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DIVISION OF SCIENCE

ELECTROPHORETIC CHARACTER OF BORATE BUFFERS IN CAPILLARIES AND MICROFLUIDIC CHANNELS

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ABSTRACT

This work describes the investigation of the character of borate buffer solutions in glass capillaries with the goal of moving towards the evaluation of EOF in microfluidic channels. EOF is a phenomenon that exists when voltage is applied to electrolytic buffers in microscale flow channels, resulting in bulk flow of buffer through the channel in response to the electric field. EOF is of great consideration because it is the dominant flow employed in capillary electrophoresis (CE) separations and an important consideration in microchip electrophoresis (ME). Many reports of integrated microfluidic analysis, with applications including protein and DNA analysis, have featured ME, but there is significantly less attention paid to EOF in favor of the more complex analysis steps. In this work, borate buffer was employed due to its widespread use in both CE and ME, monitoring current output in response to varied electrophoretic parameters (i.e., composition, surfactants). Current output was evaluated using signals from charged and uncharged analytes, providing valuable information regarding the efficiency and consistency of borate electrolytes in capillaries. This work should provide an effective basis for the study of EOF in both capillaries and microfluidic channels.

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Chapter 1

Introduction

Capillary electrophoresis (CE) is a well-established technique that continues to expand and extends applicability to a broad range of compounds such as proteins, polysaccharides, and DNA among others. CE integrates the principles of electrophoretic separation with automation, high-throughput, and miniaturization resulting in fast and efficient separations (Kemp, 1998). A significant component of CE is electroosmotic flow (EOF)—an electro-kinetic phenomenon that derives from interactions between the surface on the silica capillary and the buffer system incorporated for the separation process. EOF combined with the dynamics of solution transport, substantially influences the resolution and electrophoretic mobility of analytes as well as the time they reside within the capillary (Kemp, 1998). And with the movement from complex instrumentation to micro total analysis systems (μTAS), CE has become an important consideration in μTAS technology for genomic applications and field detection.

Primary research related to μTAS emphasized separations and sample manipulation and handling. The appeal of μTAS lies in its ability to assimilate several functional elements to produce fast and accurate analysis from reduced sample volume (Harrison et al., 1993). In the past decade, the march toward developing such integrated devices has accelerated significantly. Presently, μTAS feature multiple sample handling and processing steps that are highly integrated and automated (Vila-Planas et al., 2011). While it has been reported that advancements are still needed, particularly in areas of sample preparation, chip-to-real-world interfacing, and detection, it is the

hope that this research into buffer optimization in CE can be adapted to future application in μTAS (Easley et al., 2006).

As the number of published reports of integrated microchip electrophoresis devices has increased, it has become clear that the electrophoresis aspect of the device has received significantly less attention as the other steps (Breadmore, 2012), which is why this research is more fundamental in nature. By studying the electrophoretic behavior of borate buffer in capillaries, these findings should prove to be applicable to other microfluidic channels. Due to its common use in electrophoresis separations, borate buffer allows the optimization of a system assembled in-house to evaluate EOF. The subsequent systematic analysis of various electrophoretic parameters (i.e., buffer composition, ionic strength) should provide valuable information regarding the different factors affecting separation in capillaries and microfluidic channels, and should prove useful to other scientists in the field working on integrated devices. To execute this project, the effect of different factors affecting EOF were investigated. Variables examined include buffer type (e.g., borates), buffer characteristics (e.g., pH, ionic strength), buffer composition (e.g., anionic/cationic surfactants), applied voltage and channel substrate (e.g.,silica).

Chapter 2

Review of Literature

2.1 Electrophoresis

2.1.1 Basic Principles

In electrophoresis, an electrical field is employed to separate the components of a mixture. When given the freedom to move, charged particles seek regions, such as an electrode, having an opposing charge. In an electrophoretic system, ions separate and migrate based on charge-to-size ratio; however, neutral species are not affected. Cations (positively charged ions) migrate toward the cathode (negatively charged electrode), and anions (negatively charged ions) migrate toward the anode (positively charged electrode) (Whatley, 2001). The motion of these ions is random in the absence of an electrical field; however, once an electrical field is applied, charged species begin to move resulting in a less random distribution of charged particles (Whatley, 2001). The electric surface charge of suspended particles are strongly affected by surface adsorbed species on which an external electric field exerts an electrostatic Coulomb force (Hanover, 2012). According to the double layer theory, all surface charges in fluids are screened by a diffuse layer of ions in which forces are exerted on the ions in the diffuse layer and transferred all the way to the particle surface through viscous stress.

The most well-known and widely used theory of electrophoresis was developed by Smoluchowski (1903) and is described as follows:

$$\mu_{e=}\frac{\varepsilon_r \varepsilon_0 \zeta}{\eta}$$

where ε_r is the dielectric constant of the dispersion medium, ε_0 is the permittivity of free space (C^2 N^{-1} m^{-2}), η is the dynamic viscosity of the dispersion medium (Pa s), and ζ is the zeta potential. In real systems, numerous other factors such as the hydrodynamic radius of the molecules, the viscosity of the separation medium, and temperature influence electrophoretic separations and are important considerations in electrophoresis.

2.1.2 Brief History

Tiselius' 1937 landmark moving boundary electrophoresis (MBE) provided an effective method for separation of molecules in free solution using a U-shaped tank with an electrode at either end. In the following years, the apparatus was improved and established boundary electrophoresis as an accurate analytical method. This basic form of electrophoresis was superseded in the 1950s by zone electrophoresis (ZE), which relied on the separation of molecules in a solid support (e.g. paper, gel). Gels of gelatin and agar have been used as supports for electrophoresis for about a century; however, few papers had been published until 1955 (Grabar, 1953). From experiments conducted by Smithies (1955), it was concluded that electrophoresis with molecular sieving of a gel (agar) provided much higher resolution than electrophoresis in free solution. Zone electrophoresis in agar gels was surpassed by the introduction of agarose—a neutral component of agar—for use in electrophoresis and chromatography (Hjerten, 1961). In 1959, electrophoresis in polyacrylamide gels (PAGE) were first reported followed by the addition of sodium dodecyl sulfate (SDS) to homogenous PAGE for the purity assessment and molecular weight determination of proteins (Raymond, 1959). Further studies of proteins expanded with the development of two-dimensional gel electrophoresis in which proteins are first separated

according to charge by isoelectric focusing (IEF) in one gel and then according to size by electrophoresis in a gel slab (Svensson, 1961). After 1960, displacement electrophoresis was mostly performed in tubes with inner diameter (i.d) of 0.2-0.5 cm, which was later improved by reducing the i.d (Martin, 1967).

