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Electrochemical Glucose Biosensors: Whole Cell Microbial and Enzymatic Determination Based on 10-(4H-Dithieno[3,2-b:2′,3′-d]Pyrrol-4-yl)Decan-1-Amine Interfaced Glassy Carbon Electrodes

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ABSTRACT

The fabrication of amperometric biosensors based on whole cell *Gluconobacter oxydans* DSMZ 2343 (*G. oxydans*) and glucose oxidase (GOx) was performed for the detection of glucose. Glassy carbon electrodes (GCE) were coated with a 10-(4*H*-dithiyeno [3,2-b:2′,3′-d]pyroll-4-il)decan-1-amine (DTP-alkyl-NH_2) polymer using an electropolymerization method and the formed interface was used to connect the bacteria and the enzyme to the electrode. The transfer of electrons from enzyme to electrode was successfully demonstrated by the biocatalytic activity and unique morphology of the conducting polymer. Characterization of the biosensors was assessed using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM) analyses. The detection limits of the enzyme and microbial based biosensors for glucose were 0.022 and 0.081 mM, respectively. The broad linear dynamic ranges of the GOx and *G. oxydans* biosensors were observed to be 0.045–50.0 and 0.19–50.0 mM, respectively. The analytical performances of biosensors were compared according to the following figures of merit: detection limits, limits of quantification, pH and current response time. In addition, to demonstrate the applicability of the biosensors, real-time measurements and recovery studies were evaluated.

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Glucose biosensor; glucose oxidase; *G.* oxydans; 10-(4H-dithiyeno[3,2-b:2′; 3′-d]pyroll-4-il)decan-1-amine (DTP) conducting polymer; whole cell microbial biosensor

Introduction

Today, many studies are being carried out to increase the applicability of biosensors, especially in health, food and environmental applications (Yoo and Lee 2010; Turner 2013; Cevik et al. 2016). This technology is rapidly increasing in daily use due to the low cost, fast results, and ease of use. According to recent reports, research on biosensors continues

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to develop devices with more functionality, portability (miniaturized) and long service life (Pancrazio et al. 1998; Srinivasan and Tung 2015; Zhang and Liu 2016). These studies are mostly focused on daily follow-up of diseases, freshness of food and onsite diagnosis of environmental problems (Senel et al. 2013; Dervisevic et al. 2016).

Biosensors used for this purpose are categorized according to biomaterials: enzymatic, immunosensors, genosensors and bacterial and also by the measurement method as electrochemical, optical, piezoelectric and calorimetric. Among other biosensors, enzyme-based electrochemical biosensors have been extensively researched (Nien, Tung, and Ho 2006; Wang 2006; Jiang et al. 2011; He et al. 2012; Senel et al. 2011, 2013) and are the most commercially available biosensors in the market for biomedical devices (Malhotra and Chaubey 2003; Newman and Turner 2005).

Glucose is one of the most important components of carbohydrates found in animals and plants. Determination of the amount of glucose has an important role in health, biochemistry, and food analysis. Qualitative analysis of glucose, especially for human health, is an important point to make a precise analysis of diseases such as endocrine metabolite disorders and diabetes (Liu et al. 2000). In addition, the determination of glucose is important in many applications, such as the fermentation studies and quality analysis for the food industry.

Reports show that for the application of enzyme-based electrochemical biosensors, a group of oxidoreductase and dehydrogenase enzymes are mostly used (Wilson and Turner 1992; Heller and Feldman 2008; Deng et al. 2010; Bollella et al. 2017). Much of this research reveals that health practices such as blood sugar level determination and diabetes control are the main research areas in enzyme-based glucose biosensor research (Pohanka and Skládal 2008; Ragupathy, Iyengar Gopalan, and Lee 2009). In addition, fast and reliable glucose determinations from foods are another important area of enzymatic biosensors. Different studies have been reported for the qualitative detection from food samples (Razumien et al. 2001; Anik, Cubukcu, and Yavuz 2013).

Today, many studies have been reported on electrochemical glucose biosensors, and remarkable review articles on this subject have been published (Heller and Feldman 2008; Wang 2008; Dervisevic et al. 2017). The principles, history, and recent developments of electrochemical glucose biosensors were reviewed by Wang (Wang 2008). Another important review about electrochemical glucose biosensors and current applications was reported by Heller and Feldman (2008). These studies show that much research has been done on electrochemical glucose biosensors and now is an important field of study when these investigations become products in the market.

Bacteria-based biosensors have been developed by adhering microorganisms on the biosensor electrode as biological sensing material (Su et al. 2011). In bacterial-based electrochemical biosensors, reports say that bacteria are less selective and less sensitive than enzymes, and response times are relatively long (D'Souza 1989; Deppenmeier, Hoffmeister, and Prust 2002). However, bacterial biosensors offer important advantages over isolated enzymes that include low cost (no cost for isolation process), less time-consuming due to reduced processing, resistance to pH and temperature changes, and higher tolerance to toxic substances (D'Souza 1989).

