My High School Research Experience

By Varsha Sridhar

Introduction

One of the best presents I have ever received was a book entitled *Girls Think of Everything* on the day of my fifth grade graduation. *Girls Think of Everything* chronicles various women who have made world changing scientific discoveries. This book inspired my clueless, ten year old self to one day have such an impact on the world. Thus my passion for research began and has continued to influence my high school and college interests. During high school, I worked on two main research projects. I began the first project as a freshman in high school and studied the effects of synthetic estrogens on human health as well as the environment. The second research project used a theoretical chemistry approach to investigate the Marcus Model and *in vivo* electron transfer. In this paper, I will be discussing the integration of mathematics and science and my growth as a scientist in the context of these two research projects.

When I first began researching, I had never taken a proper science course and did not have much experience integrating mathematics with science. Because of this, I was completely overwhelmed by all of the advanced techniques and complex chemistry involved with my project. I spent many nights teaching myself chemistry and advanced math in order to better understand my procedure and results. Although I struggled back then, I value that experience because it allowed me to become a better scientist and a better student.

The best advice I can give to an aspiring researcher would be to find supportive mentors. Whether they are your teachers or parents, they are the people who will help you grow as both a scientist and as a person. I was fortunate enough to have a large support system consisting of my research mentors and family to who I owe all of my success.

Synthetic Estrogen Research

Background

During a shopping trip, I was tasked with buying baby bottles for my pregnant cousin. While looking at the different options, I noticed that some bottles were labelled "BPA Free" and some were not. I was not sure what "BPA" meant, but after a quick internet search I learned that it is an acronym for bisphenol A, an endocrine disrupting compound that has been linked to cancer, developmental issues, diabetes, and cardiovascular diseases [4]. I was surprised to discover that BPA is not only present in baby bottles but is a common ingredient in many consumer goods including polycarbonate plastic (e.g. reusable water bottles), epoxy resins [7], receipt paper [3], eyeglasses, and compact discs [1]. The compound's ubiquity has also led to environmental contamination, especially in bodies of water.

As I continued my background research, I learned that many advocacy groups have been lobbying the government to ban the use of BPA. At the time, The US and European Union had only prohibited the use of BPA in baby bottles but not in any other consumer goods. The legality of BPA was justified through the claim that a significant amount of BPA was not being absorbed or ingested by humans through these consumer products [2]. When I searched the scientific literature for studies regarding BPA and its effects on humans, to my surprise, I could not find any papers that directly measured the concentration of BPA in commercial goods. The more papers I read, the more interested I became in BPA and its effects on human and environmental safety. This inspired me to conduct my own research exploring BPA contamination from various consumer items.

However, my school did not have all of the necessary equipment for my experiment, so I contacted local researchers in the Birmingham area for feedback on my research plan and permission to use their lab. A chemistry professor at Samford University, Dr. Brian Gregory, enthusiastically responded and helped me plan my project. Under his guidance, I learned various research techniques and the corresponding mathematics. He also helped me expand my investigation of BPA to include other synthetic estrogens and a study of synthetic estrogens' detrimental environmental effects.

