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Research article

Benzodithiophene bearing conjugated polymer-based surface anchoring for sensitive electrochemical glucose detection

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Abstract. An amino-functionalized, conjugated polymer (P(BDBT)) modified glassy carbon electrode (GCE) was employed as an immobilization platform for glucose oxidase (GOx) enzyme to assemble a novel glucose biosensor. Amino groups available on the polymer backbone served as bioconjugation sites for GOx via glutaraldehyde (GA). The biosensor response to the reduction in oxygen amount because of the enzyme reaction was monitored at –0.7 V potential *versus* Ag/AgCl. The biosensor displayed a broad linear range between 0.1–1.0 mM glucose with a detection limit of 0.17 mM. The values of the apparent Michaelis-Menten constant (K_M^{app}) and sensitivity were determined as 1.74 mM, and 28.17 μA/(mM·cm$^{2)}$, respectively. GOx immobilized P(BDBT) film displayed high stability, selectivity, and reproducibility. Cyclic voltammetry (CV) and Scanning electron microscopy (SEM) techniques were utilized for the characterization of surface modifications. The fabricated biosensor was adept at determining the amount of glucose in a commercial beverage. The simple electrochemical method for the construction of P(BDBT)/GOx biosensors could pave the way to new perspectives in developing profitable biosensors.

Keywords: *molecular engineering, amperometric glucose biosensor, glucose oxidase, enzyme immobilization, conjugated polymers*

1. Introduction

Diabetes mellitus is a significant challenge to health globally. The latest International Diabetes Federation Diabetes Atlas Report presented that 537 million adults (20–79 years) have diabetes, which is estimated to rise to 643 million by 2030, and to 783 million by 2045 [1]. Only in 2021, about 6.7 million people died due to diabetes. Due to the continuously increasing prevalence of diabetes, it is crucial to check blood glucose levels frequently for its early diagnosis and prognosis. In addition, quantitative analysis of glucose is crucial, especially in some food products, for quality control. Several methods have been presented for glucose determination in food and blood samples. Among them, biosensors possess fundamental features for reliable analysis of the target molecules, such as high specificity, low cost, rapidity, sensitivity, and portability. They generate signals proportional to the analyte concentration with physicochemical transducers. Additionally, they facilitate the

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assessment of health conditions, onset, and advancement of the disease, so biosensors are widely utilized by patients for home healthcare. Especially being portable and easy to use, electrochemical biosensors are advantageous for the daily monitoring of glucose levels. The market size of biosensors is at 25.5 billion USD in 2021, which is estimated to reach 41.8 billion USD by 2028 with a compound annual growth rate of 7.9% [2]. In 2020, the medical segment had the biggest share in terms of application, and the electrochemical segment had the biggest share in terms of technology. Enzyme-based electrochemical biosensors are composed of biological recognition elements to catalyze a specific biochemical reaction. GOx immobilized amperometric biosensors have been prominent in glucose analysis in body fluids like urine and blood and in industrial solutions [3, 4]. GOx is specific for β-D-glucose and displays minimal activity with other sugars [5]. Oxidation of glucose to D-glucono-1,5-lactone is catalyzed by GOx, a dimer containing two subunits, each possessing one flavin adenine dinucleotide (FAD) molecule as a redox-active center. The FAD group of the enzyme is reduced to GOx($FADH_2$), which is accompanied by the reoxidation of the GOx($FADH_2$) by oxygen to yield hydrogen peroxide, and GOx(FAD) is regenerated [6].

