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# Characterization of Enzymatic Glucose Biosensor in Buffer Solution, in Artificial Saliva, and in Potassium Ferricyanide by Linear Sweep Voltammetry

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**Abstract.** Existing practice of glucose-level monitoring for diabetic patients involves frequent finger pricking for blood samples. This is not only constantly painful, but could make patients susceptible to infections. A non-invasive, portable, graphene-based biosensor is being developed to measure glucose levels in saliva instead of in blood, by incorporating rGO-PEDOT:PSS composites as the transducer layer on a working electrode – a screen-printed carbon electrode (SPCE) 2 mm in diameter. To evaluate the performance of the biosensor, the electrochemical technique of linear sweep voltammetry (LSV) was used. The results showed sensitivity of rGO-PEDOT:PSS-GOx/SPCE in artificial saliva: 105 μA/mM ($\sigma$ = 12.52) at no glucose to 0.2 mM glucose concentrations and 35.33 μA/mM ($\sigma$ = 5.79) at glucose concentrations of 0.2 mM to 0.6 mM, with correlation coefficient of 0.99. The sensitivity of the biosensor in 10 mM PBS (pH 6.8) is 9.88 μA/mM ($\sigma$ = 2.74), while in 100 mM potassium ferricyanide it is 10.48 μA/mM ($\sigma$ = 0.49). The high sensitivity obtained in artificial saliva suggests that the rGO-PEDOT:PSS composite is suitable for use as a transducer layer for non-invasive glucose monitoring.

**Keywords:** linear sweep voltammetry, screen printed carbon electrode, reduced graphene oxide, PEDOT:PSS, potassium ferricyanide, artificial saliva

## INTRODUCTION

Glucose monitoring using current glucose meters is an invasive approach. One needs to draw blood using a lancet to enable the glucose meter to read and give the test result after about five seconds. Although a majority of the glucose meters available on the market require a blood sample of less than one microliter, pricking one's finger four to eight times per day can be uncomfortable. In consequence, many researchers, including big companies such as Google, Abbott, and Samsung, are focusing on non-invasive glucose monitoring as the ultimate goal for glucose sensing [1]. Throughout the development of such non-invasive biosensors, many innovations have been made, by improving the biorecognition elements, enhancing the transducer layer, or using different transduction methods. Extensive research has been conducted to improve the transducer thanks to the emergence of nanotechnology over the past few decades.

In this study, a composite of reduced graphene oxide (rGO) and the conductive polymer poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) is deposited on a screen-printed carbon electrode (SPCE). The function of such composite is to enable electrochemical sensors to measure low glucose concentration in saliva; there is a strong positive correlation between glucose in blood and glucose in saliva [2]. The characterization of the rGO-PEDOT:PSS–modified electrodes was conducted using linear sweep voltammetry (LSV), in which current is measured as the voltage is swept linearly between the working electrode and reference

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electrode [3]. The technique can determine the relation of analyte concentration to current produced at the working electrode using an electrochemical three-electrode system.

We tested the rGO-PEDOT:PSS–modified electrodes in three solutions: PBS (pH 6.8), artificial saliva, and potassium ferricyanide. The PBS and artificial saliva solutions are considered to be non–redox active solutions because no distinct ions are involved in the redox reaction, while the potassium ferricyanide solution is used for comparison owing to the established redox reaction.

# METHODOLOGY

## Reagents and Apparatus

Ultra-highly concentrated single-layer graphene oxide (UHC GO), 6.2 mg/ml, was purchased from Graphene Supermarket (https://graphene-supermarket.com), USA. Screen-printed carbon electrodes (SPCEs) with 2 mm working electrodes were purchased from Pine Instruments, Grove City, Pennsylvania, USA. Poly (3,4-ethylenedioxythiophene):polystyrenesulfonic acid (PEDOT:PSS), phosphate-buffered saline (PBS) tablets, and glucose oxidase (GOx) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Artificial Saliva for Medical and Dental Research was purchased from Pickering Laboratories, Inc., Mountain View, California, USA. Potassium ferricyanide ($K_3Fe(CN)_6$) was purchased from R&M Chemicals, Selangor, Malaysia. Glucose stock solution (10 mM) was prepared using anhydrous D(+)-glucose and deionized (DI) water.