2.1.3 Slab-Gel Electrophoresis

Slab gels are designed with multiple lanes set up such that samples run in parallel with little variation in sample due to minor changes in the gel structure (Brody, 2004). Agarose and polyacrylamide gels are conventional in slab-gel electrophoresis and are specific to different types and sizes of analytes (Smisek, 1989). Polyacrylamide gels are predominantly used for protein analysis, and impart high resolution for small fragments of DNA (5-500 bp). In contrast, agarose gels offer lower resolving power for DNA yet have greater range of separation, and are therefore used for DNA fragments of 50-20,000 bp (Maniatis et al., n.d.). Slab gels are unquestionably the technique of choice for blot and auto-radiographic analyses and are now customary for laboratories performing routine nucleic acid analyses, and those employing antigenic controls (Righetti, 1998). The availability of reasonably priced commercial slab gel units has increased the use of slab gel systems, and the use of tube gels is becoming rare.

2.2 Capillary Electrophoresis

2.2.1 Basic Principles

Electrophoresis in a capillary is differentiated from other forms of electrophoresis in that it is carried out within the confines of a narrow tube with inner diameters ranging from 20–100 μm (Fig. 1). Capillaries can be constructed from various materials including fused silica, borosilicate glass, and polytetrafluoroethylene (Teflon) (Whatley). The narrowness of the capillary reduces lateral diffusion and maintains the temperature between the center of the capillary and the wall and because these two constituents move at different velocities, they can be separated. The geometry and other properties of the capillary electrophoretic separation also lead to a condition known as plug flow. Under ideal plug flow conditions, the only factor leading to sample dispersion is diffusion (Wiktorowicz, 1990). This contributes to the high efficiency of CE separations. In addition, the small diameter of the capillary and its large aspect ratio (length/width) contribute additional factors (Reijenga et al., 1992). CE offers the benefits of fast analysis time, high-resolution separation, various detection schemes, simplicity, low sample and solvent volume consumption and applicability to a wide range of biomedically important substances (Nuchtavorn et al., 2015).

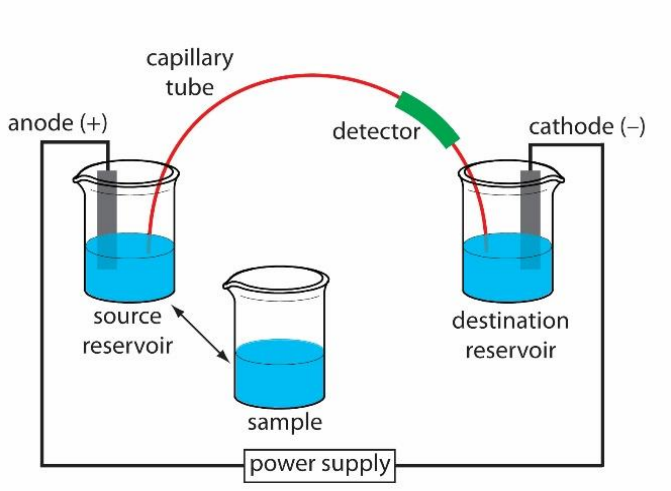

Figure 1. Diagram of the basic instrumentation for capillary electrophoresis.

The sample and the source reservoir are switched when making injections.

2.2.2 Electroosmotic Flow

Electroosmotic flow (EOF)—the motion of liquid due to applied potential across a capillary— is an essential component of CE, drives the separation process, and is contributed by the small diameter of the capillary tube (VanOrman et al., 1991). EOF exists in any electrophoretic system and occurs whenever the liquid near a charged surface is placed in an electrical field resulting in the bulk movement of fluid near that surface. Due to the high surface to volume ratio inside a capillary, EOF is a significant factor in CE (Reijenga et al., 1992).This charge arises from both the nature of the material that composes the capillary and the buffer composition (Fig. 2).

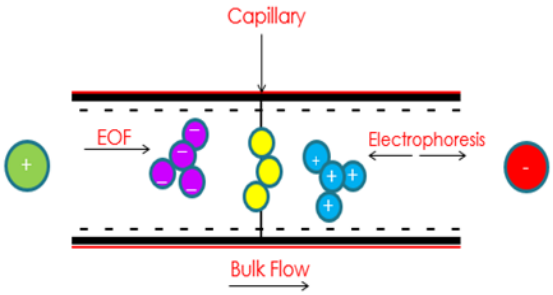

Figure 2. Schematic of EOF in capillary electrophoresis.

The separation and direction of analytes based on size to charge ratio.

2.2.3 Buffer Consideration

In the case of electrophoresis that separates on the basis of voltage applied across a conductive medium, the buffer ions represent the conductive medium. The buffer, by providing a reservoir of weak acid and base, also keeps the pH within a narrow range (Butler, 1964). This is important due to the effect of significant pH changes on the structure and charge of a protein or nucleic acid, thus preventing proper separation. Generally, the pKa value should be close to the required pH, and buffers providing species of low charge magnitude are preferred, so as not to conduct too much current (Hulanicki, 1987). Wide ranges of buffers have been employed in CE systems with phosphate, borate, citrate, acetate, and Tris (trishydroxymethylamino methane) buffers being the most commonly used. Choosing an appropriate buffer is contingent upon a multitude of factors such as pH, operating temperature, charge of the buffer relative to the analytes and the capillary wall as well as its effects on detection (Ferguson et al., 1980). A buffer that has found wide application in CE is sodium borate.

2.3 Borate Buffer

2.3.1 Boron Chemistry

Lussac and Thénard independently isolated Boron by combining boric acid with potassium (Adair, 2007). Boron exists in the earth's crust and is classified as a metalloid due its properties that reflect a combination of both metals and nonmetals. The pure element is rigid and in extremely pure form is nearly as hard as diamond, but much too brittle for practical use. Boron is an excellent conductor at high temperatures and an insulator at and below room temperature (Hasan, 2009). This behavior as well as many of its other properties earn it the classification of a metalloid. The element is prepared by the reduction of sodium tetraborate with carbon while high-purity boron is produced by electrolysis of molten potassium fluoroborate. Common compounds of boron include borax and a wide variety of mineral borates (Adair, 2007).

