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Glucose Oxidase and Prussian Blue Nanoparticles Encapsulated within a Xerogel for Electrochemical Detection of Glucose

By

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Marine Science

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Introduction

Blue pigment was once given a greater value than gold. It was near impossible to manufacture and extremely cost prohibitive. Then, by chance, in 1706 Heinrich Diesbach, a Berlin chemist, developed the world's first synthetic dye.[1] Diesbach accidently formed the pigment when experimenting with the oxidation of iron.[2] This new pigment was a deep blue color eventually to be known as Prussian Blue. The name "Prussian Blue" originated in the 18th century when the pigment was used to dye the uniform coats of the Prussian army.[1] The name, however, was converted to a variety of other options, Midnight Blue, Berlin Blue, Parisian Blue, as the average individual lost touch with who, what, and/or where Prussia was. Prussian Blue was a monumental discovery and has seen a multitude of applications across many fields since its inception in 1704. First to the artist, and eventually for the chemical and medical fields

Ferric ferrocyanide, also known as Prussian Blue, is a synthetic complex potassium compound, containing two iron ions with different charges, Fe^{2+} and Fe^{3+}. In addition, it also contains negatively charged hexacyanoferrate ions $[Fe(CN)_6]^{4-}$. The overall formula is usually written as $Fe_4[Fe(CN)_6]_3 \cdot x\ H_2O$. It is normally prepared as a colloidal suspension due to the fact the compound is not soluble in water.

Prussian Blue has seen a multitude of applications across various fields including, but not limited to, the treatment of radiation poisoning from ^{137}Cs after the Chernobyl disaster in 1986. Patients with severe internal contamination were given oral doses of up to 10g of Prussian Blue a day and the therapeutic regimen was described as successful.[3] Due to its effectiveness in the treatment of radiation exposure, the World Health Organization currently has Prussian Blue on the list of essential medical supplies to keep in surplus, in the event of a nuclear emergency.

Other scientists have found uses for Prussian Blue reduced to nanoparticles. Prussian Blue nanoparticles combined with other functional particles, such as silver, were utilized in multifunctional imaging and therapy for tumors.[4] This is only one of the many significant uses for the accidently discovered blue dye. Science has only scratched the surface of the enormous pool of potential uses of Prussian Blue, many additional uses have been discovered. For example, it is vital to patient care to make sure certain medical equipment is completely sterile and bacteria free. Recent research has demonstrated Prussian Blue may be a useful tool for this purpose. In one study, adding highly photo-thermally active Prussian Blue nanoparticles to polyvinyl alcohol films and heated up with low laser intensities produced an efficient antibacterial effect.[5]

Because Prussian Blue is one of the most commonly used electrochemical intermediaries for analytical applications, it has found wide use in the biosensor field in recent years. The deep blue dye has been found to possess considerable advantages as well as disadvantages over traditional biosensors such as platinum.[6] There is great interest in furthering Prussian Blue's usability in biosensor applications. One area of particular interest is the addition of metallic nanomaterials and an enzyme into xerogel, especially due to the unique electron transfer properties of the metallic nanomaterials.[7] Another area of increasing interest for Prussian Blue is its use as a nanostructure on electrodes which has demonstrated significantly decreased background and a low detection limit.[8]

Recent literature cites examples of electrochemical biosensor systems based on the encapsulation of enzymes and metal nanoparticles suspended in a xerogel for the detection of biologically significant compounds, such as glucose.[7] While these systems are very successful with acceptable detection limits and linear ranges, Prussian Blue modified electrodes have been

shown more effective than metal electrodes in determining glucose levels through the detection of hydrogen peroxide.[9]

The goal of this research was to gain a more comprehensive understanding of recent literature on novel Prussian Blue combined with xerogel to form a biosensor. The optimal outcome was to develop an electrochemical system that combines encapsulation of glucose oxidase within a xerogel with a Prussian Blue modified electrode. Two different locations for the modification of the Prussian Blue were also explored. The comparison between an electrode with a complete layer of Prussian Blue and an electrode with a partial layer of Prussian Blue is made in this study.

