



# Cyclic Voltammetric and Electrical Impedance Spectroscopy Studies of Graphene- and Conductive Polymer-based Enzyme Electrodes

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**Abstract:** Screen-printed carbon electrodes (SPCEs), modified with graphene oxide and poly(3,4-ethylenedioxythiophene):polystyrenesulfonic acid (GO-PEDOT:PSS/SPCEs), and SPCEs modified with partially reduced GO and PEDOT:PSS (prGO-PEDOT:PSS/SPCEs) were studied for electrochemical transduction. Randles-Sevcik analysis showed that the prGO-PEDOT:PSS/SPCE has a higher effective surface area of 219.3  $\mu\text{m}^2$  in comparison to the unreduced GO-PEDOT:PSS/SPCE (87.0  $\mu\text{m}^2$ ) and the bare SPCE (71.7  $\mu\text{m}^2$ ). Using electrical impedance spectroscopy (EIS), we determined that the prGO-PEDOT:PSS/SPCE has a lower charge-transfer resistance ( $R_{ct}$ ) of 163.82  $\Omega$  in comparison to the GO-PEDOT:PSS/SPCE (427.87  $\Omega$ ) and the bare SPCE (13.31 k $\Omega$ ). Glucose oxidase (GOx) was immobilized on all electrode types, including GO/SPCE as additional control and tested with low (0.2 mM), intermediate (0.6 mM), and high (1 mM) glucose concentrations. GOx/GO/SPCEs showed the largest change in anodic peak current ( $I_{pa}$ ), 8.5, 7.5 and 4.9  $\mu\text{A}$  for low, intermediate, and high glucose concentrations, respectively. Interestingly, GOx/prGO-PEDOT:PSS/SPCEs have no change in both anodic and cathodic peak current, although they exhibit better redox capability, while GOx/SPCEs have very low  $I_{pa}$ . The results show that the high effective surface area and low charge-transfer resistance ( $R_{ct}$ ) of prGO-PEDOT:PSS/SPCEs do not necessarily result in a sensitive glucose sensor in cases where immobilization of enzymes on the material can affect electron transfer.

**Keywords:** Cyclic voltammetry, electrical impedance spectroscopy, PEDOT:PSS, partially reduced graphene oxide, glucose oxidase, electrochemical transduction.

## 1. Introduction

Modifying electrodes by incorporating nanomaterials to enhance signal transduction is becoming a common approach in the field of electrochemical sensors. This trend is driven by ease and cost effectiveness, allowing for rapid fabrication of improved electrodes. The advent of graphene and conductive polymers has further facilitated developments in electrode modification. Numerous instances of the use of graphene and conducting polymers as composites in the development of biosensor [1–4], solar cells [5–7], and energy-storage applications [8–11] have been reported. Conductive polymer poly(3,4-ethylenedioxythiophene):polystyrenesulfonic acid, PEDOT:PSS, is the usual polymer of choice in the fabrication, owing to its flexibility, stability, and cost effectiveness [12].

The graphene-PEDOT:PSS composite exploits the synergistic properties of both graphene and PEDOT:PSS, with the former having large surface area (736.6 m<sup>2</sup>/g) [13] and abundant carboxylated functional groups [14,15] for biomolecule immobilization, and the latter high conductivity (300 S/cm) and good electrocatalytic properties for electron transfer [16]. The increased use of the nanocomposite for biosensing purposes stems from a facile fabrication process that has potential for rapid prototyping of devices [17].

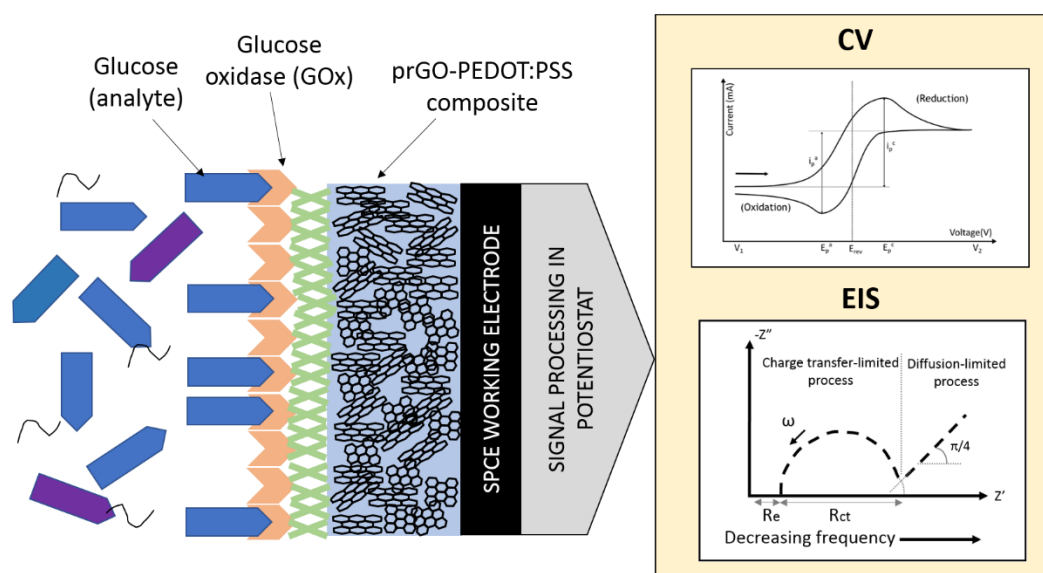
In this study, we explored the potential of drop-casting graphene and PEDOT:PSS on screen-printed carbon electrodes (SPCEs) with the intent for rapid prototyping. Although the use of graphene and PEDOT:PSS is not new for biosensors, most studies do not focus on or emphasize methods that are cost-effective with simple fabrication possibilities. The effect of these materials in either single or composite form on SPCEs is not well understood. Furthermore, when PEDOT:PSS was used, most studies looked into applications for solar cells [6,7,11,18–20]. For fabrication of reduced graphene and PEDOT:PSS on SPCEs, we drop-cast a solution containing graphene oxide (GO) and PEDOT:PSS on SPCEs, electrochemically reduced the GO to yield a partially reduced graphene and PEDOT:PSS nanocomposite (prGO-PEDOT:PSS/SPCE), and immobilized the model enzyme glucose oxidase (GOx) for glucose sensing (GOx/prGO-PEDOT:PSS/SPCE). The fabrication process does not require a cleanroom facility and was done in ambient conditions.