2.3.2 Borates

Borates are large groupings of minerals with unusually complex chemistry and crystal structure. The chemistry and reactivity of Boron gives rise to numerous oxygen compounds such as borates, which span from simple to extremely complex molecules (Delaplane, 1988). Boron consists of simply two isomers, ^{10}B and ^{11}B, which vary in nature, and has the ability to form organic compounds for various applications (Vegas, 1985). The ^{10}B isomer has a unique application and significant medical use due to its unusually large neutron capture cross section. Combinations of triangular or tetragonal negatively charged B-O structures are present in all borates (Vegas, 1985). Larger borates are composed of trigonal planar BO_3 or tetrahedral BO_4

structural units, joined together via shared oxygen atoms and may be cyclic or linear in structure (Wiberg, 2001). Boron always maintains its valence state of +3 resulting to a negative charge on the tetrahedral bonding for attachment to a cation (Delaplane, 1988). Borates are deemed as derivatives of boric acid ($B(OH)_3$)—a weak proton donor (pKa ~ 9) in the sense of a Brønsted acid, but is also a Lewis acid due to its ability to accept an electron pair (Atkins, 2010). Borates are important ingredients in a variety of household and commercial products (ie., detergents, wood treatments, fiberglass).

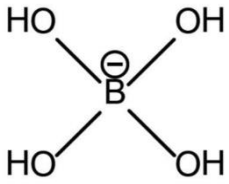

Figure 3. Tetrahedral tetrahydroxyborate anion.

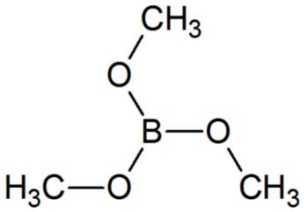

Figure 4. Trigonal planar Trimethyl Borate Molecule

2.4 Microchip Electrophoresis

2.4.1 Basic Principles

The concept of microchip electrophoresis combines the parallel analysis of gels with the automation of capillary electrophoresis and has recently undergone substantial development like

integrated microchip designs and advanced systems (Haddad, n.d.). Its advantages include speed four to ten times faster than conventional capillary electrophoresis, one order of magnitude faster than slab gel electrophoresis, simplicity and potential for automation (Jacobson, 1995). Microchips generally consist of a cross T design with a short (injection) channel and longer (separation) channel, which in itself represents a level of integration not seen with CE, where sample is simply introduced directly into the capillary (Harrison et al.). The sample is injected electro- kinetically, by applying an electric field across the sample channel. Portion of the sample present in the intersection represents the injection plug, which is subjected to separation when electric field is applied across separation channel with typical separation times of 50-200 seconds. Detection on microchip is typically made at opposite ends of the separation channel, most commonly by LIF (Laser Induced Fluorescence) due to its sensitivity (Pentoney, 1997). Miniaturization of the entire electrophoretic process dramatically decreases separation time to seconds as opposed to minutes leading to a new generation of research to manufacture μTAS or lab-on-a-chip devices.

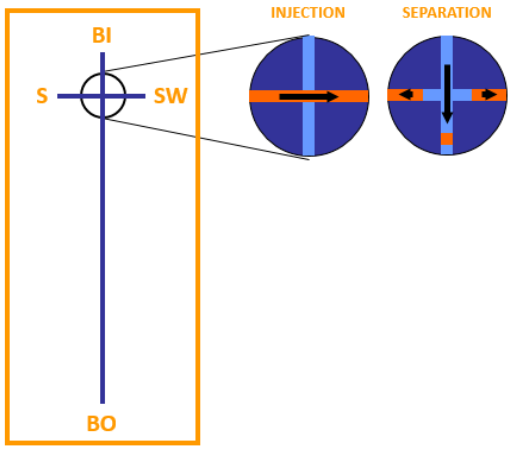

Figure 5. Basic design of micro electrophoresis device.

This device includes a T-cross section, injection and separation sites.

2.4.2 Micro Total Analysis System (μTAS)

The design of most micro fabricated electrophoresis systems is quite simple and consists of a sample injection zone, an electrophoresis separation channel, and a detection system (Harrison et al.). Separation channels, sample injection channels, reservoirs, sample preparation reactors, and pre- and post- column reactors are all fabricated onto the surface of the microchip, using photolithographic processes—the process of transferring geometric shapes on a mask to the surface of a silicon wafer (Jacobson, 1995). Some application of μTAS in literature include analysis of *E. coli* (Liu et al., 2004), biomarkers (Skelley et al., 2005), influenza (Pal et al., 2005) and anthrax (Easley, et al., 2006). It is clear from such examples that significant attention has been given to the various interfacing and integrating needs; however, research in the final separation step remains a worthwhile endeavor.

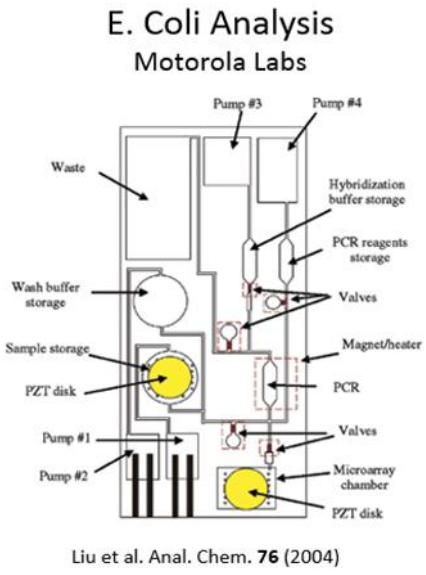

Figure 6. μTAS device developed by Motorola Labs for *E. coli* analysis.