Determination of BPA in Polycarbonate Plastic

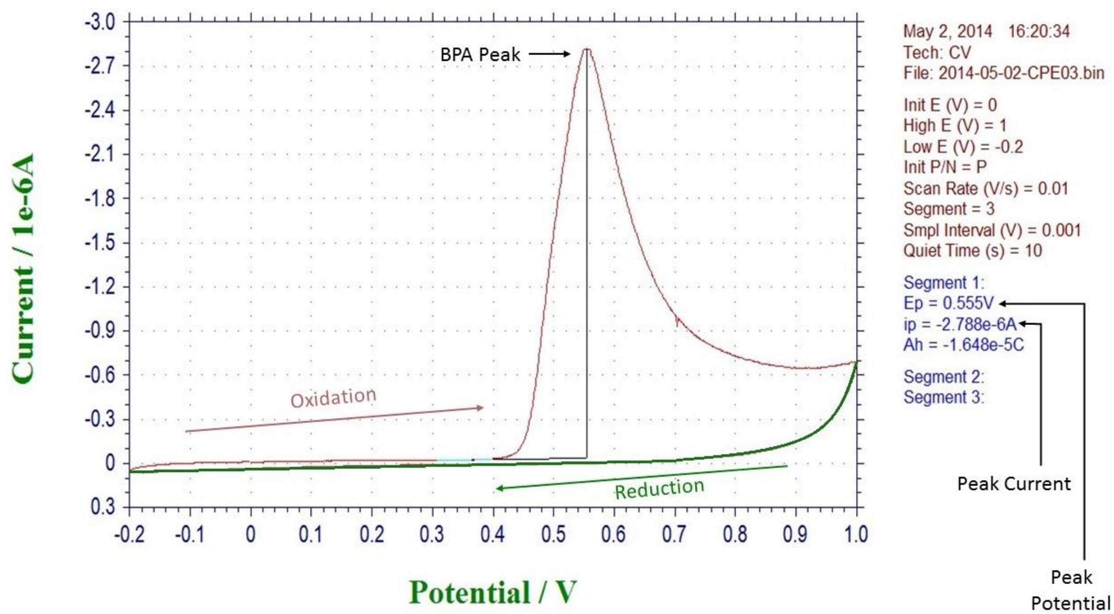


Figure 1: BPA Cyclic Voltammogram: As the potential increases, the compound in solution is oxidized at a certain reduction potential (peak potential). The oxidized compound is then reduced back to its original form as the potential decreases. The peak current can be used to find the concentration of the compound in solution.

Shortly after meeting with Dr. Gregory, I began an experiment to find the concentrations of BPA leaching out of polycarbonate plastic using cyclic voltammetry, an electrochemical method (Figure 1). The first step in this process was to create a calibration curve, or standard curve (Figure 2). This was done by first scanning stock solutions of BPA and finding their peak currents. The corresponding peak currents (A) were plotted against the known BPA concentrations (M) and the curve was very linear with an R^2 value of 0.998. The curve's equation can then calculate the BPA concentration of polycarbonate solutions using their peak currents.

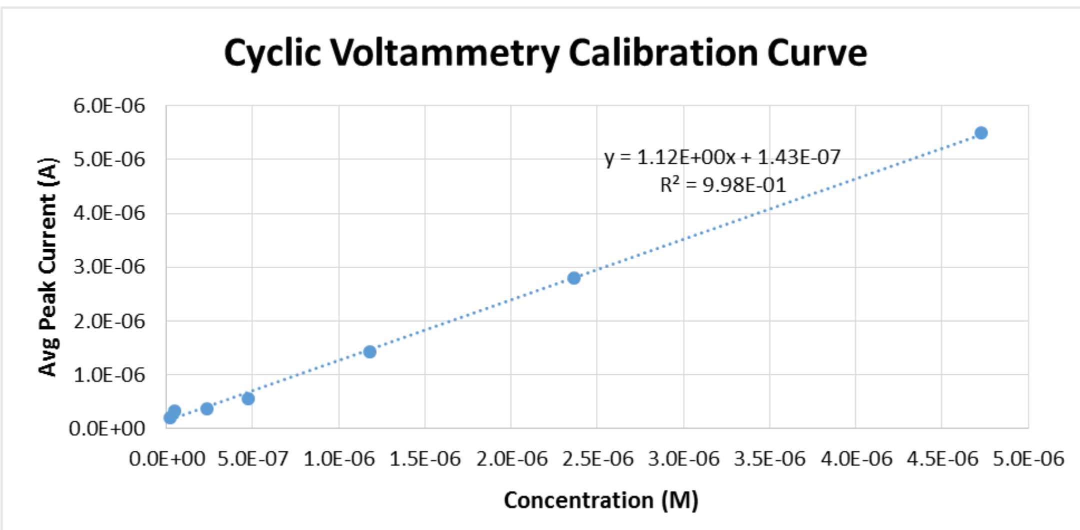


Figure 2: Calibration Curve: A calibration curve for BPA was constructed in order to determine the BPA concentrations of polycarbonate solutions. The equation "y = 1.12E+00x + 1.43E-07" was used to calculate the exact concentrations.

Using the above calibration curve (Figure 2), the BPA concentrations of polycarbonate plastic were calculated. In order to verify these results, mass spectrometry was completed with the same polycarbonate solutions by creating another calibration curve.

Determination of BPS in Receipt Paper

In recent years, the controversy surrounding the use of BPA has prompted manufacturers to abstain from using the dangerous compound and instead use bisphenol S (BPS). Many products that are labelled "BPA Free" contain BPS, which is more potent and dangerous than BPA [10]. Similar to BPA, BPS is an endocrine disruptor that mimics the hormone estrogen in the body [11] and has been linked to several serious illnesses, but 40% more BPS is used to replace BPA in consumer goods [9].