Electrochemical biosensors are one of the typical sensing devices for detecting different types of analytes because of being economical, sensitive, and highly reliable systems with compact sizes. In this type of sensors, a transducer modifier is very important for the immobilization of biomolecules and electron movement. Among numerous materials, conducting polymers (CPs) are useful enzyme immobilization materials for biosensor fabrication owing to their superior biocompatibility, ease of preparation, enhanced selectivity and sensitivity, and low cost [7–9]. Enzyme wiring directly to the electrode surface via a conjugated polymer improves the transmission of electrons to and from the electrode surface and the enzyme's redox center [6]. Biomolecules can easily be integrated into the polymer matrix. Studies employing conjugated polymers in electrochemical glucose biosensors have also been reported and summarized in Table 1. Benzodithiophene (BDT) is one of the important core structures for organic electronics while it possesses planar structure, ease of modification, and is capable of high hole mobilities when introduced to strong/highly crystalline acceptor moieties in the polymer backbone [10]. The incorporation of BDT units into CPs enhances the thermal stability and conductivity of the polymer [11]. Facile modification of BDT is advantageous for the construction of high-performance devices as its film-forming ability can be easily tuned [12]. Well investigated BDT-based polymers/small molecules have diverse applications ranging from photovoltaic applications [13–15] to electrochromics [16]. In a recently published work, heterostructure nanofilm of BDT-based polymer was utilized with conventional solar cells acceptor (6,6)-phenyl-C71-butyric acid methyl ester as a photoactive layer for fluorometric nitrite biosensor in aquaculture-relevant water samples [17]. Nevertheless, BDT-based polymers have limited research in enzyme-based biosensor applications, which are mainly introduced by our broad research team effort recently for catechol analysis in real samples. [18, 19]. In addition to these, poly(benzenediamine-bis[(2-ethylhexyl)oxy]benzodithiophene), P(BDBT) was synthesized and characterized in our previous work [20]. An electrochemical biosensor using antibodies as the biorecognition element was successfully developed for the selective and sensitive analysis of testosterone with this polymer. The analytical performance of the designed biosensor was excellent as it worked in a broad linear range, and a low detection limit was achieved for testosterone analysis. In addition, amine-functionalized P(BDBT) polymer is beneficial for the covalent attachment of biomolecules to the sensor platform and consequently improves the stability and sensitivity of the biosensor [21]. Moreover, the presence of multiple amino groups on each repeating unit of the P(BDBT) polymer may form covalent bonding with more than one amine group of each enzyme molecule at a time in the presence of GA as P(BDBT) is a suitable platform for multipoint covalent immobilization [22–24], which can lead to the rigidification of enzyme structure. Following these successful outcomes in binding a biorecognition element to an electrode surface, we applied the same platform for enzyme immobilization.

In this work, we described the fabrication of a biosensor where GOx is immobilized on P(BDBT) polymer coated on GCE via GA, for a highly efficient biosensor for glucose analysis. The preparation of the biosensor was illustrated in Figure 1. To the best of our knowledge, P(BDBT) is used as an immobilization platform for GOx for the first time. The biosensor optimization parameters: pH, crosslinker concentration, P(BDBT) and enzyme amounts were evaluated by

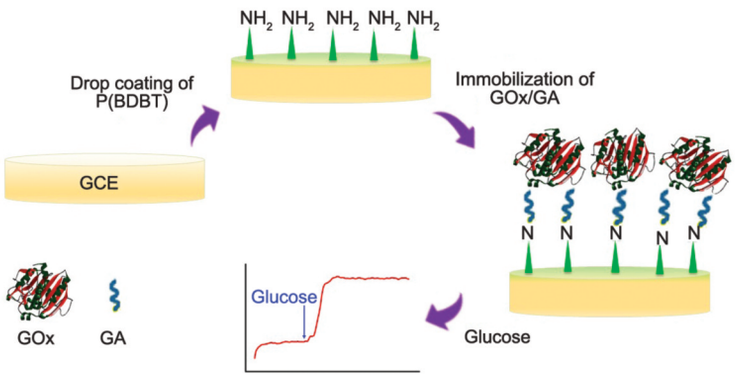


Figure 1. Illustration of the proposed biosensor.

monitoring the biosensor response as the change in current (ΔI). In addition, the biosensor was tested for possible interfering substances. Cyclic voltammetry (CV) and scanning electron microscopy (SEM) were employed to investigate electrode surface modifications. Detection of glucose content was performed with amperometry by screening the decline in oxygen amount in the medium due to the enzymatic reaction under constant potential. The changes in analytical features upon surface modifications and interactions with glucose were monitored. Finally, the confirmation of the biosensor was performed by determining glucose concentration in a commercial beverage.

2. Experimental

2.1. Chemicals and instrumentations

4,8-Bis((2-ethylhexyl)oxy)benzo[1,2-b:4,5-b0]dithiophene-2,6-diyl)-bis(trimethylstannane) was purchased from Lumtec (New Taipei City, Taiwan). 2,1,3-Benzothiadiazole was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydrofuran (THF) was used without further purification. Glucose oxidase (GOx, beta-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4, ≥100 000 units/g solid from *A. niger*), glutaraldehyde (GA, Grad II, 25% in H_2O), and tetrahydrofuran (THF) are obtained from Sigma-Aldrich (Darmstadt, Germany) and used without additional purification. D-glucose is purchased from BioFroxx (Einhausen, Germany). Sodium phosphate buffer solution (NaPBS) is prepared with 0.050 M Na_2HPO_4 (AFG Bioscience, Wood Dale, IL, USA), and sodium acetate buffer solution (NaABS) is prepared with 0.050 M CH_3COONa (Wisent Bioproducts, Quebec, Canada), and pH was adjusted with 1 M HCl in buffer solution preparations. 0.18 g of glucose is dissolved in 10 ml of relevant buffer solutions for the preparation of glucose solutions. The chemical materials used in this work are of analytical reagent grade.