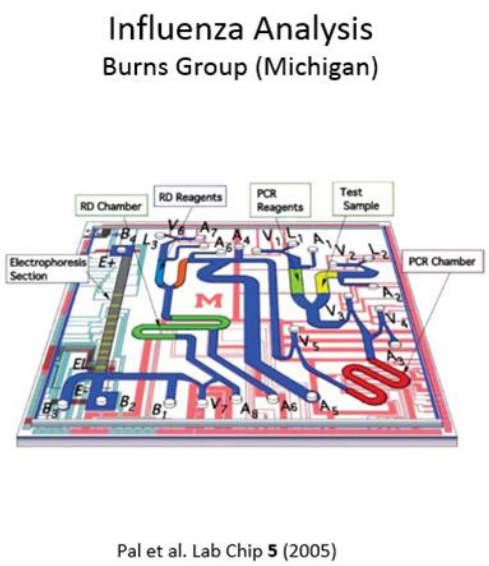

Figure 7. µTAS device developed by Burns Group (Michigan) for influenza analysis.

Chapter 3

Materials and Methods

3.1 Buffer Preparation

All reagents were purchased from Sigma Life Sciences. Stock solution of 0.05M Borax and 0.05 M Boric acid were prepared utilizing reagents of analytical grade and were used to prepare borate buffer solutions of various compositions respectively (Table 1). The solutions were all sonicated for 60 minutes at 60ºC followed by syringe filtration using a 0.45 micron filter (Thermo Scientific).

Table 1. Borate buffer compositions and concentrations used for data acquisition.

Stock Solution	Additive	pH
0.05 M Borax	0.2 M Boric Acid	8.52
0.05 M Borax	0.2 M HCl	9.26
0.05 M Boric Acid	1 M NaOH	9.22
0.05 M Borax	0.2 M Boric Acid, 1mM SDS	8.21
0.05 M Borax	0.2 M Boric Acid, CTAB	8.90

3.2 Capillary Conditions

The uncoated fused-silica capillaries employed for data acquisition were 30 cm in length, 100-µm i.d and 250-µm o.d. (Polymicro). The capillaries were conditioned preceding each CE run

using a Syringe Pump (Cole Parmer) at a rate of 86.02 mi/h with 2.00 mL of each solution in the following order:

1. Flushed with 0.1M HCl—removes residual cations
2. Flushed with Deionized H_2O
3. Flushed with 0.1M NaOH—hydrolyzes the siloxanes to create charged silanol groups to provide EOF
4. Flushed with Deionized H_2O
5. Flushed with Buffer

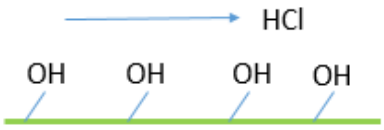

Figure 8. Flushing with HCL.

As the capillary is flushed with HCL, a strong acid, the hydroxyl groups on the capillary are deprotonated.

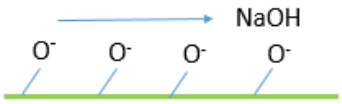

Figure 9. Flushing with NaOH.

As the capillary is flushed with NaOH a strong base, the hydroxyl groups on the capillary are protonated.

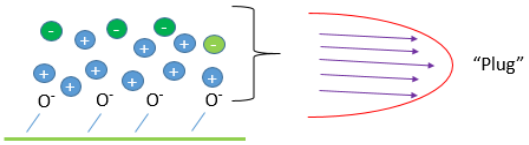

Figure 10. Flushing with Electrolyte.

Flushing the capillary with buffer creates a double layer of ions, which introduces plug flow.

3.3 Experimental Set-Up

The CE instrumental set-up consisted of two 1mL vials—filled with buffer solution—held by alligator clips on a metal stand at opposing ends. Platinum rods were submerged into each vial and connected to a PowerPac HV (Bio-Rad, Hercules, CA) power supply and a TekPower USB TRUE-RMS multimeter 6000 counts (Mont Clair, CA) via power adapter cables. Current was measured in real time using the WH500 accompanied with the TekPower multimeter. Current Data for each electrolyte was collected by applying voltage in the range of 500-4000 V across the capillary in increments of 500 V—4000 V is the maximum voltage allowed by power supply due to manufacturer safety settings.

Chapter 4

Results and Discussion

4.1 CE Parameters

4.1.1 Buffer Composition

In an electrophoretic system, the separation of analytes is considerably influenced by buffer composition amongst a host of other factors thus, the components of a buffer system are crucial for an effective separation process. To evaluate the effects of buffer composition in CE, the borate buffer system was investigated by utilizing an assortment of borate buffer solutions. Borate buffers can be made in various ways leading to some ambiguity in regards to their intrinsic chemical reactions. Borate buffers can be formulated using ortho-boric acid (H_3BO_3) and NaOH, sodium tetraborate and HCl, sodium tetraborate and H_3BO_3, or borax and NaOH (Wendell, 1940). As noted previously, the unusual chemistry of borates leads to a very complex system that has yet to be fully characterized; in fact, Trejo et al. (2012) suggest that the system undergoes ten different equilibrium reactions in solution.

The borate buffers used for this inquiry were prepared by titrating 0.05M Borax up with 0.2 M HCl, titrating 0.05M Boric Acid down with 1M NaOH and combining 0.05M Borax (4 equivalents of boron) with 0.2M Boric Acid (1 equivalent of boron). From current data obtained at varying voltages, it is evident that the change in current between the borax/HCl and borax/boric acid solutions is minimal indicating that the two acids perhaps behave similarly in the equilibrium reactions (Fig. 11, 13). However, a significant difference in current output was seen in the borax/NaOH mixture (Fig.12). Maximum currents of 75.51 µA and 83.97 µA was produced by

borax/HCl and borax/boric acid respectively at 4000V compared to 19.34 μA for boric acid/NaOH at the same voltage. This difference observed in current should be valuable in choosing the most suitable electrolyte to optimize EOF in capillaries.

Because CE is typically performed at high voltages (20kV-40kV), the relationship between current and voltage as described by Ohm's Law—V=IR , where I is the current, V is voltage and R is the resistance—is vital. Current and resistance are inversely related hence a dramatic increase in resistance will result in a decrease in current and conceivably cause the power supply to shut off due to instrument parameters. This correlation provides a plausible explanation for the reduction in current output of the boric acid/NaOH buffer solution. These data suggest that the boric acid/NaOH composite may be a less viable option for the optimization of CE than the borax/HCl and borax/boric acid solutions. It should also be noted that experimental errors—precipitation, bubble formation or fracturing of the capillary—could have attributed to a higher resistance within the capillary leading to the four fold difference in current output for the boric acid/NaOH mixture.

