Electrochemical glucose biosensing via new generation DTP type conducting polymers/gold nanoparticles/glucose oxidase modified electrodes

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ABSTRACT

The synthesis of derivatives of dithionepyrrole is a hot subject that has been studied extensively. In this study, a novel approach for constructing different glucose biosensors using conducting polymers of 4-(4H-dithiyinol[3,2-b:2′,3′-d]pyroll-4-yl)aniline and 4-(4H-dithiyinol[3,2-b:2′,3′-d]pyroll-4-yl)-[1,1′-biphenyl]-4-amine is proposed. After the synthesis and characterizations of the dithionepyrrole type monomers, they were used in glucose bio-sensing applications. The surface of gold electrode was modified with mercaptoethane sulfonic acid and p-aminothiophenol functionalized gold nanoparticles and aniline modified GOx. Electrochemical measurements were carried out by following the consumed oxygen due to the enzymatic reaction of glucose oxidase. *DTP-Ph-NH$_2$/AuNP/GOx* and *DTP-Ph-Ph-NH$_2$/AuNP/GOx* biosensors showed very good linearity between 0.1 and 2.5 mM, and 0.05 and 1 mM for glucose, respectively. LOD value was obtained for p*DTP-Ph-NH$_2$/AuNP/GOx* as 5.00×10^{-2} mM and for p*DTP-Ph-Ph-NH$_2$/AuNP/GOx* as 9.86×10^{-5} mM using S/N ratio. Optimization of molar ratio amount of *AuNP/GOx*, cycles amount to immobilize *AuNP/GOx*, conducting polymer thickness were examined. Finally, under optimized conditions, the amount of glucose in spiked human serum samples and re-covery experiments were conducted.

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1. Introduction

Scientists have been trying to develop biosensors with great efforts for the wide fields of application including medical diagnostics, environmental monitoring, and food analysis for many years [1]. The biosensors can be described as chemical sensors in which the recognition system utilizes a biochemical mechanism [2–4]. The responses recorded are related to the concentration of a specific chemical or chemicals called as substrate [5,6]. The signal can be explained in terms of changes in resonance unit [7,8], UV–Vis-IR absorption [9–11], and mass [12,13], electrical [14,15] and photoelectrical properties [16]. The development of biosensors has become increasingly important in molecular diagnostics. They need new methods continuously to get higher sensitivity, lower cost, and better reproducibility. Because of their high sensitivity property, fluorescence [17] and chemiluminescence [18,19] are widely used in clinical diagnostic laboratories. However, the high instrument cost is a major disadvantage for these methods. Compared to optical detection methods, electrochemical methods are much simpler and not expensive. Among the various electrochemistry-based biosensors

developed so far, the amperometric glucose sensing approach has attracted a great deal of attention [20].

Conducting polymers, CPs, are used as suitable matrices supplying common properties for biomolecule immobilization [21]. Their compatibility with biological molecules, easy preparation, high reproducibility and electrochemical properties make them fascinating in biosensor design. Their fascinating features for biosensor design can be listed as their consistency with biological molecules, easy and fast preparation, high reproducibility and electrochemical properties [22]. CPs have been being used widely in several applications such as electrochromic devices [23], light emitting diodes [24], solar cells [25], enzyme immobilization matrices [26], drug release systems [27], and rechargeable batteries [28]. CPs show a well-organized molecular structure so that they are able to be used to function as a three dimensional matrix for immobilization of biomolecules. They provide as suitable microenvironments for the immobilization to get fast electron transfer [29–30].

In recent years, CPs have also been started to be utilized in the construction of photoelectrochemical biosensors and biofuel cells [31]. CP's have unique electronic and photovoltaic properties. Because of these, especially last years, the compounds of dithionepyrrole have been synthesized and optical, electronical and photovoltaic properties have been investigated in the preparation of OLEDs and OFETs. p(*DTP-*

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Ph-NH$_2$) and p(*DTP-Ph-Ph-NH$_2$*) are two of the novel new generation DTP (polydithienopyrrole) type conducting polymers which have been investigated as new units in conjugated materials because of their planar structure and fused ring systems [32]. These organic materials with conjugation properties have received considerable attention, since they can be processed in simpler and more cost-effective ways than their inorganic counterparts. Dithione [3,2-b:2′,3′-d] pyrroles (DTP) as fluorescent thiophene material has been the application of first reported by Zanirato [33,34]. Functionalization of DTPs with electron-releasing group also supports the development of high-energy electronic transitions based on materials such as the preparation of OLEDs, OFEDs as well as biosensors. This property helps to get fast direct electron transfer to electrode.

