Proposing a New Study in Non-Invasive Amperometric Glucose Sensing Technology through the NYCLSAMP Summer Fellowship Program

Sunghoon Jang
Department of Electrical & Telecommunications Engineering Technology
New York City College of Technology of CUNY
300 Jay Street
Brooklyn, NY 11201

Peter Spellane
Department of Chemistry
New York City College of Technology of CUNY
300 Jay Street
Brooklyn, NY 11201

Mohammad Razani
Department of Electrical & Telecommunications Engineering Technology
New York City College of Technology of CUNY
300 Jay Street
Brooklyn, NY 11201

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Sunghoon Jang+, Peter Spellane*, and Mohammad Razani+
+ Department of Electrical & Telecommunications Engineering Technology
* Department of Chemistry
New York City College of Technology of CUNY
300 Jay Street
Brooklyn, NY 11201

Abstract: We have conducted an undergraduate research project "Proposing a New Study in Non-Invasive Amperometric Glucose Sensing Technology through the NYC-LSAMP Summer Fellowship Program" – with a freshman student. Since a non-invasive method of monitoring blood glucose would present major advantages over existing methods which use invasive technologies, our group has studied the possibility of using a novel sensing technology, an amperometric glucose sensor, based on the information derived from the relationship between glucose molecules and their electrochemical impedance within physiologic glucose levels. Changes in glucose concentrations can be monitored by measuring the impedance within a wide range of frequencies in order to optimize the impact of glucose solution via a DC/AC electrical current. We will also investigate the impedance and electrical current variation in terms of the glucose concentration within a given physiologic range. The impedance of tap water and distilled water is (50 ml each) and will be also measured by using an EIS (electro-chemical impedance spectroscopy) system. The proposed amperometric technology is sensing tear glucose levels, potentially blood glucose monitoring, may be useful as a new approach for non-invasive glucose sensing. Our amperometric glucose sensor, integrated with a disposable contact lens embedded with a glucose sensing metal, may be more economical and safe than existing methods. In this study, we will discuss how to initiate the undergraduate research and how to solve some challenging issues due to the freshman student's level of engineering knowledge and skills. Our student (research assistant) is currently persuing a Bachelor of Technology dgree in Telecommunications Engineering Technology at NY City College of Technology of CUNY.

Key words: undergraduate research, NYC-LSAMP program, monitoring blood glucose, noninvasive method, amperometric glucose sensor, impedance, electrical current, EIS system, contact lens, NYCCT

Introduction

Diabetes mellitus is a serious disease in which the body doesn't produce or properly use insulin and represents one of the major health problems in society and a chronic disease that requires long-term medical attention. Often, diabetes can lead to many serious medical problems. These include blindness, kidney disease, nervous system disease, limb amputations, stroke and cardiovascular disease (CVD). According to Data from the 2011 National Diabetes Fact Sheet, an estimated 25.8 million children and adults in the United States—8.3 percent of the population— have diabetes and the estimated cost of diabetes-related health care in the United States is approximately $174 billion annually, including $116 billion in direct medical costs [1, 2]. It is a disproportionately expensive disease; in the United States in 2002, the individual cost of health care was $13,243 for people with diabetes, while it was $2,560 for those without diabetes [3].

The recent multi-center NIH studies have indicated that the health risks associated with diabetes are significantly reduced when the blood glucose levels are well and frequently

controlled, indicating that it is prudent to measure the blood glucose as often as five or six times a day. Thus it is very important that proper monitoring be done by diabetics at home or at work [4]. At present all existing methods of home blood glucose monitoring require drawing a blood sample by piercing the skin (typically, on the finger). This method strongly discourages patients' compliance and has serious drawbacks because the procedure is invasive.

Since a non-invasive method of monitoring blood glucose would present major advantages over current existing methods which are using invasive technologies, our group has explored a noninvasive glucose sensing technique and reports preliminary results, finding a relationship between the concentration of glucose in aqueous solution and the solutions' impedance up to concentration of 1,000 mg/dl. The proposed glucose sensing amperometric system in current study may prove capable of monitoring very low glucose levels with the accuracy and precision that would satisfy medical use criteria at a cost that is significantly lower than costs for existing methods. In addition, the patient acceptance for this methodology is expected to be high due to its non-invasive nature, and its simple and safe testing procedure.

Background

There has been an increasing demand for continuous, non-invasive glucose monitoring techniques due to the increasing number of people diagnosed with diabetes and the recognition of the fact that the long-term outcome of these patients can be dramatically improved by a careful frequent and accurate glucose monitoring and control. In a previous study, we reviewed several of the newest minimally invasive and non-invasive glucose monitoring technologies under development or introduced to current market such as near infrared (NIR) spectroscopy, mid infrared (MIR) spectroscopy, radio wave impedance, optical rotation of polarized light, fluid extraction from the skin, interstitial fluid harvesting, and glucose sensing contact lens with fluorescence detection. Although recent advances in technology, research, and clinical applications in the noninvasive glucose monitoring are very encouraging and promising, we believe the non-invasive glucose sensing techniques are still a little far from satisfying requirements for clinical use. Therefore, it is necessary to develop a new technique satisfying the criteria such as accuracy, low cost, simplicity in testing, portability, and safety in use [5, 6].

