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Development of Electrochemical Methods to Enzymatically Detect Lactate and Glucose Using Imaginary Impedance for Enhanced Management of Glycemic Compromised Patients

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ABSTRACT: Lactate is an important biological marker that can provide valuable information for patients who have experienced a traumatic injury. Additionally, when coupled with glucose, the severity and likely prognosis of a traumatic injury can be determined. Because monitoring various markers proves useful in diagnosis and treatment of trauma patients, monitoring both glucose and lactate simultaneously may be especially useful for diabetic patients who have suffered a traumatic injury. Previously, using electrochemical impedance spectroscopy (EIS), a sensor capable of measuring two affinity-based biomarkers simultaneously was demonstrated using the biomarker's specific optimal frequency to develop a deconvolution algorithm, which allowed for the measurement of two biomarkers from a single signal. Herein, while developing an EIS lactate sensor, dual enzymatic biomarker detection of lactate and glucose via EIS was also attempted. Both biomarkers were validated individually with the lactate sensor being additionally validated on whole blood samples. The EIS lactate biosensor achieved a range of detection from 0 to 32 mM of lactate and the glucose sensor a range of 0–100 mg/dL of glucose, which are representative of the likely physiological ranges that trauma patients experience. However, the preliminary attempt of dual marker detection was unsuccessful due to suspected accumulation of reduced redox probe on the surface of the self-assembled monolayer (SAM). Individually, the optimal frequency of lactate was determined to be 69.75 Hz and that of glucose was determined to be 31.5 Hz. However, when combined onto one sensor, no discernable optimal frequency could be determined which again was suspected to be due to the accumulation of the reduced redox probe at the surface of the SAM.

KEY WORDS: lactate, electrochemical impedance spectroscopy, imaginary impedance, lower limit of detection, point-of-care, quality control, gold disk electrode, type 1 diabetes, type 2 diabetes, glycemic control

ABBREVIATIONS: AC, alternating current; **EIS**, electrochemical impedance spectroscopy; **GDE**, gold disk electrode; **SAM**, self-assembled monolayer; **TBI**, traumatic brain injury

I. INTRODUCTION

Various glycolytic intermediates and enzymes serve unique purposes in the body's overall metabolic pathway. Specifically, glucose and lactate represent the beginning and end products of the anaerobic metabolic pathway and can provide valuable information from blood level concentrations.

Lactate, the ester form of lactic acid, is a biomarker typically associated with illness, septic shock, and trauma. Lactate is an excellent clinical biomarker for traumatic injuries and waste, as well as being an important gluconeogenic precursor.[1] In times of metabolic stress, such as in traumatic injuries, levels of lactate are much higher in patients with diabetes mellitus because it is a final product of energy production.[2] Each year, traumatic injuries are responsible for 41 million emergency room visits, two million hospital admissions, and $671 billion in the United States.[3] The lab standard of lactate measurement takes at least an hour to obtain test results depending on each hospital's turnaround time,[4] which is excessive con-

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sidering the recommended turnaround time of a few minutes.[5] Because of the slow turnaround time, a solution of point-of-care (POC) testing of lactate has received great emphasis and many POC technologies have been implemented in various clinical settings, including the testing of handheld lactate analyzers.[4]

Lactate, being the end result of anaerobic respiration, could provide great detail about the metabolic activity in a patient with diabetes mellitus. The body mainly uses anaerobic respiration during periods of prolonged physical exercise, in which lactate levels are indicative of the intensity of a patient's physical exercise.[6] There has been substantial evidence that physical exercise increases lactate levels and is correlated with increased blood glucose concentrations in patients with type 1 diabetes mellitus.[7] Lactate is an important biomarker for monitoring patients who have sustained a traumatic injury; however, lactate level concentrations can also be beneficial to measure the intensity of physical exercise for patients with diabetes mellitus.

Like with many complex diseases, it is essential to measure multiple biomarkers in addition to lactate when dealing with various kinds of traumatic injuries. Monitoring multiple biomarkers has shown enhanced performance in the accuracy of disease diagnosis, prognosis, management, and treatment.[8–11] In regard to trauma patients, it is important to measure blood glucose and lactate levels because they can determine the likely prognosis of a traumatic brain injury (TBI)[12] as well as the expected mortality for patients who have sustained a severe trauma.[13]

Glucose, a six-carbon sugar, is the primary precursor to the human body's metabolic pathway and frequently catabolically created from other carbohydrates. Glucose is primarily used by clinicians for the management and diagnosis of diabetes.[2] Patients who have experienced a severe trauma also need their glucose levels measured because they are frequently hypoglycemic, which often results in a worse outcome than if they had a tight glycemic control.[14] As a result of the high prevalence of diabetes, there are many POC devices that measure glucose concentrations, typically using a reaction between glucose and glucose oxidase, which ultimately results in the generation of a current from free electrons.[15] However, under anaerobic conditions in the body, glucose is ultimately transformed into lactate in order to generate ATP, which cells use as their primary energy source in order to perform a multitude of functions.[16]

