A Summer in Spain: Developing a Biosensor for Differential Stroke Diagnosis

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Introduction

Through the Science Influencers internship program, I was able to travel to the University of Oviedo in Spain and spend my summer participating in research being conducted on a biosensor for differential stroke diagnosis. This life-changing opportunity would not have been possible without the help of the Science influencer program directors, Dr. Holli Leggette, Dr. Gary Wingenbach, and Dr. Barbra Gastel. As well as Texas A&M professors Dr. Frances Ligler and Dr. George Ligler. And most importantly, my host professor, Dr. María Teresa Fernández-Abedul.

The topic for my summer research was focused on the vertical flow electrochemical immunoassay for determination of a protein biomarker of differential diagnosis of stroke. Broken down, my work focused on immunoassays, electroanalysis, and device fabrication.

Purpose and Objectives

Stroke is a leading neurological health problem in countries all over the world. Worldwide, over 12 million people have a stroke each year and about 6.5 million people die as a result (Impact of Stroke). The two types of strokes that lead to these high mortality rates are ischemic and intracerebral hemorrhagic stroke. Being able to properly differentiate between these strokes is extremely challenging, but being able to do so will improve patient diagnosis and treatment. My team in Spain had already partnered with neurologists at the Central University Hospital of Asturias (HUCA) and found that a protein, GFAP, that released in nasal exudate is a useful biomarker in differential stroke diagnosis. Where a higher concentration of GFAP correlates to hemorrhagic stroke, and a lower concentration correlates to an ischemic stroke (García-Cabo et al.).

Once the connection between stroke diagnosis and a biomarker was established, my team moved on to developing a biosensor for fast, decentralized and differential stroke diagnosis. The end goal is to have a biosensor that can be used in pre-hospital settings to determine if a patient had a hemorrhagic or ischemic stroke.

Methods

As previously stated, the focus of my summer research was vertical flow electrochemical immunoassay for protein detection. To achieve this goal, my research was split into four key sections: ELISA optimization, electrode testing, vertical flow optimization, and hospital sampling.

First, I conducted two immunoassays to determine the impact of BSA (bovine serum albumin) in blocking buffer protein detection. In the first ELISA, a blocking buffer made up of PBS, BSA, and tween was used. While this is the typical solution used for protein dilution, the concern was that the BSA would incorrectly increase the amount of protein being read in a

sample. In the second ELISA, washing buffer made up of only PBS and tween was used. The goal was for the washing buffer to work to eliminate any incorrect protein measurements.

Next, my work focused on testing paper-based electrodes (PBEs) against screen printed electrodes (SPEs). First, I conducted flow tests using water on paper-based electrodes. I drew lines with a hydrophobic pen and wax cray to determine which stopped the flow of water. Once the flow tests were completed, electrochemical tests were performed on both electrode types using potassium ferricyanide.

Following this, vertical flow tests were completed using the two types of electrodes. First, chronoamperometric testing was performed on the SPEs then the PBEs. Different layer thickness combinations of the sample pad and absorbent pad were tested using a potassium ferrocyanide solution. Based on the chronoamperometry' results, the layer combinations were adjusted to allow for optimal sample flow.

Results

After analyzing and compiling the results, the washing buffer, albeit showing a smaller slope and thus less sensitivity, is a reliable substitute for blocking buffer in this step of the ELISA. This is supported by the graph in Figure 1. The R^2 values for both buffer solutions indicate that they both work well in the ELISA. Additionally, the small difference between these values, 0.001, also supports the conclusion that washing buffer is a valid substitute for blocking buffer.