The NYC-LSAMP Summer Fellowship Program

The NYC Louis Stokes Alliance for Minority Participation (NYC-LSAMP) is an alliance of 16 CUNY Colleges and the CUNY Graduate Center. The Alliance goal is to substantially increase the number of underrepresented minority students who pursue and graduate with Baccalaureate Degrees in Science, Technology, Engineering and Mathematics (STEM). The Alliance provides various financial incentives for both faculty and students and supports research training opportunities and academic support services for students to encourage greater participation to CUNY students majoring in the STEM disciplines [7].

The NYC-LSAMP Summer Fellowship Program provides a research experience program for a period of 10 weeks during the summer. Students receive a scholarship of $5,000.00 for the summer. AMP research assistantships are awarded on the basis of students' overall GPA, academic achievement, and the recommendation of their faculty mentor. Students give written reports of weekly progress and a final report on their research; they present results at a poster session at the end of the summer [8].

Initiating Undergraduate Research

In a previous study, we described problems in initiating research with freshman students who

lack adequate theoretical and experimental knowledge and skills in manipulating highly elaborate equipment and materials. Since The NYC-LSAMP Summer Fellowship Program is designed to complete student's project within a period of 10 weeks during the summer, we began with a series of lectures to help students to gain depth of knowledge in Electrical and Biomedical engineering principles and project - related theoretical and experimental background information, along with problem-solving and trouble-shooting skills.

Plan of Study

Elevated tear glucose levels during hyperglycemia were first demonstrated by Michail and his coresearchers [9]. According to their study, the tear glucose concentration follows blood glucose level with the glucose between blood and tissue fluid exists in an analogous manner to the equilibrium. Many scientists have reported that actual glucose concentrations in tears are low and in the range of 50-500 μM. More recently, Chatterjee et al. [10] and Zhu et al. [11] successfully demonstrated the relationship between tear and blood glucose concentrations and to develop a rapid method of detection of tear glucose level semi-quantitatively with glucose oxidase enzyme impregnated strips and to evaluate its role as an indicator of blood glucose level.
The concept of using tear fluid glucose will be employed as a way to follow the level of blood glucose in this study.

Since the purpose of our study is to investigate a technology that measures the relationship between concentration of glucose molecules and electric current due to changes in impedance. During investigation of literature, we found that there were impedance variations in glucose – water solutions with different concentration values that mimic glycemic levels in human blood [12, 13]. When glucose molecules are dissolved in solutions, the impedance of the solution changes and a relationship between them occur, which also allows the verification of its relationship with current. This finding can be applied to develop a new technology for the noninvasive monitoring of tear glucose levels, potentially plasma glucose monitoring in tears, using a disposable contact lens embedded with glucose sensing metal that is fast and simple sensor for diabetics.

The aim of our study at this preliminary stage was to examine the impedance of glucose solution at different glucose concentrations within the physiological range (100 – 500 mg/dl). We will also investigate various glucose concentrations from both distilled water and tap water to see which solution generates a reasonable amount of current (nano/pico amperes) needed for the data to be used for further studies. Our tear glucose is based on measuring a very low current, most likely in a nano-or pico-ampere scale. Since the concept of using tear fluid glucose will be employed as a way to follow the level of blood glucose in future studies, and test much lower concentration of glucose and introduce other ions that would mimic the physiological composition of human tears (e.g, Na^+, K^+, and Cl^- ions).

Materials and Methods

We employed a Gamry Potentiostat/ Galvanostat Reference 600 system as shown in Figure 1 to measure the levels of impedance in tap and distilled water. The system was first calibrated with a 2-kΩ dummy cell as shown in Figure 2 in order to test accuracy of the system and found 99.8% accurate result. Water was distilled in a Barnstead/Thermolyne model A1015-C still. Samples of two different solutions- tap water and distilled water- were also tested to find their impedance by using the EIS system. We then further applied the system to investigate

impedance variation in the 50 ml of two solutions presented (tap and distilled water) at wide range of frequencies ranging from 100 mHz to 1MHz at temperature of 23.9°C and 24.3°C respectively. Dextrose glucose, Anhydrous from Fisher Scientific was used to create glucose solution at room temperature with physiologic range concentration including 100 to 400 mg/dl. We also took measurements of higher concentration of 500mg/dl and 1000mg/dl for further determination of the relationship between impedance and glucose molecule. Each sample consisted of 50ml of distilled water and remarkable relationship was observed during our preliminary research.