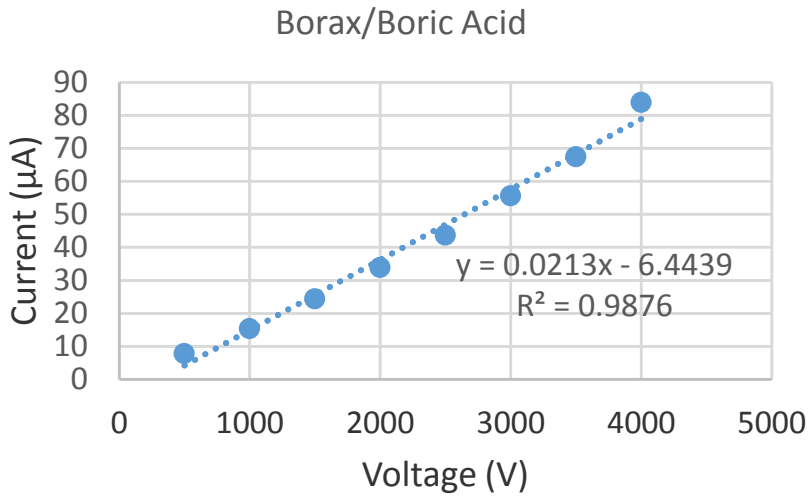

Figure 11. Current output of Borax/Boric acid mixture.

Current was measured with an ammeter with voltage of 500-4000 V in 500 V increments. Current reaches a maximum of 83.97 µA.

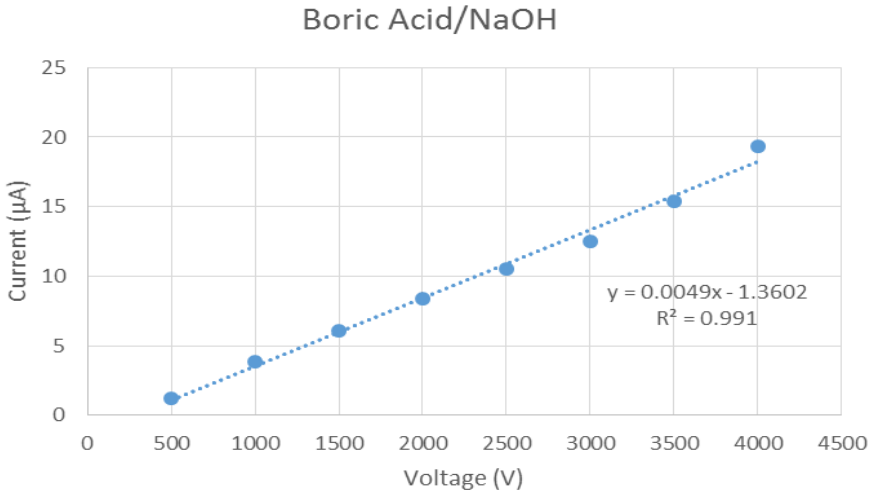

Figure 12. Current output of Boric Acid/NaOH mixture.

Current was measured with an ammeter with voltage of 500-4000 V in 500 V increments. Current reaches a maximum of 19.34 µA.

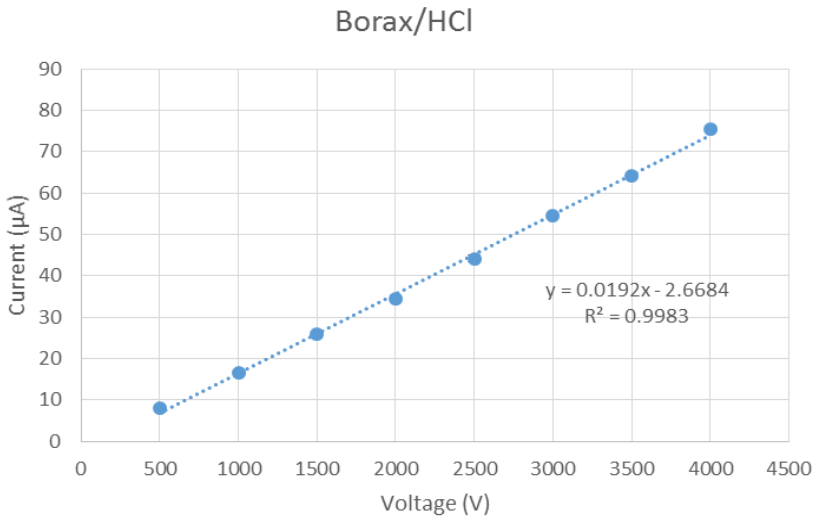

Figure 13. Current output of Borax/HCl mixture.

Current was measured with an ammeter with voltage of 500-4000 V in 500 V increments. Current reaches a maximum of 75.51 µA.

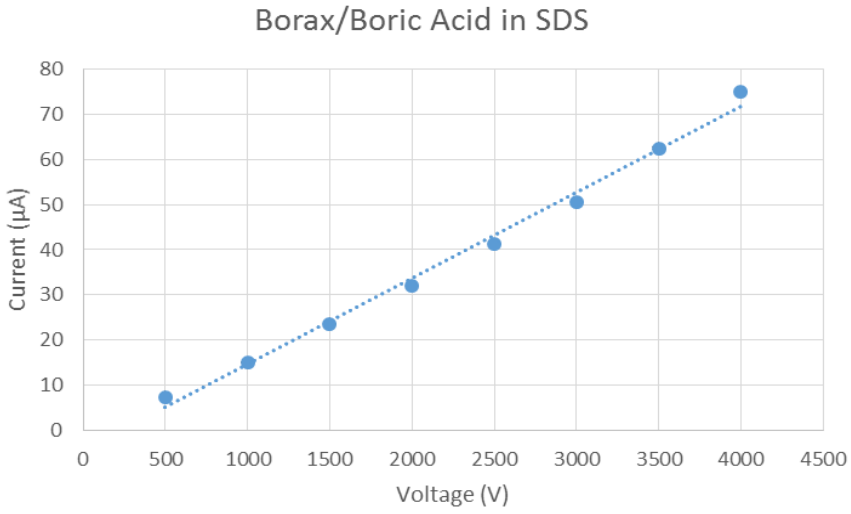

Figure 14. Current output of Borax/Boric Acid in SDS mixture.