Experimental

Chemicals:

All solutions were made in class A glassware and Milli-Q water (18.2 MΩ cm). Sodium phosphate dibasic, potassium phosphate monobasic, and aspergillus glucose oxidase were obtained from VWR chemicals. Potassium chloride and hydrochloric acid were obtained from BDH chemicals. Potassium ferricyanide and ferric nitrate nonahydrate were obtained from Amresco. Tetrahydrofuran (THF) came from Sigma-Aldrich. Trimethoxy(methyl)silane (3-MPTMS), glucose was acquired from TCI. All chemicals were used as received.

Electrochemical Equipment:

The WaveNow along with AfterMath (version 1.2.5966) were used for both deposition of the complete single-layer of Prussian blue as well as the deposition of the partial-layer of Prussian blue via cyclic voltammetry. Additionally, the WaveNow along with AfterMath

(version 1.2.5966) were used for data collection via bulk electrolysis. The type of electrode used was a Pine Research screen printed carbon electrode with a 5mm x 4mm working electrode.

***Solutions*:**

Buffer solution with a pH of 6.5 was made from potassium phosphate monobasic, sodium phosphate dibasic and potassium chloride. The solution used for the deposition of Prussian Blue in this experiment was made up of approximately 5 mM of potassium ferricyanide ($K_3Fe(CN)_6$), and 5 mM of ferric nitrate ($Fe(NO_3)_3$) mixed with 10 mL of 6 M hydrochloric acid in a 100 mL volumetric flask and diluted to volume with phosphate buffer.

Preparation of glucose solutions was carried out by forming a series of solutions with decreasing concentrations containing $1.0x10^{-2}$ M, $5.0x10^{-3}$ M, $1.0x10^{-3}$ M, $5.0x10^{-4}$ M, $1.0x10^{-4}$ M, $5.0x10^{-5}$ M, $1.0x10^{-5}$, and $1.0x10^{-6}$ M, in the buffer solution.

***Xerogel Deposition*:**

Xerogel was created using 9.0mg of glucose oxidase mixed with 75.0µL of water in a centrifuge vial and vortexed for approximately 10 minutes. In a separate centrifuge vial 25.0µL of 3-MPTMS was mixed with 100.0µL of THF and then vortexed for approximately 10 minutes. After both were vortexed separately, 50.0µL of the glucose oxidase mixture was added to the silane mixture and vortexed again for approximately 10 minutes. The newly formed xerogel is pipetted onto the surface of the electrode. The deposited xerogel is dried for at least 24 hours in a refrigerator (4.0 ˚C).

***Prussian Blue Complete-Layer Deposition*:**

The formation of the complete layer of Prussian Blue on an electrodes surface was done by running the electrode through electrolysis, with a potential set at 400 mV for a duration of 40 seconds, while in the deposition solution. The electrode was then run through cyclic voltammetry, with the potential set from -50 mV to 350 mV and a sweep rate of 40 mV/second. This was repeated ten times in the deposition solution and ten additional runs in the buffer solution. After this process was done the electrodes were dried in an oven set a 100 ˚C for one hour. Upon completion, the electrodes were ready for the xerogel deposition.

***Prussian Blue Partial-Layer Deposition*:**

For the formation of the partial layer of Prussian Blue on an electrode, each electrode was run through a cyclic voltammetry five to twenty times with the potential set at -50 mV to 350 mV and a sweep rate of 40 mV/seconds while in the deposition solution. After the electrode was run through the deposition solution it was placed in the phosphate buffer and run through cyclic voltammetry ten times under the same conditions. The electrodes were then dried in an oven at 100˚C for one hour.

Results and Discussion

***Xerogel deposition*:**

The formation of the xerogel on the electrodes surface is demonstrated by the mixing of the THF and 3-MPTMS and allowing the mixture time to solidify into its porous structure. This was verified by appearance and viewing the solidified xerogel through a microscope. Visual

inspection of the electrode surface confirmed deposition of xerogel across the entire electrode surface.