Gamry 600+ potentiostat (Gamry Instruments, Warminster, PA) is used for all electrochemical measurements. Scanning electron microscopy (SEM) (Hitachi SU 1510, Japan) is utilized for surface characterization studies. GCEs (3 mm diameter) are utilized as working electrodes, platinum wire as the counter, and silver/silver chloride as reference electrodes. All electrodes were purchased from Gamry (Warminster, PA, USA).

The data are presented as an average of three measurements. The standard deviations are listed as ±SD. Ambient conditions (25 °C) are maintained for all measurements.

2.2. Synthesis of the polymer

P(BDBT), was synthesized via Stille polycondensation of a commercially available benzodithiophene derivative and 3,6-dibromobenzene-1,2-diamine using a palladium catalyst (Figure 2), as described previously [20].

2.3. Biosensor fabrication

Before starting any measurements, the working electrode surfaces were cleaned using THF and buffed using 15 µm diamond, 3 µm diamond, and 0.05 µm alumina slurry. A 10.0 µl of P(BDBT) solution (0.75 mg/ml) dissolved in THF was drop coated on the GCE and left to dry at room temperature. 5.0 µl

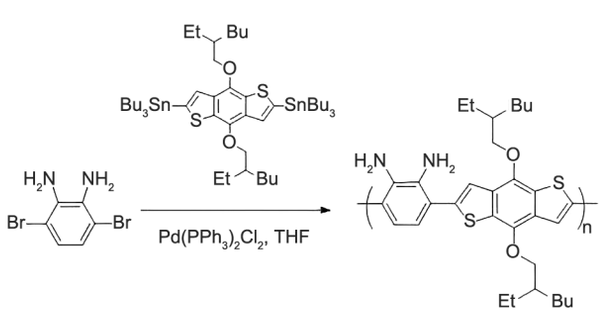


Figure 2. Synthetic pathway of P(BDBT).

GA and 5.0 µl GOx solutions were applied onto the polymer-modified electrode surface separately, and dried under ambient conditions. The electrode surface was left to dry for 1.5 hours and rinsed with distilled water thoroughly for the removal of possible impurities. Amperometry was used to record the biosensor measurements at –0.7 V *vs*. an Ag/AgCl electrode. Glucose solution was added in different concentrations into the three-electrode electrochemical cell.

2.4. Electrochemical measurements

A three-electrode cell was set up and used throughout this work. All chronoamperometric studies were carried out in 10 ml NaABS (50 mM, pH 5.5) under a constant potential (–0.7 V) *vs.* Ag/AgCl electrode at room temperature. CV studies were performed in the potential range of –0.4 to 0.6 V in a solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$, 0.1 M KCl, and 50.0 mM NaABS of pH 5.5 with a scan rate of 100 mV/s. All electrodes and the electrochemical cell were rinsed with distilled water and filled with fresh buffer solution in consecutive measurements. During each measurement, equilibrium was reached before and after the addition of glucose, and the signal changes are calculated as the difference between the current values at each equilibrium, which corresponds to the biosensor response. The change in current is monitored due to the decline in oxygen amount in the medium.

2.5. Sample application

P(BDBT)/GOx biosensor was employed for glucose detection in a commercially available beverage, Diet Coke®. The glucose content was determined by amperometry after spiking the cell containing 10 ml of 50 mM NaABS, pH 5.5 with 50 µl diet soda at first and then with various concentrations of glucose. The amperometric measurements were taken at –0.7 V *versus* Ag/AgCl electrode.

3. Results and discussion

3.1. Optimization studies

The stability and reproducibility of a biosensor are affected by certain parameters which need to be optimized. Accordingly, the amounts of polymer, crosslinker, and enzyme as well as the pH values, were probed. In every optimization experiment, the parameter being optimized was altered while keeping all other parameters constant.

The charges on the amino acids in the enzyme's active site change as the pH of the medium changes. It was shown that the catalytic activity of GOx is observed between pH 3.0 and pH 8.0 [25]. In order to attain optimum enzyme activity with proper enzyme conformation, the impact of pH on the biosensor response was studied at a pH range of 4.5–7 (Figure 3a). The change in the current signal increased as the enzyme reaction accelerated as the pH value was raised and came to a maximum at pH 5.5, which then decreased gradually. The greatest change in current response was recorded in the buffer solution with a pH value of 5.5, which is within the catalytic activity pH range of GOx, as given in the literature [25].

