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# A novel glucose oxidase biosensor based on poly([2,2′;5′,2″]-terthiophene-3′-carbaldehyde) modified electrode

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ABSTRACT

In the study, the electrochemical behavior of glucose oxidase (GOx) immobilized on poly([2,2′;5′,2″]-terthiophene-3′-carbaldehyde) (poly(TTP)) modified glassy carbon electrode (GCE) was investigated. The biosensor (poly(TTP)/GOx/GCE) showed a pair of redox peaks in 0.1 M phosphate buffer (pH 7.4) solution in the absence of oxygen the co-substrate of GOx. In here, Poly(TTP)/GOx/GCE biosensor acts as the co-substrate instead of oxygen. Upon the addition of glucose, the reduction and oxidation peak currents increased until the active site of GOx was fully saturated with glucose. The apparent m was estimated 26.13 mM from Lineweaver–Burk graph. The biosensor displayed a good stability and bioactivity. The biosensor showed a high sensitivity (56.1 nA/mM), a linear range (from 0.5 to 20.15 mM), and a good reproducibility with 3.6% of relative standard deviation. In addition, the interference currents of glycin, ascorbic acid, histidine, uric acid, dopamine, arginine, and fructose on GOx biosensor were investigated. All that substances exhibited an interference current under 10%. It was not shown a marked difference between GOx biosensor and spectrophotometric measurement of glucose in serum examples. UV–visible spectroscopy and scanning electron microscopy (SEM) experiments of the biosensor were also performed.


## 1. Introduction

Biosensors are widely used in determination of some biological substances such as glucose, acetylcholine, alcohol and cholesterol. For this purpose, many studies have been performed on enzyme sensors since the beginning of the 1960s. Among these studies, conjugated polymers (CPs) such as polipirols [1], polythiophenes [2], and polyanilines [3] have been used as enzyme immobilization matrix. The physical and chemical properties of CPs have significant influences on biosensor performance [4]. For example, CPs have an organized molecular structure which provide opportunity them to function on a three dimensional shape for enzyme immobilization protecting biological activity of enzyme for a long time [5,6]. CPs have the ability to transfer electric charge produced by the biochemical reaction. Moreover, conducting polymers allow rapid electron transfer. Conjugated π electron backbones ensure free movement of electrons throughout the lattice [7].

Among conducting polymers, poly(thiophene) and its derivatives have been a growing interest in the electrochemical studies because of a unique combination of original electronic properties, environmental stability, and structural variability [8,9]. The terthiophene type monomers are interesting for designing new biosensors, because of their long-term stability and lower oxidation potential than that of thiophene and bithiophene monomers [10].

Glucose oxidase (GOx) with a molecular weight of 152,000–186,000 Da is an oxido-reductase catalyzing the oxidation of glucose to hydrogen peroxide and D-glucono-1,5-lactone and contains flavin adenine dinucleotide (FAD) redox center.

Many studies on glucose biosensor have been carried out so far. Among these studies, the direct electron transfer between immobilized GOx and modified electrode has been reported using amperometric and cyclic voltammetric techniques [11]. In the third generation glucose sensor, GOx($FADH_2$) transfers two protons and two electrons to the oxidized form of the polymer on which GOx is immobilized. In this case the voltammetric and amperometric methods can be used to determine glucose in blood samples. An artificial mediator is used in the construction of the third generation glucose sensors that carries electrons between the redox center of GOx and electrode surface (Scheme 2b).

In the study, [2,2′;5′,2″]-terthiophene-3′-carbaldehyde was synthesized as matrix for GOx immobilization (Scheme 1). Glassy carbon electrode was coated with poly([2,2′;5′,2″]-terthiophene-3′-carbaldehyde) and GOx was immobilized on the polymer coated electrode. GOx was immobilized on poly(TTP)/GCE surface through

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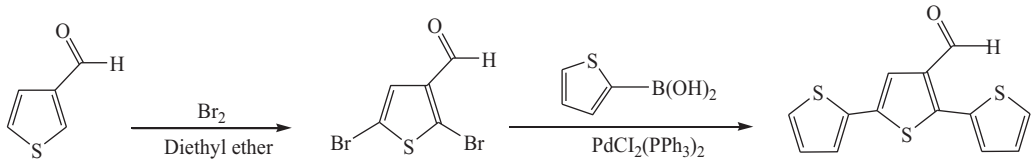
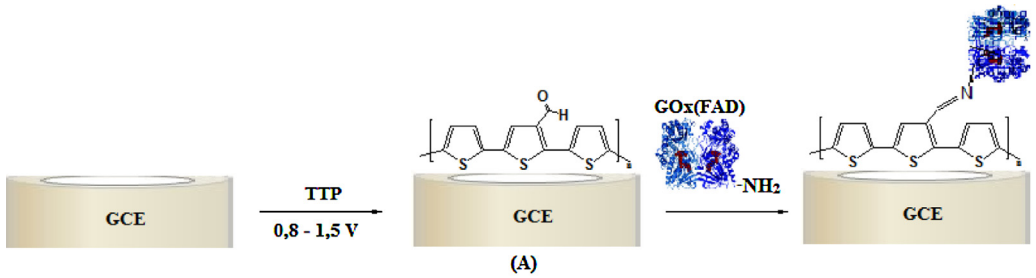



**Scheme 1.** Synthesis of [2,2′;5′,2″]-terthiophene-3′-carbaldehyde.




**Scheme 2.** (A) Systematic representation of poly(TTP)/GOx/GCE. (B) Electron transfer reactions with the redox cycles occurring on the working electrode surface.

the covalent bond formed between aldehyde group and amino group (Scheme 2a).