There are well-established methods for the measurement of glucose that can be done within minutes using amperometric techniques.[15] However, recent advancements have shown that electrochemical impedance spectroscopy (EIS) is effective at measuring concentrations of a reagent based on its binding and has the potential to measure different reagents in the same sample.[17,18] In short, EIS works by applying a voltage across the electrochemical cell containing the sample. The voltage is then measured as a current, and using Ohm's law, the impedance is calculated.[19] The benefit of EIS is that it is rapid, nondestructive, and uses an alternating current (AC), which sweeps through frequencies from 1 Hz to 100 KHz. After running EIS on a known concentration gradient and plotting the R^2 (RSQ) values and the slope of the impedance versus the frequency, the optimal frequency can be determined. The optimal frequency is where the slope is at a maximum and there is high RSQ, which can be used for reliable and reproducible measurement of the reagent. RSQ is the coefficient of determination and tells the proportion of variance in a dependent variable compared to the independent variable. A high RSQ will be a good indicator that the model fits the data points very well and is useful in determining where the optimal frequency lies. By using an AC current, EIS can be used to generate a Nyquist and Bode plot, which allows for simple and tractable analysis of the results.[20] Furthermore, since optimal frequency is unique to each reagent, it is proposed that multiple biomarkers can be measured on one sensor by using EIS since each biomarker has its specific optimal frequency. Previous work has demonstrated the ability of EIS to detect two affinity-based biomarkers via their optimal frequencies;[21] however, detecting two enzymatic biomarkers simultaneously using their optimal frequencies has not been successfully demonstrated. The aim of the present study is to contribute additional knowledge to the field of dual enzymatic biomarker detection of glucose and lactate. Because glucose has been studied extensively in the past,[17,22] the emphasis of this study is on categorizing lactate in blood before attempting purified dual marker detection.

By using a 16-carbon chain self-assembled monolayer to immobilize lactate dehydrogenase to a gold disk electrode (GDE), EIS can be used to determine the amount of lactate bound to the lactate dehydrogenase. A concentration gradient of lactate in a buffer solution will be used to determine the optimal frequency. In addition, by adding a Nafion film, lactate levels in blood can also be measured by using EIS on a GDE. Since a similar method is used to immobilize glucose oxidase for the measurement of glucose, it is likely that both enzymes can be immobilized on a single sensor and both glucose and lactate can be successfully measured from one sample.[21]

II. MATERIAL AND METHODS

A. Chemicals and Reagents

All chemical reagents were purchased from Sigma or Sigma-Aldrich (St Louis, Missouri) unless stated otherwise. In addition, 130 mM NaCl, 10 mM phosphate buffer (PBS), and 3 mM KCL tablets were purchased from Calbiochem (Gibbstown, New Jersey); pH electrode storage solution was purchased from Thermo Electron Corporation (Beverly, Massachusetts); potassium hexacyanoferrate (III) was purchased from EMD Chemicals (Billerica, Massachusetts); and N-hydroxysulfosuccinimide sodium salt (NHS) was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). The YSI biochemical analyzer was obtained from YSI Incorporated (Yellow Springs, Ohio). The blood samples used in the lactate detection were donated from the Mayo Clinic Hospital in Scottsdale, Arizona. The redox probe reagent used for the lactate dehydrogenase testing was potassium hexacyanoferrate (III) dissolved in 10 mM phosphate buffer saline. The redox probe reagent used for the glucose oxidase testing was potassium hexacyanoferrate (III) dissolved in 10 mM phosphate buffer saline.

B. Sensor Preparation and Development

The sensor used in the study consists of GDEs, silver/silver chloride (Ag/AgCl) reference electrodes, and platinum (Pt) counter electrodes (CH Instrument, Austin, TX, USA). All EIS measurements were performed at room temperature using a CHI660C electrochemical analyzer from CH Instrument. The GDEs were cleaned by first rinsing them with ethanol. Following the ethanol rinse, the GDEs were polished with 10 figure-eight motions on a Buehler felt pad using 0.05 μm grit alumina oxide in distilled (DI) water. Finally, the GDEs were sonicated in DI water for 20 min. After polishing, individual formal potentials were obtained by performing cyclic voltammetry from –1.0 to 1.0 V using a three-electrode setup (gold working electrode, silver-silver chloride reference electrode, and platinum counter electrode) suspended only in redox probe solution. EIS was then performed using the calculated formal potential and a 5 mV AC sine wave sweeping from 1 Hz to 100 kHz to measure the bare impedance of GDEs.

C. Immobilization of Lactate Dehydrogenase Enzyme

After rinsing the GDEs with DI, 100uL of 1 mM of 16-mercaptohexadecanoic acid (16-MHDA) in ethanol was incubated onto the GDEs for 1 hr to form a self-assembly monolayer (SAM). Incubating the sensor with a solution consisting of 100 uL of 40 mM EDC and 20 mM NHS for 1 hr activated the carboxylic acid group of 16-MHDA (see Fig. 1). After washing the sensor with DI, 50 ug/mL of the lactate dehydrogenase enzyme was then immobilized onto the sensor at room temperature for 1 hr. Following the immobilization period, the sensors were washed with PBS. To ensure any unbound activated carboxylic acid did not spontaneously react among other active groups, the remaining reactive sites were blocked with 100 uL of 1% ethanolamine in deionized water that were incubated for 30 min. Furthermore, the ethanolamine binds to potential binding sites on the gold surface. After the final incubation period, the sensors were rinsed with PBS, stored at 277.15 K, and ready for testing. Post-MHDA impedance was measured at each GDE's specific formal potential for quality control.

D. Immobilization of Glucose Oxidase Enzyme

The immobilization of the glucose oxidase enzyme followed a protocol similar to that of the lactate dehydrogenase. Using fresh and cleaned GDEs out-

