if left within the PDMS during the curing process.

Once the coverslip is placed onto the SU8 mold, the electrode must be aligned with the microchannel shape. Using a stereomicroscope at a magnification of 3x, the electrode is aligned so that the electrode fingers run perpendicular to the microchannel with the first electrode finger crossing the microchannel at the beginning of the separation channel, close to the injection-t. After completion of the electrode and microchannel alignment, a thin clear plastic is placed covering the coverslip and a 1Kg weight is carefully placed on top ensuring no change to the alignment occurs. The PDMS microchannel is left to cure for 24 hours before removal is attempted. The alignment of the electrode at the injection-t can be seen in Figure 3.5.

3.2.5 PDMS Channel Removal

Once curing has completed, the microchannel can be removed for further fabrication. SU8 mold and coverslip are immersed in acetone to swell the PDMS and allow for removal from the SU8 mold. After immersion for 10-15 minutes, the coverslip, with PDMS channel attached, is removed by running a sharpened toothpick along the
edges of the coverslip. The flexibility of the coverslip allows this removal to proceed as the glass will bend as the PDMS releases from the silicon and SU8 master mold. This process must be completed slowly and carefully as the coverslip is very fragile and can break under too much stress. The master mold can be cleaned using acetone and isopropyl alcohol and reused multiple times as long as the SU8 mold shows no signs of damage. Figure 3.6 shows a representation of what the PDMS channel looks like on top of the thin glass slide.

Figure 3.5 - An image taken of the electrodes aligned perpendicular to the separation channel with the first electrode crossing the injection beginning at the injection-t.
3.3 Thick Glass Electrode Preparation

3.3.1 Drilling for Microchannel Access

As the glass slide and electrode will form the ceiling of the enclosed microchannel, access holes must be drilled into the thicker glass slide to allow for the introduction of buffer and sample into the microchannel. The PDMS microchannel has 1mm diameter wells that align with where the holes should be drilled into the thick glass slide as seen in Figure 3.7. Using the previously fabricated microchannels the thick glass slide can be aligned and marked where the holes need to be drilled into the slide.
Alignment is completed using a stereomicroscope and by first aligning the electrodes on the coverslip and the electrodes on the thick glass slide. Once this alignment is completed, the back side of thick glass slide is marked where the 1mm diameter wells will come in contact with the thick glass slide.

![Diagram](image)

Figure 3.7 – (A) The diagram illustrating the 1mm wells created by the SU8 mold that align with drilled holes in the thick glass slide. (B) A drawing of the thick glass slide with holes drilled.

Circuit board drill bits of diameter 1.05mm (Kyocera) and a drill press are used to carefully create access holes in the thick glass slide. To assure that the glass doesn’t crack or break during the drilling process, each hole must be drilled approximately halfway into the glass and then flipped, aligned, and drilled to finish the hole from the opposite side.
3.3.2 Cleaning and Electrical Connectivity

The drilling process leaves the electrode and glass surface dirty and must be cleaned before attempting to bond the PDMS channel to the glass surface. Sonication for 5 minutes in acetone clears the holes that have been drilled into the glass and a brief rinses with distilled water followed by isopropyl alcohol. Connectivity to the electrodes that are patterned onto the glass is done through the use of conductive silver paint. The electrodes found on the glass slide must be connected and extended around the edge of the slide so that after the PDMS channel the electrodes can be connected to an external voltage supply. Minimal silver paint is applied to the gold electrode face and also extended to the vertical side of the thick glass slide, seen in Figure 3.8.

Figure 3.8 - A drawing of the thick glass slide with the silver paint extended onto the vertical edges to allow for connectivity once bonded to the thin glass slide.
3.3.3 PDMS Layer Application

To create a separation channel that is influenced by voltage but free from chemical reactions, a PDMS layer can be applied to the electrode surface before bonding to the PDMS channel on the other side. To maximize the influence of the electrodes on sample, this PDMS layer needs to be less than 100nm in thickness. Various methods were used to try and obtain this desired thickness which will be discussed further, but the most effective method was to dilute the PDMS into hexane before application. The PDMS is diluted to form a solution of 98 percent hexane by mass. This solution is applied to the prepared glass slide and electrode using a spin coater at 4000rpm for 30 seconds. After application the slide is placed on a hot plate at 100° C for 1 hour to cure the polymer surface.