Current was measured with an ammeter with voltage of 500-4000 V in 500 V increments. Current reaches a maximum of 74.90 µA.

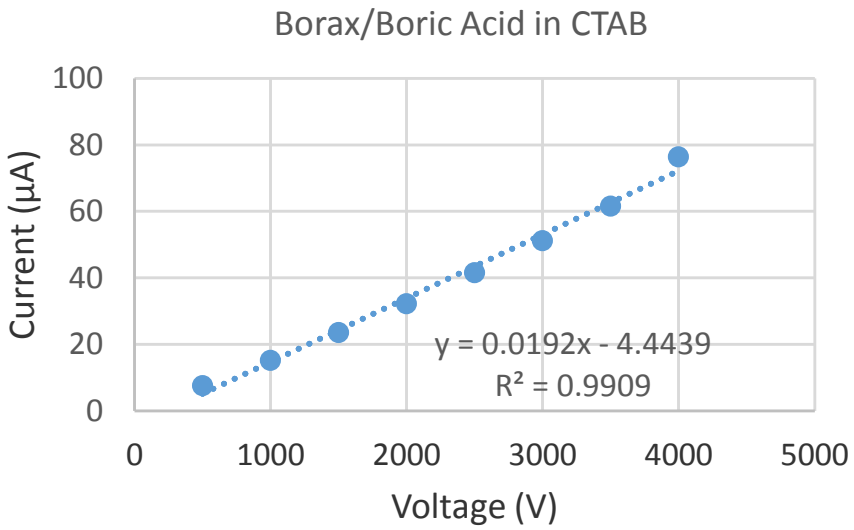

Figure 15. Current output of Borax/Boric Acid in C-TAB mixture.

Current was measured with an ammeter with voltage of 500-4000 V in 500 V increments. Current reaches a maximum of 76.43 µ4.1.2 pH

4.1.2 pH

A buffer resists variations in pH upon the addition of acidic or basic components by neutralizing small amounts of added acid or base, resulting in a relatively stable solution pH. To effectively maintain a pH range, a buffer must consist of a weak conjugate acid-base pair, a weak acid and its conjugate base, or a weak base and its conjugate acid. These reactions are approximated by the Arrhenius-Ostwald model:

$$pH = pK + \log(\frac{[A^-]}{[HA]})$$

HA and A^- represent the conjugate acid and conjugate base, respectively, of a buffer system (Thorsten, 2013). For optimal buffering capacity, the pH of a buffer system should + or – 1 of the pKa of the buffering species. The pKA of the boric acid / borate system is ~9 at room temperature

and is therefore most operable between pH 8 and 10. This system, however, does not comply with the Arrhenius model. Rather than donating a hydrogen ion, boric acid accepts an OH^- from water. Furthermore, borates in solution yield polymeric species that do not form readily balanced equations (Trejo et al., 2012)

$$B(OH)_3\,(aq.) + H_2O \longleftrightarrow B(OH)_4^-\,(aq.) + H^+\,(aq.)$$

The pH values considered for the borate buffer solutions were between 8 and 10, to ensure deprotonation of the silica walls of the capillary, for a consistent negative charge. It is expected that changing buffer pH will have drastic effects on EOF. In some cases, EOF is crucial for separation and in others, it can be detrimental to the stability of the analytes. There have been reports of the use of capillary coatings to eliminate or reverse EOF contingent on the experiment at hand (Doherty, 2003). Polydimethyl siloxane (PDMS) is one of many polymeric compounds used to coat the wall of capillaries and is seen as a better alternative to using an extremely acidic pH—as the low pH may affect the viability of the sample—to eliminate EOF (Doherty, 2003).

4.1.3 Surfactants

Due to the extensive use of surfactants or detergents in CE to manipulate the charge on the capillary wall as well as to separate the neutral species unaffected by EOF, sodium dodecyl sulfate (SDS) and Cetyl-trimethylammonium bromide (C-TAB) were added to the borax/boric acid solution to investigate the effect of surfactants on current. As seen in Figures 14 and 15, the presence of surfactants in the buffer system, independently, had little impact on current. The current outputs for the surfactants were comparable to those seen in the other buffer systems excluding the boric acid/NaOH solution. C-TAB, a cationic detergent, can be used to form a

positively charged surface and force EOF towards the anode instead of the cathode (Cifuentes, 1997). SDS, an anionic detergent, introduces the micellar electro-kinetic chromatography (MEKC), a mode that allows the separation of analytes without charge (Cifuentes, 1997). MEKC is made possible by micelles formed by SDS at concentrations above the critical micelle concentration (cmc) and neutral molecules are separated based on their associations within the micelles. Other than the separation of neutrals, micelles also increase the solubility of extremely hydrophobic compounds, improve resolution and are useful in the separation of drugs, biopolymers and more (Kuhr, 1992) .

4.1.3 Ionic Strength

The complexity of the borate buffer system—yielding to both trigonal planar and tetrahedral species—makes it difficult to attribute ionic strengths to the borate solutions (Garrett, 1998). Ionic strength is affected by concentration and charge for example: using 1M NaOH for titration affects ionic strength less than a 1M $Mg(OH)_2$ solution. This is due to the higher charge carried by magnesium (Mg^{+2}) as compared to that of sodium (Na^{+1}).

$$\mu = \frac{1}{2}\sum C_n Z_n^2$$

Furthermore, research performed at SUNY-Buffalo found that the size of the ion will also affect the electrophoretic performance (Saint-Fort et al.). This relation is elaborated by the activity of an ion which is dependent upon the activity coefficient (γ) that takes into account ion size ($\propto$), charge (Z) and ionic strength (μ) (Ref.). A cation such as Na^+, which has a radius of 450 pico-meters (pm), will have a higher γ than the anion Cl^- with a radius of 300 pm (Harris, 2003).

$$log\ \gamma = -\frac{0.5Z^2\sqrt{\mu}}{1 + (\propto \sqrt{\mu}/305}$$

Similar to the sodium thiosulfate-copper (II) system the boric acid-borate system is highly convoluted and incites a new opportunity for research pertaining to the presence of ionic species under numerous circumstances (Thorsten, 2013).

