Development of a Non-Enzymatic Electrochemical Glucose Sensor using Copper Oxide

Michelle Shimberg

March 23, 2021

Submitted for Dean's and Chancellor's Awards 2020.

The methods and results of this paper will be sent out for journal review.

Department of Biomolecular Engineering

Univeristy of California, Santa Cruz

Jack Baskin School of Engineering

Abstract

This project was conducted in order to develop a simple, non-enzymatic method of glucose detection. Glucose serves as a primary source of energy for almost all organisms. Diabetes, a disease characterized by chronic hyperglycemia, affects millions of people worldwide, and particularly in low-income countries. This increases the pressure to develop a stable, reproducible, time, and cost-effective glucose biosensor. Traditional biosensors use glucose oxidase to catalyze the oxidation of glucose in electrochemical sensors, but enzymes have been found to be less stable and more expensive than alternative methods. Copper oxide has been explored as a potential alternative to traditional enzymatic sensors due to its highly catalytic properties. In this paper, copper oxide was deposited onto a platinum electrode and was used to detect glucose under alkaline conditions. Glucose was detected in the range of 0.625μM to 30mM, and the sensor showed no response against other sugars or potentially interfering species.

1 Introduction

Glucose functions as the primary source of energy for all organisms that undergo energetic metabolism. It is typically stored in the liver and muscles, with its levels regulated by the feedback loop between two hormones released by the pancreas: insulin and glucagon [7]. Normal levels of glucose in the body range from 72-108 mg/dL, or 4-6mM. When glucose metabolism is interrupted, it is characterized as either hyperglycemia, elevated levels of blood glucose, or hypoglycemia, decreased levels of blood glucose. [5].

Diabetes mellitus is a chronic, metabolic disease characterized by chronic hyperglycemia affecting over 420 million people worldwide. Other symptoms include excessive urination, thirst, hunger, sudden weight loss, vision changes, fatigue, and nerve sensations. Over time, the disease can cause severe damage to the heart, blood vessels, eyes, kidneys, and nerves. As of late, the disease has been particularly prevalent in middle and low-income countries due to environmental/societal factors and inaccessibility to medical care [14]. Diabetes can be divided into three categories: type I, type II, and gestational. Type I diabetes is caused by deficient insulin production, leading to an immune response where insulin generating cells in the pancreas are attacked. A daily dose of insulin is required to maintain proper cellular metabolism. Type II diabetes is the most common form, and is typically caused by external factors, such as obesity, physical inactivity, genetics, age, or other health problems. In this case, the individual may have either insufficient production or inefficient use of insulin [13]. Gestational diabetes occurs in pregnant women, usually as a result of changes in hormone levels, weight, and diet. Genetic history can also contribute to the onset of gestational diabetes. The development of chronic hyperglycemia during pregnancy increases the chance of potential complications, as well as increasing the risk for post-pregnancy diabetes for both mom and baby [14]. At this point in time, there is no cure for any form of diabetes mellitus. Patients are required to regularly monitor blood glucose levels, undergo changes in diet and physical activity, and receive insulin doses. With the rise of diabetes across the world, and particularly in middle and low-

income countries, this increases the pressure to develop a stable, reproducible, time, and cost-effective glucose biosensor.

Electrochemical techniques have been employed in the biosensor industry due to their high sensitivity, wide range of detection limits, low cost, and simple instrumentation [9]. Traditionally, glucose sensors have used glucose oxidase as the basis for their redox reactions, oxidizing glucose into gluconolactone, thereby releasing electrons that are then used to quantify the levels of glucose. Glucose oxidase, compared to other enzymes with similar properties, has been proven to be relatively stable and cost-effective [11]. Despite the success of these sensors, the intrinsic nature of all enzymes, including that of glucose oxidase, in response to changes in pH, humidity, and temperature makes them prone to overall lower stability, stringent operating conditions, high cost, and interference from other oxidative species. Additionally, natural enzymes can also be expensive to purchase or develop [1], [15]. Alternatively, metal oxides, polymers, and noble metals have been used as catalysts in non-enzymatic sensor development [6], [11], [12].