## Fabrication of rGO-PEDOT:PSS-GOx/SPCE

The composite was prepared by mixing 500 µl PEDOT:PSS solution with 500 µl UHC GO solution. This mixture was stirred using a magnetic stirrer and sonicated at 30℃ for 10 min in order to form a well-mixed GO-PEDOT:PSS composite. Next, 40 µl GO-PEDOT:PSS composite was mixed with 60 µl GOx solution. This solution was sonicated at 30℃ for 30 min. Then, 3 µl of the mixture was drop-cast onto the working SPCE. The electrode was dried at room temperature before reduction and characterization.[4]

## Electrochemical Reduction and Characterization

A portable potentiostat known as pocketSTAT (IVIUM Technologies, Eindhoven, the Netherlands) was used for electrochemical reduction and characterization. The reduction process was performed via repetitive cyclic voltammetry (CV) with potential range from 0 V to -1.5 V at 0.05 V/s in 10 mM PBS (pH 5.0) for 15 cycles. The reduced electrode, denoted as rGO-PEDOT:PSS/SPCE, was rinsed with DI water and dried at room temperature for 24 hours [4].

## Linear Sweep Voltammetry

LSV testing was performed on the rGO-PEDOT:PSS/SPCE in 10 mM PBS (pH 6.8), in artificial saliva, and in 100 mM potassium ferricyanide with different glucose concentrations (0.1 mM to 0.6 mM) and different scan rates (25, 50, 100, 150, and 200 mV/s). The concentrations were chosen to be in the range for salivary glucose levels. The PBS, artificial saliva, and potassium ferricyanide test solutions were prepared prior to the characterization tests by dilution of 10 mM glucose stock solution with the test solutions.

LSV with different glucose concentrations was conducted in 10 mM PBS (pH 6.8) and in artificial saliva solution at a potential range from -2 V to 2 V. For 100 mM potassium ferricyanide, the potential range used was from -0.5 V to 1 V. LSV with different scan rates was conducted in 10 mM PBS (pH 6.8) and in artificial saliva solution at a potential range of -0.5 V to 0.5 V and in 100 mM potassium ferricyanide solution at a potential range of -0.5 V to 1 V.

## Statistical Test

Data collected from the LSV are further analyzed using statistical tools to provide inferences on the effects of changing glucose concentration and sweep scan rates on the electrochemical performance of the electrode. The

analysis first investigates the homogeneity of variance across each dataset, as well as its normality, using the Fligner-Killen test. This helps to determine whether the subsequent analyses should follow a parametric or non-parametric test for significance.

If the variances are not significantly different ($p > 0.05$), the statistical analysis would then proceed to significance tests using one-way ANOVA. Otherwise, the Kruskal-Wallis method shall be used, followed by Dunn's Post-hoc tests.

Significance tests were performed on each dataset by segregating the regions based on the potential range in the sweep. For statistical analyses of the glucose concentration effect, the potential window was separated at the point where the faradaic current starts to increase from baseline, whereas for the scan-rate statistical analyses, the two test regions are separated at the intersection point where the current is zero.

## RESULTS AND DISCUSSION

Figure 1 shows the linear sweep voltammetry (LSV) curves of rGO-PEDOT:PSS/SPCEs in 10 mM PBS (pH 6.8), in artificial saliva , and in 100 mM potassium ferricyanide together with the corresponding calibration curve.

As can be seen in Fig. 1, the current decreases with increasing glucose concentration for measurements made in 10 mM PBS (pH 6.8) or in artificial saliva. However, the result is different for LSV ran in 100 mM potassium ferricyanide, where the current increases with glucose concentration. It is also notable that there was no peak current from LSV in 10 mM PBS (pH 6.8) and in artificial saliva, while a peak current was observed near a voltage of 0.2 V for the ferricyanide. The calibration curve with the highest correlation coefficient ($R=0.99$) was obtained at a potential of 0.49 V in 10 mM PBS (pH 6.8); the linear plot, represented by the equation $y = -9.88x + 42.39$, gave the sensitivity of the biosensor as 9.88 μA/mM with average standard deviation of 2.74 ($n=3$). The LSV of the rGO-PEDOT:PSS–modified electrode in artificial saliva is represented by two equations respective to the concentration of glucose. For artificial saliva with no glucose (0 mM) to 0.2 mM glucose, the straight line is represented by the equation $y = -105x + 100.06$, which gave the sensitivity of the biosensor as 105 μA/mM ($\sigma = 12.52$). From 0.2 mM to 0.6 mM glucose in artificial saliva the sensitivity was lower at 35.33 μA/mM ($\sigma = 5.79$). The existence of two linear operating ranges suggests how the transducer material interacts with glucose in the saliva solution; the larger standard deviation of 12.52 at the lower glucose concentrations in comparison to 5.79 at the higher glucose concentrations suggest more interference from competing ions in the saliva at lower glucose concentrations. For measurements in artificial saliva, the highest correlation coefficient is obtained at a potential of 0.34 V. The calibration curve for increasing glucose concentration when current is measured in 100 mM potassium ferricyanide shows a linear profile represented by the equation $y = 10.48x + 3.48$, with a correlation coefficient 0.99 and average standard deviation 0.49 ($n=3$). From the linear equation, the sensitivity obtained for the rGO-PEDOT:PSS-GOx/SPCE is 10.48 μA/mM at 0.16 V.