G. oxydans are frequently used bacteria for the determination of substances such as disaccharides, aldoses, ketoses and mono- and poly-alcohols in electrochemical

biosensors (Katrlík et al. 2007). *G. oxydans*-based electrochemical biosensors were also used in the analysis of fluid systems for beverages (Yılmaz et al. 2012). It has also been reported that *G. oxydans* were utilized to monitor 1,3-propanediol (Katrlík et al. 2007), 2-phenylethanol (Schenkmayerová et al. 2015), and ethanol (Indzhgiya et al. 2012) for biotechnological process.

Conductive polymers have been extensively reported in biosensor studies, especially in electrochemical applications due to their easy synthesis and functionalization and the presence of a biocompatible surface for biomaterials and high performances in electron transfer (Vidal, Garcia, and Castillo 2002; Khan et al. 2008; Senel, Cevik, and Abasıyanık 2010; Senel 2013; Soylemez et al. 2015). Among the conducting polymers, the π-conjugated systems attract the attention of researchers with their high conductivity, as well as their optical and electronic properties (Abdelwahab, Mi-Sook, and Yoon-Bo 2010). Besides, flexible properties and ability to form bonds with inorganic materials increases their potential to use in different applications (Wang et al. 2012).

Among the π-conjugated systems, dithienopyrrole (DtP) structures composed of two rings of thiophene attached to the pyrrole ring are a good example of these polymers (Parameswaran et al. 2009; Rasmussen and Evenson 2013) and have been reported in many bioelectronics and biosensor applications (Dervisevic et al. 2016; Azak, Yildiz, and Carbas 2018; Cevik et al. 2018). In a urea biosensor study, graphite electrode was modified with the DTP polymer functionalized with ferrocene and a successful application was performed for the enzyme immobilization (Dervisevic et al. 2016). A DTP polymer was also used as a cathode in a bioelectrochemical fuel cell by coating on a gold electrode (Cevik et al. 2018). In addition, a highly sensitive cholesterol biosensor was produced using the DTP polymer coated on a carbon electrode. The resulting electrode was used for the enzyme immobilization and electron transfer for cholesterol detection. (Cevik et al. 2018).

Herein, a new biosensor system based on *G. oxydans* immobilized on the DTP polymer was constructed for the first time and utilized to detect glucose. Another biosensor was constructed with GOx. The comparison of biomaterials for the usage as a biological recognition agent in the biosensing applications were also tested in this study. Biosensors were designed based on p(DTP-alkyl-NH_2) polymeric mediator served as a host matrix on the glassy carbon electrode. Analytical characteristics of the biosensors were also performed by determining glucose in food samples.

Experimental

Materials

The 10-(4*H*-dithiyeno[3,2-b:2′,3′-*d*]pyroll-4-il)decan-1-amine (DTP-alkyl-NH_2) monomers was supplied and also reported in previous work (Udum et al. 2014). D-glucose, glucose oxidase (E.C. 1.1.3.4), dichloromethane, toluene, tetrabutylammonium hexafluorophosphate ($TBAPF_6$), succinyl chloride toluene, $NaBH_4AlCl_3$, 2,2-dithiophene, and sodium citrate were purchased from Sigma Aldrich (Germany). All other chemicals used in the measurements (not mentioned here) were analytical grade and used without any extra purification. A Millipore Milli-Q system was used as the deionized water source for the experiments.

Apparatus

The electrochemical measurements in this work were performed with a Palmsens electrochemical device (The Netherlands). A standard three-electrode measurement system was used for room temperature measurements. A glassy carbon electrode (GCE) with a diameter of 3 mm (Bioanalytical Systems) was used as the working electrode in cyclic voltammetric and amperometric measurements. A reaction cell with a 10 mL container was used for the electrochemical measurements. A Ag/AgCl electrode (3 M potassium chloride, Metrohm, Switzerland) was used as the reference. A platinum electrode (Metrohm, Switzerland) was used as the counter electrode.

Bacterial culturing

The bacterial culture used in this study (*G. oxydans* DSMZ 2343) was supplied from the German collection of microorganisms and cell cultures (Germany). The bacterial culture medium was prepared by mixing of glucose (100 g/L), yeast extract (10 g/L), calcium carbonate 20 g/L) and agar (20 g/L) in a glass bottle. The bacteria were added into a bottle containing yeast extract (5 g/L), glucose (5 g/L), and placed on a shaker at 28 °C (Tuncagil et al. 2009a, 2009b).