4.2 Simple Linear Regression Analysis with R

4.2.1 Basic Principles

Simple linear regression (SLR) is a statistical method that allows one to evaluate the relationship between two continuous variables. One variable (voltage) is held as the independent variable and the other (current) as the response or dependent variable. To evaluate which borate composition was the most effective for CE, simple linear regression analysis was used to compare and contrast the different borate solutions. An SLR model was created for each borate buffer with voltage and current as the variables of interest. There are various approaches to analyzing a simple linear regression but for the purpose of this experiment, four elements will be considered: the P-value of the regression coefficient, the multip-R^2, the ANOVA of the model and the residual standard error—all of which are described in detail below as adapted from Probability & Statistics with R for Engineers and Scientists.

4.2.2 P-value of the Regression Coefficient

The p-value, or calculated probability, is the probability of finding the observed, or more extreme, results when the null hypothesis (H_0)—typically a hypothesis of "no difference" (e.g. no difference between diet in group A and group B)—of a study question is true the definition and depends on how the hypothesis is being tested. P is also described in terms of rejecting H_0 when it is actually true but is not a direct probability of this state. A variable with a p-value of 1 is expected to have zero effect on the model or independent variable. As p-value decreases, the probability of the variable affecting the model increases. Therefore, a p-value greater than 0.1 indicates that the variable has little significance and perhaps should be excluded from the model. The value that indicates the variable's significance is Alpha and is set by the evaluator. Alpha is interpreted in two ways: there is either a 10% chance that the variable is affecting the model by random chance or 90% confidence—the congregate of alpha—that the variable is affecting the model.

4.2.3 Coefficient of Determination (R2)

R^2 is a measure of the variation of the dependent variable that is explained by the regression line. A high or low R^2 value is again determined by the significance of the variables within a model. It increases when variables are of high significance and degrades as significance decreases. For example, $R^2 = 0.98$ implies that 98% of the variation of current is explained by voltage. In an ideal situation, $R^2=1$, or as close to this value as possible, indicating that all variables in the model are highly significant.

4.2.4 ANOVA Test of the Model

A one-way analysis of variance (ANOVA) determines the significance in difference between two models by comparing the p-value to alpha (measure of certainty, normally 0.05). Two models are significantly different if p is less than alpha and not significantly different if p is greater than alpha. When performing stepwise regression, a dependent variable is added or removed from a model followed by a test of difference between the new and previous model. An ANOVA that shows no significant difference between two models suggests that the more simplistic model is preferred or in the case of SLR—the one with the more favorable R^2.

4.2.5 Residual Standard Error

The residual standard error describes the standard deviation of points formed around a linear function, and averages the difference between observed (measured in the lab) and predicted (by the model). An important factor in residual standard error is the degree of freedom. In statistics, the degree of freedom (df) refer to number of independent ways by which a dynamic system can move, without violating any constraint imposed on it.

4.2.6 SLR Evaluation of Borate Buffers

As seen in Table 2, the adj-R^2 and multiple R^2 values for each borate solution was significantly high with little difference seen between the two values. Furthermore, the p-values for solutions were all less than 0.01. This finding suggests that the borate solutions all have significant effects on the model and serve as important considerations for choosing a buffer system; however,

the borax/HCl model seems to be the most optimal. With a p-value of 1.654e-9 and a difference of 0.0003 between the multiple R^2 and adj-R^2 –the smallest values of the group—the borax/HCl model displayed the highest probability of predicting current at the specified voltages.

In contrast, the borax/boric acid solution, though quite significant when evaluated independently, was the least significant. Furthermore, the residual standard error for the borax/boric acid model, at 3.162 on 6 degrees of freedom was the most concerning. The boric acid/NaOH model showed the lowest residual standard error but is still considered less significant than the borax/HCl model and for these reasons; the borax/HCl model was further analyzed. According to the one-way ANOVA, there is a significant difference between borax/HCl and Boric acid/NaOH—$F(1, 6) = 3437.4$, $p = 1.654e\text{-}09$.

The residual plots were used to check assumptions of the SLR model of borax/HCl. The residuals vs fitted plot showed consistent variance about the line of best fit (Fig. 16). In an SLR model, a straight line is expected instead of the quasi-hyperbola fit seen in the residual vs. fitted plot. This finding leads to the possibility of creating transformed or segmented models. The normal Q-Q plot shows a moderate left skew to the distribution of the residuals; normality assumption is invalid according to Cook's distance in the Residuals vs Leverage plot also indicating that a transformation to linearity or segmented model should be used (Fig. 16)

Table 2. SLR values obtained using R Software

Buffer	Residual Std. Error	Mutiple R^2	p-value
Borax/HCl	1.059 on 6 df	0.9983	1.654e-9
Boric acid/NaOH	0.6164 on 6 df	0.991	2.253e-7
Borax/Boric Acid	3.162 on 6 df	0.9876	5.986e-7
Borax/boric acid in SDS	2.203 on 6 df	0.9924	1.366e-7
Borax/Boric acid in CTAB	2.429 on 6 df	0.9909	2.351e-7

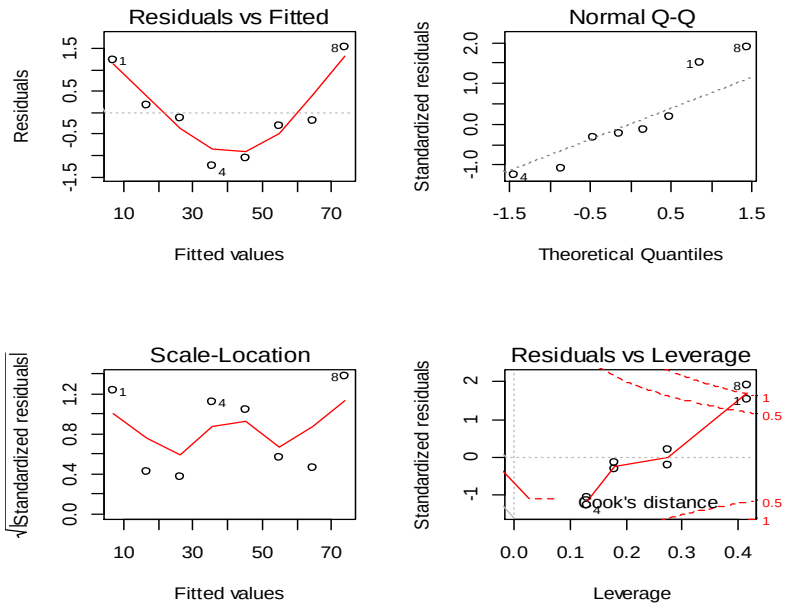

Figure 16. Residual plots of SLR Model for Borax/HCl obtained from R.