The decrease in current with increasing glucose concentration for measurements made in both PBS and artificial saliva solutions could be attributed to the slow electron-transfer rate relative to the voltage scan rate, owing to the weak redox properties of PBS and artificial saliva solutions as electrolytes. These weak electrolytes could also result in irreversible reactions on the surface of the WE, confirmed by the nonexistence of a peak. Potassium ferricyanide solution is redox active and a good electrolyte to mediate electron transfer efficiently at the WE, facilitating transfer of electrons that result from the glucose–glucose oxidase reaction at the electrode surface.

Statistical analysis of the different glucose concentrations on the three analytes show that changes in the glucose concentration do not have any significant effect on the current output for measurements taken in PBS (pH 6.8) and artificial saliva at a scan rate of 30 mV/s. However, increasing the glucose concentration to 0.6 mM in 100 mM potassium ferricyanide has a significant effect on the current measured, with a *p*-value of 0.035, relative to the current output at 0 mM glucose. This suggests that presence of glucose could be discriminated by the electrode, thus supporting the contention that the glucose oxidase is contributing to the electrochemical reaction on the electrode. However, the absence of any supporting electrolyte in the solution may have masked the actual activity of the electrode, thus avoiding redox to take place at the solution-electrode interface.

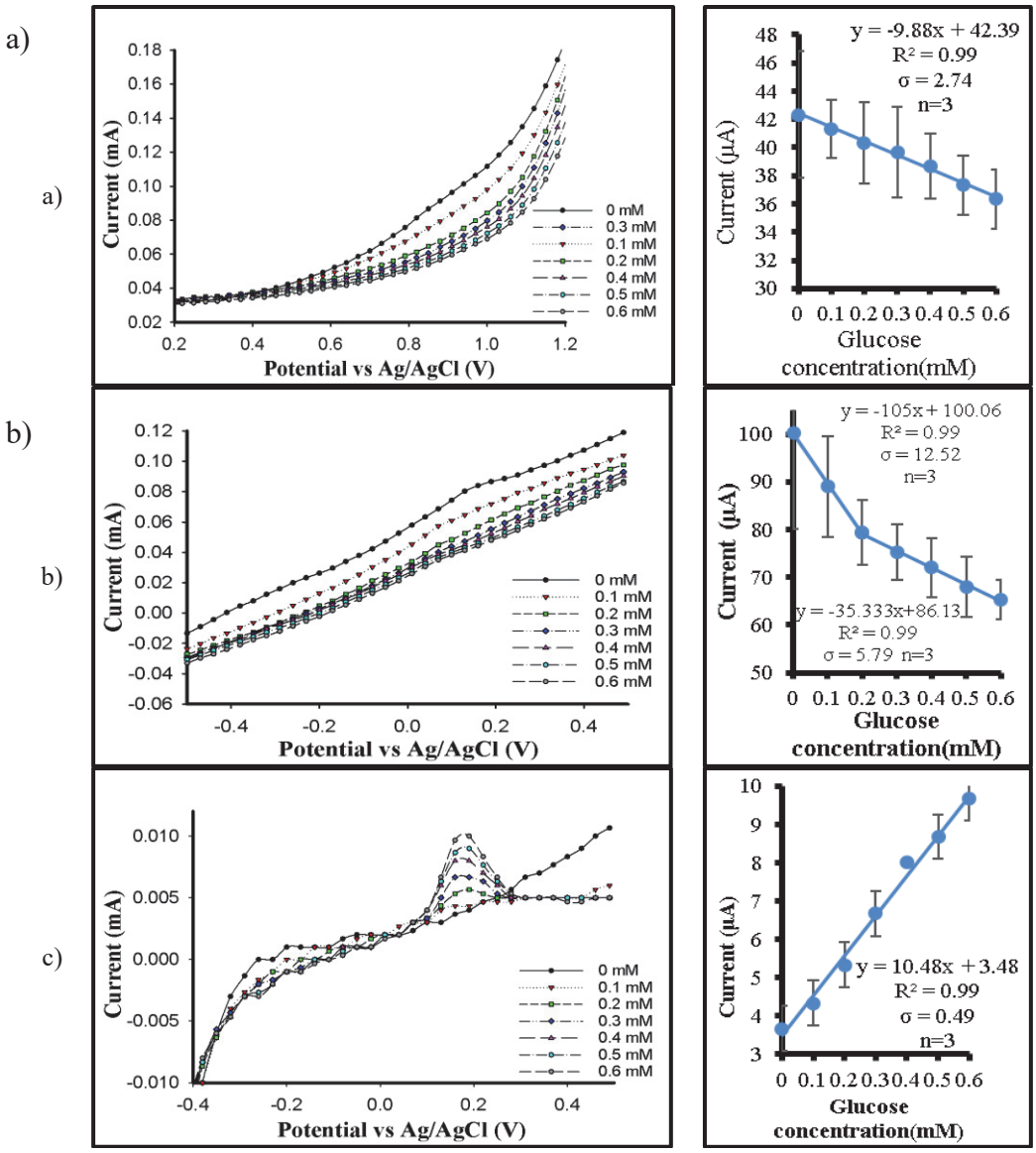


**FIGURE 1.** LSV of rGO-PEDOT:PSS-GOx/SPCE with increasing glucose concentration from 0.1 mM to 0.6 mM at a scan rate of 30 mV/s for the voltage range with the most sensitive change in current in a) 10 mM PBS (pH 6.8), b) artificial saliva, and c) 100 mM potassium ferricyanide, with the corresponding curve of current versus glucose concentration. The sweep was run at a potential range from -2 V to 2 V in 10 mM PBS (pH 6.8) and in artificial saliva solution, while a potential range from -0.5 V to 1 V was used in 100 mM potassium ferricyanide.