After the testing and optimization of pH values, concentrations of polymer, enzyme amount, and GA% were altered at the optimum pH. To immobilize the enzyme well onto the surface of the electrode, coating the surface with CPs helps to create a suitable immobilization platform for the biomolecules. To find the optimum polymer concentration, P(BDBT) solutions in the range of 0.1–1.75 mg/ml were prepared in THF, and 10 µl of the related solutions was coated onto a clean GCE surface while all other parameters were kept constant. As a result, 0.75 mg/ml polymer produced the highest enzyme response to glucose, meaning the highest amount of enzyme immobilization had been achieved (Figure 3b).

For the effect of enzyme amount on biosensor response, 0.5, 0.7, 1.0, 1.25, 1.5, and 1.75 mg of GOx were immobilized on different electrodes, and amperometric measurements were carried out while the other parameters were kept constant (Figure 3c). The experiments demonstrated that the biosensor prepared with 1.0 mg enzyme gives the enzymes high stability and the minimization of leaching. Glutaraldehyde (GA), as the crosslinker, has been considered a crucial component for biosensor design because of its low cost, high reactivity, and commercial availability. It promptly reacts with the enzyme's amino groups and maintains the enzyme conformation [26]. Moreover, using a crosslinker improves the compact structure of the enzyme attached to the polymer. 0.25, 0.5, 1.0, 1.25 1.5, and 2.5% GA values were assessed, and the optimum value of GA was evaluated as 1% (Figure 3d). Subsequently, pH 5.5 buffer solution, 0.75 mg/mL polymer, 1% GA, and 1.0 mg GOx were chosen as the optimized parameters before proceeding with calibration studies.

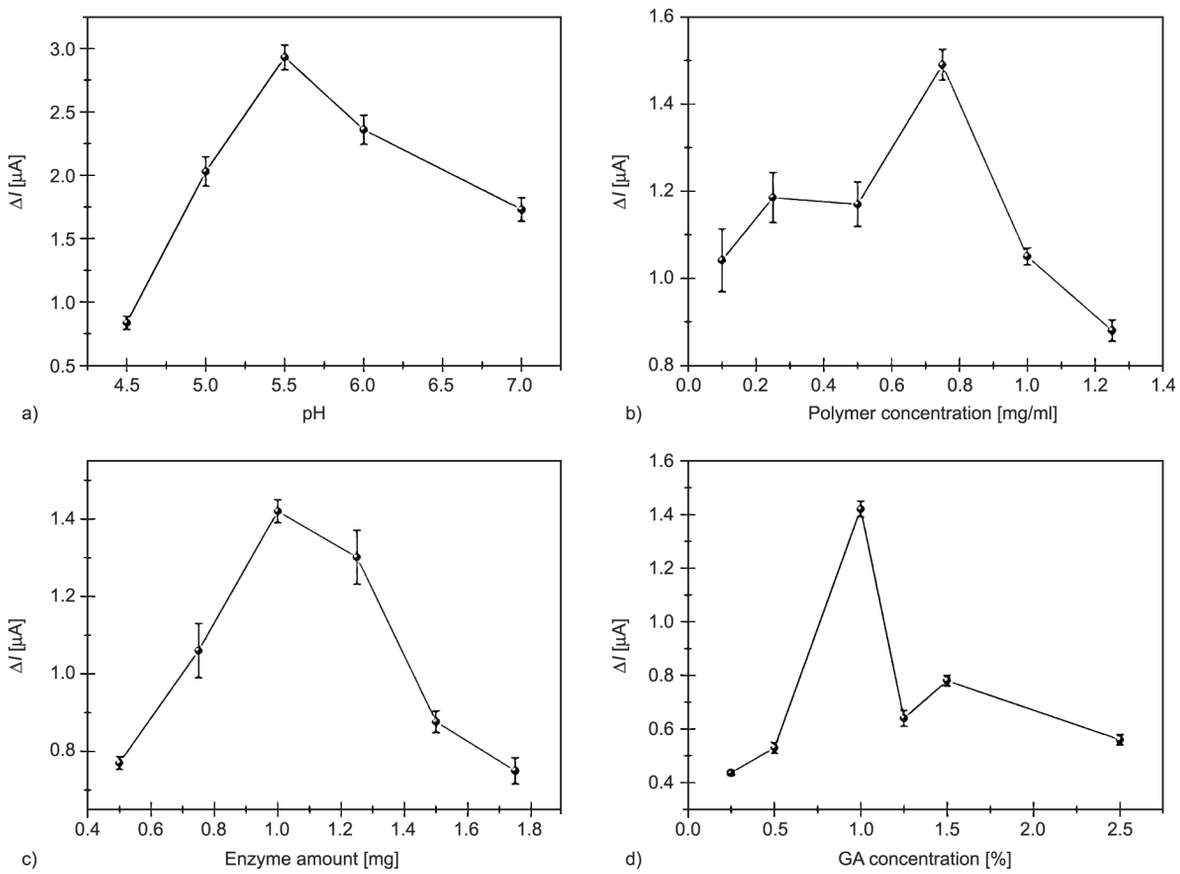


Figure 3. Impact of (a) pH (50 mM NaABS at pH 4.5, 5.0, 5.5, and 50 mM NaPBS at pH 6.0 and 7.0); (b) polymer concentration; (c) enzyme amount, and (d) crosslinker concentration on biosensor response (in 50.0 mM NaABS, pH 5.5, 25 °C). (Error bars computed from the SD of three measurements.)