## 2. Materials and methods

### 2.1. Materials

Glucose oxidase (GOx, EC 1.1.3.22 from *Aspergillus niger*), glucose, tetrabutilammonium hexaflourophosphate ($TBAPF_6$), dimethylformamide (DMF), 2-thienylboronic acid, 3-thiophenecarboxaldehyde, bis(triphenylphosphine) palladium (II) dichloride ($PdCl_2(PPh_3)_2$) were purchased from Sigma chemical. All other chemicals were obtained from Merck. Autolab PGSTAT 128N potentiostat was used for all electrochemical measurements performed in the study. Ag/AgCl reference electrode, platinum (Pt) wire counter electrode and glassy carbon working electrode (GCE) were obtained from BASi Corporation. The scanning electron microscope (SEM) and nuclear magnetic resonance (NMR) studies were carried out at Selcuk University Advanced Technology Research and Application Center. UV–vis spectroscopy was performed using UV-1800 spectrophotometer (Shimadzu Co., Japan).

### 2.2. Synthesis of 2,5-dibromothiophene-3-carbaldehyde

3-thiophenecarboxaldehyde (0.25 g, 0.93 mmol) was added into a 100 mL round-bottomed flask containing 20 mL diethyl ether. This mixture was stirred about 30 min under $N_2$-saturated medium. After that 3 mL of diethyl ether containing $Br_2$ (3.14 mL, 61.30 mmol) and $HBr/H_2O$ were added into the reaction mixture. The last mixture was stirred in ice water about 2 h. The mixture was then heated to 50 °C and stirred until the starting material was finished. After completing the reaction, the mixture was poured into cold water so as to eliminate the effect of $Br_2$ and extracted several times using diethyl ether. Finally, the product was purified (80% yield) using column chromatography [12]. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.80 (s, 1H), 7.34 (s, 1H) [13].

### 2.3. Synthesis of [2,2′;5′,2″]-terthiophene-3′-carbaldehyde

In order to obtain [2,2′;5′,2″]-terthiophene-3′-carbaldehyde (TTP), 2,5-dibromothiophene-3-carbaldehyde (500 mg, 1.85 mmol), 2-thienylboronic acid (710 mg, 5.55 mmol), $PdCl_2(PPh_3)_2$ (140 mg, 0.2 mmol), and $KHCO_3$ (200 mg, 2.0 mmol) were put into a 100 mL round-bottomed flask containing 10 mL of $DMF/H_2O$ (4:1). The mixture was stirred about 30 min at room temperature. The solution was then heated to 100 °C under $N_2$-saturated medium until the starting material was finished. The mixture was extracted several times with dichloromethane. The organic phase was then dried with $MgSO_4$ and filtrated. The solvent was removed using rotary evaporator. The oily yellow residue was purified (73% yield) by means of column chromatography. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.1 (s, 1H), 7.56 (s, 1H), 7.49 (dd, $j$ = 5.1 Hz, $j$ = 1.1 Hz, 1H), 7.30 (m, 2H), 7.21 (dd, $j$ = 3.62 Hz, $j$ = 1.1 Hz, 1H), 7.15 (dd, $j$ = 5.2 Hz, $j$ = 3.7 Hz, 1H), 7.04 (dd, $j$ = 5.1 Hz, $j$ = 3.7 Hz, 1H); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ 185.2, 146.1, 137.8, 136.9, 135.7, 132.2, 129.4, 128.9, 128.5, 128.2, 125.9, 125.1, 122.5 [13].

### 2.4. Electropolymerization of [2,2′;5′,2″]-terthiophene-3′-carbaldehyde (TTP)

Prior to polymerization of TTP monomer, GCE was polished with 0.3 μm alumina slurry and washed ultrasonically in bi-distilled water and ethanol for 5 min and washed with bi-distilled water again. After that, TTP monomer was polymerized on the cleaned GCE surface via cyclic voltammetry (CV) between 0.8 V and 1.5 V potentials. The polymerization of TTP was carried out in 5 mL acetonitrile solution containing 0.02 M TTP and 65 mM $TBAPF_6$ (Fig. 1).

In order to explain the redox behavior of the polymer, poly(TTP)/GCE was washed with acetonitrile to remove the unbound species and then it was placed into electrochemical cell containing acetonitrile and 65 mM of $TBAPF_6$. Poly(TTP) film showed a reversible redox peaks. The peak currents were proportional to the scan rate. This indicates a non-diffusional redox

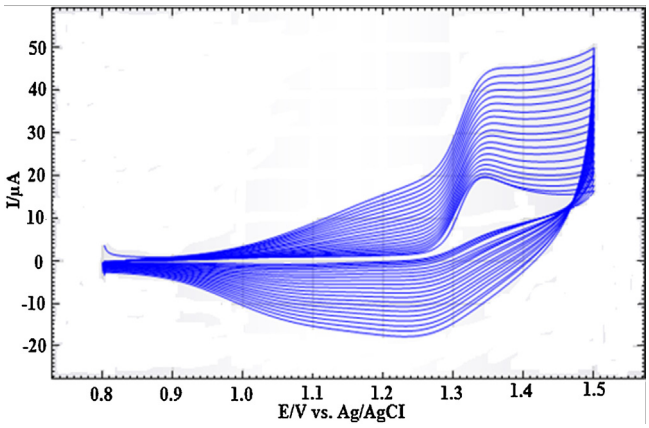



**Fig. 1.** Electrochemical polymerization of TTP on GCE electrode in 65 mM of $TBAPF_6$/Acetonitrile electrolyte-solvent system at 0.1 V/s of scan rate.