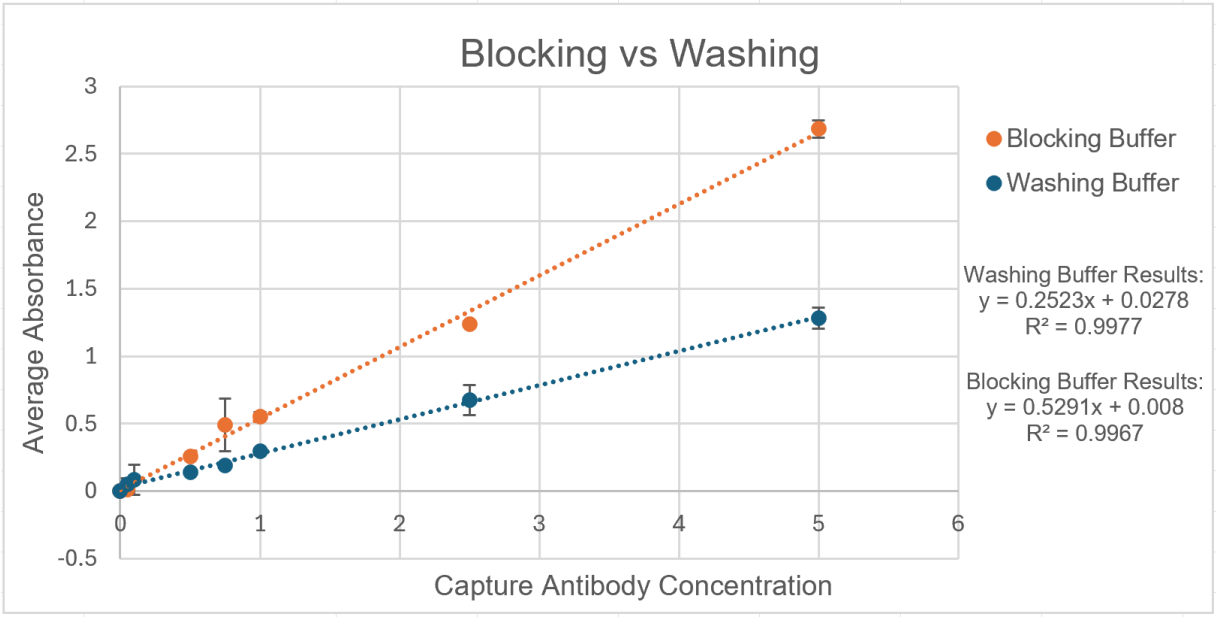

Figure 1: Blocking vs Washing ELISAs

After comparing the hydrophobic pen and wax crayons, it was determined that the wax would be more effective for use on the PBEs. The results of the flow tests can be seen in the figures below. In Figure 2. seen below, there are four paper test strips displayed with lines using the hydrophobic pen. The first strip, 1, had one line drawn on one side and the water leaked through. The second strip, 2s, had two lines drawn on one side and successfully held the water to one side. The third strip, 2ss, had one line drawn on either side of the paper and the water leaked

through. The final strip, 3, had three lines drawn on one side of the paper and the water leaked through.

In Figure 3, seen below, three paper tests strips are displayed with wax lines. All lines were melted at 70°C but for different durations of time. This creates a wax barrier that goes all the way across the paper strip section. The orange line was melted for 30 minutes, the blue line was melted for 10 minutes, and the purple line was melted for 5 minutes. Despite the various melting times, all the wax lines successfully held the water to one side of the paper. After comparing the hydrophobic pen and wax crayons, it was determined that the wax crayon would be more effective for use on the PBEs.