3.4 Electrode Alignment and Device Completion

3.4.1 Electrode Alignment and Bonding

The two surfaces, glass slide and PDMS channel, are rinsed using isopropyl alcohol to clear any extraneous dirt or dust which prepares the surfaces for plasma oxidation. The PDMS channel and glass slide are placed inside a plasma oxidation chamber for 15 seconds to oxidize the two surfaces and allow for stronger bonds between the silicon substrates.

Using a stereomicroscope and an x-y-z stage with a vacuum chuck, the coverslip complete with PDMS channel and the glass slide are aligned so that the electrode’s
fingers on the top and bottom electrodes are parallel and are equally spaced along the separation channel, seen in Figure 3.9. The two substrates are slowly brought into contact forming bonds between the substrates. To ensure good bonding, the now completed TWE separation channel is placed under 1 kg of weight and placed inside of an oven overnight at 100° C. Alignment and interdigitation of the electrodes on the top and bottom of the channel can be observed in Figure 3.9 with the addition of fluorescein to the microchannel.

![Figure 3.9 - An image of the separation channel with a solution of fluorescein added in order to view to alignment of the top and bottom electrodes.](image)

3.4.2 Reservoir Addition

Anything can be used as a reservoir to hold samples, but the reservoir must have a tight seal to the glass surface and be able to withstand suction from a syringe pump. Multiple reservoir types were used in this study, but NanoPorts assemblies (NanoPort) were best suited for the vacuums that were applied. NanoPort reservoirs are added to the TWE device by placing the rubber gasket, adhesive ring, and reservoir around the access holes in the top glass slide. Weights, 200g, are placed onto the reservoirs and the
device is placed into an oven at 170° C for 1 hour to complete the reservoir adhesion process. The addition of the reservoirs can be visualized in Figure 3.10. Figure 3.11 shows the full assembly of all parts of the TWE device.

3.4.3 Electrical Connectivity

The microfluidic channel is now completed and the electrodes must be extended off of the glass substrates in order to connect to the voltage supply. As the electrodes on the thin glass slide are covered in PDMS, seen in Figure 3.6, the excess PDMS covering the edge of the electrode is removed using triethylamine (Fischer). Silver paint is applied directly to the exposed gold, before copper tape placed and covered with more silver paint. The copper tape and silver paint are strengthened using a conductive silver epoxy. This silver epoxy assures that the connection is durable and will last throughout multiple tests and will not be torn off the TWE device. The extra copper tape extending out of the epoxy are where the leads from the voltage supply can be connected. The connections with silver paint and silver epoxy are shown in Figure 3.12.
Figure 3.10 - An exploded diagram showing the addition of reservoirs (red), rubber gaskets (green), and adhesive rings (white) onto the thick glass slide.

Figure 3.11 - The full exploded assembly of the TWE device showing the alignment of the thin slide (gray), electrodes (yellow), PDMS layer and channel (magenta), thick slide (tan), and reservoir assembly (white, green, and red).
3.5 Traveling-Wave Electrophoretic System Characterization

3.5.1 Voltage Wave Analysis

The idea behind traveling-wave electrophoresis is to apply a wave of voltage to the separation channel instead of the customary direct voltage that is normally applied to an electrophoretic system to achieve this setup the TWE device has four separate electrodes to simulate different areas of the wave across any given wavelength. These four electrodes can be programmed to produce any frequency of wave system within the parameters of a given voltage supply.

Two different voltage supplies were tested in this process, one a HVS448 3000LC from Labsmith and the second a digital voltage output supply NI9472 (National
Instruments). As we were tried to replicate the results from the paper found in Analyst, one of the voltage supplies was similar to the one used in that paper, the HVS448 3000LC. The other supply was chosen as we wanted to not just try and approximate a sine wave but actually apply a sine wave of voltage. The digital output supply, NI9472, allowed for that type of voltage application.

Using an oscilloscope, voltage waves that were applied to the TWE device were measured to verify the accuracy of the voltages being applied. These voltages can be seen in Figure 3.13. The voltages supplied by the Labsmith voltage supply used a sequencing software that allowed a simulation of a sine wave. This was accomplished by dividing a sine wave into four and six parts and creating a stepwise function as seen in Figure 3.13. The voltage supply provided by national instruments created the voltage wave that should be ideal for the TWE process, which can be seen in Figure 3.13.