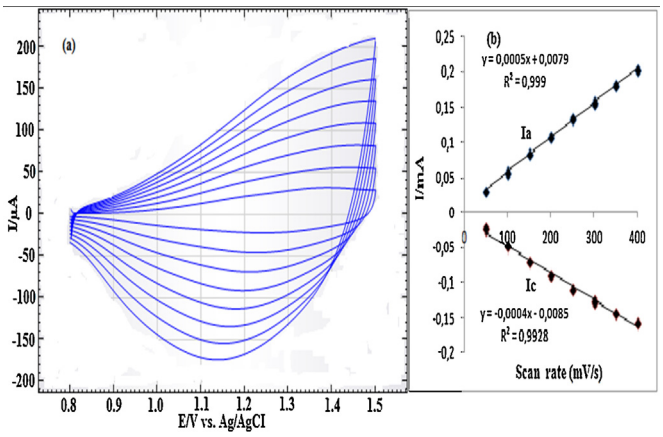



**Fig. 2.** (a) Cyclic voltammograms of the poly(TTP)/GCE electrode in acetonitrile containing 65 mM of $TBAPF_6$ at different scan rates (50, 100, 150, 200, 250, 300, 350, 400 mV/s), (b) the plots of anodic ($I_a$) and cathodic ($I_c$) current peaks vs. scan rates.

reaction and a good adhered electrochemically active polymer film (Fig. 2).

### 2.5. Preparation of GOx biosensor

The prepared TTP polymer film was rinsed with acetonitrile to remove unbound monomer and reagents before GOx immobilization. Then 5 μL of GOx solution (10 mM of sodium phosphate buffer pH 7.4 containing different amount of GOx) was spread over the poly(TTP) modified electrode and allowed to dry at 4 °C. Poly(TTP)/GOx/GCE was rinsed with 0.1 M of sodium phosphate buffer (pH 7.4) to purge unbound enzyme molecules and stored at 4 °C when not in use.

## 3. Result and discussion

### 3.1. Surface characterization of poly(TTP)/GOx

The electron transfer between poly(TTP) and electrode is crucial, especially in biosensor applications. The properties of conducting polymers depend on their morphologies, in addition to their