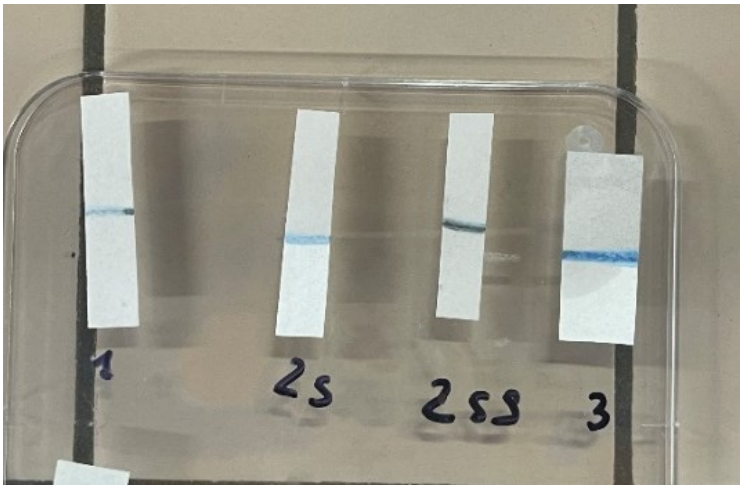

Figure 2: Hydrophobic pen test strips

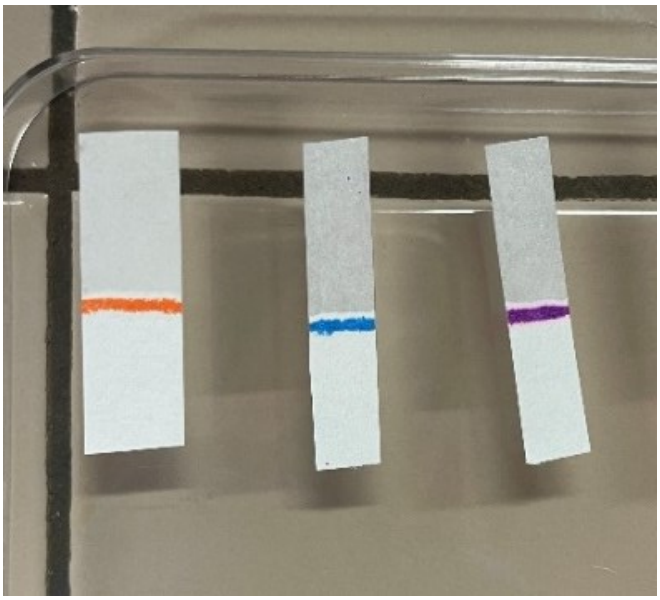
Figure 3: Wax crayon test strips

Once it was determined that wax worked best to limit flow, PBEs were prepared and used in electrochemical testing. After performing chronoamperometric testing, the results of the oxidation and reduction reactions that occurred on both electrodes were plotted against each other. The results from this test can be seen in figures 4 and 5 below. The hypothesis for this

experiment was that the PBE measurements would be as accurate as the SPE measurements. This hypothesis was proved true, with an oxidation R^2-value of 0.9992 and a reduction R^2-value of 0.9790. A comparison of the oxidation and reduction reactions from the screen-printed and paper-based electrodes can be seen below in Figures 4 and 5. In comparing these figures it is important to note that the PBEs present a higher sensitivity than the SPEs.