3.5.2 Applied Voltage Effects on Microfluidics

In order to verify if the voltages were affecting the separation channel as we desire an optical test is performed inside the separation channel. To visualize what is happening inside the separation channel, a negatively charged fluorescent molecule, fluorescein, is put into solution and used to fill the separation channel. Under a fluorescent microscope the fluorescein illuminates the microfluidic channel and allows the visualization of the electrodes that cross the channel. When voltages are applied to the electrodes that cross the separation channel, fluctuations in the intensity or concentration of the fluorescein can be witnessed. The negatively charged ions are
attracted by a positive voltage and consequently repelled by a negative voltage. This is what causes the fluctuations that can be visualized and used to determine if the voltage is reaching the separation channel.

Figure 3.13 - (A) The voltage sine wave of amplitude 0.5V that was produced by the national instruments voltage supply. (B) A simulated voltage sine wave approximated by a four step function with an amplitude of 0.5V.

3.5.3 Voltage Supply Analysis

The digital output voltage supply was first tested as we expected it to able to produce better results using an actual wave of voltage as compared to the simulated wave of voltage that the other supply produced, see Figure 3.13. After months of testing with this supply, it was determined that we could not affect the separation channel the way that is desired. Various amplitudes of voltage ranging between 1.2V and 0.5V were applied to the electrodes and several devices were tested, but ultimately no fluctuations in the fluorescein concentrations could be seen under the fluorescent microscope.
The high voltage supply from Labsmith was tested next to verify that the functionality of that voltage supply. This high voltage supply is designed to produce much higher voltages that are desired for the TWE experiments. As such the stability of the voltages below 50 volts is not very strong. In order to increase the stabilities of the voltages that are produced from this voltage supply a voltage dividing system was used to step down from the higher and more stable voltages that are supplied from the Labsmith system. Similarly to the tests performed using the other voltage supply, voltages being applied to the electrodes varied in voltage amplitude between 1.2V and 0.5V. These tests showed more positive results than those tests that were performed with the other voltage supply. Fluctuations in concentration of the fluorescein within the separation channel were seen very clearly. We verified that the voltages that were applied to the TWE electrodes were affecting the molecules within the separation channel. This evidence shows that the fabrication of the TWE device was at least partially successful and also shows that the voltage supply also produces results consistent with previous experiments.

The question arises about why one voltage supply was functional for TWE while the other was not. Since we used the same TWE devices with both supplies, any variables that could arise from differences in the devices are eliminated. After a thoughtful analysis of both of the voltage supplies, it was determined that the digital output voltage supply for NI was not functional due to the fact that it could not sync any current. Diodes are used in the making of the digital output supply that only allow for
current to go in one direction. This type of setup causes charging to occur at all electrodes at inhibit the desired effects of the TWE system. Since the frequency of the waves that were applied to the system were 1 Hertz, the TWE electrodes would be fully charged and the system would not be functional within 1 second of the beginning of the process. As all electrodes after 1 second into the process are fully charged and no current can escape through syncing, there is no voltage differential within the TWE system. Voltage differential is the driving force behind charged ion movement and since the digital output voltage supply cannot sync current, this voltage differential cannot be achieved using this supply.

3.5.4 Plug Injection and Plug Movement

Analysis of chemical separations are measured similar capillary electrophoresis. It is a measurement recorded as a measure of fluorescent intensity verse time. As such the resolution of the results are often determined by the width or length of the bands being analyzed. The smaller the band, or plug, the higher the resolution becomes as concentration remains unchanged. The microfluidic channel is designed to facilitate the creation of small plugs using a t-junction at both ends of the separation channel, see Figure 3.14.

The injection-t has four channels that feed into the junction. As seen in Figure 3.14, four channels consist of a sample channel, a waste channel, a running buffer channel, and a separation channel. Before the injection of the sample into the
separation channel, the whole device is filled with a phosphate buffer and all bubbles are removed using a vacuum pump.

![Diagram of microfluidic channel](image)

Figure 3.14 - (A) A diagram of the microfluidic channel showing the top and bottom injection-t and the separation channel. (B) A diagram illustrating the various channels that feed into the injection-t.

The creation of the sample plug is created using both a hydrodynamic pump and electro osmotic flow (EOF). Initially the sample is loaded into the sample reservoir with equal levels of buffer solution placed inside all of the other reservoirs. Since all of the reservoir heights are equal, there is no hydrodynamic pumping unless a vacuum is applied to one of the reservoirs. To begin the process a vacuum is applied to the waste reservoir creating a pinching effect at the injection-t as seen in Figure 3.15. The pinching
that occurs creates the opportunity for a small injection plug. As the vacuum is released from the waste reservoir voltages are applied to the solutions in the running buffer reservoir, the waste reservoir, and the sample reservoir. These voltages are applied to the reservoirs by using platinum electrodes that are placed inside the open reservoirs in contact with the solutions inside the reservoirs. Voltages of 10V, 25V, and 10V are supplied to the sample, running buffer, and waste reservoirs respectively, as seen in Figure 3.15. The voltage differentials between the reservoirs that are closely connected to the injection-t drives the electro osmotic flow towards both the sample and the waste reservoirs. Although the majority of the sample and buffer flow away from the separation channel, a small sample plug is also pushed into the separation channel. Although the separation channel has no voltage associated with it, the electro osmotic flow produces a residual force down the separation channel that emanates from the injection-t. This process can be seen in Figure 3.15. Using this process a consistent sample plug can be produced for analytical purposes.