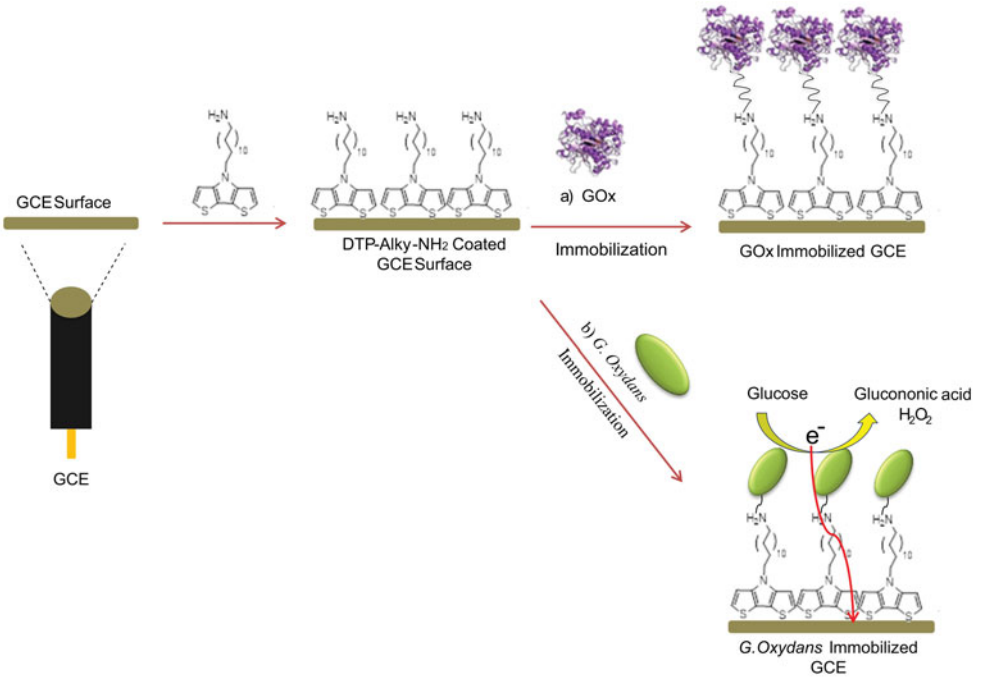
The resulting cells were obtained by centrifugation at 6000 rpm for 15 min. A repeated centrifugation process was applied to the cells after washing with 0.9% NaCl. During bacterial culture study, growth of cells was monitored spectrophotometrically at 600 nm by measuring the optical density (Tuncagil et al. 2009a, 2009b).

Preparation of GCE/p(DTP-alkyl-NH_2)/GOx and GCEp(/DTP-alkyl-NH_2)/*G. oxydans* biosensors

Biosensing electrode preparation was performed by using two biological recognition elements; GOx and *G. oxydans*. Prior to any modifications, GCE was cleaned and rinsed with deionized water. In the electrochemical studies, the three-electrode cell measurement system used and a GCE working electrode, platinum wire counter electrode and Ag/AgCl reference electrode were used. The electropolymerization of DTP-alkyl-NH_2 was performed by cyclic voltammetry method in the potential range between 1.0 to –1.0 V at a scan rate of 100 mV/s. The polymer (DTP-alkyl-NH_2) coating on the electrode surface was applied using 20 voltammetric cycles in the presence of (0.1 M) TBAPF6/dichloromethane/acetonitrile (ratio 1:1:1).

The GOx biosensor electrode structure used for glucose determination was obtained by applying the steps shown in Scheme 1(a). 5.0 μL of GOx solution (1.5 mg/mL) prepared in 10 mM pH 5.5 phosphate buffer were spread on polymer-coated GCE and 5.0 μL glutaraldehyde solution (1.0%) prepared in 10 mM pH 5.5 PBS were added to the surface. The electrodes were subsequently incubated at +4 °C for 90 min and rinsed with deionized water.

The whole cells of *G. oxydans* immobilization studies were evaluated using 5.0 µL of *G. oxydans* suspension (a cell titer of 2.72×10^9) prepared in 10 mM phosphate buffer at pH 5.5. Bacterial cells were crosslinked to the p(DTP-alkyl-NH_2)-coated GCE surface



Scheme 1. Schematic of the biosensing electrodes: (a) GOx and (b) *G. oxydans* DSMZ 2343.

using 5 µL of 1.0% glutaraldehyde solution (Scheme 1(b)). After modification with *G. oxydans,* the GCE was allowed to incubate in a humid environment for 1 h and the electrodes were rinsed with deionized water. The fabrications of the microbial biosensors were performed using fresh bacterial cells and all biosensing electrodes were daily prepared unless otherwise stated.

Electrochemical measurements

Electrochemical measurements were started by characterizing the unmodified GCE by CV with constant stirring at room temperature. Polymer modification and subsequent enzyme immobilization were characterized by CV and EIS. After each step, the electrode was washed with deionized water. In the amperometry studies, after each measurement the electrode was left in 10 mM pH 5.5 phosphate buffer until a steady state was reached. This process was carried out in 10 mM pH 5.5 phosphate buffer for bacterial electrodes.

The determination of the applied potential were carried out across a potential range of 0.1 to −1 V. The optimum current response of biosensor was recorded in microamperes (μA) by following the oxygen consumption at −0.7 V due to the biological activity of the immobilized material (GOx and *G. oxydans*) (Figure 1(c)). For electrochemical measurements, glucose standard solutions and samples were used as the substrates. The coefficient of variance along with standard deviations of the biosensors were obtained by averaging the values from three independent measurements.