Partially reduced graphene (prGO) is produced by mild electrochemical reduction of GO in a slightly acidic buffer solution (pH 5) to prevent the loss of all GO oxygenated functional groups [21–24]. For prGO, the  $\pi$  conjugation is partially restored, while the conductivity of the material is maintained [25]. Electrochemical reduction of GO to prGO is a safe and environmentally friendly process in comparison to thermal and chemical reduction [26].

Electrochemical analysis by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) was conducted on SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs to investigate the feasibility of the materials as transducers. In CV, we look into the current versus square root of scan rate and into the effective surface area to reveal electrode electrocatalytic activity and electron-transfer reversibility, while in EIS, we look into the charge-transfer resistance ( $R_{ct}$ ) at the electrode-solution interface [27,28]. We report on the aforementioned parameters from CV and EIS to identify the potential of the prGO-PEDOT:PSS as an ion-to-electron transducer, with SPCEs and GO-PEDOT:PSS/SPCEs as controls.

In addition to the choice of materials for transducers, enzyme immobilization on the transducer surface can affect sensitivity and limit of detection. We have immobilized glucose oxidase on electrodes through methods of physical adsorption [29–31]. However, adsorption can result in enzyme leaching, reducing the sensitivity of detection. Immobilization of glucose oxidase with glutaraldehyde as crosslinker proved successful for glucose sensing [1,32–34]; such crosslinking provides a stable template for enzyme immobilization and prevents enzyme leaching [34–37]. A solid immobilization strategy exposes the active enzyme site to the analyte and retains biological activity, resulting in highly sensitive biosensors. Therefore, we immobilized GOx through glutaraldehyde crosslinking on prGO-PEDOT:PSS/SPCEs, and controls of SPCEs, GO/SPCEs and GO-PEDOT:PSS/SPCEs.

We fabricated and tested the functionality of prGO-PEDOT:PSS/SPCEs and controls of SPCEs, GO/SPCEs and GO-PEDOT:PSS/SPCEs at low (0.2 mM), intermediate (0.6 mM), and high (1 mM) glucose concentration for glucose sensing. In glucose sensing, GOx is exposed to glucose to form an enzyme-analyte complex. The active site of an enzyme is reduced by accepting electrons at the transducer, where the signal is measured by a potentiostat (Figure 1). Although many studies have reported on highly sensitive biosensors, we believe that our facile fabrication method with no requirement for any cleanroom facility can lead to commercialization of electrochemical biodevices for noninvasive glucose sensing. This study will report on the fabrication process, electrochemical characterization of the transducer, and sensing of glucose.



**Fig. 1 - Detection mechanism for glucose biosensing.** The glucose oxidase (GOx) immobilized on the electrode is exposed to glucose and forms an enzyme-analyte complex that is detected by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Electrons successfully transduced through the electrode surface are reported as current (I) against voltage (V). For successful detection, the electrons must shuttle through the enzyme-analyte layer. EIS measures resistive behavior against electrochemical reaction at the electrode-solution interface where the real ( $Z_{re}$ ) and imaginary ( $Z_{im}$ ) impedances are obtained.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

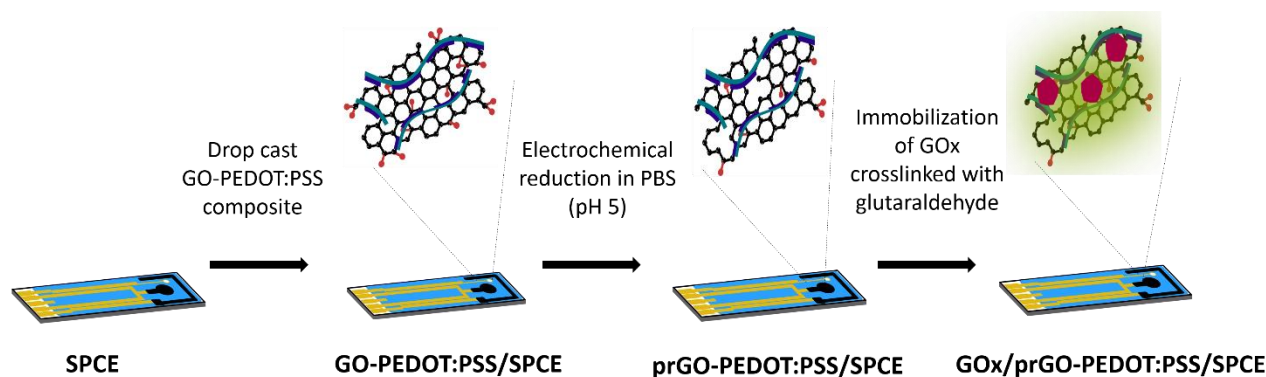
Chemicals used in this work were analytical grade and is described in [40]. For glucose measurements, glutaraldehyde, glucose oxidase (GOx, 15,000 units/g solid), and D-(+)-glucose ( $C_6H_{12}O_6$ ) were purchased from Sigma-Aldrich, St. Louis, Missouri, USA.