Another advantage of these materials is their simple and low-cost processing as well as their potential for electronic, optoelectronic and biosensing applications. The charge transport properties of p-conjugated materials play a key role in the design and fabrication of biosensors [22,23]. Therefore, these types of conducting polymers as immobilization matrices can have amazing properties in the design of biosensors. Considering the advantages of its homogeneous and manageable film character, tuneable physical and optical properties, stability and biocompatibility, and its reproducible and easy production, it is thought that DTP-NH$_2$ can be effectively used in biosensors. The application of polymers to the fabrication of biosensors is useful.

In this study, a novel approach for constructing highly sensitive two types of electrochemical glucose biosensors called as p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* and p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* is demonstrated. For the preparation of the biosensors, first of all, synthesis of the conducting polymer synthesis was achieved. These conducting polymers have not only aryl and alkyl groups but also —NH$_2$ functional groups which are used as electron supplying agents. 2-Mercaptoethane sulfonic acid and p-aminothiophenol functionalized gold nanoparticles and thioaniline modified glucose oxidase were also synthesized. Immobilization of aniline modified glucose oxidase, (GOx), onto conducting polymer modified gold electrode was achieved by electropolymerization technique. Optimization of molar ratio amount of *AuNP/GOx*, cycles amount to immobilize *AuNP/GOx*, conducting polymer thickness were examined. Finally, the amount of glucose in spiked human serum samples was determined using designed glucose biosensors.

2. Material and Methods

2.1. Reagents

Glucose oxidase (GOx, E.C. 1.1.3.4; 21,200 U/g), LiClO$_4$, NaClO$_4$, NaBH$_4$AlCl$_3$, succinyl chloride toluene, 2,2′-dithiophene D-glucose, HAuCl$_4$, and sodium citrate were purchased from Sigma (St. Louis, USA, www.sigmaaldrich.com). Dichloromethane and toluene were bought from Merck (Darmstadt, Germany, www.merck.com). N-(maleimidocaproyloxy) sulfosuccinimide ester (sulfo-EMCS) was obtained from PIERCE. Human serum from human male AB plasma was purchased from Sigma-Aldrich (St. Louis, MO, no need for ethical committee permission). Milli-Q water was obtained from purification system and all solutions were prepared with ultra-pure water from a Millipore-Milli-Q system.

2.2. Synthesis of Monomers and Polymers of DTP-Ph-NH$_2$ and DTP-Ph-Ph-NH$_2$

The monomer, 4-(4H-dithyinol[3,2-b:2′,3′-d]pyroll-4-yl)aniline, (DTP-Ph-NH$_2$) was synthesized from 0.308 mmol dibromo thiophene complex and 0.308 mmol 1,4-diaminobenzene. 0.616 mmol K$_2$CO$_3$, 0.074 mmol CuI and 0.12 mmol L-pyrolin were added to this mixture and it was dissolved in 4 mL dimethyl sulfoxide. The resultant mixture was stirred and refluxed for 24 h under argon. When the starting

materials were finished, which was controlled by TLC, the reaction has been canceled and the solution was saturated with saturated NaCl, the extraction was made (EtOAc (3 × 30 mL) and after it was dried with MgSO$_4$. The solvent was removed under vacuum and eluation was achieved with methanol using silica column. After removing the organic phase, with 20% efficiency the monomer DTP-Ph-NH$_2$ was obtained [35].