3.2. Analytical characterization

A calibration curve was plotted of current change (ΔI) versus glucose concentration under optimum conditions (Figure 4). The response of the biosensor was found to be linear between 0.1 and 1.0 mM, using the equation $y = 2.0902x - 0.0829$ ($R^2 = 0.996$). The limit of detection (LOD) and the sensitivity values were computed as 0.167 mM and 28.17 μA/(mM·cm^2), respectively. In addition, the apparent Michaelis-Menten constant (K_M^{app}) was determined from the Lineweaver–Burk graph, which is the plot of the reciprocal reaction rate *vs*. the reciprocal substrate concentration. A small value of K_M^{app} implies a strong affinity of the enzyme to the substrate [27]. The value of K_M^{app} for the proposed biosensor was found as 1.74 mM, which is either lower than or similar to reported values in the literature (Table 1), demonstrating a high affinity to glucose.

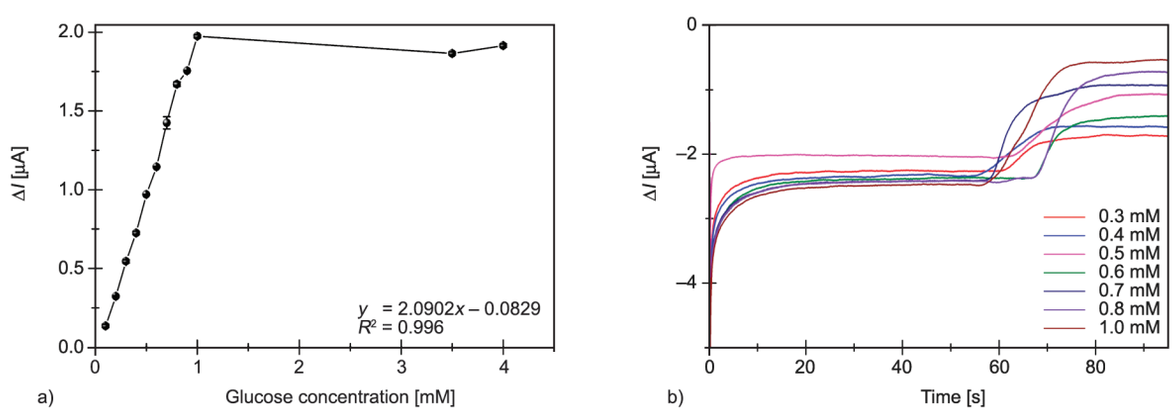


Figure 4. (a) Calibration graph for glucose analysis (in 50 mM NaABS pH 5.5 buffer, 25 °C, –0.7 V). (b) Amperometric responses of P(BDBT)/GOx biosensor for several glucose concentrations under optimum conditions.

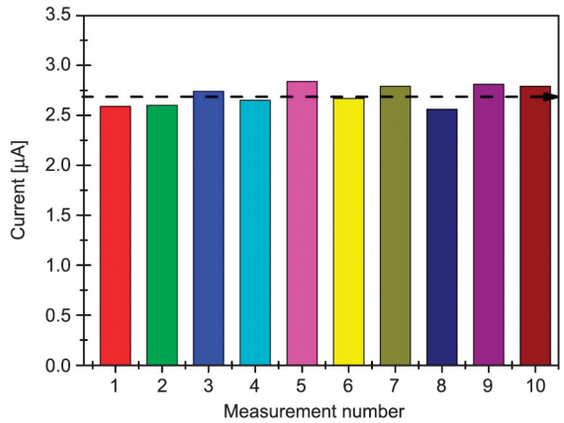


Figure 5. Operational stability of the P(BDBT)/GOx biosensor.