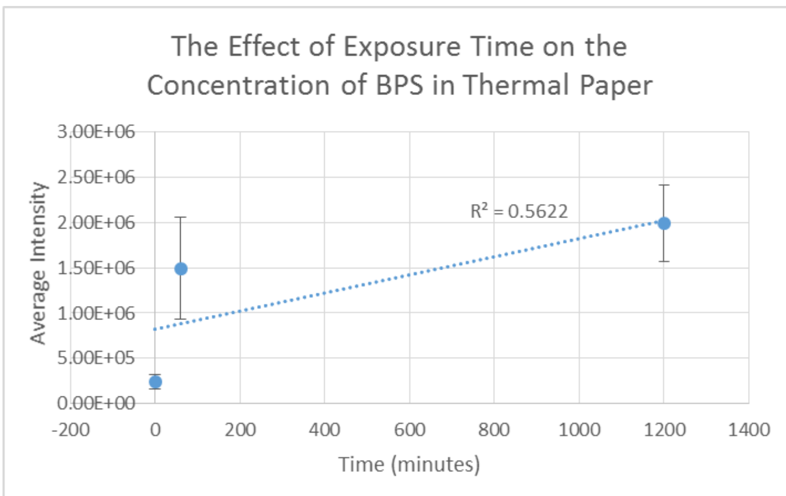


Figure 3: Change in BPS Concentration: The average relative intensities of the thermal paper solutions were plotted against the submergence time in solution. Error bars represent 95% confidence intervals.

For this experiment, receipt paper, or thermal paper, was analyzed using mass spectrometry. Receipt paper solutions were made with 5 second, 1 hour, and 20 hours submergence times in order to determine whether the concentration of BPS in solution increases with time. Since BPS is a loose, powdery coating on thermal paper, I hypothesized that all the BPS would instantaneously move into the solution and there would be no increase in concentration over time. Five trials of each thermal paper solution were scanned using an Ion Trap mass spectrometer. The results indicated that, conversely to what I hypothesized, the concentration of BPS in thermal paper increases with time. However, the correlation is not very linear (R^2=0.562) and the chosen time intervals are very large (Figure 3).

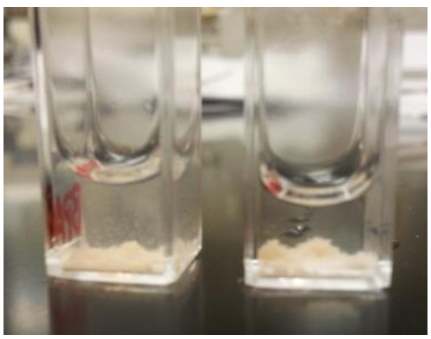
The results can be applied to the increasing absorption of BPS through contact with thermal paper as well as the increasing concentrations of BPS present in wastewater and surface water when thermal paper is discarded as trash in landfills and as litter. This experiment gives insight into the movement of thermal paper BPS in solution, but several more time intervals need to be tested to make more refined conclusions.

Mathematics plays an integral role in this experiment because of the need for statistical analysis. The data alone could not be used to definitively conclude whether there is a significant change in the concentration of BPA in solution. An ANOVA test, as well as several other statistical analyses including the Q-test and standard deviation, had to be completed in order to make this determination

Environmental Applications

BPA and BPS are known environmental pollutants that can damage the health of ecosystems through bioaccumulation and biomagnification [10]. To combat this problem, I sought out to create a method of removing BPA from contaminated water without using harmful, residual chemicals. *Trametes versicolor* (TvL) is a laccase derived from turkey tail mushrooms, a type of white-rot fungi that has been known to be able to remove estrogenic compounds from solution [6].

The TvL laccase was added to a special BPA solution and the reaction was allowed to proceed for 24 hours or until precipitate formed (Figure 4). The precipitate was dissolved in solution and scanned using a mass spectrometer. The resulting spectrum had several large peaks spanning from mass to charge ratios (m/z) of 200 to 1800. In order to determine the compound that correlates to each of the peaks, I had to calculate the masses of several different structures and polymers of BPA. Even though the math was simple, this was a tedious task that required organization to ensure that the calculations were accurate. When the precipitate was dissolved and analyzed using the Ion Trap mass spectrometer, there were peaks at mass to charge ratios correlating with BPA polymers (i.e. m/z 454, m/z 680, m/z 905, Figure 5).



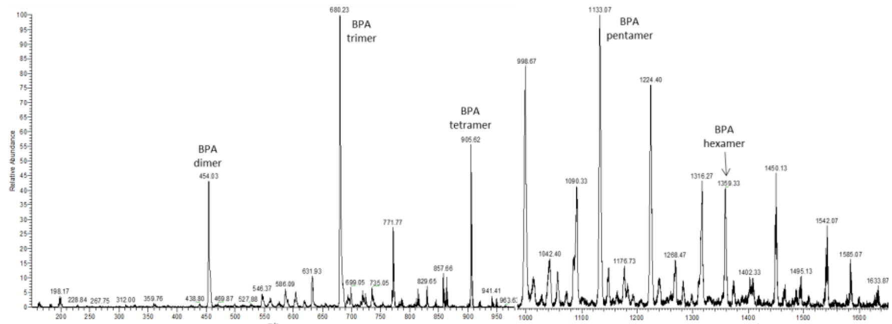


Figure 4 (Left): BPA Precipitate: After 24 hours of exposure to the TvL laccase, the BPA in solution polymerizes to form a cloudy, white precipitate. The precipitate can then be analyzed using mass spectrometry.
Figure 5 (Right): BPA Precipitate MS Scan: The precipitate from the BPA experiment was scanned using mass spectrometry. The resulting scan showed multiple BPA polymers and fragments of those polymers.