The monomer, 4-(4H-dithyinol[3,2-b:2′,3′-d]pyrol-4-yl)-[1,1′-biphenyl]-4-amine, (DTP-Ph-Ph-NH$_2$), was synthesized from 0.700 mmol dibromothiophene complex and 0.673 mmol benzidine. 0.06 mmol Pd$_2$dba$_3$, 0.148 mmol 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP), and 3.649 mmol NaO*t*Bu were added to this mixture and in 10 mL toluene, this solution was stirred and refluxed for 48 h under argon. When the starting material was finished, which was controlled by TLC, the reaction has been canceled and the solution was saturated with saturated NaCl, the extraction was made with EtOAc (3 × 30 mL) and after it was dried with MgSO$_4$. The solvent was removed under vacuum and eluation was achieved with methanol using silica column. After removing the organic phase, with 45% efficiency the monomer DTP-Ph-Ph-NH$_2$ was obtained. (Scheme 1.)

Polymerization of DTP-Ph-NH$_2$ and DTP-Ph-Ph-NH$_2$ was performed in the presence of tetrabutylammonium hexafluorophosphate in dichloromethane and acetonitrile medium using scan rate of 100 mV/S on gold electrode with cyclic voltammetry.

2.3. Synthesis of 2-Mercaptoethane Sulfonic Acid and Sulfonic Acid/p-Aminothyophenol Functionalized Gold Nanoparticles

2-Mercaptoethane sulfonic acid and p-aminothiophenol functionalized gold nanoparticles were synthesized with the following procedure. 10 mL of ethanol solution containing 197 mg HAuCl$_4$ was mixed with 5 mL of methanol solution containing 42 mg Mercaptoethane sulfonate and 8 mg p-aminothiophenol. 2.5 mL of glacial acid was added to this mixture and it was stirred in ice bath for 1 h. After that, 7.5 mL of 1 M NaBH$_4$ was added slowly to this mixture till a black solution was obtained. This mixture was stirred for 14 h in room temperature and washed with methanol, ethanol and diethylether solutions [36].

2.4. Modification of Glucose Oxidase

Glucose oxidase was dissolved in phosphate buffer (3 mL, pH 7,4) and N-(maleimidocaproyloxy)sulfosuccinimide ester (sulfo-EMCS, 52 mL, 12 mg/mL) was added to the solution. Subsequently stirring for 40 min, 1.6 mg/mL 4-aminothiophenol in ethanol was added to the medium. After 150 min reaction time, it was purified using G-25 (GE healthcare) column with the help of 0.1 M phosphate buffer. The obtained glucose oxidase solution was dried under high vacuum at 20 °C till it becomes a pale yellow powder [37]. (Scheme 2.)

2.5. Preparation of Enzyme Biosensor

After polymerization of the conducting polymers on gold electrode surface, mercaptoethane sulfonic acid and p-aminothiophenol functionalized gold nanoparticles and modified GOx were added to the medium. In 0.1 M phosphate buffer (pH = 7.4), between −0.1 V and +1.1 V, with 100 mV/s scan rate, immobilization of the enzyme in polymer matrix with gold nanoparticles was achieved. (Scheme 3.)