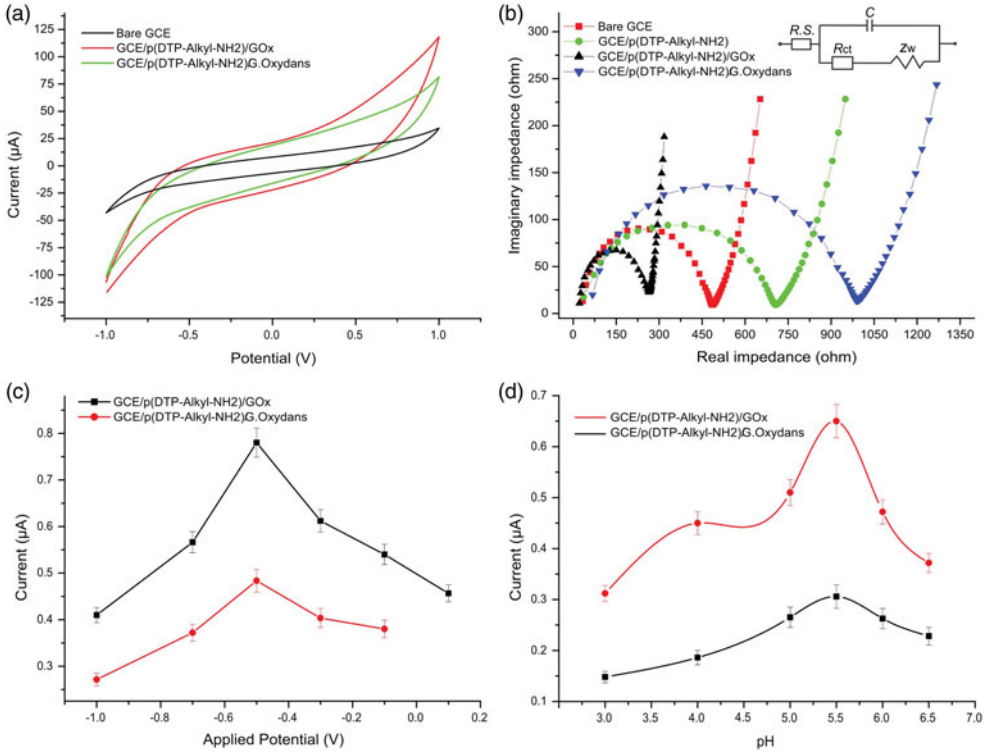


Figure 1. (a) Cyclic voltammetry of the unmodified GCE, GCE/p(DTP-alkyl-NH_2)/GOx and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors. (b) EIS of the unmodified GCE and stepwise attachment of components p(DTP-alkyl-NH_2), and p(DTP-alkyl-NH_2)/GOx. The inset shows the circuit diagram of the system. (c) Optimization of applied potential to the GCE/p(DTP-alkyl-NH_2)/GOx and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors in 10 mM phosphate buffer at pH 5.5. (d) Optimum pH of the GCE/p(DTP-alkyl-NH_2)/GOx and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors.

Results and discussion

Optimization studies of GCE/p(DTP-alkyl-NH_2)/GOx and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors

Cyclic voltammetry measurements were performed after construction of the GCE/p(DTP-alkyl-NH_2)/GOx and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors in a 10 mL phosphate buffer (10 mM at pH 5.5) at a potential range between −1 and 1 mV. As it can be seen in Figure 1(a), different CVs were obtained from GOx and *G. oxydans*. The anodic (Epa) and cathodic (Epc) peak potentials of the unmodified GCE were recorded at a scan rate of 100 mV/s and plotted with a black line in the voltammogram. An increase in Epc and Epa (shown in the red line) was observed in the voltammogram obtained after the polymer was coated and the GOx was immobilized (GCE/p(DTP-Alkyl-NH2)/GOx). The CV of a GCE/p(DTP-alkyl-NH_2)/*G. oxydans* electrode (shown in green line) exhibited a decrease in the peak potentials.

Bacterial immobilization applied to the electrode surface causes a new layer to be formed, resulting in reduced peak potential. However, this limited reduction did not

block the electron transfer between the electrode and biomaterial, indicating the formation of sufficient oxidation and reduction peaks. This result means that the biological materials are in good agreement with the crosslinking structure in the electrode architecture. The results showed that bacterial immobilization blocked some of the electrical contacts with the electrode and reduced peak potentials. Despite this decrease in peak potentials, the electrode systems showed sufficient oxidation and reduction peaks for both systems. This means that the immobilized biological materials are in good coordination with the electrode structure and allow the electrons to be transferred.

Figure 1(b) shows the EIS plots obtained from unmodified GCE, polymer-coated GCE (GCE/p(DTP-alkyl-NH2)) and biomaterial (GOx and *G. oxydans*) immobilized electrodes. The radius of the semi-circular graphs obtained in the EIS spectrum shows the charge transfer resistance (R_{ct}) of the electrodes. The charge transfer resistance for each electrode was calculated by fitting the EIS graph with an appropriate equivalent circuit shown in the inset in Figure 1(b). The components (*R.S.*, R_{ct}, *C*, and *Z*w) in the equivalent circuit show the electrolyte resistance, the capacitance of the biocomponent material, the charge transfer resistance of the electrode surface, and the Warburg impedance of the system. The lowest R_{ct} value was obtained from unmodified GCE at 265 Ohms. It was observed that the R_{ct} value after modification with electrode surface p(DTP-alkyl-NH_2) was 470 Ohms. This increase in R_{ct} value indicates that the polymer is well attached on the electrode surface. After the GOx and *G. oxydans* were immobilized on the electrode surface, the R_{ct} values were determined to be 730 and 1035 Ohms, respectively, which clearly indicates that both materials are immobilized on the electrode surface.