Figure 1: Gamry Reference 600 EIS System **Figure 2**: Dummy cell with 2 kΩ resistor. (Electrochemical impedance spectroscopy) [14]

Results

We calibrated the system several times with a dummy cell consisting of a 2 kΩ resistor. Figure 3(a) shows that the calibration result was 99.8% accuracy resulting in 1.998 kΩ impedance which gave us confidence taking further measurements with water samples. According to the experimental data plots shown in Figure 3(b) and (c) of impedance measurement, we found that the impedance level of distilled water is 45 times higher than that of tap water; the distilled water impedance at 15.79 Hz was 90.19 kΩ, while the impedance of tap water was 2.1 kΩ at the same point/ frequency range. The result indicates that the distilled water has higher purity and fewer electrically conductive ions present than tap water. We also observed a significant phase shift that occurred at high frequency around 5 kHz for distilled water and 100 kHz for tap water. However, no phase shift occurred for 2 kΩ dummy cell regardless of frequency range due to its pure resistive characteristics.

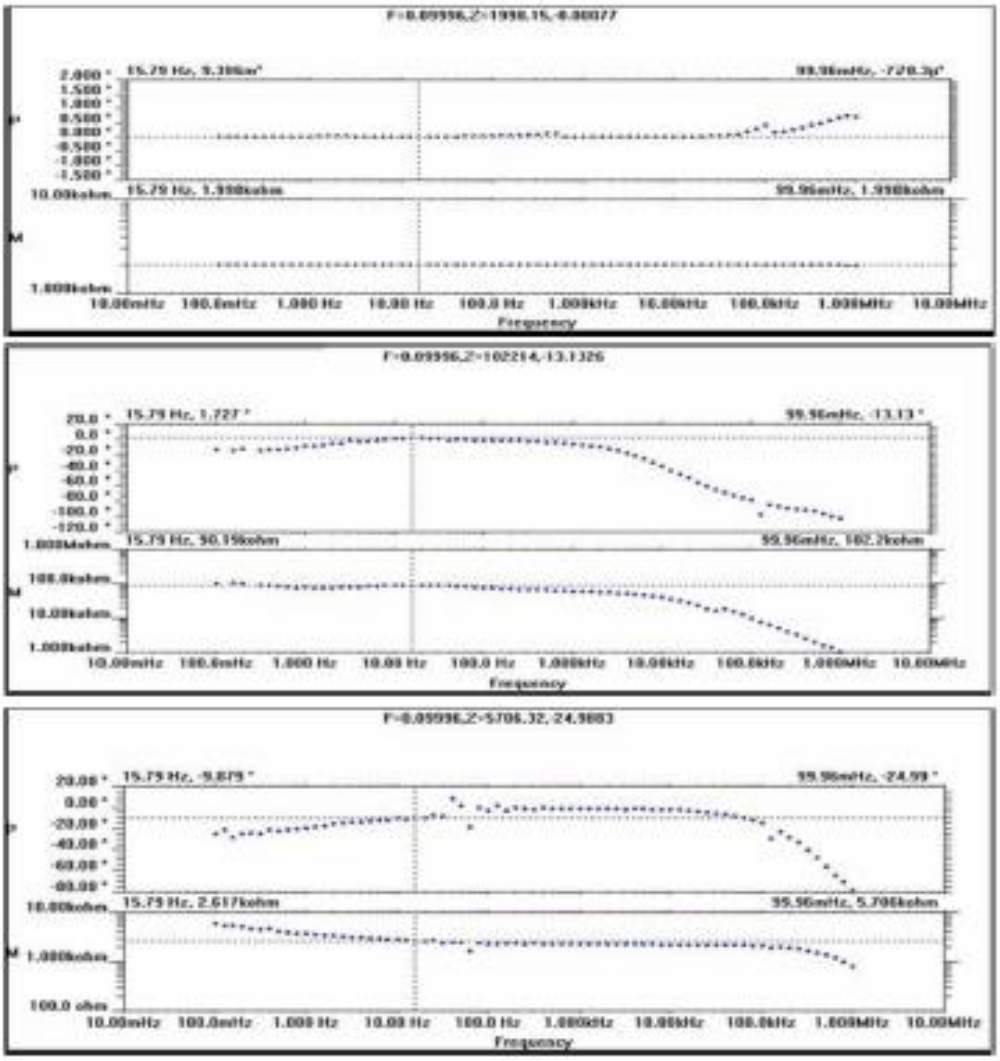
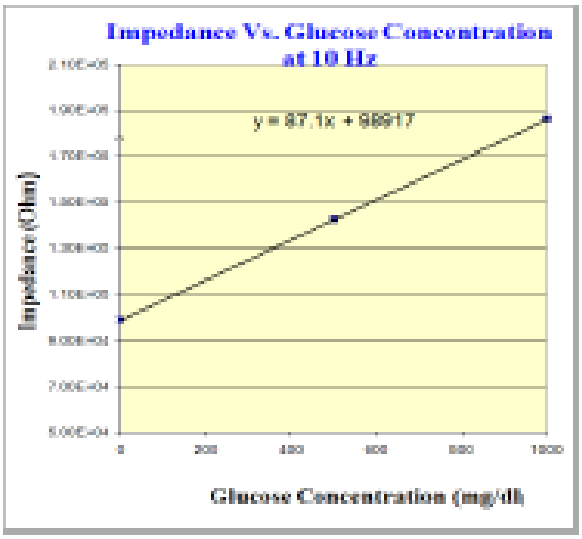