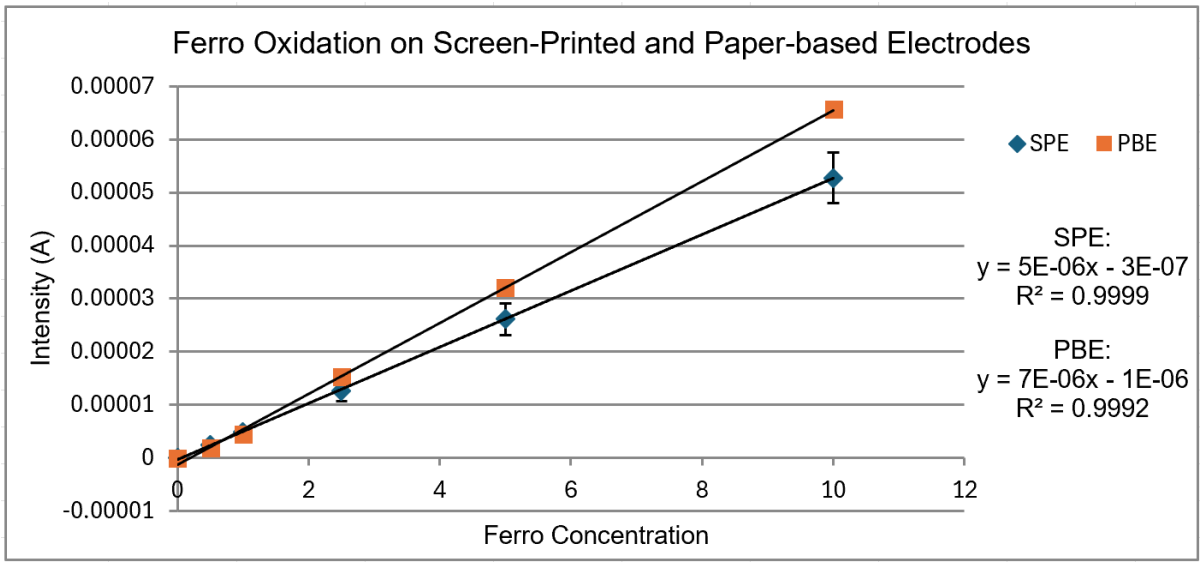

Figure 4: Comparison of Oxidation on Plastic and Paper Electrodes

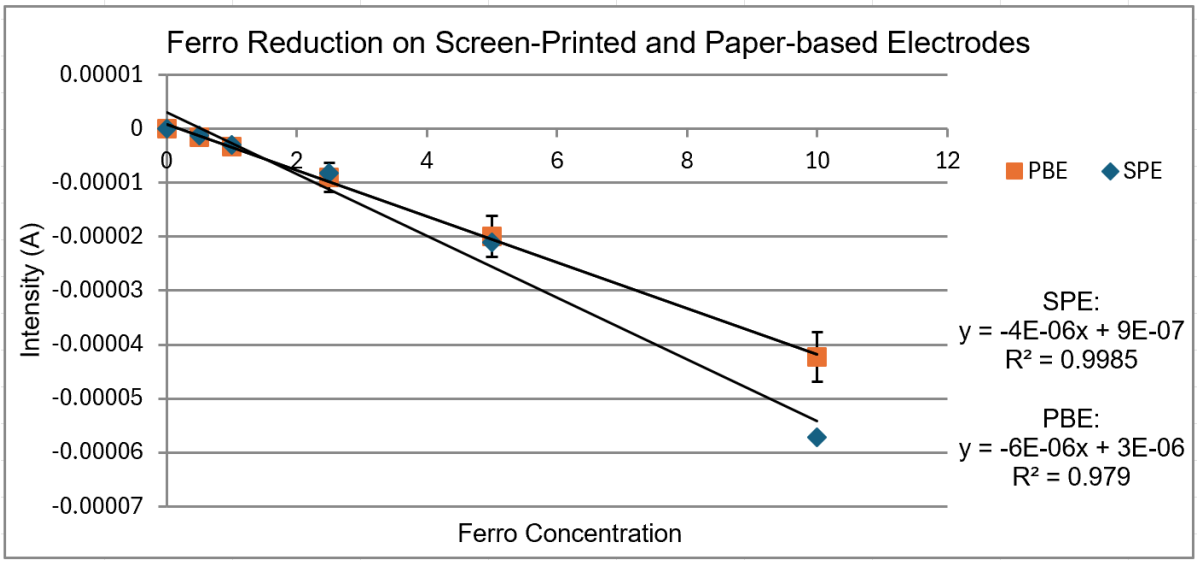

Figure 5: Comparison of Reduction on Plastic and Paper Electrodes

After analyzing and comparing the results, the various layer combinations and volumes of ferrocyanide solution used can be seen below in Table 1. It was determined that the best layer combinations were thin-thin 50, thick-thin 50, thick-thick 75, and thin-thick 50. These gave readings where an oxidation of the ferrocyanide was observed, meaning that the sample flowed into every layer of the device. These results were further compared in Figure 6. Based on the time it took each reading to occur it can be concluded that the combinations thick-thick 75 (K-K

(75)) and thin-thick 50 (N-K (50)), were the best layer and volume combinations, as they show a rather steady flow instead of an abrupt one, that can be observed in other conditions.