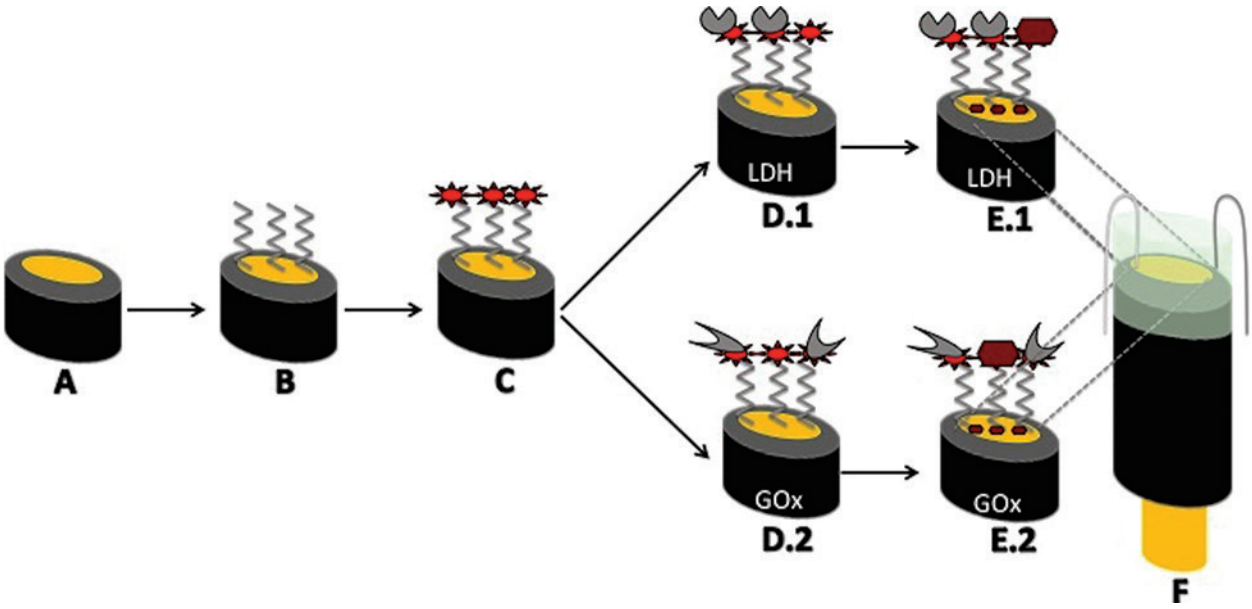

FIG. 1: The immobilization process to covalently bind lactate antibody onto the end of the 16-MHDA SAM of the GDE. The parts are as follows: A is the bare Au disk electrode; B, a 1 mM 16-MHDA linker; C, EDC/NHS coupling; D.1, 100 nM lactate dehydrogenase enzyme molecule Immobilization; D.2, 10 mg/mL of glucose oxidase enzyme molecule immobilization; E.1/E.2, 1% ethanolamine blocking; and F, Au electrode setup with Ag/AgCl electrode used as a reference for the determination of the potential using a 1000 L tube. Note that during the lactate experimentation, an NADH cofactor is used, although there was no need for a cofactor for the glucose oxidase.

lined above in Section II.B, the SAM (16-MHDA) linker was attached to the gold surface. The SAM was activated via incubation of 100 uL of 40 mM EDC and 20 mM NHS for 1 hr. Then, 10 mg/mL of glucose oxidase solution was incubated onto the GDE surface for 1 hr to effectively bind to the activated carboxylic acid of 16-MHDA. Lastly, the electrodes were incubated in 100 uL of deionized water of 1% ethanolamine. The sensors were again rinsed with PBS, stored at 277.15 K, and ready for testing. Post-MHDA impedance was measured at each GDE's specific formal potential for quality control.

E. Quality Control

Electrodes were prepared in batches of either 18 or 24, using the methods described above. Each electrode was analyzed at the formal potential of the electrode using EIS. Once EIS was performed on a batch of sensors, the sensors were placed into batches of those with a similar baseline response. To ensure there was minimal variance within the experiments, the electrodes were analyzed and placed into batches of similar baseline impedance values. Figure 2 shows a pictorial representation of placing electrodes into similar baseline values to ensure there is a variance of approximately 6–10% for each batch. Additionally, to ensure that background variance was accounted for in each concentration assay experiment, a blank or PBS-only concentration was measured three times. The average blank reading was used to subtract out the background noise in each individual concentration impedance measurement, as done in prior studies.[23] The quality control was similar for experiments involving the glucose oxidase enzyme and experiments that involved lactate dehydrogenase enzyme.

F. Glucose Assay in Purified Solutions—Electrochemically

Suspending glucose powder in PBS made a stock solution of glucose. The solution was refrigerated for 12 hr. From the stock solution concentration, dilutions were made for 0.001, 1, 8, 25, 60, and 100 mg/dL. The

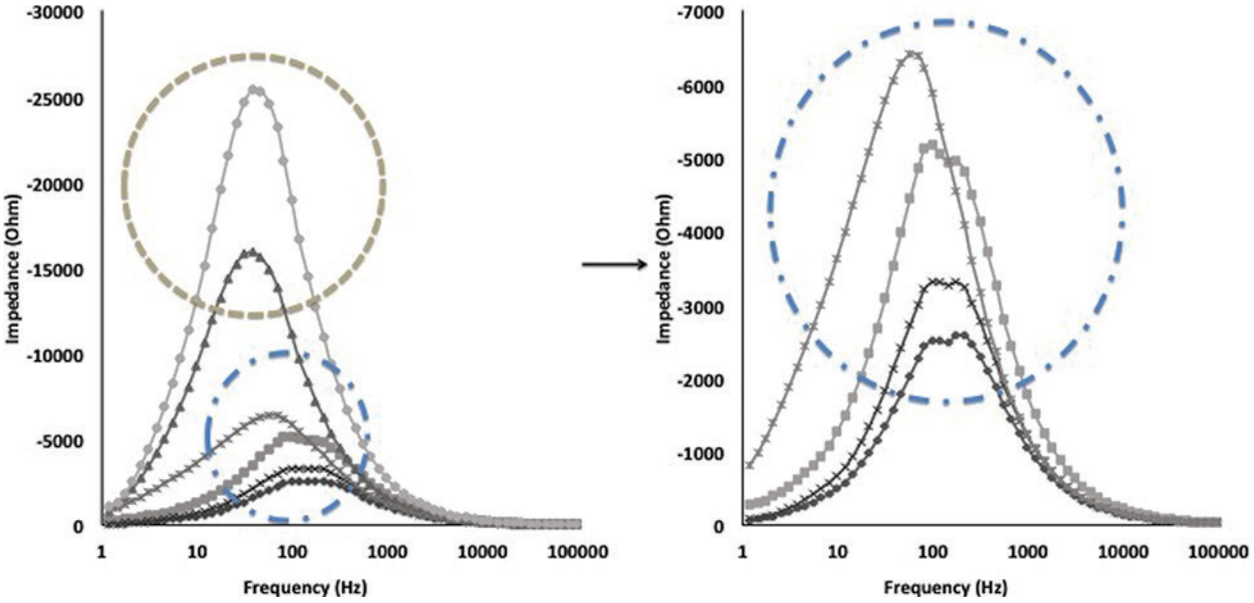

FIG. 2: Pictorial representation of the method used to batch specific electrodes into a similar impedance range to ensure low variance within experiments. Each line in the figure represents a unique electrode.