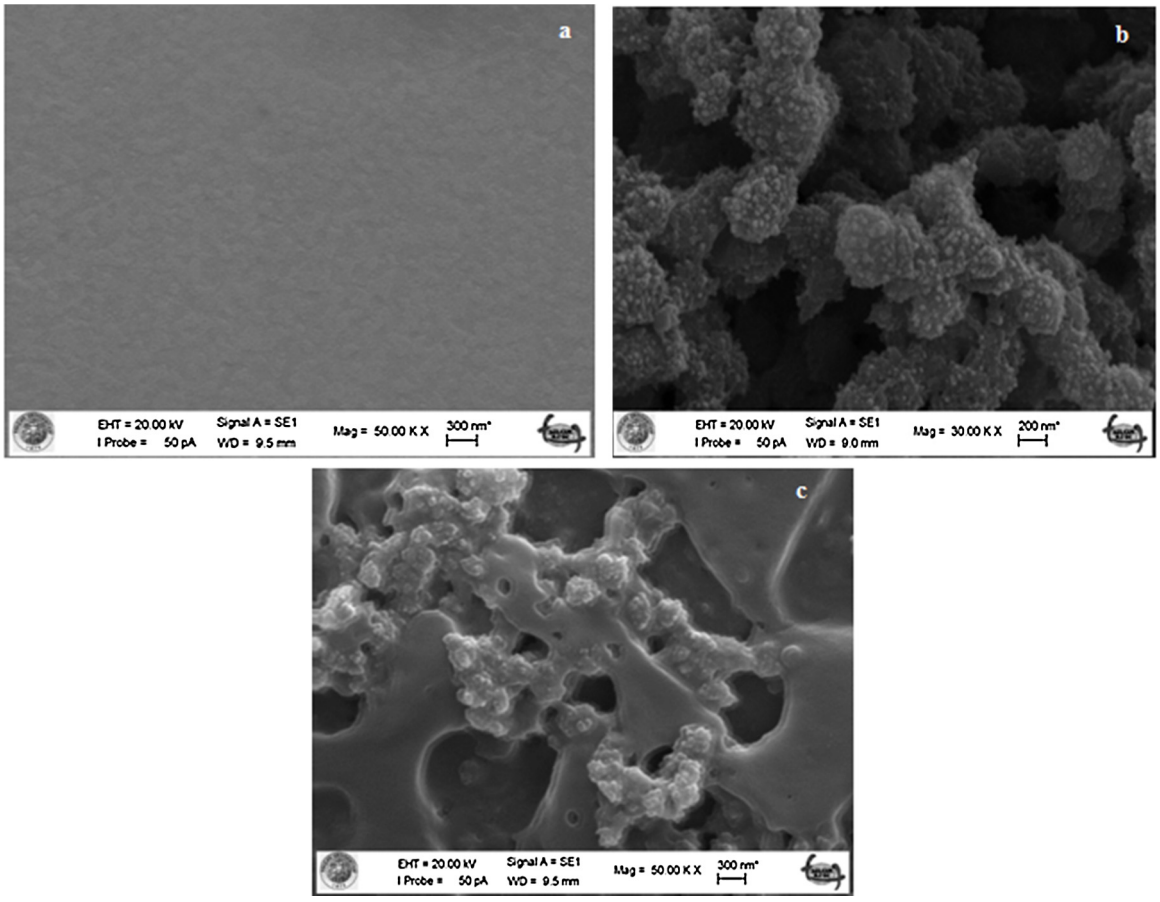



**Fig. 3.** SEM images of bare ITO (a), poly(TTP)/ITO (b), and poly(TTP)/GOx/ITO (c).

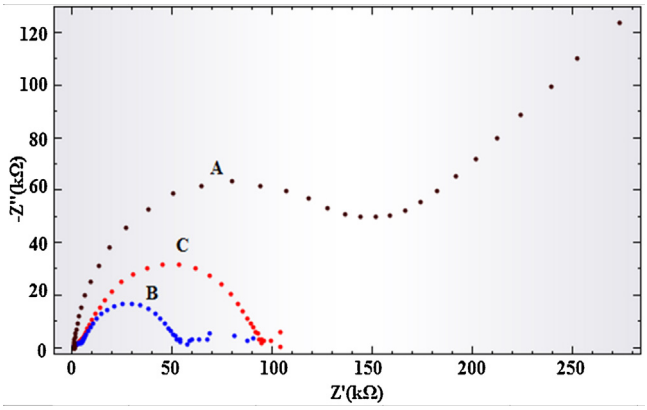



**Fig. 4.** Electrochemical impedance Nyquist plot of bare GCE (A), poly(TTP)/GCE (B), and poly(TTP)/GOx/GCE (C) in 0.1 M phosphate buffer (pH 7.4) containing 2.5 mM $[Fe(CN)_6]^{3-}$ + 2.5 mM $[Fe(CN)_6]^{4-}$ and 0.1 M KCl. Frequency range: 0.1 Hz to 100 kHz.

backbone structure. The surface characterization of poly(TTP) coated ITO was investigated before and after GOx immobilization by scanning electron microscopy (SEM). Fig. 3 shows the SEM of ITO (a), poly(TTP)/ITO (b), and poy(TTP)/ITO/GOx (c). Electrodeposited poly(TTP) film via CV showed a granular morphology (b). GOx immobilized on poly(PTT) film using chemical entrapment method resulted compact growth pattern and the glutaraldehyde is seen covering GOx units (c). GOx immobilization was achieved with and without glutaraldehyde and these two working electrode showed the same response to glucose but the working electrode with glutaraldehyde exhibited wider linear response to glucose than that without glutaraldehyde.

### 3.2. Electrochemical behavior of poly(TTP)/GOx/GCE

As mentioned above, it is known that conducting polymers have been used for enzyme immobilization and to enhance conductivity and sensitivity of working electrode. In here, electrochemical impedance spectroscopy (EIS) and CV were used to evaluate the electrochemistry of poly(TTP) and poly(TTP)/GOx at GCE. Fig. 4 shows the Nyquist plot of EIS of GCE (A), poly(TTP)/GCE (B) and poly(TTP)/GOx/GCE (C). It shows that the EIS response to 5 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ containing 0.1 M KCl at poly(TTP)/GCE much weaker than that at GCE and poly(TTP)/GOx/GCE. According to these results, it was demonstrated that the polymer enhances the response of GCE. When the enzyme is bounded to the polymer, the response of the modified GCE relatively decreases because of partial inhibition of electron transfer by GOx. The cyclic voltammogram of glucose was investigated on GCE, poly(PTT)/(GCE), and poly(TTP)/GOx/GCE, as shown in Fig. 5(a). No detectable redox peak was observed at GCE and poly(PTT)/(GCE) in the $N_2$-saturated of 0.1 M of phosphate buffer (pH 7.4), but when 5 mM of glucose solution was added into the evaluation solution, an oxidation peak at −0.27 V and a reduction peak at −0.5 V were observed at poly(TTP)/GOx/GCE. The peak potential separation ($\Delta E_p$) is about 230 mV. The cathodic peak potential is higher than those reported previously, but the anodic peak potential is close to GOx-graphene-chitosan and GOx/Au/CPE [14,16]. When this experiment was repeated in the $O_2$-saturated of 0.1 M phosphate buffer (pH 7.4), a reduction peak was observed only at the bare working electrode in the absence of glucose because of the presence of oxygen in the cell (Fig. 5(b)). In many studies the direct electron transfer between electrode and glucose oxidase center occurs in the presence of oxygen co-substrate of the enzyme taking two protons and two electrons from the enzyme center ($FADH_2$) to form hydrogen peroxide [14,15]. In our study, the direct electron transfer from