### 2.2 Fabrication of nanomaterial-modified screen-printed carbon electrodes for glucose sensing

The fabrication steps for a glucose oxidase/partially reduced graphene oxide/poly(3,4-ethylenedioxythiophene):polystyrenesulfonic acid/screen-printed carbon electrode (SPCE), denoted as GOx/prGO-PEDOT:PSS/SPCE, are shown in Figure 2. Fabrication of GO/SPCEs and GO/PEDOT:PSS/SPCEs follows a similar process of drop-casting; SPCEs are unmodified electrodes.

Pretreatment of the SPCEs was conducted via cyclic voltammetry (CV) for 3 CV cycles in 0.1 N  $H_2SO_4$  at a scan rate of 100 mV/s and potential range of -2.5 to 2.5 V [4]. Activation of SPCEs with anodic and cathodic potential increases the porosity of the electrode surface, improves voltammetry responses, and removes adsorbed oxygen [38,39]. After pretreatment, the electrodes were rinsed with DI water and dried at ambient temperature. The method for GO-PEDOT:PSS composite preparation, deposition, and reduction of GO-PEDOT:PSS/SPCEs to prGO-PEDOT:PSS/SPCEs were reported previously [40,41]. The reduced prGO-PEDOT:PSS/SPCEs were rinsed with PBS, pH 7.4, and dried in ambient conditions.

Fifty  $\mu$ l glucose oxidase (GOx) solution (1 mg/ml) was mixed with 20  $\mu$ l of 2% (vol) glutaraldehyde [1] to form GOx-glutaraldehyde solution. Three  $\mu$ l solution was drop-cast onto SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs to form GOx/SPCEs, GOx/GO-PEDOT:PSS/SPCEs, and GOx/prGO-PEDOT:PSS/SPCEs. The fabricated electrodes were dried for 24 hours in ambient conditions before further measurements. For the purpose of glucose detection, a 20 mM glucose stock solution was prepared by dissolving 0.36 g D-(+)-glucose powder in 100 ml of 0.01 M PBS, pH 7.4. Glucose solutions of 0.2, 0.6, and 1 mM were prepared by serial dilution of the prepared stock solution to the desired concentration. Electrochemical analysis of the modified electrodes was performed using a pocketSTAT (IVIUM Technologies, Eindhoven, The Netherlands).



**Fig. 2 - Fabrication steps of prGO-PEDOT:PSS/SPCEs for glucose sensing.** A screen-printed carbon electrode (SPCE) activated through CV was used as a conductive substrate. Modification of the SPCE was done by drop-casting 3  $\mu\text{l}$  GO-PEDOT:PSS composite onto the working electrode. Next, the electrical conductivity of GO was restored by electrochemical reduction in PBS, pH 5, which results in the formation of a partially reduced graphene oxide-PEDOT:PSS (prGO-PEDOT:PSS) layer on the SPCE. Subsequent immobilization of glucose oxidase (GOx) was accomplished through crosslinking with glutaraldehyde [40,41] on the prGO-PEDOT:PSS/SPCE.

### 2.3 Cyclic voltammetry and electrical impedance spectroscopy

CV and electrical impedance spectroscopy measurements is described in [40]. For glucose detection, CVs were carried out in 0.01 M PBS, pH 7.4, at a scanning potential of -0.5 to 1 V for GOx/SPCEs, GOx/GO/SPCEs, GOx/GO-PEDOT:PSS/SPCEs, and GOx/prGO-PEDOT:PSS/SPCEs. These electrodes measured glucose at low (0.2 mM), intermediate (0.6 mM), and high (1 mM) concentrations (Figure 6) at pH 7.4.