3.5.5 Traveling-Wave Electrophoresis Electrode Tests

Plug injection and movement has been produced to this point using EOF but no traveling-wave electrophoresis. To analyze if TWE is functioning as it should, the injection voltages that created the EOF must be turned off and the sequential voltages that are applied to the electrodes crossing the separation channel must be activated. If TWE is working as it should, the plug should be able to be manipulated and moved forward and backward. Multiple tests yielded similar negative results. No plug
movement could be observed and the plug seemed to disperse over time. These results show that the voltages that were being applied along the separation channel did not have a strong enough effect to produce TWE. In an effort to increase the effects of the voltages on the separation channel, a study was undertaken on the depth of PDMS covering the electrodes along the separation channel.

Figure 3.15 - Time series illustrating the injection process: (A) The pinching that occurs when a vacuum is applied to the waste channel. (B) A moment after the vacuum is released and the injection voltages haven’t been turned on. (C) The voltages are applied to sample, waste, and running buffer streams. (D) An illustration of the movement of the EOF within the microfluidic channel. (E, F) The fluorescein moves further down the microfluidic channels by virtue of electro osmotic flow.
3.5.6 Micofluidic Channel PDMS Analysis

There are two sides of the channel that could be shielded by the thickness of the PDMS. The thickness of the PDMS on the thin glass slide is affected by the size of the weight that is placed on the slide and SU8 mold during curing. Weights of 1 kg were used during the curing of all the TWE devices. Atomic force microscopy (NaioAFM, Nanosurf) was used to analyze the thickness of the PDMS at the bottom of the PDMS channel on the thin glass slide. To analyze the depth of the PDMS, a cut and tear across the separation channel was used and the AFM tip crossed the channel onto the glass slide. The results from the atomic force microscopy are seen in Figure 3.16. This analysis proves that by using 1 kg weights the thickness of PDMS at the bottom of the channel is less than 100nm. The analyst paper lists 100nm as the depth of the PDMS necessary for TWE to occur.

The thick glass slide also has PDMS covering the surface of the electrodes. This PDMS can be altered and manipulated a lot easier than the thick glass slide since this coating is done using a spin coater. To optimize the effect of the voltages on the separation channel, the coating of the PDMS was minimized. Using the maximum speed of 4000 RPM on the spin coater three different diluting agents were used triethylamine, methanol, and hexane. Hexane produced the only results that were uniform and less than 100nm in thickness. The PDMS layers were scraped off using a scalpel before being put under AFM to analyze the change in height from the top of the PDMS layer down to
the glass slide. Using a 98% mixture of hexane mixed into PDMS, a thickness between 50nm to 100nm is achieved. The results can be seen in Figure 3.16.

Figure 3.16 - (A) An AFM image measured across the separation channel on a thin glass slide. The height is 84 nm for the channel base. (B) An AFM image measured on a thick glass slide of PDMS that was spin coated with a mixture of 98% hexane. The PDMS height is measured as 55nm.

To determine if the thickness of the PDMS was the reason why TWE was not occurring in the previous TWE devices similar tests were run to witness plug movement. Despite verification of the thicknesses of the PDMS on the top and bottom of the channel, no plug movement was observed inside the separation channel. Although no
plug movement was observed, it seemed as though fluctuations inside the separation channel were strong. Since voltages seemed to be influencing the separation channel to some extent, an analysis of the double layer formation was attempted.

3.5.7 Chronoamperometry Tests

The formation of double layers within the electrical system can neutralize the effect of the voltages being applied to the separation channel. If these double layers can form quicker than the electrode voltages change, than the desired effect of TWE could be neutralize during parts of each step in voltage. To analyze how quickly these double layers are forming inside of the TWE system chronoamperometry is used.