Of some concern is the possibility of the glucose oxidase not being trapped within the xerogel. Through this study, the indication of glucose oxidase being trapped within the xerogel matrix would be the responses seen while running the electrode through the bulk electrolysis in glucose solutions and collecting data. When an electrode was run through a glucose solution it would give a reading differing from the reading given off when the same electrode was run through the standard phosphate buffer. This indicates the glucose oxidase is working to catalyze the reaction. No change in response was observed for electrodes that were created without the inclusion of glucose oxidase. In addition, an alternative method of determining the presence of glucose oxidase within the xerogel is through visual examination, as the enzyme is a bright yellow color and can be seen within the xerogel.

There are two different deposition arrangements employed in this experiment. One method involved depositing a complete layer of Prussian Blue on the surface of the electrode and then adding the layer of xerogel with glucose oxidase within it, in hopes of keeping the Prussian Blue on the surface of the electrode. The second method was to combine the Prussian Blue with the glucose oxidase within the xerogel deposited on the surface of the electrode as a suspension. These two deposition arrangements are visualized in Figure 1.

***Prussian Blue Below Xerogel*:**

The electrode tested with a complete layer of Prussian Blue on the surface produced a range of results as show in the calibration curve in Figure 2. The calibration curve produced a trend line with a coefficient of determination equal to 0.9367. The slope of the trend line is -

25.385 and the y-intercept is 0.0763. This calibration curve shows the change in of the log of the current in relation to the change in concentration of glucose.

The coefficient of determination of the calibration curve is reasonable, indicating the system works and can detect glucose over a wide range of concentrations. Another interesting aspect of this calibration curve was, the current decreased as the concentration of glucose increased. This was expected because as the concentration of glucose increases the amount of hydrogen peroxide created increases causing more Prussian Blue to react. The more the Prussian Blue reacts the more electrons are needed to satisfy its charge, these electrons come from the electrode and therefore the current decreases as the glucose concentration increases (Figure 3). This decrease lead to change from positive current to a negative current reading in the middle of the series of glucose solutions, which can be seen in Figure 2, even after taking the log of the current. Despite the levels of success there remain uncertainties with using this system to confidently predict glucose concentrations. The main concern is the ability to obtain consistent results. The reproducibility of an individual electrode was not consistent.

Looking at Figure 2, there is a cluster of points at the far left of the graph. This cluster indicates the initiation of a less significant response in current as the concentration of glucose continues to decrease at a constant rate. The detection limit of this systems is approximately 5.0 X 10^{-5} M glucose. This could also be a result of examining the log of the current verses concentration, instead of current versus concentration.

***Prussian Blue Within Xerogel*:**

Initially, the method employed for preparing the electrodes was by co-deposition of Prussian Blue and xerogel. The inconsistency of results inspired a change in methodology to the

deposition of Prussian Blue within xerogel. The method of depositing Prussian Blue within the xerogel was able to produce more accurate calibration curves as well as show better reproducibility with each electrode. This method's data shifted the focus from attempting to deposit the Prussian Blue and xerogel together, to depositing the xerogel and then introducing the Prussian Blue into the xerogel.

The electrodes tested with a partial layer of Prussian Blue on the surface also produced a range of results. The desired results were seen infrequently. Although infrequent, the desired results are shown in the calibration curve labeled Figure 4. The calibration curve shown in Figure 4 produced a trend line with a coefficient of determination equal to 0.9921. The slope of the trend line is -113.46 and the y-intercept is 0.8351. This calibration curve shows the change in the log of the current in relation to the change in concentration of glucose.

While the coefficient of determination is stronger for the results shown in the experiment with the electrode containing Prussian Blue within the xerogel than the results with the electrode containing Prussian Blue below the xerogel, it suffers from similar difficulties. The most common challenge was getting some desired results from one electrode and not another, with no clear explanation as to why. The proposed idea is the Prussian Blue is unable to consistently form from the deposition solution inside the xerogel.