Metals that have been explored for glucose sensors include platinum, gold, nickel, and copper [6]. The use of copper as copper oxides and copper nanoparticles has attracted attention in recent years due to its natural abundance and highly catalytic properties [8], [9]. Further study into nanotechnology showed copper to have a higher catalytic effect on glucose oxidation, with higher sensitivity and stability than other metals [10]. Gou, Shi, Li, et al. [4] explored the use of copper nanoclusters for glucose sensing when fixed onto an alginate gel. It has been found that using metal-oxides as opposed to pure metals enhances the direct oxidation of glucose [10]. Nguyen, Huy, Hwang, et al. [8] reports on the preparation and use of cuprous oxide particles deposited on ITO (indium tin oxide) for detection of glucose down to 0.4μM. Dai, Molazemhosseini, Abbasi, et al. [2] developed a cuprous oxide thin film for the non-enzymatic detection of glucose. Dong, Zhong, Kou, et al. [3] were able to reach a limit of detection at 0.005mM using cuprous oxide nanoarrays. Hwang, Lee, Seo, et al. [6] further provides a comprehensive review of literature available

on Cu, CuO, and Cu_2O sensors. While effective, these methods are plagued by complicated protocols to develop the necessary nanostructures, as well as limited selectivity or sensitivity [6]. In this paper, I present a simple, non-enzymatic method of glucose detection using copper oxide deposited onto a platinum electrode.

2 Materials and Methods

2.1 Materials and Instrumentation

Copper (II) sulfate was acquired from ACROS Organics. Sodium hydroxide was purchased from Fisher Scientific. All other reagents were purchased from Sigma Aldrich. Platinum and silver wire (0.005" diameter) were acquired from A-M Systems. All electrochemical measurements were taken with a 1200C Series Handheld Potentiostat/Bipotentiostat from CH Instruments Inc.

2.2 Fabrication of Copper Electrode

A platinum working electrode, platinum counter electrode, and Ag/AgCl reference electrode were immersed in a solution of 0.2M Cu_2SO_4 and run under a -0.8V potential for 40 seconds to deposit the copper onto the working electrode. The electrodes were then placed inside of a 0.3M NaOH solution and run under a +0.8V potential for 40 seconds to oxidize the copper particles.

2.3 Detection of Glucose

After successful copper deposition, linear sweep voltammograms (LSVs) from 0.2-0.8V were applied in a 0.1M NaOH solution for a baseline current measurement. Glucose was added incrementally from a 0.5M stock solution.

2.4 Specificity Test

The sensor was tested against structurally similar species, including galactose, lactose, fructose, sucrose, and maltose. Chloride, creatinine, acetamidophenol, urea, and an electrolyte solution were selected as potentially interfering species.

3 Results and Discussion

Figure 1 shows the results of LSVs taken in 0.1M NaOH solution. The highly alkaline NaOH solution favors the copper oxide, providing it greater stability and creating an environment for its reduction reaction. The formation of OH groups from the reduction of the copper oxide are capable of oxidizing the organic molecule, in this case glucose [6]. The graph in Figure 1 shows a concentration range of 0-30mM, with a lower limit of detection for glucose at 0.625μM. The oxidation of glucose occurs around 0.6 to 0.7V. Shifting of the oxidation peak can be attributed to the stripping of chloride ions from the Ag/AgCl reference electrode.

Because of copper oxide's high catalytic properties towards carbohydrates, this sensor was tested against sugars with similar structures. None of these sugars showed a significant response to the sensor. Similarly, glucose was also the only compound to give a response when compared against potentially interfering species. These results are shown in Figure 2.