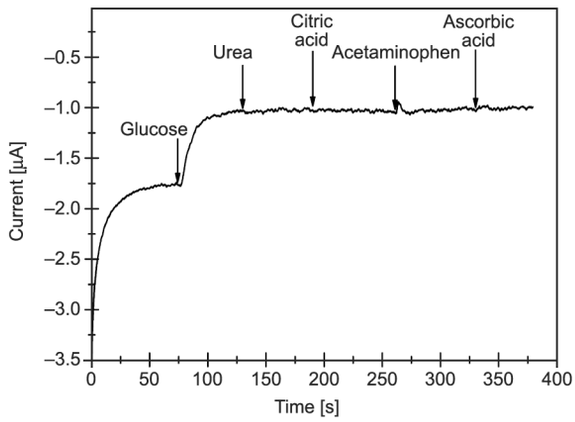


Figure 6. Effect of interfering compounds and glucose.

To test the operational stability of the biosensor, the standard deviation and RSD% values for P(BDBT)/GOx biosensor after 10 consecutive measurements were calculated and found to be ±0.0137 and 2.96%, respectively (Figure 5). Such good operational stability of the P(BDBT)/GOx biosensor could be due to the great biocompatibility of the porous P(BDBT) polymers that could help to preserve the GOx molecules without the loss of activity. The amine functionalities on the polymer backbone serve to immobilize GOx through glutaraldehyde and act as a spacer arm for better interaction of the enzyme with glucose molecules. In addition, as for the reproducibility of the devices, three independent sensors were prepared by measuring the current responses to 1.0 mM glucose at the optimized working conditions. The results showed that all devices displayed almost the same properties after each preparation, and the SD value was obtained as ±0.16. Moreover, the shelf life of the proposed biosensor was studied. Biosensor responses were measured for thirty-five days, and the enzyme electrode was refrigerated at 4 °C after the measurements. After 20 days of storage, no significant change in current was observed. Throughout 35 days of storage, the biosensor maintained its sensitivity for a long time, and approximately 26% activity loss was observed compared to its initial sensitivity at the end of the 35 day period. Hence, it was deduced that the proposed biosensor displayed long-term stability.

In the analyses with biosensors, it is imperative to get a signal solely for the target analyte. In real samples, other compounds may be present, which will lead to an interference effect. To make sure that our biosensor is impervious to other compounds, potential interfering compounds were tested with the proposed biosensor. Acetaminophen, ascorbic acid, citric acid, and urea (0.5 mM) were introduced into the electrochemical cell respectively instead of glucose, and no responses were detected for those substances, as shown in Figure 6.

Moreover, the P(BDBT)/GOx biosensor is also tested against matrix effects of a commercially available beverage. For this purpose, an artificially sweetened beverage containing no glucose was used, and the electrochemical cell was spiked with glucose during measurement. The biosensor has responded to the addition of glucose added in this manner, and the signal change achieved was correspondent to that of pure glucose addition at the same concentration. The biosensor did not give any response when only Diet Coke® was added, containing no sugar as reported on its product label. With these results, the designed novel sensor could be proposed for glucose sensing. There is a myriad of conjugated polymer-based electrochemical sensors reported in the literature with satisfactory analytical performance for glucose analysis. Zhai *et al.* [28] constructed a glucose sensor using Pt nanoparticles (NPs) – polyaniline (PAni) hydrogels, where PtNPs were deposited onto the PAni hydrogel. In another system where GOx was immobilized onto a conducting polymer with amine groups via glutaraldehyde, the biosensor surface was further coated with PMMA/clay nanocomposites [29]. Al-Mokaram *et al.* [30] electrodeposited nanocomposite films on ITO, where a solution of chitosan with uniformly dispersed Fe_3O_4 nanoparticles is linked with electropolymerized PPy through hydrogen bonding. Ayenimo *et al.* [31] developed a PPy-based bilayer glucose biosensor combined with a perm-selective layer (PPy-GOx/PPy-Cl). In another work,

graphene-coated paper-based electrodes were coated with a CP end-capped with 2,5-diphenyl-1,2,4-oxadiazole. GOx was then immobilized using gold NPs via physical adsorption [32]. Alim *et al*. [33] fabricated a bienzymatic glucose biosensor, in which horseradish peroxidase and GOx with SnO_2 multiporous nanofibers (MPNFs) were polymerized with PAni. A chronoamperometric glucose biosensor was devised by the electrodeposition of a nanocomposite film composed of electropolymerized poly(3-aminobenzoic acid) and platinum/reduced graphene oxide on a screen-printed carbon electrode (SPCE). Employing EDC/NHS chemistry, GOx was then immobilized on the surface of the modified electrodes [34]. Moreover, the composition of the modified electrodes, the methods used for analyses, and real sample applications were summarized in Table 1, along with the analytical parameters such as sensitivity, linear range, LOD, and K_M^{app} values. Most of these sensors were fabricated by assembling several materials to procure satisfactory biosensor features, whereas the proposed biosensor was constructed only with the polymer P(BDBT) and displayed a decent performance with a low LOD, and high stability and a high affinity for the substrate. It could be assumed that polymerization of the alkoxy substituted BDT monomer with the amino-functionalized monomer, 3,6-dibromobenzene-1,2-diamine, affords the copolymer in which the amine functionalities in the polymer backbone improved the interaction between the biorecognition element and the target analyte. Proper immobilization of the biorecognition element on the transducer surface was achieved with the help of the related polymer without leakage of enzymes from the immobilization medium since GA acts as an appropriate spacer between the support and the enzyme, reducing steric hindrance.