### 2.4 Statistical analysis

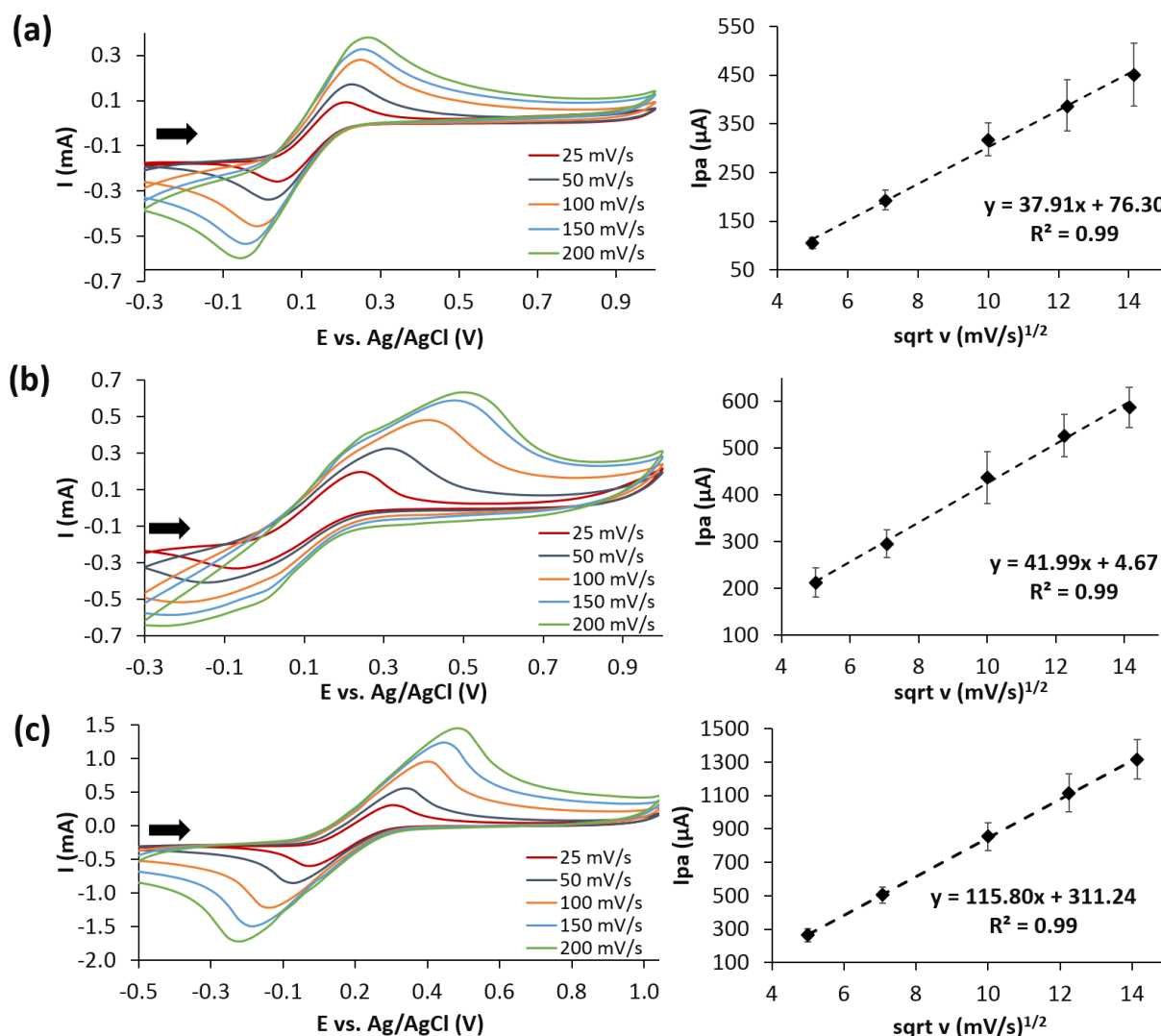
Statistical analysis conducted in this work is described [40].

## 3. Results and Discussion

### 3.1 Cyclic voltammetry for different electrode modifications

Figure 3 shows the CV measurements on SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs using 0.1 M  $\text{K}_3[\text{Fe}(\text{CN})_6]$  as the redox mediator. Linear regression plots of peak current ( $I_p$ ) against the square root of scan rate ( $v^{1/2}$ ) suggested that electron transfer at the electrode-electrolyte interface is reversible for all electrodes. The scan rates used for this study are 25, 50, 100, 150, and 200 mV/s. As can be seen in figure 3(b), there exists a shift in peak potential ( $E_p$ ) of GO-PEDOT:PSS/SPCEs, suggesting sluggish electron transfer. The linear regression plots of each electrode modification also imply the dependence of  $I_p$  on scan rate, which can be described by the Randles-Sevcik equation [3] [42].

Reversibility of electron transfer ensures that the analyte can undergo a repeatable redox process. A forward scan from -0.5 V to 1 V oxidizes  $\text{Fe}(\text{CN})_6^{4-}$  and produces the anodic current. A reverse scan from 1 V back to the initial potential of -0.5 V reduces  $\text{Fe}(\text{CN})_6^{3-}$  to  $\text{Fe}(\text{CN})_6^{4-}$  and produces the cathodic current.