The amperometric responses of the GCE/p(DTP-alkyl-NH_2)/GOx biosensor was obtained in 10 mM phosphate buffer with pH values between 3.0 and 6.5 at an applied potential of –0.7 V at room temperature. The effect of pH to the electrode response in terms of current was evaluated by the introduction of 5 mM glucose into the reaction medium. Figure 1(d) shows the increase in the current response with increasing pH values and the maximum current response (μA) was obtained from pH 5.5. The response of the biosensor response was observed to decrease at high pH values from 5.5 and this value was chosen as the optimum for the GOx biosensor.

The optimum pH studies of the GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensor were performed using the same conditions applied for the GOx biosensor. The maximum current response was observed from the bacterial biosensor to the introduction of 5 mM glucose solutions. Similar results were obtained from the bacterial and GOx biosensing electrodes, indicating pH 5.5 is compatible for the both systems. In lower and higher pH values, the reduced but comparatively broad pH range observed in the bacterial biosensor in terms of current responses indicating bacteria has more resistance to the pH changes than GOx.

Surface characterizations of fabricated biosensor were investigated by scanning electron microscopy (SEM). Figure 2(a) shows the SEM image of the unmodified GCE. The conductive p(DTP-alkyl-NH_2) polymer was coated on the GCE surface using electropolymerization and the image is presented in Figure 2(b). The SEM images of the GOx and *G. oxydans* immobilized GCE/p(DTP-alkyl-NH_2) electrodes are shown in Figure 2(c,d), respectively. The images clearly show from that enzyme and bacteria are well incorporated with the polymer and attached to the electrode surface.

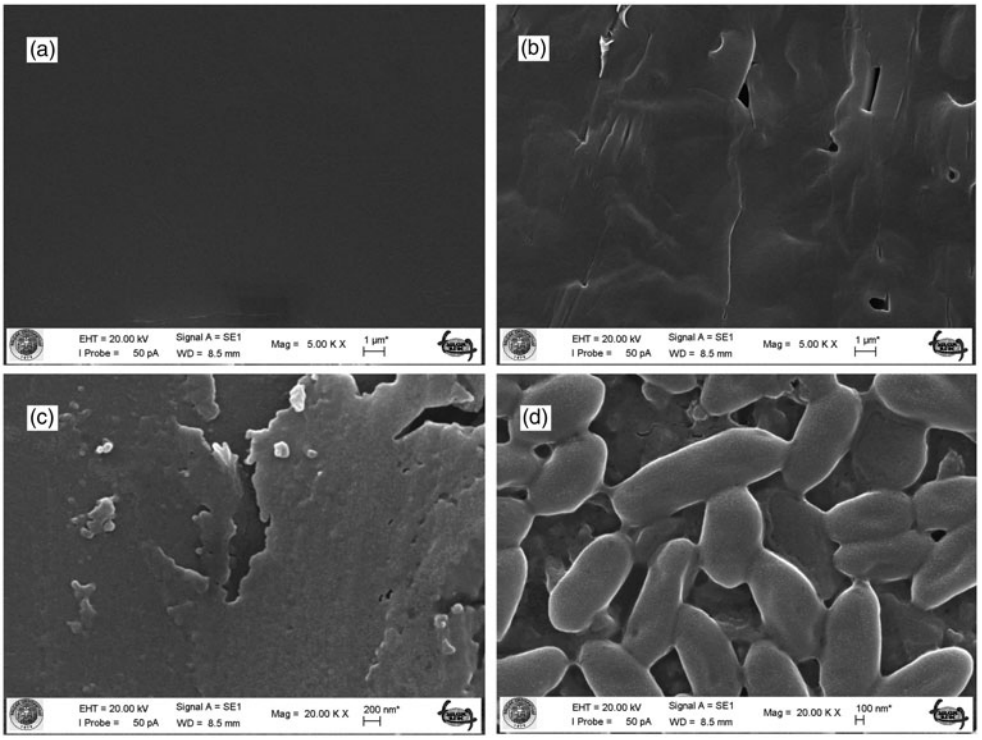


Figure 2. SEM images of the (a) unmodified GCE, (b) p(DTP-alkyl-NH_2) coated GCE, (c) GCE/p(DTP-alkyl-NH_2)/GOx, and (d) GCE/p(DTP-alkyl-NH_2)/*G. oxydans*.

Effect of scan number on the biosensor response

The thickness of the polymer interface in terms of the number of scans between the enzyme and the electrode surface is an important parameter for biosensor performance by affecting the electron transfer capacity. The electron transfer property directly affects the biosensor performance by changing the sensitivity. The effect of polymer film thickness on biosensing electrodes decorated with GOx and *G. oxydans* was evaluated by amperometric measurements.