3.3. Surface characterization

A study of the individual electrode modifier in a solution of 5 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl and NaABS (50 mM, pH = 5.5) from –0.4 to 0.6 V was conducted. In this part, three different electrodes, a bare glassy carbon electrode, P(BDBT), and P(BDBT)/GOx were prepared. The aim is to observe how modification of the electrode affects the behavior of the redox couple in the solution. As seen in Figure 7, the P(BDBT) modified electrode displayed a higher current response compared to the P(BDBT)/GOx electrode. The effective surface areas for the electrodes were calculated with the Randles-Sevcik equation [37] (Equation (1)):

$$I_p = 2,69 \cdot 10^5 n^{3/2} A D^{1/2} C v^{1/2} \quad (1)$$

Table 1. Analytical properties of conjugated polymer-based biosensors for glucose analysis.

Modified electrode	Method	Linear range [mM]	LOD [mM]	Sensitivity [μA/(mM·cm²)]	K_M^{app} [mM]	Application	References
GOx/PtNP/PAni/Pt	Amperometry	0.01–8.0	$7.0 \cdot 10^{-4}$	96.1	2.35	NR	[28]
Poly(BEDOA-6)/PMMA-Laponite nanocomposite/GOx	CV and Amperometry	$2.8 \cdot 10^{-3}$–1.2	$1.99 \cdot 10^{-3}$	37.16	1.31	Human blood serum	[29]
ITO/PPy–CS–Fe_3O_4 nanocomposite	CV and Amperometry	0.1–16	0.234	12	NR	NR	[30]
PPy-GOx/PPy-Cl	CV and Amperometry	0.5–24	$2.69 \cdot 10^{-4}$	3.5	8.4	Fruit juices	[31]
PFLO/AuNPs/graphene/GOx	Amperometry	0.1–1.5	0.081	7.36	0.229	Beverage	[32]
GOx-HRP/PAni/SnO_2-NFs/Ch	CV and Amperometry	$5.0 \cdot 10^{-3}$–0.1	$1.8 \cdot 10^{-3}$	NR	NR	Urine	[33]
GOx/Pt/rGO/P3ABA	Chronomperometry	0.25–6.0	0.0443	22.01	3.54	Human serum	[34]
Pt/PB/GOx-PPy	CV	NR	NR	1.30	17.3	NR	[35]
Graphite/p(BTP)/GOx	CV and Amperometry	0.034–1.0	0.034	9.43	NR	Beverage	[36]
P(BDBT)/GOx	CV and Amperometry	0.1–1.0	0.17	28.17	1.74	Beverage	This work

NR: Not reported

Pt: Platinum, PtNPs: Pt nanoparticles, PPy: polypyrrole, PAni: polyaniline, rGO: reduced graphene oxide, P3ABA: poly(3-aminobenzoic acid), Ppy–CS–Fe_3O_4: Polypyrrole–Chitosan–Iron oxide, ITO: Indium tin oxide, PB: Prussian Blue, PFLO: poly(9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl)-end capped with 2,5-diphenyl-1,2,4-oxadiazole, p(BTP): poly(5-amino-N^1,N^3-bis(2,5-di(thiophen-2-yl)-1Hpyrrol-1-yl)isophthalamide), HRP: horseradishperoxidase