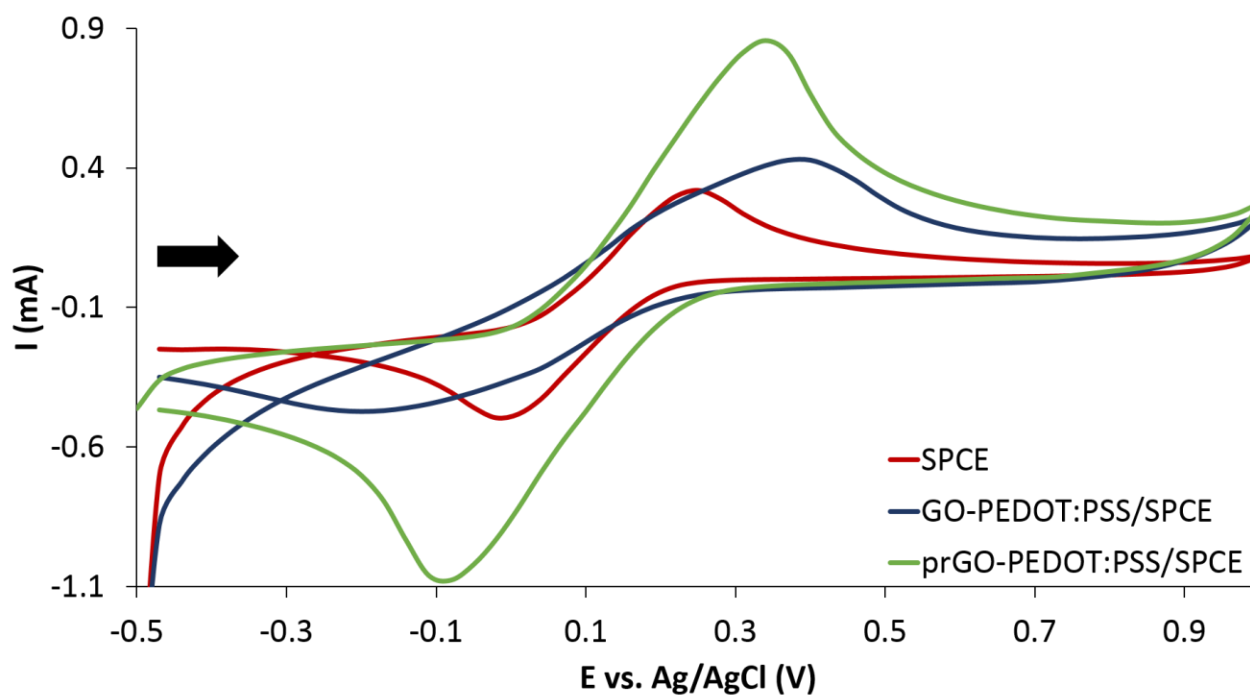


**Fig. 3 - Cyclic voltammograms (CVs) of SPCEs (a), GO-PEDOT:PSS/SPCEs (b), and prGO-PEDOT:PSS/SPCEs (c) in 0.1 M  $K_3[Fe(CN)_6]$  at scan rates of 25, 50, 100, 150 and 200 mV/s with corresponding linear regression between the peak current ( $I_p$ ) and the square root of the scan rate ( $v^{1/2}$ ). The slope ( $k$ ) of the linear plot is used to calculate the electroactive surface area of SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCE using the Randles–Sevcik equation as reported in [40].**

Figure 4 shows CVs of SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs at a scan rate of 100 mV/s. The anodic and cathodic peak potentials, respectively, are  $E_{pa} = 0.25$  V and  $E_{pc} = -0.02$  V for bare SPCEs,  $E_{pa} = 0.40$  V and  $E_{pc} = -0.2$  V for GO-PEDOT:PSS/SPCEs,  $E_{pa} = 0.34$  V and  $E_{pc} = -0.08$  V for prGO-PEDOT:PSS/SPCEs. The peak-to-peak potential difference ( $\Delta E_p = E_{pa} - E_{pc}$ ) of SPCEs is 0.27 V, while  $\Delta E_p$  for GO-PEDOT:PSS/SPCEs and prGO-PEDOT:PSS/SPCEs is 0.42 V. The similar  $\Delta E_p$  for GO-PEDOT:PSS/SPCEs and prGO-PEDOT:PSS/SPCEs suggests that the nanocomposites shifted the peak potential without altering the electrode surface.

The anodic peak current ( $I_{pa}$ ) for SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs is 318.3  $\mu A$ , 428.7  $\mu A$ , and 856.3  $\mu A$ , respectively. The electroactive surface area for SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs is 71.7  $\mu m^2$ , 87.0  $\mu m^2$ , and 219.3  $\mu m^2$ , respectively. The increase in the electroactive surface area of prGO-PEDOT:PSS/SPCEs from GO-PEDOT:PSS/SPCEs could be due to the reduction of GO to prGO. The structure of prGO is made up of some oxygenated functional groups on the edges and basal plane of carbon atoms [15,22]. Furthermore, prGO has a semi-restored  $\pi$  conjugation and  $sp^2$  lattice, which increases the conductivity of the material compared to GO [25].

PEDOT:PSS has excellent stability in aqueous media [43], high conductivity (1 S/cm), and transparency. Furthermore, PEDOT:PSS was found to retain its electrocatalytic activity even after electrochemical reduction [44] which explains the high peak current in prGO-PEDOT:PSS/SPCEs (856.3  $\mu\text{A}$ ). When combining with graphene, PEDOT:PSS could inhibit the stacking of the graphene layer [4]. The synergistic interaction of prGO and PEDOT:PSS could assist the electron transfer between the redox analyte and the electrodes interface. The aforementioned properties indicate that prGO-PEDOT:PSS composite could be useful as a solid-state transducer for glucose sensing.