2.6. Measurements

Three electrode system including a graphite rod (d = 5 mm) counter electrode, calomel reference electrode and gold working electrode was used. All electrochemical experiments were carried out using an electrochemical analyzer with potentiostat (EG&G Model 263). Amperometric experiments were examined in room temperature (25 °C) using 10 mL 50 mM phosphate buffer. The current density was
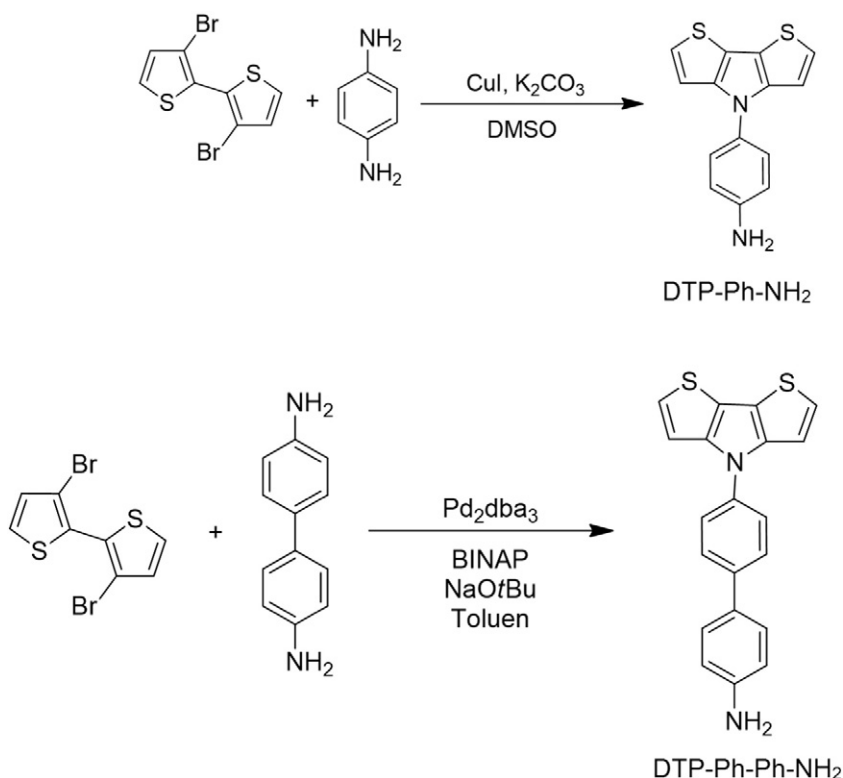
Scheme 1. Synthesis and structures of DTP-Ph-NH$_2$ and DTP-Ph-Ph-NH$_2$.

calculated with the oxygen consumption after adding glucose under −0.7 V. In characterization experiments of the synthesized monomers, ^{1}H NMR Bruker 400 MHz, ^{13}C NMR Bruker 100 MHz, ^{1}H NMR Varian 200 MHz, ^{13}C NMR Varian 50 MHz, Perkin Elmer 1000 FTIR Spectrometers were used.

2.7. Analytical Characterization of the Biosensors

Calibration curves were obtained by plotting photocurrent vs. substrate concentration and y = mx + n equations were obtained where y is the sensor response in current (nA) and x is the substrate concentration. Limit of detection (LOD) was calculated from the equations of LOD = 3S/N using the standard deviation of response (s) and the slope of the calibration curve.

2.8. Real Sample Application

The proposed amperometric biosensors were tested to analyze glucose in spiked human serum. No sample pretreatment was required for the analysis. Known amount of glucose and acetonitrile was added to the human serum samples and it was ultrasonicated for 10 min at 2500 rpm. Supernatant part was taken and glucose response was recorded for different concentration. The glucose detection from human serum (human male AB plasma) was achieved by recovery tests after addition of known amounts of glucose to the human serum plasma and dilution with phosphate buffer. Applicability of the proposed

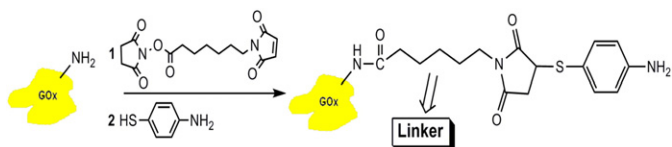**Scheme 2.** Modification of glucose oxidase.

method was confirmed by utilizing recovery studies in spiked human serum analyses.

3. Results and Discussions

In order to determine the surface morphologies of p(DTP-Ph-NH2) and p(DTP-Ph-Ph-NH2) films and, p(DTP-Ph-NH2)/AuNP/GOx and p(DTP-Ph-Ph-NH2)/AuNP/GOx electrodes, scanning electron microscopy (SEM) technique was used. Scanning electron micrographs of polymer films; p(DTP-Ph-NH2) and p(DTP-Ph-Ph-NH2) films and p(DTP-Ph-NH2)/AuNP/GOx and p(DTP-Ph-Ph-NH2)/AuNP/GOx electrodes are given in Fig. 1A, B, C, D respectively. It can be seen that the surface morphologies of the polymer films were completely different compared to p(DTP-Ph-NH2)/AuNP/GOx and p(DTP-Ph-Ph-NH2)/AuNP/GOx structures.