Figure 3(a) shows the relationship between the GCE/p(DTP-alkyl-NH_2)/GOx and Figure 3(b) shows the GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors responses with increasing numbers of scans. The figures show that when the number of cycles is increased to 20, the electron transfer rate is observed to increase for both biosensors. Further increases in the number of cycles caused a dramatic decrease in the current response and showed that a thick polymer layer was formed on the surface of the electrode, which adversely affecting electron transfer. When the polymer deposition was less than 20 cycles, the resulting composition has fewer functional groups on the electrode surface. The decrease in the functional group led to a reduction in the number of immobilized enzymes, which resulted in a low biosensor response. Similar properties were observed for both electrodes. Therefore, 20 scans were found to be optimum for both glucose biosensing electrodes.

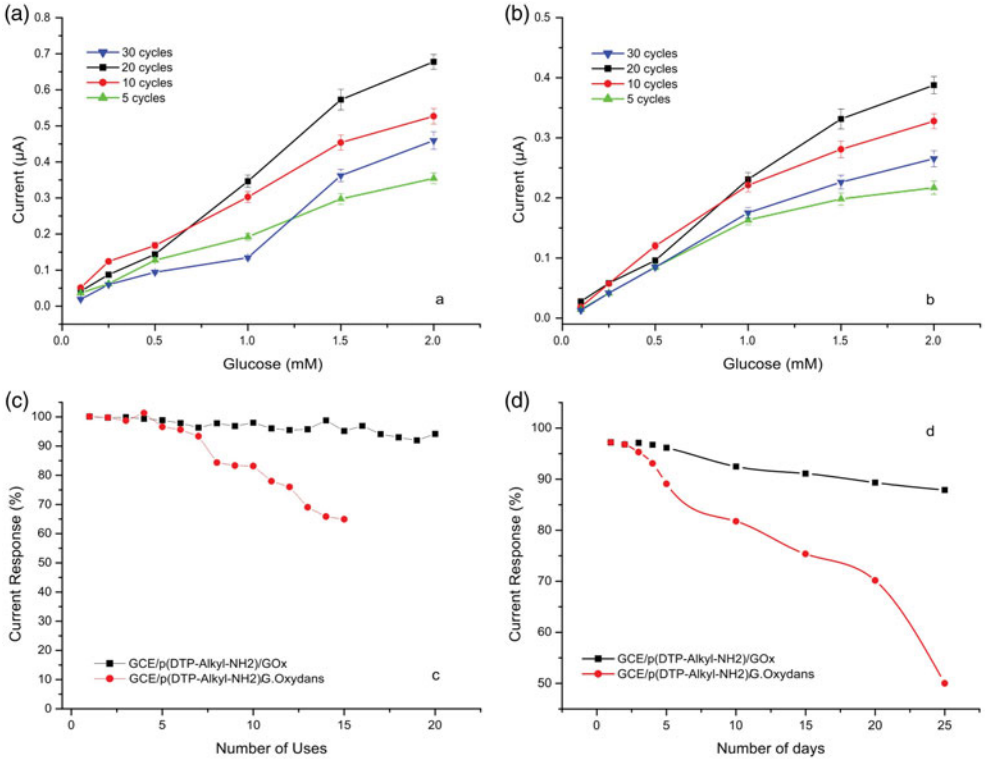


Figure 3. Influence of the number of scans on the biosensor response of the (a) GCE/p(DTP-alkyl-NH_2)/GOx and (b) GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors. (c) Reusability of the biosensing electrodes upon the addition of glucose at an applied potential of –0.7 V. (d) Amperometric response of biosensing electrodes obtained for 10 days in 10 mM phosphate buffer at pH 5.5 at an applied potential of –0.7 V.

Analytical characterization of the GCE/p(DTP-alkyl-NH_2)/*G. oxydans* and GCE/p(DTP-alkyl-NH_2)/GOx biosensors

The analytical characteristics of biosensors linear conditions and linear equations were investigated under the optimum conditions. Calibration plots were obtained as a function of the current response versus concentration.

The analytical performance of the amperometric GOx and G. *oxydans* biosensors was characterized under the optimized conditions using the same glucose solutions in the range of 0.05–10.0 mM. Glucose injections were applied to the reaction medium for both biosensors and amperometric current responses were obtained. Different behaviors were observed in the calibration curves of the two systems. The GCE/p(DTP-alkyl-NH_2)/GOx system showed a linear range between 0.05 and 8.0 mM and a regression equation of $y(\mu A) = 0.4339$ [glucose mM/mL] $+ 0.1572$, with a regression coefficient of 0.992 (Figure 4(a)). The biosensor reached its maximum steady-state current response in approximately 3 s. The detection limit of the GCE/p(DTP-alkyl-NH_2)/GOx biosensor was calculated at a signal-to-noise ratio of three to be 0.035 mM/mL. The limit of quantification was determined to be 0.095 mM.