conversion for 18 mg/dL of glucose is equivalent to 1 mMol of glucose, and the specific concentrations values of 0.001, 1, 8, 25, 60, and 100 mg/dL were carefully picked to demonstrate detection sensitivity with imaginary impedance, because previous work has shown that strong signal correlations are associated with high concentrations and are more easily detected.[17]

G. Lactate Assay in Purified Solution—Electrochemically

The lactate dehydrogenase enzyme was stored at 253.15 K and thawed just prior to use. Lactate gradients were prepared through a serial dilution with PBS. The NADH cofactor, which is required for the lactate dehydrogenase to work properly, was dissolved in PBS to make a concentration that would follow a 1:1:1 ratio to form a total volume of 100 μL of each solute. The concentration gradients were made for 0.001, 2, 4, 8, 16, and 32 mMol to establish a purified calibration curve.

H. Lactate Assay in Complex Solution—Electrochemically

Similar to the steps outlined above, 10% whole blood samples were developed with the same lactate concentrations. The concentration gradients were made for 0.001, 2, 4, 8, and 16 mMol to establish a complex calibration curve [see Fig. 6(c) in Section III]. The parameters were performed similar to that of purified lactate, and each sensor had three blanks ran to evaluate the baseline. One of the key differences was that the sensors were dipped into Nafion to follow commercial protocol standards.[17] The blood samples followed the same protocol of 1:1:1 ratio of redox probe, NADH cofactor, and 10% blood to form a total volume of 100 μL of each sample.

I. Calibration Curve from Characterization of Purified Glucose

Following the serial dilution of the glucose in making the glucose concentration assay, the GDEs were pulled from the fridge, where they were stored and allowed all to reach a constant temperature of 277.15 K and then brought to room temperature of 293.15 K. The glucose assay was tested in a gradient using concentrations of 0.001, 1, 8, 25, 60, and 100 mg/dL. The purpose of the calibration was crucial at determining the optimal frequency of the reaction between the glucose oxidase and the glucose. The imaginary impedance values for each concentration was collected,

and a correlation to each frequency was determined. The correlation involved graphing the slope and R^2 value against the frequency sweep of 0–100 KHz. The optimal binding frequency would occur at a point that correlates to the maximal slope and R^2.[24] As seen in Fig. 3(A), the glucose optimal binding frequency occurs at 31.5 Hz and that has the highest correlated slope and R^2.

J. Calibration Curve from Characterization of Purified and Clinical Lactate Sample

Determination of the optimal binding frequency began with a set of sensors different from the glucose assay. Similar protocol was enacted. All sensors were brought to room temperature before each test. The optimal binding frequency was determined based off the data obtained from the purified lactate gradients. To simply state again, the gradients were prepared through serial dilution with PBS, and mixed well with 200 mM potassium ferricyanide, and NADH at 1:1:1 ratio to form a total volume of 100 µL of each sample. The sample range measure was 0.001, 2, 4, 8, 16, and 32 mMol to establish a purified calibration curve. The same principle of correlating the slope and R^2 value against the frequency sweep of 0–KHz was utilized to determine the optimal binding frequency. Figure 3(B) shows the optimal binding frequency of 69.75 Hz.

When testing the complex solution, similar parameters were performed to that of purified lactate. Each sensor had three blanks run to evaluate the baseline, and then each sensor was used three times on the complex blood solution.

K. Lactate Assay in Blood Solution—YSI

Blood samples were collected from the Mayo Clinical Hospital in Scottsdale, Arizona. IRB approval was granted through Mayo Clinical Hospital IRB #14-008935. The blood samples were provided after unspecified traumatic injuries and used within 24 hr. The YSI (Lab Version) biochemical analyzer was used on each sample with three repetitions to help evade any human error or outlier data. The YSI was performed as per instructions that came with