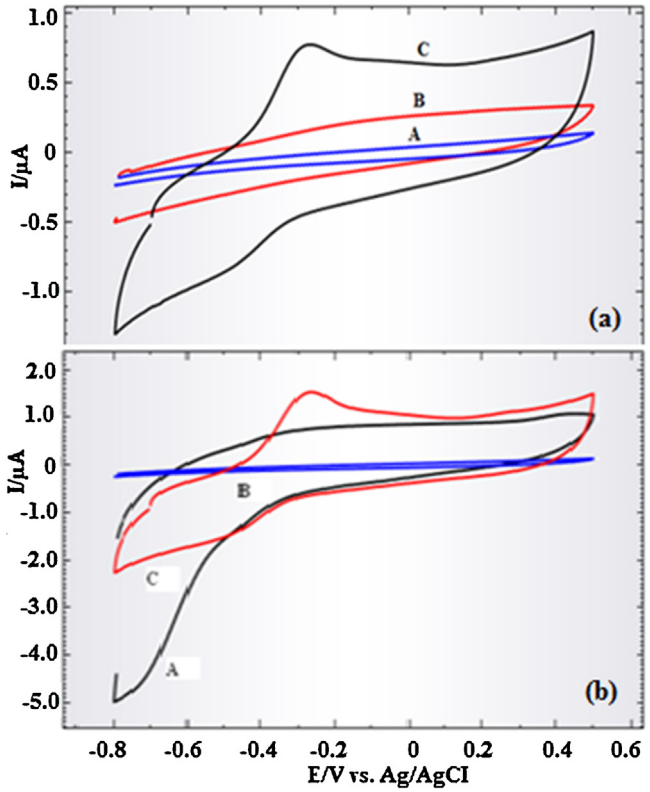



**Fig. 5.** (a) Cyclic voltammograms, GCE (A), poly(TTP)/GCE (B), and poly(TTP)/GOx/GCE (C) in $N_2$-saturated 0.1 M phosphate buffer (pH 7.4) containing 5 mM of glucose (scan rate: 0.1 V/s), (b) cyclic voltammograms, GCE (A), poly(TTP)/GCE (B), and poly(TTP)/GOx/GCE (C) in $O_2$-saturated 0.1 M phosphate buffer (pH 7.4) containing 5 mM of glucose (scan rate: 0.1 V/s).

the enzyme center to the polymer has occurred in the absence of oxygen. In here, the polymer acts as a co-substrate.

### 3.3. Effect of glucose on poly(TTP)/GOx/GCE biosensor

In here, different glucose concentrations were used to determine the biosensor response to glucose. When glucose was added into the electrochemical cell containing $N_2$-saturated 0.1 M of phosphate buffer (pH 7.4), a reduction and an oxidation current occurred at −0.5 V and −0.27 V respectively. In the increase of the oxidation and reduction peaks increased with increasing of glucose concentration. Fig. 6(A) shows the cyclic voltammograms of poly(TTP)/GOx/GCE with addition of glucose into $N_2$-saturated 0.1 M of phosphate buffer. The calibration range of glucose was determined from 0.5 to 24.72 mM. It was found that poly(TTP)/GOx/GCE response to glucose is linearly increased with increasing concentration of glucose from 0.5 to 20.15 mM with a correlation coefficient of 0.998 (Fig. 6(B)). Thus, this biosensor can be used for determination of diabetic glucose concentration because the diabetic glucose concentration is higher than 7.0 mM [17]. The linear range of this biosensor is wider than 0.08–12 mM for GOx/Graphene/Chitosan [16], 0–7.8 mM for GOx/MWCNTs/Chitosan [18], 0.08–0.28 mM for GOx/Au/CPE [14], 0.5–11.1 mM for GOx immobilized on CdS nanoparticles [15] and 0.01–5.5 mM for GOx/Polyaniline nanotubes [19]. The detection limit of the biosensor was calculated 0.1 mM. This value is lower than of GOx/poly(3,4-ethylenedioxythiophene)/Platinum [20], Pd–GOx–Nafion CNT electrode [21] and PC membrane/Au/2-mercaptoethylamine/glutalaldehyde/GOx [22]. The calculated sensitivity of the biosensor to glucose was 56.1 nA/mM. The apparent Michealis Menten constant ($K_M$) was estimated 26.13 mM (Fig. 7).

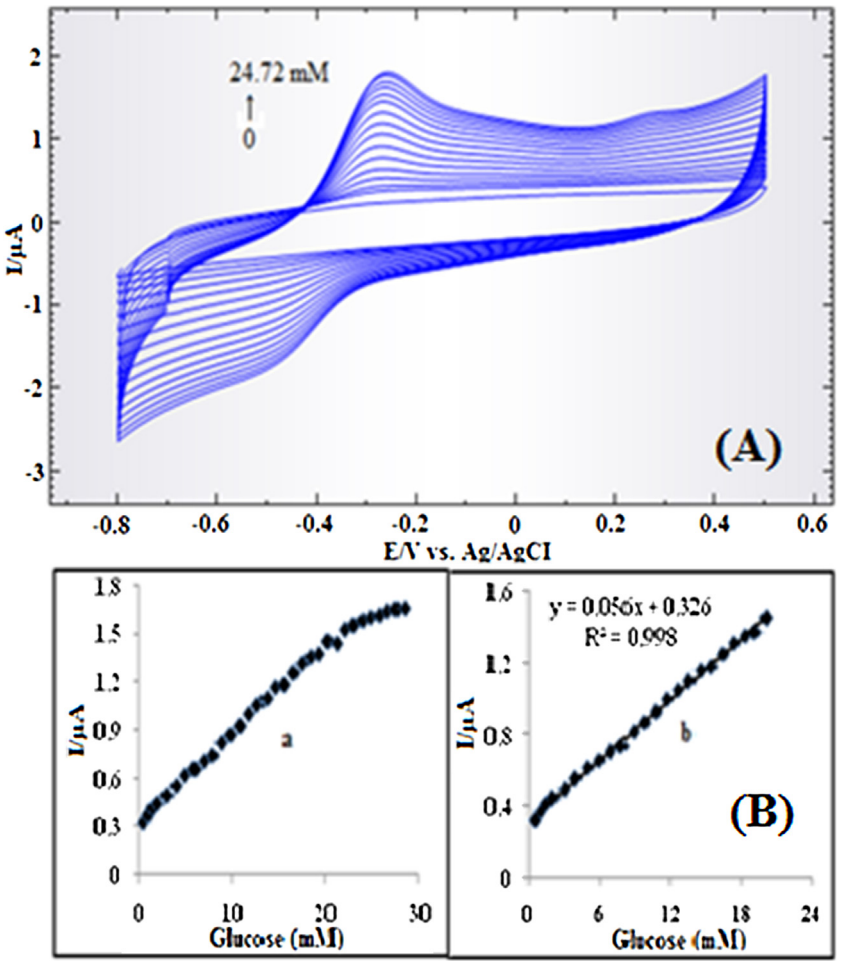



**Fig. 6.** (A) CVs of poly(TTP)/GOx modified GCE in the $N_2$-saturated 0.1 M of phosphate buffer (pH 7.4) after addition of glucose (scan rate 50 mV/s). (B) (a) The increased current of the oxidation peak of poly(TTP)/GOx/GCE electrode vs. glucose concentration in 0.1 M of phosphate buffer (pH 7.4), (b) linear plot.