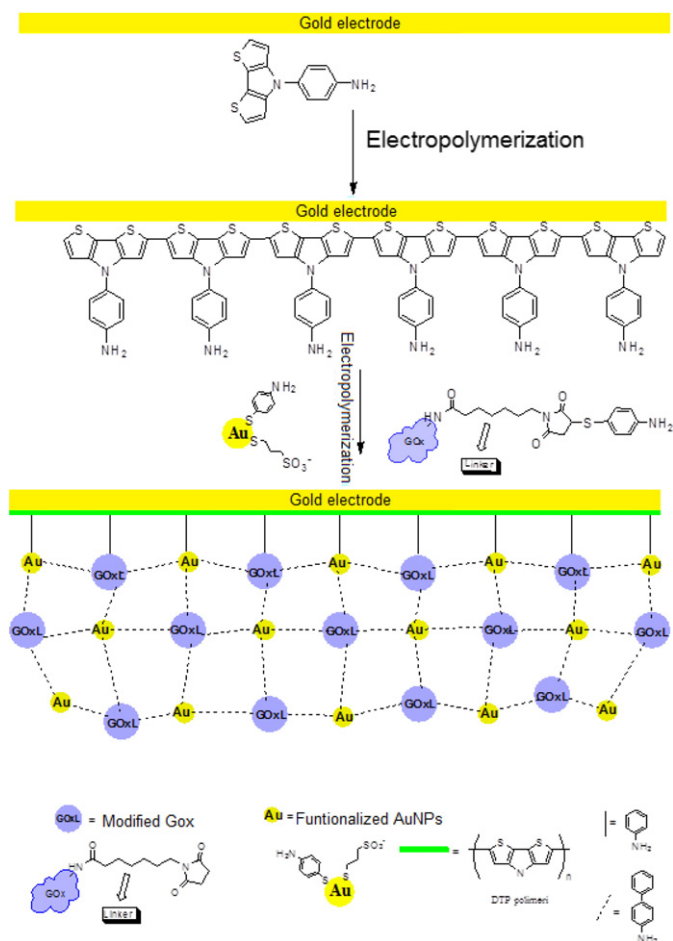### 3.1. Synthesis of the DTP-Ph-NH$_2$ and DTP-Ph-Ph-NH$_2$

In the first step, 3,3′-Dibrom-2,2′-dithiophene was synthesized. 30.80 mmol brom was slowly added to the mixture of 2,2′-dithiophene and glacial acetic acid. This mixture was stirred in room temperature for 16 h, and in oil bath for 24 h at 90 °C. After cooling at room temperature, 6.33 mmol Zinc powder was slowly added. This mixture was also stirred in room temperature for 16 h at 90 °C. Using vacuum, glacial acetic acid was removed. The mixture was dissolved in 100 mL of CH$_2$Cl$_2$, and washed with 10% KOH (2 × 50 mL) and distilled water (1 × 50 mL). Organic phase was dried using MgSO$_4$ and solvent was removed using vacuum system. The product was characterized by ^{1}H NMR (400 MHz, CDCl$_3$ (δ 7.40 (d, J = 5.4 Hz, A part of AB system, =CH, 2H), 7,12 (d, J = 5.4 Hz, B part of AB system, =CH, 2H))), ^{13}C NMR (100 MHz, CDCl$_3$, δ 130.8, 128.9, 127.5, 112.7), IR (cm^{-1}, 3105.2, 3087.0, 2925.0, 2854.8, 2519.0, 2351.0, 2146.4, 1737.1, 1712.7, 1550.9, 1444.0, 1340.2, 1157.0, 857,6). The spectrum was given in supporting information as figure SI 1.

Scheme 3. Preparation of the biosensors.