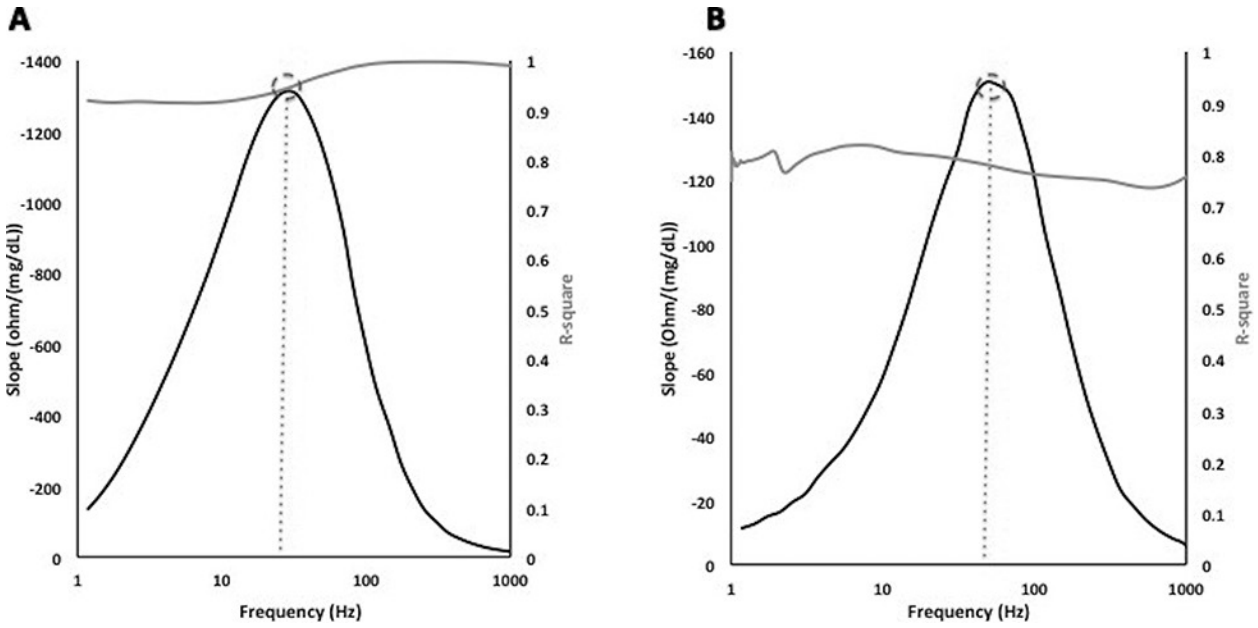

FIG. 3: (A) The plot shows the dual relationship between slope against frequency and R^2 against frequency. The relationship is for the glucose marker and showed an optimal binding frequency of 31.5 Hz. (B) The plot shows the duel relationship between the slope against frequency and R^2 against frequency. The relationship is for the lactate marker and showed an optimal binding frequency of 69.75 Hz.

the equipment, including using all new buffers and lactate membranes to help avoid variance between samples.

L. Correlation Study of YSI and EIS Values

Blood samples delivered from the Mayo Clinical Hospital in Scottsdale, Arizona were used for comparison testing between the YSI, gold standard for lactate detection, and our EIS platform.[25] The parameters were performed similar to that of purified lactate, each sensor had three blanks run to evaluate the baseline, and one GDE was used for each human blood sample, replicating it three times. The blood samples followed the same protocol of 1:1:1 ratio of redox probe, NADH cofactor, and 10% blood to form a total volume of 100 μL of each sample.

A calibration curve was derived as a correlation between the impedance value of the 10% blood and the value determined by the YSI (Lab Version) biochemical analyzer. The relationship of input concentration and output impedance at the optimal frequency of 69.75 allowed a concentration equation, the slope was −32279x with x being the concentration of YSI lactate and the intercept being 36585, to be developed that correlated the two values (see Fig. 4). Using the linear fit equation and the output impedance at the specific frequency we anticipated being able to accurately determine lactate concentrations based on the impedance results seen in Table 1.

M. Preliminary Attempt of Dual Immobilization

To immobilize both glucose oxidase and lactate dehydrogenase, the GDEs were polished, had a SAM applied, and had the SAM activated with EDC and NHS, as previously described above. Next, 9.4 and 10 mg/mL of lactate dehydrogenase and glucose oxidase, respectively, was added to the GDE and allowed one to incubate for 1 hr to ensure proper binding. The GDEs were finally incubated in 100 uL of deionized water containing 1% ethanolamine. The electrodes were stored in PBS at 277.15 K until they were ready for testing. The impedance was measured on each GDE to determine the specific formal potential of the electrode and as a quality control test. While testing the dual marker electrodes, lactate levels were held constant at 1, 8, and 30 mM, while each lactate concentration was conducted with a glucose gradient consisting the following concentrations: 0, 8, 25, 60, and 100 mg/dL. Additionally, a lactate gradient, concentrations 0, 2.2, 4.4, 8.8, 15, and 30 mM, was conducted holding glucose concentrations constant at 8 mg/dL, 60 m/dL, and 100 mg/dL for three separate gradients.

III. RESULTS

Using the methods described above, the optimal frequency determination is crucial for any subsequent tasks. Figure 3 shows the process that was used to determine the optimal binding frequency for glucose and lactate. The optimal binding frequency for glucose was determined to be 31.5 Hz, and the optimal binding frequency for glucose was determined to be 69.75 Hz. The red circle will point to the region where the slope and regression are maximal.

Table 1 shows the unique relationship determined by graphing average impedance and YSI concentrations to derive an equation that would allow for calculation of the EIS lactate concentration from three unique samples. The slope for the equation is –32,279 Ω/mMol with an intercept of 36,585 and R^2 of 0.8596. The samples were compared to YSI determined lactate concentration and compared by determining the error in the two values. Sample 1 found the lactate concentration to be 0.92 mMol when the YSI machine determined it to be 1.51 mMol, which alludes to a 39.4% error in the sample. However, as the concentration is increased, the error drastically decreases, as shown by Sample 3, with a percent error of 8.18%.

Figure 4 shows two unique images pertaining to the glucose assay results. Figure 5(A) shows the range of frequency corresponding to the average amplitude of impedance. This relationship was evident in all four electrodes, suggesting to effective quality control of the electrodes used in the glucose assay experiment based on the optimal binding frequency aligning in the four electrodes. Figure 5(B) presents the correlations between the impedance and concentrations of 0.90 and the slope of –56.896x, with x be