Figure 3: The experimental data plots of impedance measurement using a Gamry Potentiostat/

Galvanostat reference 600 system (a) 2.0 kΩ dummy cell (b) Distilled water (c) Tap water.

Figure 4 presents the impedance of distilled water and of glucose solutions at various concentrations: 500 mg/dl, and 1,000 mg/dl; we found the best sensitivity at 10Hz. The slope at the point was 87.1 (87.1x + 98917) which means that for every 1 mg/dl increase of glucose concentration, there is about 87.1 Ω increase in impedance. We further investigated the impedance at lower concentrations between 100-400 mg/dl and found the best sensitivity point at 0.1Hz. The slope of this measurement was 554.2 (554.2x – 30980) and we noted that effects are more relevant at frequencies below the 1 MHz band. i.e. higher frequency resulted in lower impedance and starts to fall at 100kHz (the frequency variation was nonlinear). Our preliminary experimental results showed that glucose concentration has direct proportional relationship to impedance. Linear relationships between glucose molecule and water are presented in Figures 4 and 5.



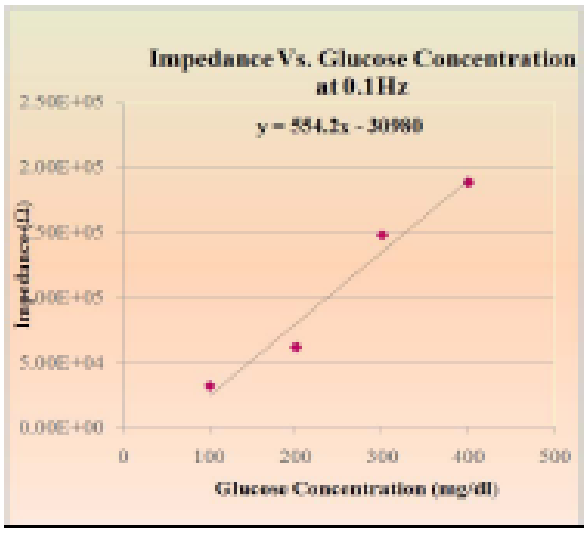


Figure 4: Graph showing a linear relationship between 0mg/dl, 500 mg/dl and 1000mg/dl.

Figure 5: Graph showing a linear relationship between 100 mg/dl - 400 mg/dl.

Conclusions

We have successfully demonstrated the relationship between glucose concentration and electrochemical impedance. These findings will be applied to develop a new technique for the non-invasive amperometric sensor monitoring of tear glucose concentrations for application in plasma glucose monitoring. According to our experimental data, we can conclude that there is enough system sensitivity to identify glucose concentration of solution. This is a very significant finding because as far as we know this is the first time to present the numerical relationship between glucose concentration and impedance. We also confirmed that impedance variations due to changes of glucose concentrations are certainly more evident at lower frequencies most especially close to DC. This result basically agrees with previous findings from other research groups, since Tura and Iguchi demonstrated that the variations of voltage and current which are related to the conductivity of solutions due to changes in glucose concentration can be observed [15, 16]. We strongly believe our preliminary research is very significant and important step to our ultimate goal of study in developing a non-invasive pure electrical current glucose sensing device for diabetics. Optically pure D-glucose while others used racemic (mixed D and L) glucose
is it reasonable that the impedance INCREASES with increased glucose concentration? I would have thought that glucose served to facilitate conductivity, and therefore DECREASE impedance

The NYC-LSAMP Summer Research Program truly helped our student in gaining intellectual and practical knowledge of research. Through the various research activities required by program, the student also gained confidence with completing research project. Our student (research assistance) is currently persuing a Bachelor of Technology dgree in Telecommunications Engineering Technology at NY City College of Technology of CUNY. With the guidance and help of one of the co-authors of this paper, she also worked for NSF REU

program provides opportunities for undergraduate students of CUNY to become active participants in Remote Sensing research at the NOAA Cooperative Remote Sensing Science and Technology Center (NOAA-CREST) over last summer at CCNY.

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