LSV were conducted at various scan rates in all solution types. Measurements in all solutions show that the current increases with increasing scan rates; the higher the scan rate, increasing the current. There is no peak observed from the current profile of LSV in 10 mM PBS (pH 6.8) or in artificial saliva, whereas a peak near 0.2 V was observed in 100 mM potassium ferricyanide. The existence of a peak suggests a reversible reaction, while the opposite is true for measurements made in PBS (pH 6.8) and artificial saliva. The current for 10 mM PBS (pH 6.8) increases more rapidly at higher scan rates than does the current for artificial saliva. The rapid increase shows that electron transfer for glucose in 10 mM PBS solution for the rGO-PEDOT:PSS-GOx/SPCE biosensor is relatively slow in comparison with that in artificial saliva; again, this is a result of more interfering ions in artificial saliva.

Statistical analysis for the scan-rate experiment shows significant effect of scan rate on the cathodic current at the positive potential range seen in all three solvent conditions. In potassium ferricyanide solution, the effect of scan rate at the right-hand side of the intersection point is statistically significant ($p$ <<< 0.0001), but the effect is

statistically non-significant at lower potentials. Most significant here is the effect of scan rate at 150 mV and 200 mV (both with *p*-value < 0.0001); at 100 mV the effect is statistically less significant ($p$ = 0.011). Similarly, the effect of scan rate in PBS also shows significant behavior at higher potential ranges ($p$<<<0.0001), while its effect at lower potentials at the left-hand side of the intersection point is not significant.