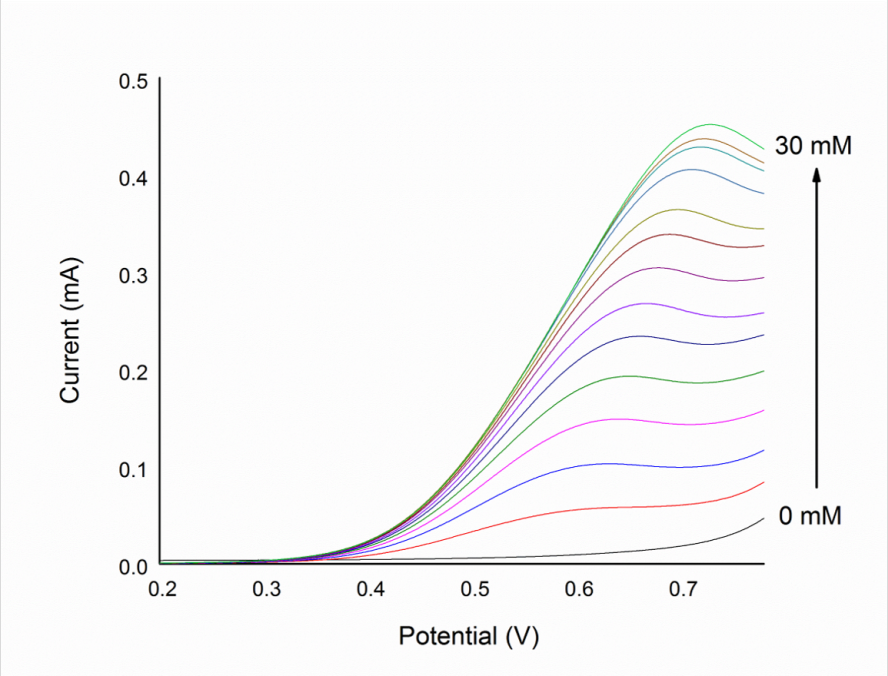

Figure 1: Linear sweep voltammograms taken in 0.1M NaOH solution with glucose added incrementally. The glucose oxidation peak can be seen between 0.6-0.7V. The range of detection was calculated to be 0.625μM-30mM.

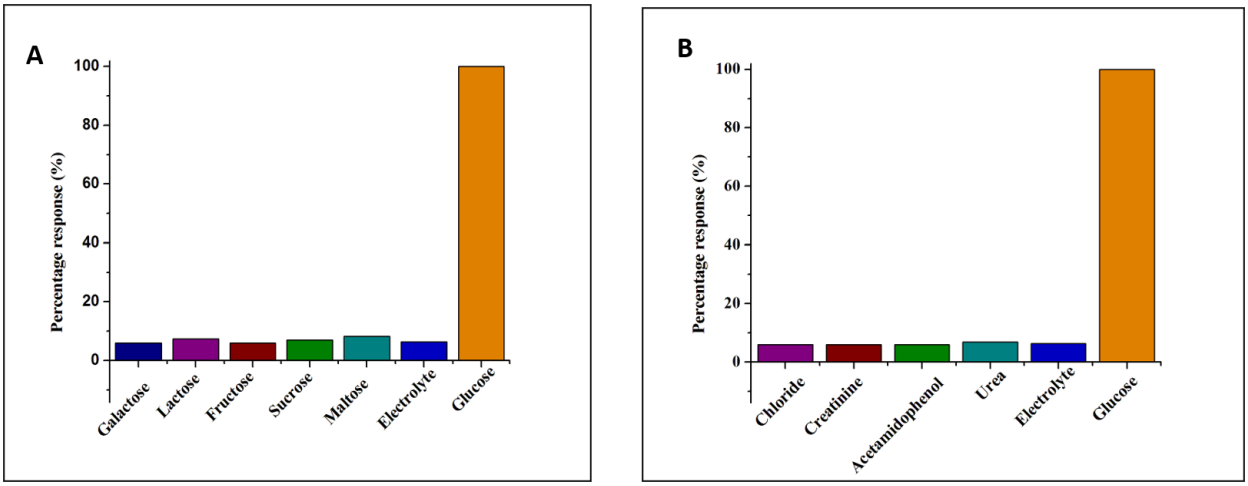

Figure 2: Results of specificity test with the copper oxide sensor. Figure A shows the response of other sugars: galactose, lactose, fructose, sucrose, maltose, and an electrolyte. Glucose was found to be the only sugar that responded to oxidation. Similarly, Figure B shows the responses of potentially interfering species found in the body: chloride, creatinine, acetomidophenol, urea, and an electrolyte. None of these species showed a significant response.

4 Conclusion and Future Work

In this work, I was able to develop a non-enzymatic method for the detection of glucose using copper oxide to catalyze the oxidation of glucose in an alkaline environment. The upper limit of detection was shown to be 30mM, while the lower limit of detection was calculated at 0.0625μM. Testing other species, glucose was the only sugar shown to be responsive to this sensor when compared against other sugars, as well as potentially interfering species. Thus, this sensor shows a very high level of both specificity and sensitivity. In the future, I hope to transfer this sensor into the nanopipette to achieve increase sensitivity, as well as the ability for *in vitro* diagnostic studies.

5 Acknowledgements

I would like to acknowledge the principal investigator of this project, Dr. Nader Pourmand, for his material contributions and support of this project. Similarly, I would like to express thanks to Dr. John Stanley for his extensive collaboration on this project, both in proof of concept and experimental execution. Additionally, I would like to express my gratitude to all members of the Pourmand Lab from 2019-2021 for their continued support and input.

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