Chronoamperometry measures the amount of current passing into an electrode over time. This is an ideal test as once the double layer is fully formed, current approaches zero. The charging time is defined as the amount of time that it takes for the current to decay to 33% of its maximum height. Initial tests were positive and yielded charging times longer than the step times in the sine wave sequence. After several tests yielding results that were almost identical, suspicions arose as to the validity of the chronoamperometry readings. To verify that the results were measurements of the TWE system, the chronoamperometry tests were performed with nothing connected to the working and counter electrodes. Results were almost identical from the measurement with no connection and the measurement with the TWE connection as shown in Figure 3.17. Results suggest that chronoamperometry is not a good measure to analyze charging time using this electrochemical detection system. In order to find accurate
charging times using chronoamperometry, an electrochemical detection system must be able to resolve charging times on the order of microseconds.

3.5.8 Traveling Voltage Wave Testing

All tests to this point seem to prove that the voltage sequences provided by the TWE electrodes have no ability to move a sample plug alone. Since the TWE cannot move the sample plug alone, an analysis was performed to determine if the TWE electrodes and voltages had any effect on the separation channel. To analyze the effect of the TWE voltages on the separation channel, three experiments were completed by looking at the plug velocity in the separation channel. Using EOF, a sample plug of fluorescein was injected and propelled down the separation channel using voltages similar to those seen in Figure 3.15. Plug velocities were calculated for a sample plug without TWE voltages, with TWE voltage waves applied in the direction of EOF, and with TWE voltage waves applied against the direction of EOF. Table 3.1 gives the results from the experiment using plug velocities.
Figure 3.17. Two graphs of chronoamperometry measurements taken with (A) air in the channel as well as with (B) solution in the channel. The similarities in the shape and charging times in the graphs prove that the chronoamperometry tests were unsuccessful in the resolution of the charging times in the TWE system.
Table 3.1 shows that the velocities with TWE voltage wave sequences applied in the direction of the EOF are very similar to the plug velocities with no TWE voltage wave sequences applied at all. This result suggests that the majority of the plug movement that was observed was due to EOF pressure. When TWE voltage wave sequences are applied in the direction opposite of the EOF the plug velocities decrease. By observing the changes in plug velocity as a function of the direction of the TWE voltage waves, it indicates that TWE is having an effect on the separation channel. Although the TWE voltages affect the separation channel, the electrodes don’t have significant enough impact to move a sample plug down the channel without the help of EOF. As TWE is reliant on the ability to propel charged molecules down a separation channel it is impossible to produce a chemical separation unless the effect of the electrodes on the channel is increased significantly. To increase the effect of the electrodes in the TWE process a variety of solutions could be realized.

Table 3.1 - The calculated plug velocities of fluorescein within the separation channel.

<table>
<thead>
<tr>
<th>TWE Voltage Wave Direction</th>
<th>Plug Speed Down the Channel (µm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No TWE</td>
<td>29.8</td>
</tr>
<tr>
<td>Down Channel</td>
<td>29.2</td>
</tr>
<tr>
<td>Up channel</td>
<td>24.7</td>
</tr>
</tbody>
</table>
The voltage supply device that was used in these experiments was a low current voltage supply, which is different from the high current voltage supply that was successfully used previously. The high current supply may make charge transfer within the system larger and allow for plug movement strictly using the TWE electrodes. Another solution to strengthen the effect of the TWE electrodes on the system is to take away the PDMS layer between the electrodes and the separation channel. That would involve a change in the design of the TWE device that would allow the metal to be plated inside the separation channel. Further analysis of the system could also be completed to monitor what is happening within the system electrochemically. Advanced analytical techniques such as electric impedance spectroscopy could also be used to understand the functionality of the electrochemical system.
CHAPTER 4
CONCLUSION AND FUTURE WORK

4.1 Conclusion

Electrochemistry is a promising technique useful in the analysis and detection of contaminants in water. Given the recent improvements in microfluidics, small electrochemical sensors can be cheaply manufactured and incorporated into a portable device. The electrochemical system can detect several different contaminants within an aqueous system.

Using photolithography a microelectrode array was designed and fabricated capable of the aqueous detection. Cyclic voltammetry tests proved the capabilities of the electrodes to perform both oxidation and reduction reactions. All tests were measured using potassium ferricyanide, a common reagent for electrochemical tests. The CV tests also showed a remarkable amount of reproducibility as the faradaic current peaks on all six working electrodes at a concentration of 5mM ferricyanide solution showed a standard deviation of 7.7 percent of the average peak current. The reproducibility of the CV curves are best visualized by looking at Figure 2.6.