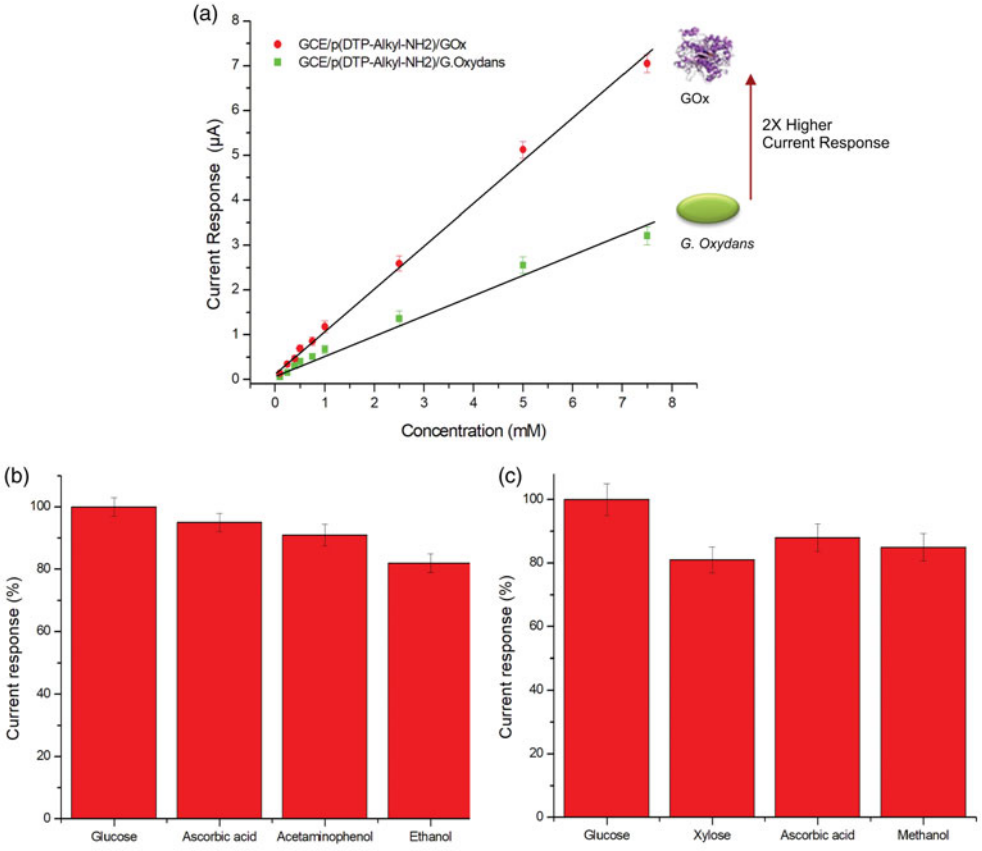


Figure 4. (a) Calibration curves of the GCE/p(DTP-alkyl-NH_2)/GOx (●) and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* (■) biosensors. Influence of interferences on the electrode response on the (b) GCE/p(DTP-alkyl-NH_2)/*G. oxydans* and (c) GCE/p(DTP-alkyl-NH_2)/GOx biosensors.

The GCE/p(DTP-alkyl-NH_2)/*G. oxydans* microbial sensor exhibited a linear range between 0.10 and 8.0 mM defined by the equation: $y(\mu A) = 0.9441[\text{Glucose mM/mL}] + 0.1604$, with a regression coefficient of 0.982 (Figure 4(a)). The biosensor reached its maximum steady-state current response in approximately 11 s. The limit of detection of the designed GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensor was 0.10 mM and the limit of quantification was 0.27 mM.

To evaluate the operational stability, repeated measurements were performed. The responses obtained from the glucose biosensors were recorded and the results were determined as the percentage. The stability of biosensors was characterized with 0.5 mM glucose ($n = 25$) applying the regular amperometric assays. It was observed that there was an activity loss of about 5–8% in the GOx biosensor response after 25 measurements (Figure 3(c)).

Operational test studies of the microbial biosensor were obtained by performing 15 amperometric measurements of the same electrode. The biosensor retained 75% of its initial activity in first 15 measurements (Figure 3(c)).

The long-term stability of the of GCE/p(DTP-alkyl-NH_2)/GOx and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors were studied by taking measurements for 25 days.

Table 1. Comparison of the analytical performance of the glucose biosensors.

Electrode	Response time (s)	Detection limit (mM)	Linear range (mM)	References
GCE/p(DTP-alkyl-NH2)/*G. oxydans*	11	0.081	0.19–50	This study
GCE/p(DTP-alkyl-NH2)/Gox	3	0.022	0.045–50	This study
GCE/carbon nanotube/poly(ethylenimine)/Gox	–	0.05	0–0.3	Deng (2010)
Indium tin oxide/chitosan-ionic liquid/multiwall carbon nanotubes/Au/Gox	6	–	1–10	Ragupathy (2009)
Au/Pt/ordered mesoporous carbon nanocomposite/Gox	7	0.05	0.05–3.70	Jiang (2011)
GCE/poly(3,4-ethylenedioxythiophene)/Gox	4–10	0.13	0.1–10	Nien (2006)
GCE/polymethylmetacrylate-bovine serum albumin/Gox	8	–	0.2–9.1	He (2012)
GCE/chitosan-ferrocene/*G. oxydans*	70	0.797	2.0–25.0	Katrlík (2007)
GCE/poly(anilineboronic acid-*co*-aniline)/Gox	12	0.24	1–17.0	Şenel (2011)
Au/mercaptopropionic acid/ferrocene-dendrimer/Gox	7	0.23	1–5	Malhotra and Chaubey (2003)

The same biosensor electrodes were used for all measurements and stored at +4 °C when not in use. Regular amperometric experiments were performed with injections of 0.50 mM glucose and the results are shown in Figure 3(d). The GOx biosensor retained almost 90% of its initial activity after 25 days. However, *G. oxydans* biosensor lost 25% of initial activity in the first 15 days and lost almost 50% of the total activity after 25 days (Figure 3(d)).