Table 1: Sample and Absorbent Pad Thickness Combinations

Sample Pad	Absorbent Pad	Volume (µl)
Thick	Thin	40
Thick	Thin	50
Thick	Thick	50
Thick	Thick	75
Thin	Thin	40
Thin	Thin	50
Thin	Thick	50

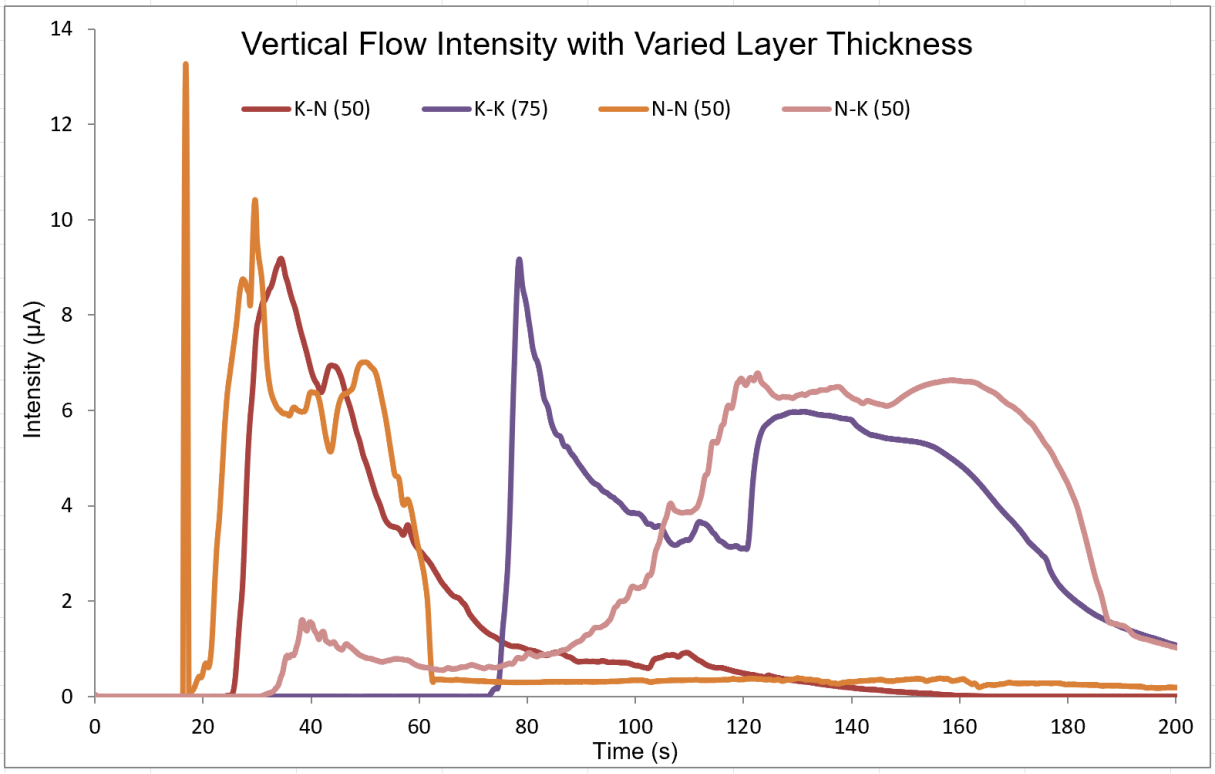

Figure 6: Time vs Intensity of Flow through Different Layers

Conclusions

The results from my experiments are now being used in research for the final biosensor. Being able to contribute a summer's worth of work to a medical device that will change lives was extraordinary. I cannot wait to see future publications about the final device and see how my small contributions were used to progress the project.

My summer spent in Spain was truly life changing. I grew as an individual in professional and personal ways that are invaluable. Once again, I want to thank all of the people who believed in me, invested in my future, and had enough confidence in my abilities to send me to Spain; thank you Dr. Holli Leggette, Dr. Gary Wingenbach, Dr. Barbra Gastel, Dr. Frances Ligler, Dr. George Ligler and Dr. María Teresa Fernández-Abedul.

References

García-Cabo, Carmen, et al. “Measurement of GFAP in Nasal Exudate in the Differential Diagnosis between Ischemic Stroke and Intracerebral Hemorrhage.” *Cerebrovascular Diseases*, vol. 53, no. 5, 25 Oct. 2023, pp. 515–518, karger.com/ced/article/53/5/515/868353/Measurement-of-GFAP-in-Nasal-Exudate-in-the, https://doi.org/10.1159/000534693. Accessed 18 Oct. 2024.

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