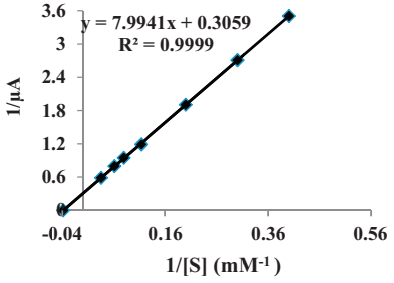



**Fig. 7.** Lineweaver–Burk graph of poly(TTP)/GOx/GCE enzyme electrode.

In here, the estimated $K_M$ value is lower than that reported in some studies [1,23,24].

### 3.4. Determination of optimum poly(TTP) thickness

As well-known, the polymer film thickness on the working electrode affects both the response time of the biosensor and conductivity of conducting polymer. If the film is too thick, the electron transfer between electrode and GOx center will not take place. Thus, the film thickness can be controlled by varying the thicknesses to a desired charge rate using CV method [25]. In the study, four different working electrodes were prepared with different thicknesses for determination the best film thickness of poly(TTP) on the electrode surface in the same monomer concentration and scan rate. Poly(TTP)/GOx/GCE showed a linear range of glucose concentration from 0.25 to 10.60 mM for 15 scans, from 0.90 to 6.50 mM for 20 scans, from 0.75 to 9.15 mM for 25 scans and from 0.80 to 12.55 mM for 30 scans. The optimum thickness of poly(TTP) was provided with 30 scans, but Poly(TTP)/GOx/GCE with 25 scans was used in the study because it showed better linear range than those with 15 and 20 scans and better reproducibility than that with 30 scans.

### 3.5. Determination of optimal GOx concentration

Enzymes can easily change the electron transfer rate because they are not conductive molecules and they can inhibit the electron transfer if the concentration is too much. In order to study the effect of GOx concentration on the working electrode, four different GOx concentrations (375, 625, 875, 1125 and 1375 U/cm$^2$) were immobilized on poly(TTP) coated GCE with 25 scans. Poly(TTP)/GOx/GCE showed a linear range of glucose concentration from 0.8 to 12.55 for 375 U/cm$^2$, from 0.5 to 12.75 for 625 U/cm$^2$, from 0.5 to 20.15 for 875 U/cm$^2$ and from 0.98 to 1.99 for 1125 U/cm$^2$. It was demonstrated that 875 U/cm$^2$ of GOx immobilized on GCE coated with 25 scans of poly(TTP) is the best enzyme concentration for a wide linear range of glucose detection.

### 3.6. Effect of pH

The pH dependence of poly(TTP)/GOx/GCE response in 0.1 M of phosphate buffer (pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5) was investigated. The response current increases with increasing

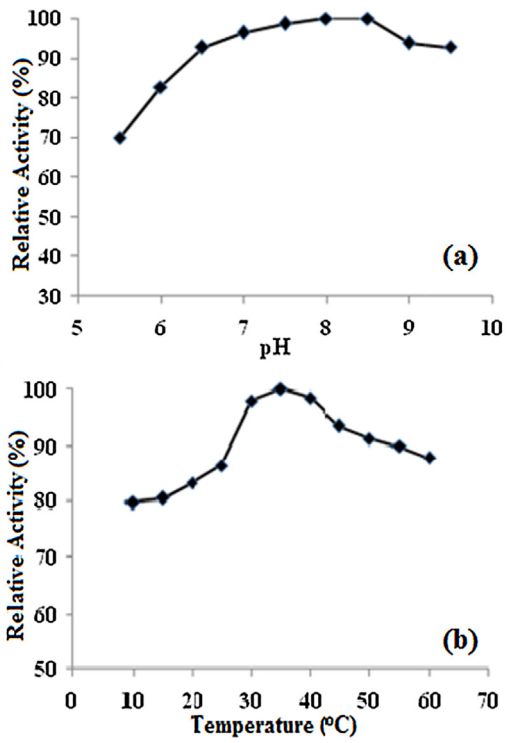



**Fig. 8.** (a) Effect of pH on poly(TTP)/GOx/GCE enzyme electrode, (b) effect of temperature on poly(TTP)/GOx/GCE enzyme electrode in 0.1 M of phosphate buffer (pH 7.4) containing 10.0 mM glucose.

pH from 5.5 to 8.0 and then decreases slightly in the increase of pH from 8.5 to 9.5. Fig. 8(a) shows that the maximum response current can be observed at pH 8.0 in 0.1 M of phosphate buffer, but the buffer with pH of 7.4 was used because of the physiological pH. The pH optimum of the biosensor is closer to the physiological pH than those reported in literatures [19,26].

### 3.7. Effect of temperature

As well-known, temperature can easily affect the enzyme activity. To determine the effect of temperature on the biosensor response, the voltammetric response of the biosensor was studied from 10 to 60 °C in 0.1 M phosphate buffer (pH 7.4). The response current of the biosensor increases with increasing temperature to 35 °C. The maximum response current is observed at about 35 °C (Fig. 8(b)). At temperatures above 35 °C, the biosensor response decreases because of the denaturation of GOx. This optimal temperature of poly(TTP)/GOx/GCE is close to that of GOx based biosensors reported earlier [19,27,28].