Recovery studies were performed by measurements with five similarly arranged electrodes for each type. Relative standard deviations for GOx and *G. oxydans* biosensors were determined to be 1.034% and 1.254%, respectively. The results were evaluated by measuring 0.5 mM glucose with five biosensors constructed from the same electrodes.

Analytical comparison of the GCE/p(DTP-alkyl-NH_2)/*G. oxydans* and GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensors with similar literature devices is provided in Table 1. The GCE/p(DTP-alkyl-NH_2)/GOx electrode has a fast response time, wide linear range and comparable detection limit with respect to the other glucose biosensors. The GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensor was also shown to provide comparable results to enzyme-based biosensors. The performance of the biosensors included fast response, high sensitivity and large linear range, demonstrating the compatibility of the biological material with the biosensor platform and the successful transfer of electrons.

Substrate specificity for GCE/p(DTP-alkyl-NH_2)/*G. oxydans* and GCE/p(DTP-alkyl-NH_2)/GOx biosensors

Possible interfering substances were used to assess the glucose sensitivity of the biosensors. For the GCE/p(DTP-alkyl-NH_2)/GOx biosensor, the effects of ascorbic acid, ethanol, and acetaminophenol solutions (1 mM) containing a glucose standard solution (1 mM) were investigated in 10 mM phosphate buffer at pH 7.0. The results obtained from the biosensor electrodes were recorded as current responses and converted to percentages. Figure 4(b) shows the maximum percentage of current response obtained

Table 2. Real sample application of the GOx and *G. oxydans* biosensors.

Beverage	Spectrophotometric Method [Glucose](g 100/mL)	GOx Biosensor [Glucose](g 100/mL)	*G. oxydans* Biosensor [Glucose](g 100/mL)
Fruit juice	4.9 ± 0.8	5.1 ± 0.7	4.5 ± 1.5
Sprite	4.2 ± 0.9	4.4 ± 0.4	4.7 ± 2.1
Control sample	4.9 ± 0.10	5.1 ± 0.6	4.7 ± 0.9

from the glucose standard solution. Current reductions of 5%, 9% and 18% were observed with the addition of ascorbic acid, ethanol and acetaminophenol, respectively.

The substrate specificity of the developed GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensor was evaluated for glucose, xylose and methanol in 10 mM phosphate buffer at pH 5.5 (Figure 4(c)). The amperometric response obtained in the presence of only glucose were assigned to be 100%, and the response to other substrates was calculated as the percent sensing. The loss of current response for methanol was 19%, xylose was 12%, and ascorbic acid was 15%. Higher substrate response was recorded for the bacteria-based biosensor because the *G. oxydans* cells possess high dehydrogenase activity and therefore have a broad substrate range.

In order to validate the applicability of the designed biosensor, real samples were analyzed. The glucose concentrations in commercially available fruit juice and a lemon-lime carbonated beverage and one control sample prepared in the laboratory containing 5 g glucose in 100 mL were tested using the biosensors. Before the measurements, sample pretreatments (dilutions) were applied to evaluate the linear ranges of the GOx and *G. oxydans* biosensors. All electrochemical and spectrophotometric measurements obtained in triplicate and average measurements are provided in the Table 2. The results obtained using the biosensors were in good agreement with a standard spectrophotometric method. The enzyme-based biosensor provided better performance than the bacterial biosensor.

Conclusion

Amperometric biosensor systems based on GOx and *G. oxydans* were developed using an amine functionalized conductive polymer interface. Biological materials were immobilized with a glutaraldehyde crosslinker. Biosensor optimization of the pH, operational stability, polymer thickness and interference studies were performed for both biosensors and the analytical characteristics were found to be favorable based on the obtained results. The results also showed that the microbial and enzymatic biosensors with the conductive polymer structure were biocompatible due to the amine functional group.

Since the linear range is wide for both biosensors (0.045–50 mM) and the limit of detection is very low (for GCE/p(DTP-alkyl-NH_2)/GOx biosensor, limit of detection of 0.022 mM, for GCE/p(DTP-alkyl-NH_2)/*G. oxydans* biosensor, limit of detection of 0.081 mM), these biosensors have practical applications for glucose biosensing. The microbial sensor can survive hard sensing conditions since it is not strongly affected by interferences compared with glucose oxidase-based biosensor. However, the GOx based biosensor showed better sensing performance. These biosensors have application for practical glucose sensing.

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