Similar to the electrode with Prussian Blue below the xerogel, the electrode with Prussian Blue within the xerogel shows a grouping of data points near the y-axis. Possible explanations for this grouping could be, examining the data for the current in the log form, or a decrease in significant differentiation between concentrations as they become very small. The calibration

curve also shows the decrease in current as the concentration of glucose increases (Figure 4). Again, this is expected based on reactions taking place (Figure 3)

Since this calibration curve appears like that of the previous calibration curve, the same approach is taken when looking for detection limits (Figure 4). The curve shows a grouping of points near the y-axis. This grouping could be showing the approximate detection limit of the system similar to the other method, the approximate detection limit of this system is 1.0×10^{-4} M glucose. It is also possible, presenting the y-axis data in the log form is shifting the points visually. The limit of detection for the upper end is well defined, based on the curve.

Conclusion

The results from this study show that similar calibration curves can be achieved when using the method of a partial layer deposition compared to a complete layer deposition of Prussian Blue. The xerogel plays an important role in this as it creates many locations for the Prussian Blue to form within its porous structure. The time required to prepare individual electrodes is significantly different due to the fact the partial layer takes much less time to prepare. Confirming the complete coverage of xerogel on the electrode surface can be validated with the use of a higher-powered microscope or with instrumentation allowing for the examination of the surface of the electrode and xerogel. The information gathered from this study is valuable in that it will help the development of glucose biosensors utilizing Prussian Blue nanoparticles within xerogel. The next step in creating an electrode with good reproducibility will be understanding how to control the spontaneous formation of Prussian Blue within xerogel.

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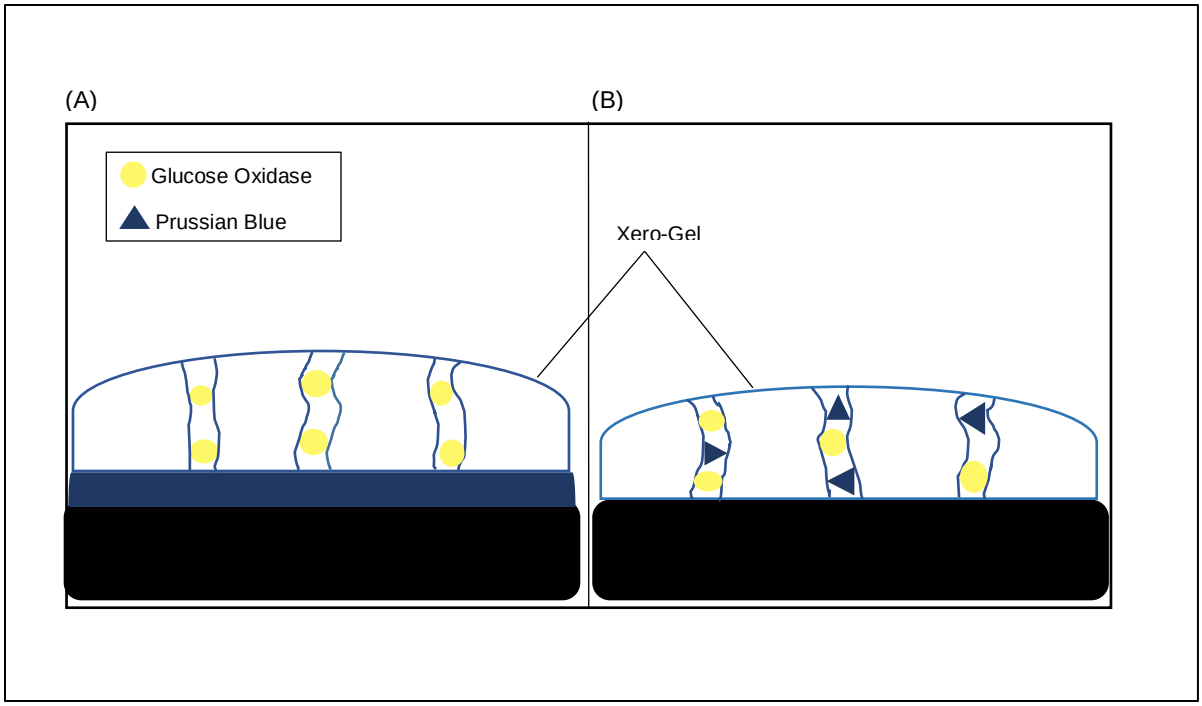