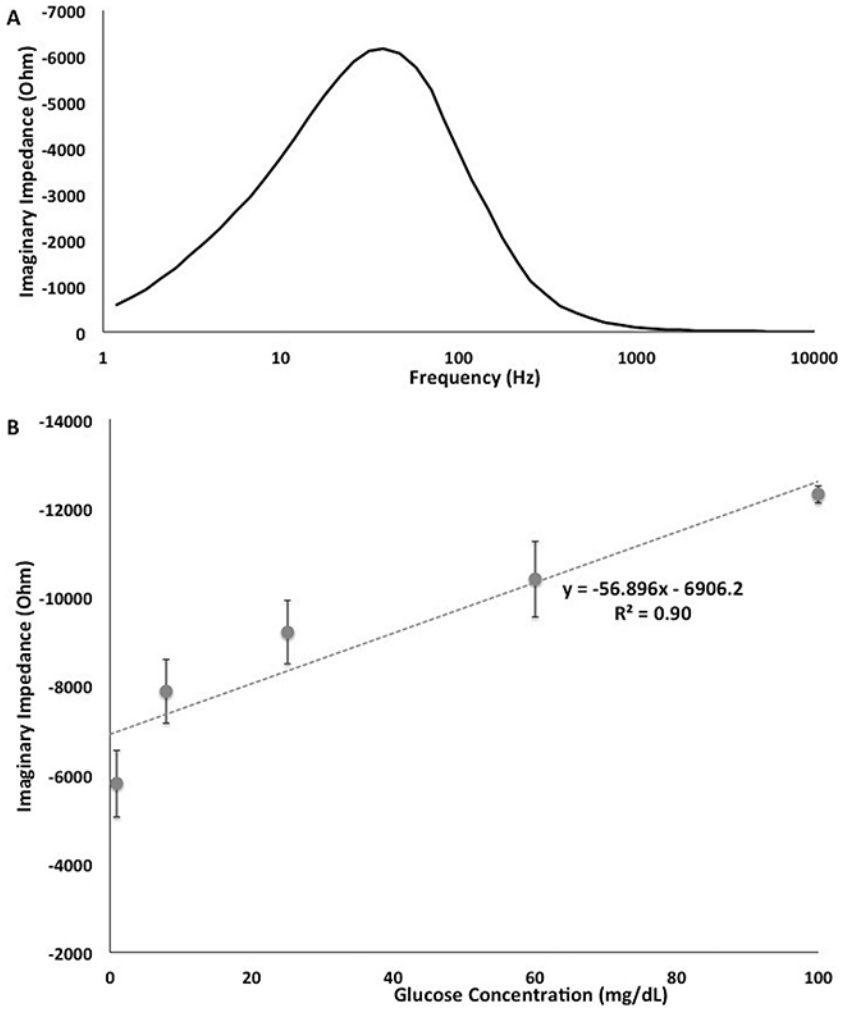

FIG. 4: (A) Shown is a representation of the average peak location and amplitude of that frequency demonstrating the quality control for the wanted electrodes and (B) Graph shows the glucose assay of 0.001, 1, 8, 25, 60, and 100 mg/dL of impedance as a function of glucose concentration in purified solution at an optimal frequency of 31.5 Hz ($N = 4$). The slope for the linear fit equation is –56.896 Ω/(mg/dL) with an intercept of –6906.2 and R^2 of 0.90.

TABLE 1: Lactate concentration obtained from EIS experiments using a unique sample and calculating the lactate concentration using an equation derived from the average impedance ($N = 3$) graphed against the average YSI concentration ($N = 3$). The slope for the equation is –32279 Ohms/mMol with an intercept of 36585 and R^2 of 0.8596. A comparison was made to the YSI determined concentration and the percent error was calculated and is shown in the table.

Electrodes	EIS Lactate Concentration (mMol)	YSI Lactate Concentration (mMol)	Percent Error (%)
Sample 1	0.92	1.51	39.40
Sample 2	1.86	1.63	14.31
Sample 3	2.29	2.12	8.18

ing the concentration of glucose concentration and the intercept being –6906.2.

Figure 5 shows three unique images pertaining to purified and complex lactate assay results. Figure 5(A) shows the range of frequency corresponding to the average amplitude of impedance. The relationship between frequency and impedance amplitude is key in ensuring consistency and quality control among the four electrodes used in the lactate assay experiment. The same principle was used to ensure quality control among the electrodes in the complex assay of Fig. 5(c). Figure 5(b) presents the correlations between the impedance and concentrations of 0.95 and the slope of –164.69x with x being the concentration of lactate concentration and the intercept being –822.5. Additionally, Figure 5(c) presents the 10% whole blood complex solution correlations between the impedance and concentrations of 0.94 and the slope of –34.63x