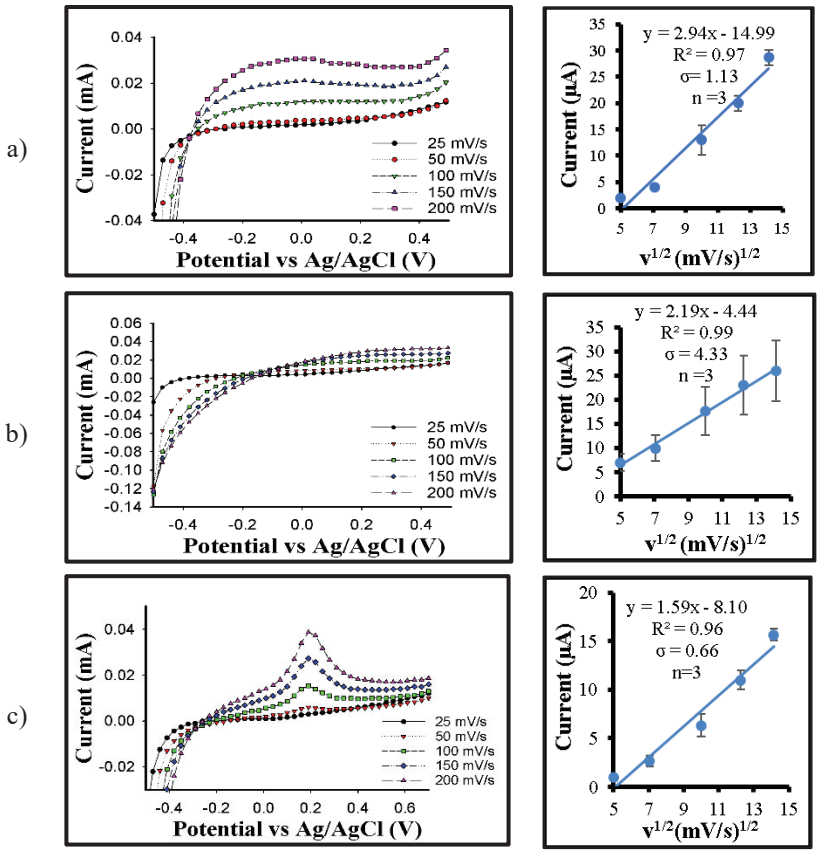


**FIGURE 2.** LSV of rGO-PEDOT:PSS-GOx/SPCE for 0.3 mM glucose at different scan rates (25, 50, 100, 150, 200 mV/s) and corresponding Cottrell plot in (a) 10 mM PBS (pH 6.8), (b) artificial saliva, and (c) 100 mM potassium ferricyanide. The sweep was run at -0.5 V to 0.5 V in 10 mM PBS (pH 6.8) and in artificial saliva solution, while a potential range from -0.5 V to 1 V was used in 100 mM potassium ferricyanide solution.

Interestingly, the effect of scan rate on current measured in artificial saliva shows a significant difference on both sides of the potential range (left-hand *p*-value = 0.00236 and right-hand *p*-value = $3.211 \times 10^{-10}$). The increase in current is most significant at 150 mV/s and 200 mV/s. The results suggest that the activity of the electrode in artificial saliva is sensitive to the scan rates, and it is likely to operate on both low and high potentials.

The Cottrell plot for LSVs in all solutions show a linear relationship between the current measured and the square root of the scan rate. The highest value of slope, 2.94, was obtained from a Cottrell plot of the rGO-PEDOT:PSS-GOx/SPCE in 10 mM PBS (pH 6.8), followed by artificial saliva with slope of 2.19; the smallest slope of 1.59, is in 100 mM potassium ferricyanide.

According to Shi et al. (2011), the Randles-Sevcik equation for peak current ($I_p$) can be used to calculate effective surface area of the electrodes. Effective surface area means the electrode area that is involved in redox reactions at the electrode-solution interface. Note here that the Randles-Sevcik equation can be used only when redox species are known in the solution, which in this case, only the potassium ferricyanide solution, where ferricyanide ions ($Fe(CN)_6^{-3}$) can be reduced to ferrocyanide ($Fe(CN)_6^{-4}$) upon applied voltages, or oxidized, depending on the polarity of the applied voltages.

As the Cottrell plot in Fig. 2(c) is linear, the slope can be used to calculate the effective surface area of the rGO-PEDOT:PSS-GOx/SPCE. The calculated effective surface area of the rGO-PEDOT:PSS electrode in potassium ferricyanide is 8.836 $mm^2$ [5][6][7]. The effective surface area is found to be ~ 2.8 times larger than the electrode diameter (ø=2 mm, surface area, $\pi*r^2$ = 3.14 $mm^2$), suggesting that the transducer material rGO-PEDOT:PSS enhances the surface of electrodes for effective and fast electron transfer.

## CONCLUSIONS

The goal of the study is to fabricate a non-invasive glucose sensor that can detect the low glucose concentration in saliva, which is twofold lower than that of blood. To ensure that electrochemical sensors can detect glucose in such lower concentrations, a new transducer material needs to be developed. In our study, rGO-PEDOT:PSS was used as a transducer material that can enhance transduction. The performance of rGO-PEDOT:PSS-GOx composite deposited on a SPCE in 10 mM PBS (pH 6.8), in artificial saliva, and in 100 mM potassium ferricyanide was observed using LSV, and the sensitivity and linear range of the composite-modified SPCE was determined for all solutions. The results show highest sensitivity of rGO-PEDOT:PSS-GOx/SPCE in artificial saliva at lower glucose concentrations from 0 to 0.2 mM, but with the highest standard deviation. The higher sensitivity obtained in artificial saliva shows that the rGO-PEDOT:PSS composite is suitable for use as a transducer layer for non-invasive glucose monitoring; however, the larger standard deviation at the lower glucose concentration suggests that sensitivity of the transducer towards glucose needs to be enhanced. Future work will use the results to design a device for non-invasive glucose sensing with saliva.

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