Figure 1: A simplified schematic of the two types of biosensors developed in this work. A) On the glassy carbon screen printed electrode, a complete layer of Prussian Blue is deposited over which the xerogel is formed that has glucose oxidase trapped within the pour matrix. B) On the glass carbon screen printed electrode, the xerogel is deposited with glucose oxidase contained in the pores and then nanoparticles of Prussian Blue were deposited within the remaining pore space.

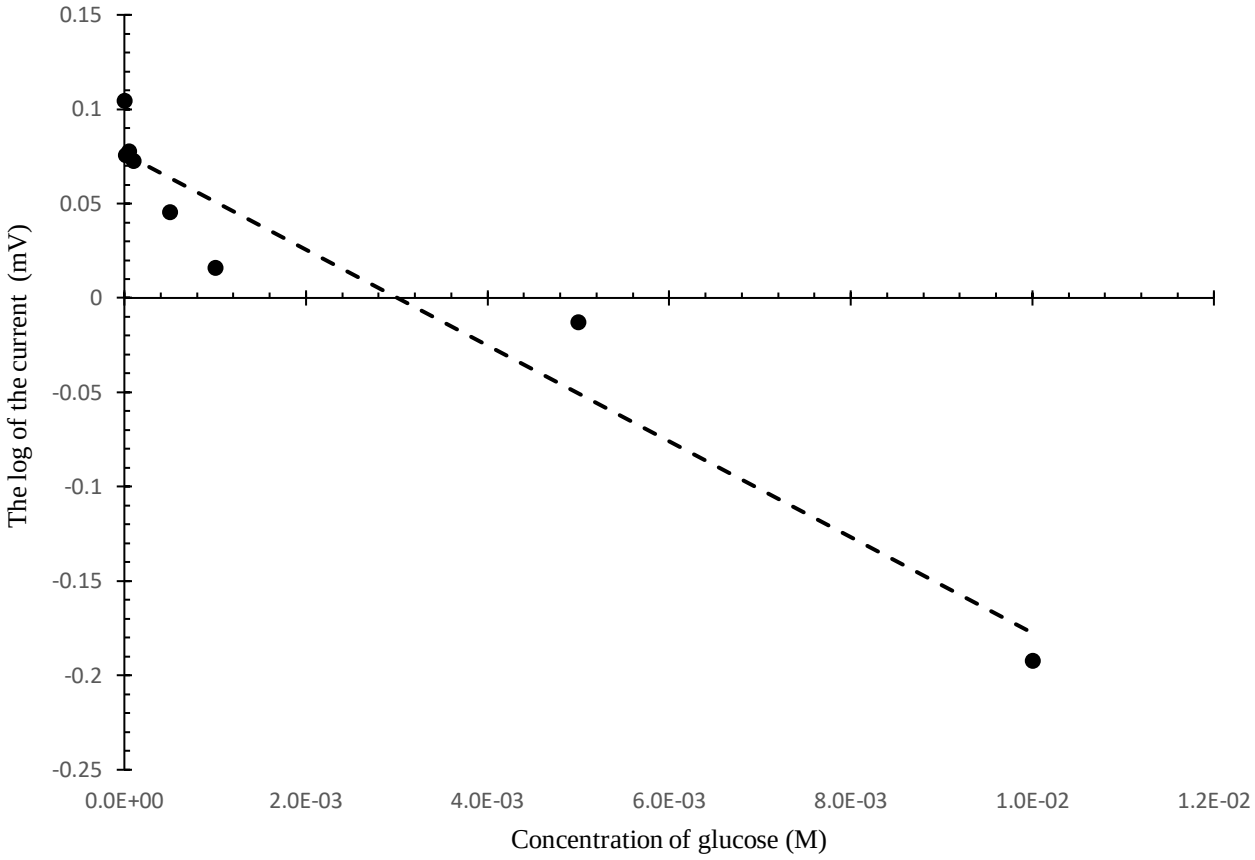

Figure 2: Calibration Curve for Electrode with PB-SL