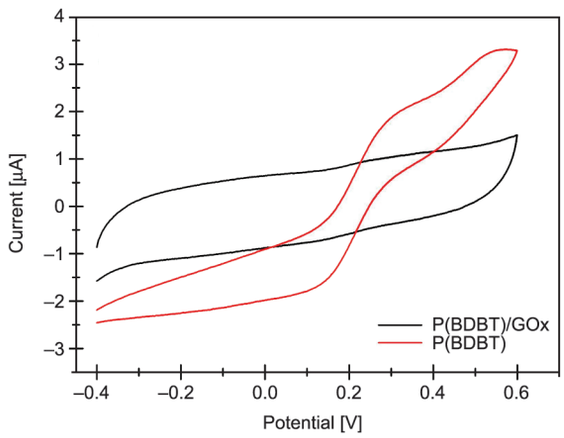


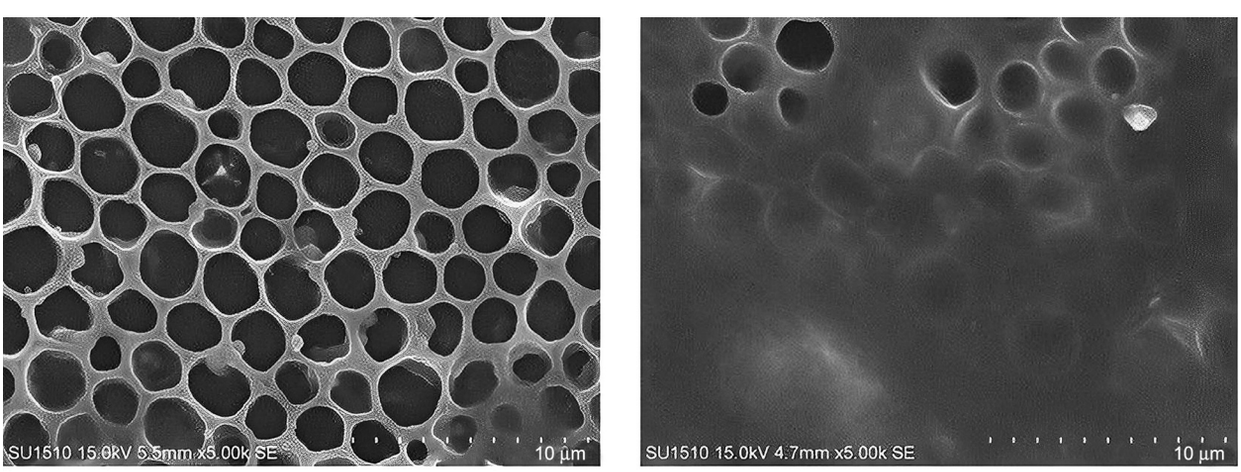
Figure 7. Cyclic voltammograms of P(BDBT) (—) and P(BDBT)/GOx (—) in 5 mM $Fe(CN)_6^{3-/4-}$ – 0.1 M KCl, pH 5.5 NaABS.

where I_p is the peak current [A], n is the number of electrons participating in the redox reaction (n = 1), A is the area of the electrode [cm^2]; D is the diffusion coefficient of the molecule in solution [cm^2/s] which was the average value of the diffusion coefficient of $[Fe(CN)_6]^{3-}$ ($7.6 \cdot 10^{-6}$ cm^2/s) and $[Fe(CN)_6]^{4-}$ ($6.3 \cdot 10^{-6}$ cm^2/s) [38], C is the concentration of the probe molecule in the bulk solution ([mol/cm^3]; 5.0 mM), and v is the scan rate [100 mV/s]. Using the related equation, the effective surface areas of the P(BDBT) and P(BDBT)/GOx electrodes were estimated as 0.174 and 0.096 mm^2, respectively. Successful modification of the electrode surfaces led to different interfacial structures. After GOx was immobilized on the polymer surface, the decline in oxidation current confirmed that the biomolecule was effectively attached to the electrode. Despite the decrease in current, the area of the electroactive surface was adequate for the efficient transfer of electrons.

Surface modifications of electrodes were also investigated with scanning electron microscopy. SEM images of polymer-coated electrodes before and after GOx immobilization were provided (Figure 8). As seen in Figure 8, the surface morphology of the P(BDBT) coating showed a homogeneous porous surface. After enzyme immobilization, enzyme molecules covered all the polymer-coated surfaces, which shows the enzyme was well-immobilized onto the polymer film. Moreover, it was clearly shown that the enzyme molecules penetrated the polymer film. This may increase the access of the substrate to the biorecognition part.

4. Conclusions

A novel, rapid, low-cost, practical, and sensitive amperometric glucose biosensor was designed and constructed. The sensing platform was fabricated by immobilizing GOx onto a benzenediamine-benzodithiophene conjugated polymer-modified GCE surface via glutaraldehyde in a single step. The analytical performance of GCE/P(BDBT)/GOx was excellent with a low LOD, a high affinity, sensitivity, and selectivity for glucose analysis as well as repeatability and long-term stability. The biosensor displayed no interference effect, making it convenient for real sample analysis. The biosensor performance was demonstrated in a commercial beverage spiked with glucose. Compared to similar works reported in the literature, the P(BDBT)-modified GCE biosensor shows superior properties for glucose sensing. Furthermore, it offers potential use for the fabrication



a) b)

Figure 8. SEM images of P(BDBT) (a) before and (b) after GOx immobilization at optimum conditions.

of biosensors with different enzymes with high accuracy results.

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