**Fig. 4 - Cyclic voltammograms (CV) of SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs at a scan rate of 100 mV/s and potential range of -0.5 to 1 V in 0.1 M  $\text{K}_3[\text{Fe}(\text{CN})_6]$ . The anodic peak current ( $I_{pa}$ ) of the SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs was measured as 318.3  $\mu\text{A}$ , 428.7  $\mu\text{A}$  and 856.3  $\mu\text{A}$ , respectively. The shift in the anodic peak potential was observed and measured as 0.25 V, 0.40 V and 0.34 V for SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs, respectively.**

### 3.2 Electrical impedance spectroscopy for different electrode modifications

Electrical impedance spectroscopy (EIS) was used to determine the charge-transfer resistance ( $R_{ct}$ ) for the electrodes. Figure 5 shows the Nyquist plot of SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs over a frequency range of 100 kHz to 0.01 Hz. The impedance spectroscopy was fitted to the Randles equivalent circuit (inset of figure 5a) using the IviumSoft™ electrochemistry software (<https://www.ivium.com/software/>). A typical Randles equivalent circuit is described in [45–48]. The Nyquist plot fitted with a Randles equivalent circuit was typically characterized by a 45° linear region at lower frequency, which indicates a mass transfer-limited process. For an electron transfer-limited process, the semicircular region at high frequency of the Nyquist plot shows that  $R_{ct}$  becomes dominant [49].

Figure 5b shows the charge-transfer resistance for SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs to be 13.31 k $\Omega$ , 427.87  $\Omega$  and 163.82  $\Omega$ , respectively. For unmodified SPCEs, a large semicircular shape is formed across the scanned frequency range. At high frequency, a small semicircular region followed by a linear region is observed in GO-PEDOT:PSS/SPCEs. The prGO-PEDOT:PSS/SPCE plot has a smaller semicircular region and a more prominent linear region, which suggests a mass transfer-limited process based on the Warburg impedance,  $Z_w$  [45–47,49–51].

The prGO-PEDOT:PSS/SPCE has the lowest  $R_{ct}$ , which is preferable for electron transfer and effective electrochemical transduction. Several studies have showed that PEDOT:PSS is a stable hole conductor that, when combined with GO, lowers  $R_{ct}$  [6,52]. A graphene oxide that is partially reduced was found to improve electron transfer at the electrode-solution interface [10]. For glucose biosensors, a prGO-PEDOT:PSS/SPCE can improve sensitivity and limit of detection. Immobilization of enzymes onto transducer materials can increase  $R_{ct}$  as a result of reduced electron transfer [50,53].

Statistically, the differences in  $R_{ct}$  values for modified electrodes in comparison with an unmodified SPCE were highly significant, with adjusted  $p$ -values  $< 0.0001$ . The differences in  $R_{ct}$  for GO-PEDOT:PSS/SPCEs and prGO-PEDOT:PSS/SPCEs were moderately significant, with  $p$ -values  $< 0.05$ .