A calibration curve formed from an electrode with a single layer of Prussian Blue deposited and then run through a cyclic voltammetry five times with the deposition solutions and five times with the phosphate buffer. The figure shows the log of the current versus the concentration of glucose. The slope of the trend line is -25.385 and the y-intercept is 0.0763. The R^2 of the figure is 0.9367. Given the identification PB 11/6 PB gel B58 OG S2 PB-SL C.V. dep 3.0 (5x) Phos. buff. (5x)(1)

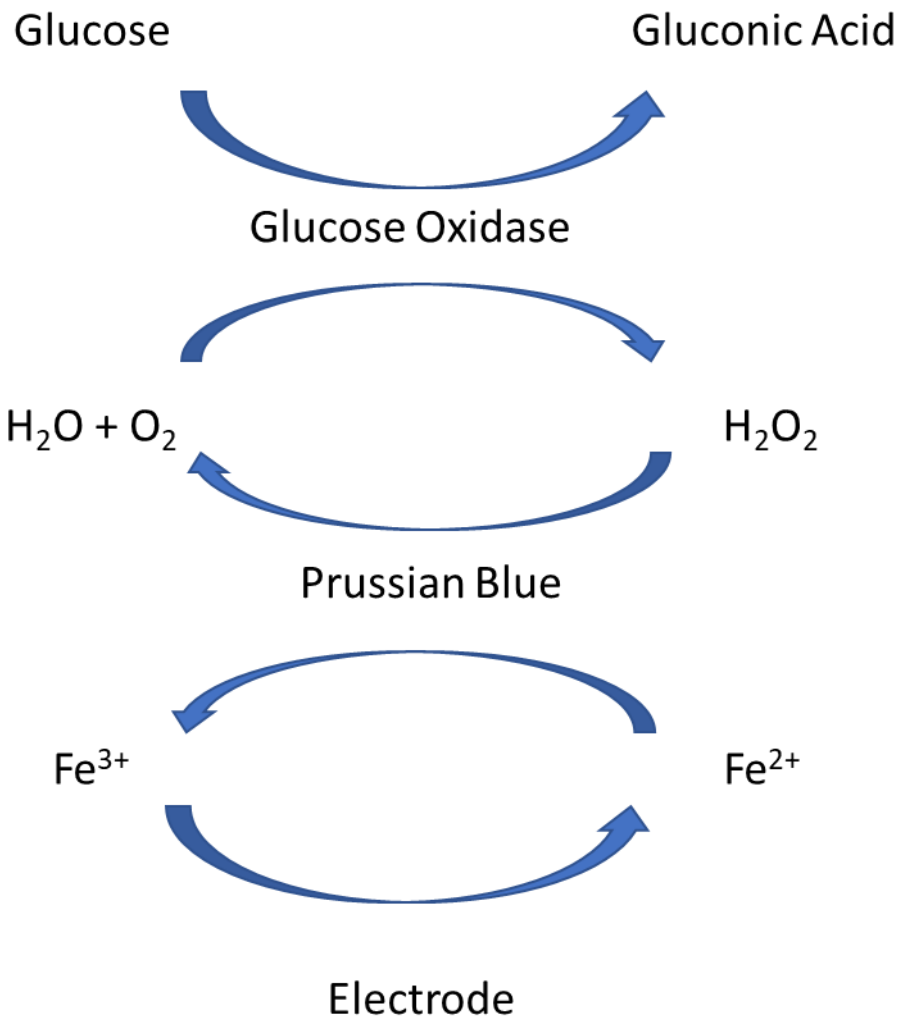

Figure 3: The reactions occurring between glucose and Prussian Blue that prompt an electrochemical response from the electrode.