### 3.8. Interference studies

There are some electroactive species such as ascorbic acid (AA), uric acid (UA) and dopamine (DA) coexisting in real samples. They can affect the biosensor response due to the overlapping of the peak which is proportional to the concentration of the analyte. In here, the interference effects of glycin, AA, histidine, UA, DA, arginine, and fructose on the biosensor were investigated to test their effect on glucose determination. For this purpose, 0.98 mM of each substance higher than physiological level of these interfering substances (0.2 mM) [19] was added into electrochemical cell separately containing 5 mM of glucose. The interference current was calculated as 5.85% for ascorbic acid, 4.3% for glycin, 5.01% for histidine, 7.2% for uric acid, 5.5% for dopamine, 1.5% for arginine and 4.3% for fructose using the equation of the interference percentage $((I - I_o) \times 100/I_o)$. Where $I$ is the response of glucose + test compound and $I_o$ is the response of glucose only obtained by CV. It can be concluded that the biosensor has proved to be of good selectivity to glucose [27,29].

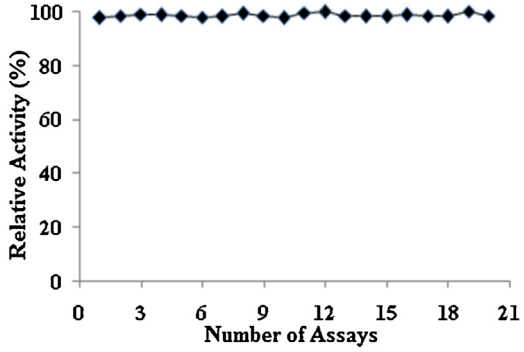



**Fig. 9.** Operational stability of poly(TTP)/GOx/GCE enzyme electrode in 0.1 M of phosphate buffer (pH 7.4) containing 10.0 mM glucose.

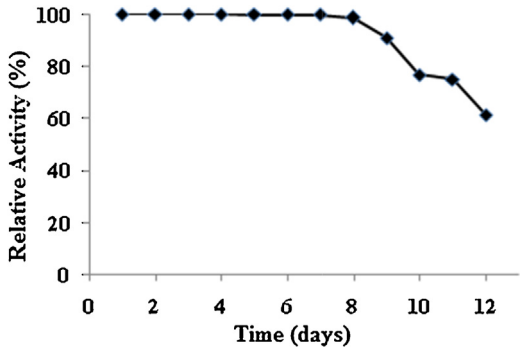



**Fig. 10.** Stability of poly(TTP)/GOx/GCE biosensor in 0.1 M of phosphate buffer (pH 7.4) containing 10.0 mM glucose.

### 3.9. Stability of poly(TTP)/GOx/GCE biosensor

The reproducibility of poly(TTP)/GOx/GCE has been evaluated by investigating the current response of the same working electrode for 10 mM concentrations of glucose. The average, standard deviation, and relative standard deviation of the enzyme electrode were 98.63, 3.55 and 3.6% after 20 sequential measurements. This result is better than that of the PEDOT-Pd/GOx-ME [27,29,30] (Fig. 9).

One of the most important properties of an enzyme electrode is the storage stability. In the study, the storage stability of the biosensor was determined using the same electrode under the same conductions within 12 days. The biosensor retains 61.52% of its initial activity for the same glucose concentrations after using over a 12 days period (Fig. 10).

### 3.10. UV–vis spectroelectrochemical studies

In this section, indium tin oxide (ITO) was used for evaluation of UV–vis spectroelectrochemistry of the biosensor. For this, TTP monomer was polymerized on the surface of ITO via cyclic voltammetry (CV) between 0.8 V and 1.5 V potentials. Then the absorbances of the cuvette in 0.1 M of phosphate buffer (pH 7.4) containing 0, 2.49, 4.98, 9.80, 14.56 and 19.23 mM of glucose were measured at 0.7 V respectively. When glucose was added into the cuvette, an increase was observed at 532 nm (Fig. 11). This result demonstrates that the chemical reaction catalyzed by GOx occurs in the electrochemical system.

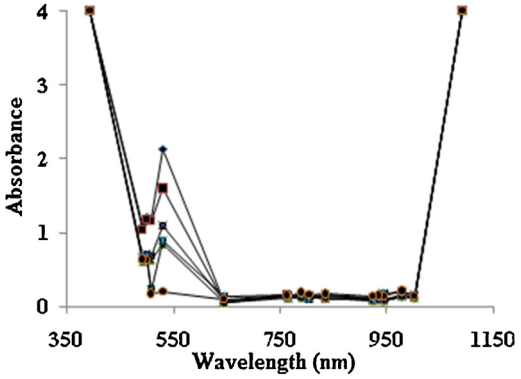



**Fig. 11.** UV–vis spectrophotometric measurement monitoring the increase of glucose (0, 2.49, 4.98, 9.80, 14.56 and 19.23 mM) in 0.1 M of phosphate buffer (pH 7.4).

**Table 1**
Quantitation of glucose in serum samples using Poli(TT)/GOx/GCE enzyme electrode and spectrophotometer.

| Serum samples | Glucose concentration (mg/dL) | |
|---|---|---|
| | Poli(TT)/GOx/GCE electrode* | Spectrophotometric |
| I | 73.6 ± 3.52 | 76 |
| II | 86.12 ± 3.71 | 88 |
| III | 93.9 ± 3.84 | 97 |
| IV | 101.1 ± 4.13 | 106 |
| V | 165.9 ± 5.24 | 160 |

* Each result is the average of three measurements.