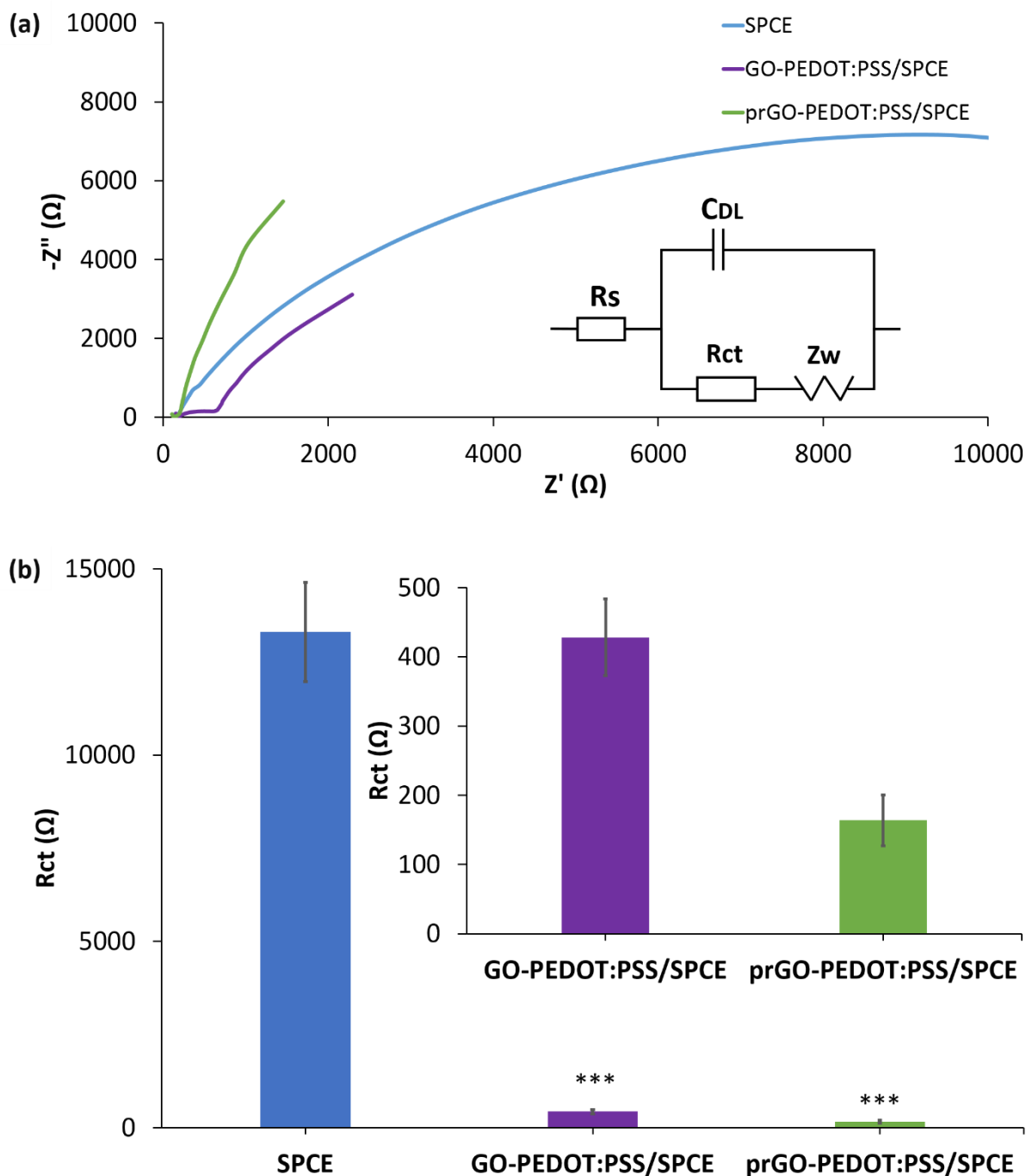


Fig. 5 - (a) Nyquist plot of SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs measured over a frequency range of 100 kHz to 0.01 Hz with applied potential of 0.25 V and amplitude 10 mV. The electrochemical impedance spectroscopy was measured in 0.01 M PBS, pH 7.4, containing 5 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  (1:1) as redox probe. Inset shows Randles equivalent circuit that was used to fit the enzyme-based electrochemical biosensor. (b) Graph of charge-transfer resistance for SPCEs, GO-PEDOT:PSS/SPCEs, and prGO-PEDOT:PSS/SPCEs. Statistical analysis in comparison with SPCEs shows \*\*\* for  $p < 0.001$  ( $n=3$ ).



### 3.3 Cyclic voltammetry of different electrode modifications for glucose detection

Electrochemical signals at the electrode-solution interface for glucose detection are dependent on the surface characteristics of the electrodes. To minimize the effect of adsorbed residual oxygen on the electrode surfaces, which can affect CV measurements, an electrode pretreatment process was conducted as described in section 2.2. As can be seen from figure 6, broad peaks with corresponding peak current ( $I_p$ ) exist for all electrodes except GOx/GO-PEDOT:PSS/SPCEs, which have no identifiable anodic or cathodic peak current. Note also that these samples exhibit broader peaks because measurements are made in PBS, 7.4, and not in potassiumferricyanide ( $K_3[Fe(CN)_6]$ ). The broad anodic/cathodic peak current for GOx/SPCEs, GOx/GO/SPCEs, and GOx/prGO-PEDOT:PSS/SPCEs resulted from oxidation/reduction of oxygen in measurement solution, or an oxygen layer adsorbed on the electrode surfaces [54]. Any peaks observed could be the result of glucose oxidase catalyzing the glucose reaction with oxygen and producing gluconic acid and  $H_2O_2$ , where  $H_2O_2$  can result in Faradaic signal. The shift in peaks for all electrodes could be the result of different mass- and charge transfer resistance ( $R_{ct}$ ) of the different materials used for enzyme immobilization [54]. Spiking the buffer solution with glucose at low (0.2 mM), intermediate (0.6 mM), and high (1.0 mM) glucose concentrations does not result in obvious change in  $I_p$  for GOx/SPCEs, GOx/GO/SPCEs, and GOx/prGO-PEDOT:PSS/SPCEs, while GOx/GO/SPCEs resulted in the highest change in anodic and cathodic  $I_p$  at the different glucose concentrations (Table 1). In a normal reaction, addition of glucose should decrease the peak current owing to the consumption of oxygen when glucose oxidase catalyzed the oxidation of glucose. Note here that only for every 2 moles of  $H_2O_2$  resulted in 1 mole of oxygen, and oxygen will often leave the electrode surface through diffusion, which should result in lowering of the  $I_p$ . However, it seems that the resulting change is not obvious for GOx/prGO-PEDOT:PSS/SPCEs. Larger changes in  $I_p$  for GOx/GO/SPCEs could be the result of better glucose immobilization at the electrode surfaces.

It is interesting to note here that the PEDOT:PSS samples retain  $I_p$  values with addition of low, intermediate, and high glucose. This could be the result of PEDOT:PSS having a capacitive effect as a result of the electrical double layer at the interface of PEDOT and PSS nanoscale grains, where charges are retained on PEDOT:PSS instead of being measured by the SPCE [55]. The capacitive effect of PEDOT:PSS is further confirmed by the square-shaped CV curve as shown in figure 6. Reduced graphene oxide (rGO) was also found to be a good oxygen barrier owing to its multilayer structure and nano interlayer distance between the sheets [56]. The combination of both partially reduced graphene (prGO) and PEDOT:PSS in GOx/prGO-PEDOT:PSS/SPCEs could affect transport of oxygen at the electrode-solution interface. Further studies are needed to confirm this claim.