Some assumptions are violated in the model indicating that a transformation is needed.

4.3 Suggestions for Future Work

4.3.1 EOF and Sensitivity Detection

EOF determination using the current-monitoring method is insufficient and leaves much room for improvement.– The fluctuations in current, in addition to the rise and fall times, in the current high voltage system make it difficult to get accurate EOF measurements. Different power supplies will be considered for future work. Currently, two different optical detection methods are in development, including a laser-induced fluorescence (LIF) system featuring a 532 nm diode laser and a photo-multiplier tube (PMT) and a modified slab gel viewer with 470 nm LED excitation and a digital camera for a larger viewing window all of which will allow sensitivity detection.

4.3.2 Progression towards a Microchip Device

With the goal of moving toward poly (dimethyl siloxane) (PDMS) microchips, EOF will first be studied in PDMS-coated capillaries. PDMS microchips will then be fabricated using two different techniques to create a patterned mold, either by removing material using a CNC (computer numeric-controlled) mill or building up material using a 3-D printer.

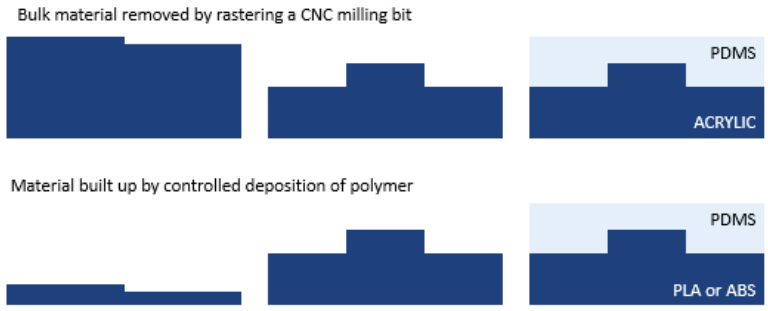

Figure 17. Fabrication of microchip device.

Two different methods and machinery by removing or adding material.

4.3.3 Segmented Linear Regression Analysis

Because joule heating is ubiquitous in electro-kinetic separations, it can alter the physical properties of the buffer and the efficiency of the separation (Whatley, 2001). To determine the voltage and current at which joule heating becomes a factor, the SLR models will be further analyzed via segmented Linear Regression Analysis. A segmented regression partitions the independent variable into intervals and a separate line segment fits to each interval (Ritzema, 1994). Segmented linear regression can be useful in quantifying unexpected changes in current with varying voltages. Breakpoints approximated by such analysis should help to determine the voltage at which the models deviate from Ohm's Law and initiate joule heating.

Chapter 5

Conclusion

Microchips are still in their infancy stages and are lacking in more ways than one thus further research is necessary for the development of more operational systems which could revolutionize sample analysis for field detection particularly in areas where resources are scarce. Based on results obtained in glass capillaries using borate buffer solutions, a legitimate starting point for microfluidic evaluation was established. Initial efforts resulted in the development of a CE instrument with manual switching of solution vials and real time current monitoring. This enabled the ability to compare various CE parameters and initiate an investigation into buffer optimization for electrophoresis in glass capillaries. Future work can now focus on PDMS-coated capillaries with an eye toward PDMS microchips and sample analysis. This study also brought light to a puzzle that has yet to be solved; the intrinsic chemical reactions of borates. Due to the complexity of this system, it was difficult to determine the form and/or amount of borate ions within any given reaction and because borate buffers are widely used in CE, understanding their behavior could potentially lead to an increase in the efficiency of CE separation. Furthering this research provides new grounds for developing ME devices while taking into consideration an essential constituent of electrophoresis, buffers, that has been overlooked compared to the other features of the technique.

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EDUCATION

Pennsylvania State University, Reading, PA August 2012-Present
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Honors Physical Chemistry-Quantum Mechanics
Honors Calculus I
Honors Physical Chemistry-Thermodynamics
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Inorganic Chemistry
Physics I* & II*
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Formal course was given and taken to Norway to explore, in depth, the Idea of the Castle in relation to chemistry.

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Chemistry Tutor

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Assisted students in the Penn State Berks Learning Center, in solving problems in general, organic and physical chemistry as well as helping students to develop more effective study tactics.

Chemistry Laboratory Assistant

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Prepared solutions, performed standardizations, assisted with chemical inventory and waste

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Operated autoclave, prepared bacterial growth media, performed aseptic cell culture, and assisted with biohazard waste disposal; set-up instructional cell biology and microbiology labs.

Chemistry Supplemental Instructor
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Assisted professors during lecture by answering student questions and clarifying lecture material; held exam reviews; provided individualized tutoring on personal time in General and Organic Chemistry.

Certified Nursing Assistant
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Maintained patient stability by ambulating, turning, and positioning patients; assisted with meals by feeding patients as necessary. Provided personal hygiene support, checked vital signs and weight; tested urine, recorded intake and output information.

PROJECTS

Curriculum Development Assistant
Pennsylvania State University, Reading, PA June 2014
Assisted with the curriculum development for Penn State Berks with regards to the College of Earth & Mineral Sciences standards.

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Best REU Research Poster Award-University at Buffalo (SUNY)	2015
NIH Undergraduate Scholarship Program Scholar	2015
Sieg Trustee Scholarship	2015
American Chemical Society Scholar	2015
Schreyer International Scholarship for Norway	2015
Pennsylvania Commonwealth Education Abroad Scholarship for Norway	2015
Bonsall Trustee Scholarship	2014
R. Walker Society Trustee Scholarship	2014
Penn State Berks Undergraduate Research Award	2014, 2015, 2016
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Boscov Academic Excellence Achievement Award	2013-Present
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Penn State Berks Honors Club	2013-Present
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