The TvL precipitation method to remediate BPA from solution can be used to create a more practical and convenient way to remove BPA, as well as other synthetic estrogens, from water. It could also be used to create new sources of potable water. In developing countries, the estrogenic chemical benzene, resulting from petroleum pipelines, is a major source of water contamination that negatively affects both plants and animals [5]. TvL could alleviate this problem by remediating benzene and other estrogenic contaminants from water.

Another application I addressed with my research is the effect of BPA and BPS on plant development. By exposing *Vigna radiata* seeds (mung beans) to BPA and BPS during early stages of growth, I was able to determine how the synthetic estrogens affected germination and maturation. BPA lowered the rate of germination the most significantly while the plants exposed to BPS only had slightly lower germination rates than the control. This pattern was consistent throughout the maturation of the plant.

Conclusion

As I studied synthetic estrogens, I had to learn how to use different instruments and the corresponding chemistry. Throughout the course of my research project, I came across several problems that I had to resolve. Although I was not excited about these hindrances when they occurred, I now have a better understanding of the instruments and the chemical processes involved because of these setbacks. I would not have learned so much if all my experimentation had proceeded smoothly. I also realized that despite appearances, scientific research is largely a creative endeavor. A scientist must use his or her imagination to develop a unique research question that attempts to solve a real world problem. I am proud to have applied creativity in my research through the development of distinct, interesting questions and novel approaches to answering them.

Generalization of the Marcus Model for *in vivo* Electron Transfer

In the spring of my junior year, I was selected to participate in the Research Science Institute, a prestigious summer research program for high schoolers around the world. In my application, I discussed my research experience and interests in chemistry, so I was paired with Dr. Udayan Mohanty, a theoretical physical chemistry Professor at Boston College. Under his guidance, I completed a research project on *in vivo* electron transfer and the Marcus Model.

Dr. Mohanty introduced me to topics such as the Frank-Condon principle and Brownian motion, and he provided me with a transcript of Rudolph Marcus's Nobel Prize Lecture in which Marcus discusses his research on electron transfer and the discovery of the inverted effect. While reading the transcript and Marcus's other publications, I observed that all of his research was done in a laboratory setting rather than a biological environment. I began wondering if his results would hold true in an intracellular environment [8]. Upon further research on *in vivo* electron transfer and crowded environments, I discovered that there was very little current research on *in vivo* electron transfer despite it being thought to be "the future of renewable energy." The potential applications and my own curiosity inspired me to conduct this research.

In order to answer this question, I had to learn many challenging mathematical techniques including probability distributions and the Laplace transform. In my junior year of high school, I took a math course that combined calculus I, II, and III. My mathematical knowledge made the calculations involved with my project much easier, but conducting completely theoretical research was still difficult. Before this project, my research experience primarily consisted of wet lab work. I never understood how theoretical research could contribute to society. To me, it appeared to be just abstract calculations with little significant meaning outside of the scientific community. Once I started this project I discovered that it had a plethora of applications that have the capacity to change the world.

Currently, the availability and environmental effects of energy have become critical issues. Non-renewable resources are scarce, yet there is no viable replacement that satisfies all parties (governments, businesses, and environmental activist groups). Bacterial fuel cells have the ability to resolve this problem because they are able to produce renewable energy through *in vivo* electron transfer [6]. Upon discovering this application, I became even more excited about my research. Bacterial fuel cells have the potential to improve the lives of millions people. Applications like these motivate me to continue researching and reinforce my overall passion for science.

Acknowledgements

I would like to thank the following people for their help and guidance throughout this project:

- **Dr. Udayan Mohanty** from Boston College for his time and patience, as well as his guidance throughout this project
- **Dr. Brian Gregory** from Samford University gave me permission to work in his lab as well as his time and guidance
- **Dr. Corey Johnson** from Samford University gave me permission to work in his lab and guided me through the laccase experimentation
- **Dr. David Johnson** from Samford University pointed me in the right direction by referring me to Dr. Gregory
- **Dr. Morgan Ponder** from Samford University for giving me permission to work in his lab
- **Ms. Kelly Breland** and **Mr. Ryan Reardon** from Jefferson County IB for their support
- The **Research Science Institute (RSI)** and the **Center for Excellence in Education (CEE)** for giving me the opportunity to conduct research

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