Differential pulse voltammetry allowed for the quantification of a detection limit for the six working electrodes. The lowest measurable concentration using the microelectrode array for the potassium ferricyanide solution was 0.2mM. A calibration curve for the potassium ferricyanide solution verified the performance of the electrodes over a varying range of concentrations. The calibration curve produced an $R^2$ value of
0.9941. The reproducibility of the DPV tests is easiest visualized by looking at Figure 2.8.

Given the positive results of the reproducibility of the working electrodes in the microelectrode array, further tests and experiments are planned and will continue forward.

Traveling-wave electrophoresis is an analytical chemical separation technique capable of separating charged molecules based on the different electrophoretic mobility of particles. The fabrication of TWE devices is a complicated process requiring multiple steps. It involves the same photolithography process to create the microelectrodes for TWE as was used for the microelectrode array. Once the microelectrodes are made, the microfluidics is created through the use of PDMS and more photolithography.

The fabrication process for the TWE device was completed and analyzed for performance. The initial tests showed that the TWE electrodes were not having the desired effect on the microfluidic chamber. To improve the performance of the TWE device an analysis of the voltages that were supplied to the system was performed using an oscilloscope. Although the correct voltages were applied to the TWE channel, the electrodes were still not having the desired effect. An analysis of the thickness of the PDMS was next undertaken to understand if it was having an undesirable effect. Using atomic force microscopy and creative PDMS thinning techniques, the thickness of the PDMS layers were minimalized to less than 100nm. Finally an attempt to quantify the charging times within the system was undertaken using chronoamperometry. Due to
limited capabilities of the electrochemical device used, all tests using chronoamperometry were deemed unsuccessful.

The fabricated TWE devices were not capable of moving bands of charged particles down a microfluidic channel, but the fabricated electrodes did have an effect on the separation channel. By analyzing the wave speeds of sample injections with TWE working with and against the electro osmotic flow, it was determined that the TWE electrodes are having an effect. Despite the evidence of the TWE electrodes having some effect, the electrodes don’t have a strong enough effect to propagate any charged molecules down the separation channel. Future experiments can be developed to try and increase the strength of the TWE electrodes.

The strength of the electric field seems to be the main factor that contributes to the success of the TWE device. There are a few factors within the fabrication of the device that contribute to the electric field strength. The microchannel height seems to have a large impact on the strength of the electric field. A functioning device has been fabricated using a 15 micron microfluidic channel height, but channel heights lower than this would seem to have a higher electric field strength and improve the functionality of the TWE system. The thickness of the PDMS layers covering the electrodes also has a large impact on the functionality. The thinner you can fabricated the PDMS the stronger the electric field strength is within the system. The last contributing factor to the electric field strength is the voltage supply. It seems that the voltage supply has to be capable of syncing current for the TWE system function properly. All electrodes have to be able to
function as a current sync and voltage supply within the system. Although electrode alignment will affect the wave shape and performance it seems that minimal electrode displacement has limited impact on TWE performance.

4.2 Future Work

- Photolithography techniques can be performed to fully incorporate both reference and counter electrodes into the microelectrode array and to test the reproducibility.
- The microelectrode array can be used for multiple heavy metal detection.
- The microelectrode array can be used to analyze multiple single stranded DNA samples and identify specific genotypes that could be dangerous to human health.
- Further development of fabrication techniques to bring TWE electrodes into direct contact with the aqueous sample inside the microfluidic chamber.
- A high current voltage supply could be tested to verify if the TWE can be reproduced as the voltage supply used in these tests was a low current supply.
- DNA, peptide, or and other ion separation can be done with a working TWE device.

4.3 Engineering Significance

There is significance in the success of this project for multiple engineering fields such as biological engineering and chemical engineering. The microelectrode array would allow for on-site detection of a variety of chemical constituents. This could
further biological engineering techniques as the exact chemical composition of samples could then be altered to improve biological components inside any system. Traveling-wave electrophoresis is of specific significance inside of chemical engineering. TWE through the use of separative and non-separative transport theoretically the capability of higher resolution or infinite separations. The improvements that TWE could provide within the analytical chemistry field are profound if higher resolution or infinite separations are realized. Engineering benefits clearly with the increase in knowledge that higher resolution separations would create.
References


Additional Electrochemistry Results

Figure A.1 - Cyclic voltammetry curves measured using a variety of concentrations of potassium ferricyanide and working electrode number one.
Figure A.2 - Cyclic voltammetry curves measured using a variety of concentrations of potassium ferricyanide and working electrode number two.
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