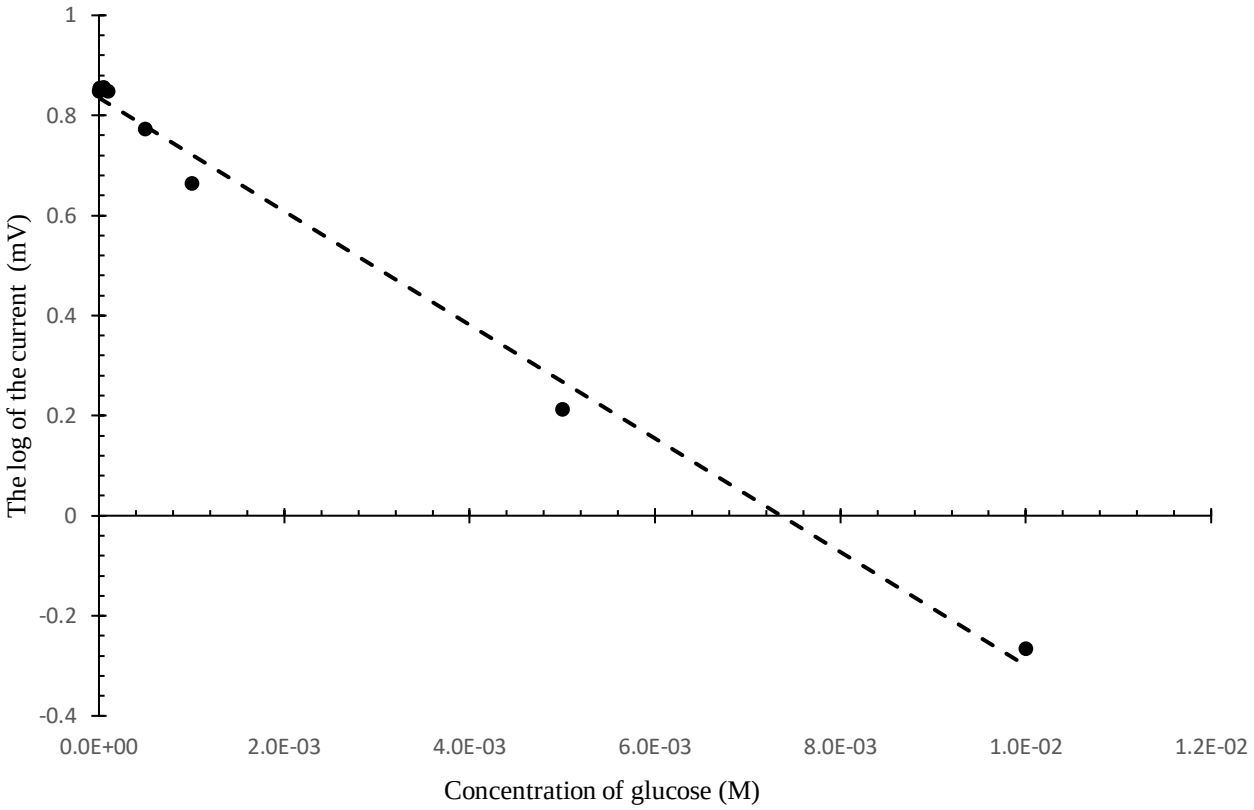

Figure 4: Calibration Curve for Electrode without a PB-SL

An electrode with Prussian Blue deposited into the xerogel by running through a cyclic voltammetry five times with the deposition solutions and five times with the phosphate buffer. The electrode was run through bulk electrolysis for 30 seconds. The figure shows the log of the current versus the concentration of glucose. The slope of the trend line is -113.46 and the y-intercept is 0.8351. The R^2 of the figure is 0.9921**.** Given the identification PB 11/6 PB gel B58 OG S1 C.V. dep 3.0 (5x) Phos. buff. (5x)(3)

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