### 3.11. Glucose determination in serum samples

The reliability and practical use of poly(TTP)/GOx/GCE were examined by determining the concentration of glucose in human serum samples obtained from a private hospital and compared with the values measured in the hospital. For this purpose, the methods of standard addition and standard curve were performed for determination of glucose in the samples. The samples were diluted to half of its initial concentration using 0.1 M phosphate buffer (pH 7.4). Then the glucose concentration in the samples was measured using the biosensor. The serum samples and measurements are summarized in Table 1. The results demonstrate that the biosensor can be applied to serum sample for glucose determination.

## 4. Conclusions

It would be desired to construct a glucose biosensor without mediator and determine glucose concentration a wide linear range and the operating potential needs to close the redox center of GOx. In the study, we have developed a novel glucose biosensor. Poly(TTP) was used for GOx immobilization. The biosensor showed a wide linear range glucose response without any mediators. In addition, the electrochemical reaction catalyzed by GOx occurred in the absence of oxygen the co-substrate of the enzyme. This is a good result because the oxygen is limited in the measuring cell and it can easily degrade the polymer matrix. The biosensor provides both a good environment for the enzyme entrapment and the direct electron transfer between the enzyme and electrode. The biosensor showed a good sensitivity, stability and reproducibility. It was not exhibited a certain difference between GOx biosensor and spectrophotometric measurement of glucose in serum examples. Consequently, poly(TTP)/GOx/GCE biosensor can be used for glucose determination in real samples.

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## References

[1] J.H. Cho, M.C. Shin, H.S. Kim, Sens. Actuators B 30 (1996) 137–141.
[2] C. Liu, T. Kuwahara, R. Yamazaki, M. Shimomura, Eur. Polym. J. 43 (2007) 3264–3276.
[3] A.A. Karyakin, M. Vuki, L.V. Lukachova, E.E. Karyakina, A.V. Orlov, G.P. Karpachova, V. Wang, Anal. Chem. 71 (1999) 2534–2540.
[4] B. Adhikari, S. Majumdar, Prog. Polym. Sci. 29 (2004) 699–766.
[5] Y. Xian, Y. Hu, F. Liu, Y. Xian, H. Wang, L. Jin, Biosens. Bioelectron. 21 (2006) 1996–2000.
[6] F.R.R. Teles, L.P. Fonseca, Mater. Sci. Eng. C 28 (2008) 1530–1543.
[7] E. Bakker, M. Telting-Diaz, Anal. Chem. 74 (2002) 2781–2800.
[8] M. Gerard, A. Chaubey, B.D. Malhotra, Biosens. Bioelectron. 17 (2002) 345–359.
[9] S.B. Adeloju, G.G. Wallace, Analyst 21 (1996) 699–703.
[10] T. Abdiryim, R. Jamal, A. Ubul, I. Nurulla, Molecules 17 (2012) 8647–8660.
[11] P. Krzyczmonik, E. Socha, Chemik Int. 67 (2013) 801–810.
[12] R. Satapathy, H. Padhy, Y.H. Wu, H.C. Lin, Eur. J. 18 (2012) 1606–1672.
[13] B.B. Çarbaş, A. Kivrak, M. Zora, A.M. Önal, React. Funct. Polym. 71 (2011) 579–587.
[14] S. Liu, H. Ju, Biosens. Bioelectron. 19 (2003) 177–183.
[15] Y.X. Huang, W.J. Zhang, H. Xiao, G.X. Li, Biosens. Bioelectron. 21 (2005) 817–821.
[16] X. Kang, J. Wang, H. Wua, I.A. Aksay, J. Liua, Y. Lin, Biosens. Bioelectron. 25 (2009) 901–905.
[17] Z.H. Dai, J. Ni, X.H. Huang, G.F. Lu, J.C. Bao, Bioelectrochemistry 70 (2007) 250–256.
[18] Y. Liu, M.K. Wang, F. Zhao, Z.A. Xu, S.J. Dong, Biosens. Bioelectron. 21 (2005) 984–988.
[19] Z. Wang, S.N. Liu, P. Wu, C.X. Cai, Anal. Chem. 81 (2009) 1638–1645.
[20] P.C. Nien, T.S. Tung, K.C. Ho, Electroanalysis 18 (2006) 1408–1415.
[21] S.H. Lim, J. Wei, J. Lin, Q. Li, J.K. You, Biosens. Bioelectron. 20 (2005) 2341–2346.
[22] M. Delvaux, S. Demoustier-Champagne, A. Walcarius, Electroanalysis 16 (2004) 190–198.
[23] F. Palmisano, P.G. Zambonin, D. Centonze, M. Quinto, Anal. Chem. 74 (2002) 5913–5918.
[24] M.J. Rogers, K.G. Brandt, Biochemistry 10 (1971) 4624–4630.
[25] K. Grennan, A.J. Killard, C.J. Hanson, A.A. Cafolla, M.R. Smyt, Talanta 68 (2006) 1591–1600.
[26] X.L. Luo, J.J. Xu, Y. Du, H.Y. Chen, Anal. Biochem. 334 (2004) 284–289.
[27] M. Şenel, M. Dervisevic, E. Çevik, Curr. Appl. Phys. 13 (2013) 1199–1204.
[28] S. Fabiano, C.T. Minh, B. Piro, L.A. Dang, M.C. Pham, O. Vittori, Mater. Sci. Eng. C 21 (2002) 61–67.
[29] P. Santhosh, K.M. Manesh, S. Uthayakumar, S. Komathi, A.I. Gopalan, K.P. Lee, J. Bioelectrochem. 75 (2009) 61–66.
[30] S. Zhang, L. Han, C. Hou, C. Li, Q. Lang, L. Han, A. Liu, J. Electroanal. Chem. 742 (2015) 84–89.