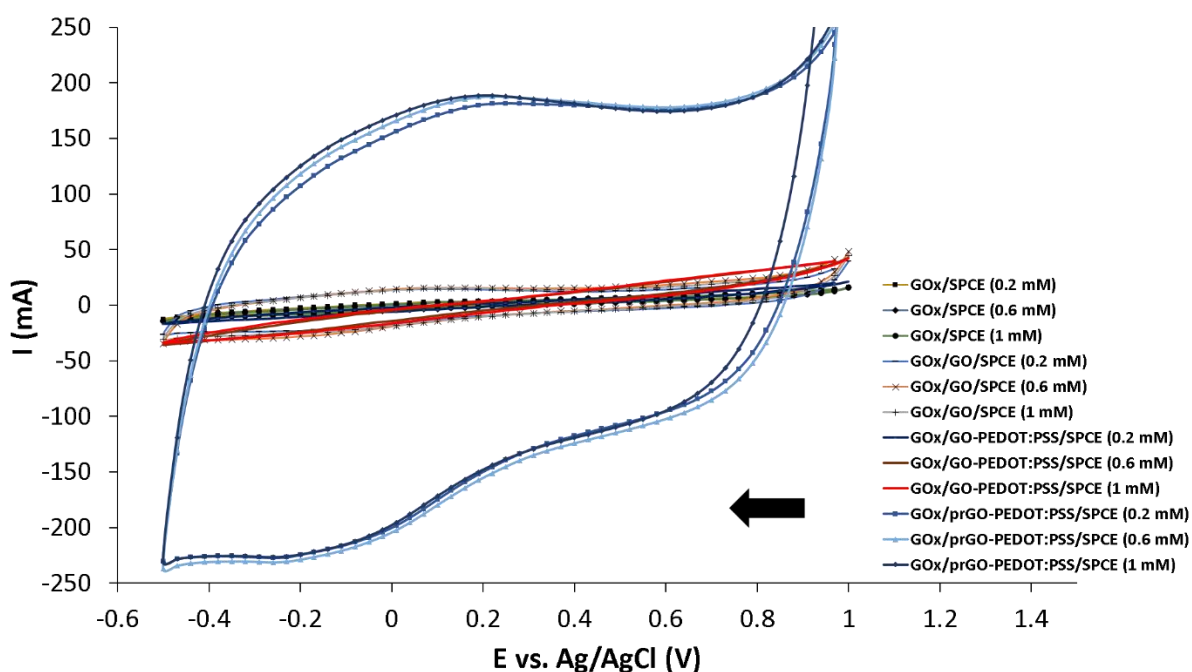


Fig. 6 - Cyclic voltammograms of GOx/SPCEs, GOx/GO/SPCEs, GOx/GO-PEDOT:PSS/SPCEs, and GOx/prGO-PEDOT:PSS/SPCEs in 0.01 M PBS, pH 7.4, with glucose concentrations of 0.2, 0.6 and 1.0 mM at a scan rate of 100 mV/s. Arrow corresponds to the direction of forward scan as reduction, and reverse scan as oxidation.



**Table 1 - Peak current of GOx/SPCEs, GOx/GO/SPCEs, and GOx/prGO-PEDOT:PSS/SPCEs at glucose concentrations of 0.2, 0.6 and 1.0 mM at a scan rate of 100 mV/s.**

Glucose concentration (mM)	GOx/SPCE			GOx/GO/SPCE			GOx/prGO-PEDOT:PSS/SPCE					
	E <sub>pa</sub> (V)	I <sub>pa</sub> (μA)	Std. error	E <sub>pa</sub> (V)	I <sub>pa</sub> (μA)	Std. error	E <sub>pc</sub> (V)	I <sub>pc</sub> (μA)	Std. error	E <sub>pa</sub> (V)	I <sub>pa</sub> (μA)	Std. error
0.2	0.08	0.000693	0.000415	0.09	8.58	0.558	-0.26	-227.38	3.686	0.07	181.48	0.446
0.6	0.11	0.000519	0.000113	0.14	7.56	1.395	-0.26	-231.32	4.091	0.13	187.62	0.358
1.0	0.10	0.000154	0.000135	0.10	4.93	0.373	-0.26	-226.56	2.229	0.10	187.72	0.406

#### 4. Conclusion

CV measurements in a redox solution of potassium ferricyanide ( $K_3[Fe(CN)_6]$ ) showed prGO-PEDOT:PSS/SPCEs to have the largest effective surface area of  $219.3 \mu m^2$  compared to SPCEs and GO-PEDOT:PSS/SPCEs. In EIS, prGO-PEDOT:PSS/SPCEs also have the lowest charge transfer resistance ( $R_{ct}$ ) of  $163.82 \Omega$ .

However, the non-directed immobilization of enzymes on prGO-PEDOT:PSS/SPCEs could compromise enzymatic conformation, thus reducing GOx catalytic activity. This can be seen when GOx/SPCEs, GOx/GO-PEDOT:PSS/SPCEs, and GOx/prGO-PEDOT:PSS/SPCEs were tested in glucose concentration of 0.2, 0.6 and 1 mM; the least obvious change in peak current ( $I_p$ ) was seen for GOx/prGO-PEDOT:PSS/SPCEs, and the most obvious change in  $I_p$  for GOx/GO/SPCEs. Although prGO-PEDOT:PSS/SPCEs have a larger surface area involved in the redox reaction in  $K_3[Fe(CN)_6]$ , and that increase of  $R_{ct}$  suggests immobilization of enzymes, GOx/prGO-PEDOT:PSS/SPCEs could not differentiate between the low, intermediate, and high glucose concentrations, where there is no obvious change in  $I_p$ . The results could be due to the GOx structure when immobilized on electrode surfaces, which could impede enzyme catalytic activity.

Future studies will focus on increasing the changes in  $I_p$  for all electrode types at different glucose concentrations through optimizing enzyme immobilization on different materials and statistical tools such as machine learning to better understand the effect of materials on  $I_p$ .

#### Acknowledgment

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