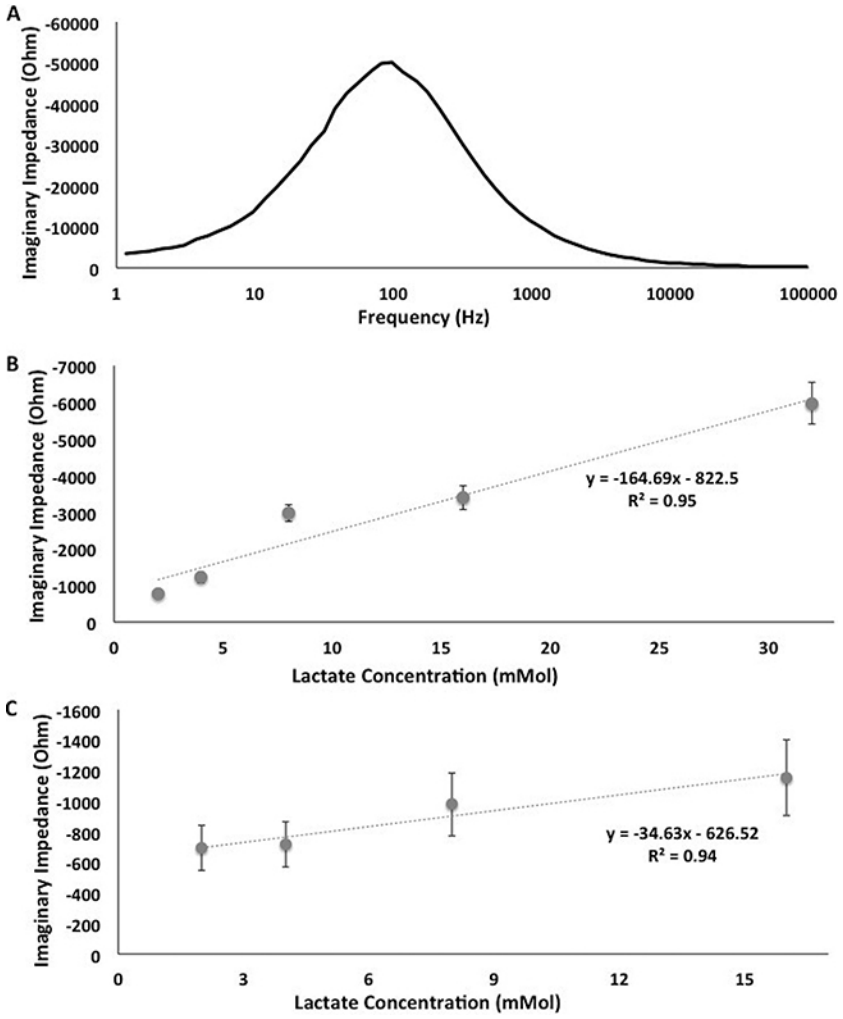

FIG. 5: (A) Representation of the average peak location and amplitude of that frequency demonstrating the quality control for the wanted electrodes for lactate marker. (B) Graph showing the lactate assay of 0.001, 2, 4, 8, 16, and 32 mMol of impedance as a function of lactate concentration in purified solution at an optimal frequency of 69.75 Hz (N = 4). The slope for the linear fit equation is –164.69 Ω/(mMol) with an intercept of –822.5 and R^2 of 0.95. (C) Graph shows the lactate assay with 10% whole blood of 0.001, 2, 4, 8, and 16 mMol of impedance as a function of lactate concentration in 10% whole blood solution at an optimal frequency of 69.75 Hz (N = 3). The slope for the linear fit equation is –34.63 Ω/(mMol) with an intercept of –626.52 and R^2 of 0.94.

with x being the concentration of lactate concentration and the intercept being –626.52.

Figure 6 shows the slope and RSQ versus frequency for each gradient of glucose with lactate held constant as well as the lactate gradients where glucose is held constant. Using the same method, the optimal frequency was determined to generate a calibration curve for the biomarkers. The optimal frequency was determined to be 31.5 Hz for glucose and 97.77 Hz for lactate. However, further analysis of the data to generate a calibration curve of lactate and glucose individually was unsuccessful. Developing a deconvolution algorithm for these signals was unsuccessful because no correlation could be drawn to either biomarker.

Figure 7 is a calibration curve generated using EIS to measure glucose a carbon screen-printed electrode. The optimal frequency of glucose was determined to be 31.5 Hz. Glucose oxidase was immobilized onto the sensor via physical absorption and cross-linked with glutaraldehyde to avoid the need for a SAM.

IV. DISCUSSION

A. Electrochemical Impedance Results

The optimal frequencies of detection were determined by plotting the slope and R^2 values versus frequency. These curves are shown in Fig. 3. The optimal frequency of detection was determined to be the point at which the slope was the highest. The calibration curves for each biomarker were then plotted at their respective optimal frequencies (Figs. 4 and 5).

As shown in Figs. 4 and 5, the impedance magnitude increases with increasing concentrations of glucose and lactate. The increasing trend was not anticipated because, according to Ohm's law, when voltage is held constant, increasing current from the electrons generated from enzymatic reactions should lead to a decrease in impedance. The increase in impedance was suspected to be due to the redox probe being unable to penetrate the monolayer. To examine such a hypothesis, an alternate method of directly immobilizing the glucose oxidase on screen printed carbon electrodes via physical absorption was used and the impedance trend was reversed (Fig. 7). The impedance trend of the carbon sensors suggests that the increase in impedance is likely due to the accumulation of ferrocyanide at the surface of SAM. Because the SAM is electron-limiting, the ferrocyanide could not penetrate it and deposit electrons on the electrode surface, which resulted in a higher impedance. Perhaps the buildup of ferrocyanide was what prevented the detection of lactate and glucose simultaneously, as seen in Fig. 6. An accumulation

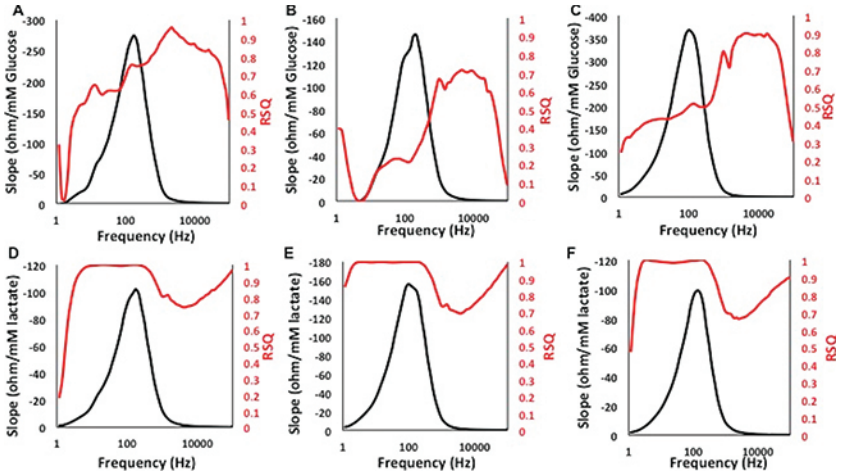

FIG. 6: Co-immobilization of lactate and glucose: (A) Glucose gradient in the presence of 1 mM lactate, (B) glucose gradient in the presence of 8 mM lactate, (C) glucose gradient in the presence of 30 mM lactate, (D) lactate gradient in the presence of 8 mg/dL glucose (E) 60 mg/dL glucose, and (F) 100 mg/dL glucose

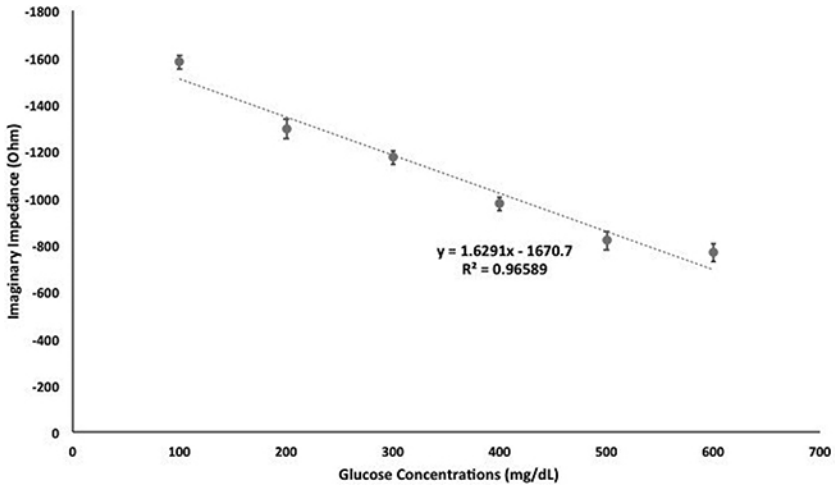

FIG. 7: Calibration curve of glucose sensors prepared by physical adsorption at 31.5 Hz. Error bars were calculated as 1 standard deviation.

of ferrocyanide on the surface of the SAM perhaps leads to a small signal-to-noise ratio when attempting dual-marker detection. From Figs. 6(A)–6(C), it appears that glucose can only be detected in the presence of 1 mM lactate. Perhaps, electrons generated from both the glucose and lactate reactions caused a large ferrocyanide buildup and confounded the signal so much that back-calculating the glucose concentration was impossible. Figures 6(D)–6(F) on the other hand, showed that lactate can be accurately detected regardless of glucose concentrations. Perhaps the detection limitation of glucose is due to lactate being at a much higher concentration than glucose and thus generating a significantly larger number of electrons. However, ferrocyanide buildup can still occur, invalidating the potential of detecting both biomarkers using SAM-prepared electrodes. As suggested from Fig. 7, future work of dual enzymatic detection should focus on sensor preparation without the use of a SAM and thus have little to no impedance for the ferrocyanide to reach the electrode surface.

The lactate measurements obtained using EIS were compared to that of the YSI gold standard as shown in Table 1. Measurements were taken using both a CHI potentiostat and a YSI bioanalyzer, and the percent error between both methods was calculated. The calculated error for each concentration ranged from 8.18 to 39.40%. However, further optimization is required to obtain clinical utility.

Although lactate and glucose levels can indicate the severity of the trauma as well as the likely prognosis,[12,13] lactate levels in combination with glucose levels are frequently not properly utilized due to the extensive time it often takes to receive results of laboratory studies in a hospital setting.[4] Here we show a reliable technique to measure lactate and glucose in a matter of minutes rather than hours. Since the immobilization techniques onto the GDEs are similar for both lactate and glucose, it is highly plausible to combine these techniques into one sensor to simultaneously measure both lactate and glucose with one blood sample. Individually, the lactate and glucose sensors functioned well at detecting their respective target; however, the dual marker was unsuccessful. Figure 7 indicates that using physical absorption rather than a monolayer may result in a successful dual enzymatic sensor. A dual marker sensor will bring the amelioration of treatment for trauma patients as their severity and prognosis can immediately be determined. However, dual marker detection was unsuccessful likely due to the sensor platform and immobilization technique. It is believed that the accumulation of reduced redox probe on the surface of the SAM confounded the signal such that a deconvolution algorithm could not be generated.

B. Clinical Implications

By using EIS on a GDE, the concentration of lactate can be measured in a matter of minutes rather than an hour. Coupled with glucose detection, clinicians can therefore use such data to accurately assess and determine the likely prognosis for patients who have suffered traumatic injuries. However, a dual lactate and glucose sensor is not only useful for traumatic patients, but is also useful for patients who suffer from diabetes. As it is the medical standard, blood glucose levels are not only used to diagnose but also manage diabetes. Additionally, lactate levels are also useful for diabetics because it is not only indicative of a traumatic injury, but also the intensity of physical exercise. Because exercise will alter a patient's glucose levels and is recommend for those with type 2 diabetes, it is necessary and useful to determine the quality of physical exercise. An elevated lactate level will indicate that the exercise performed was physically demanding to the individual who is being tested.

V. CONCLUSION

An increasing impedance magnitude trend was observed for lactate on GDEs. Because the reaction is enzymatic, the trend would have been that of decreasing impedance magnitude according to Ohm's law. The most probable reason for the decreasing magnitude is that the enzymatic generation of electrons affects the rate of ferricyanide reduction to ferrocyanide, and SAM is electro-insulated. Therefore, the electrical signal observed most likely did not come from enzyme-substrate binding, but rather from a change in the ratio of ferricyanide to ferrocyanide. The SAM most likely prevented the ferrocyanide from depositing electrons, which resulted in an accumulation of ferrocyanide. Therefore, the signal of lactate and glucose could not have been successfully deconvoluted due to the accumulation of ferrocyanide even though the sensors were able to accurately measure both lactate and glucose on individual platforms using similar techniques. Because of the suspected accumulation, the dual marker sensor was unsuccessful as the accumulation confounded the signal and inhibited the development of a deconvolution algorithm. However, the accumulation of redox probe will likely be avoided by immobilizing the electrodes without a SAM, which can still provide accurate measurements.

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