Fig. 1. SEM images of A) p(DTP-Ph-NH$_2$), B) p(DTP-Ph-Ph-NH$_2$), C) p(DTP-Ph-NH$_2$)/AuNP/GOx and D) p(DTP-Ph-Ph-NH$_2$)/AuNP/GOx.
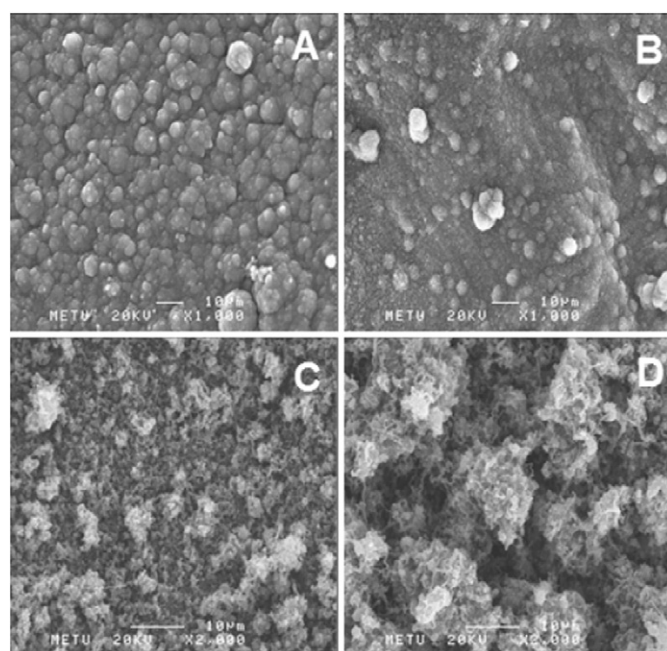
After that *DTP-Ph-NH$_2$* and *DTP-Ph-Ph-NH$_2$* were synthesized as mentioned in experimental section. These conducting polymers were characterized by ^{1}H NMR, ^{13}C NMR and IR. The spectrum was given in supporting information as figures SI2 and SI3.

For *DTP-Ph-NH$_2$*; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.37 (d, J = 8.6 Hz, A part of AB system, =CH, 2H), 7,16 (d, J = 5.3 Hz, A part of AB system, =CH, 2H), 7.10 (d, J = 5.3 Hz, B part of AB system, =CH, 2H), 7,84 (d, J = 8.6 Hz, B part of AB system, =CH, 2H), 3.84–3.78 (bs, NH2, 2H), ^{13}C NMR (100 MHz, CDCl$_3$): δ 144.94, 144.61, 131.15, 124.51, 123.07, 115.92, 115.80, 112.08, IR (cm^{-1}): 3737.3, 3622.2, 3366.7, 3057.3, 3024.9, 2924.1, 2856.7, 2337.6, 1707.9, 1600.0, 1571.2, 1495.6, 1448.8, 1398.4, 1304.9 [35].

For *DTP-Ph-Ph-NH$_2$*; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.68 (d, J = 8.8 Hz, A part of AB system, =CH, 2H), 7.60 (d, J = 8.8 Hz, B part of AB system, =CH, 2H), 7.46 (d, J = 8.7 Hz, A part of AB system, =CH, 2H), 7.20 (d, J = 5.3 Hz, A part of AB system, =CH, 2H), 7.18 (d, J = 5.3 Hz, B part of AB system, =CH, 2H), 6.79 (d, J = 8.7 Hz, B part of AB system, =CH, 2H), 3.87–3.70 (bs, NH$_2$, 2H), ^{13}C NMR (100 MHz, CDCl$_3$) δ 145.85, 138.95, 128.26, 127.88, 127.52, 123.63, 123.41, 122.89, 122.83, 115.54, 112.30, 112.26. IR (cm^{-1}) 3661, 3397, 3327, 3201, 2988, 2901, 1888, 1605, 1497, 1409, 1263, 1174, 1066.

3.2. Electropolymerization of DTP-Ph-NH$_2$ and DTP-Ph-Ph-NH$_2$

Electropolymerization of *DTP-Ph-NH$_2$* and *DTP-Ph-Ph-NH$_2$* was performed in the presence of tetrabutylammonium hexafluorophosphate in dichloromethane and acetonitrile medium using scan rate of 100 mV/S on gold electrode with cyclic voltammetry. Ag|AgCl was used as the reference electrode and DTP-Ph-NH$_2$ has an oxidation

peak at +1.25 V [32]. Detailed electrochromic studies were performed in previous study [32]. As it can be clearly seen from Fig. 2, for DTP-Ph-Ph-NH$_2$ it has oxidation peaks at +0.3 V and +1.1 V. Continuous cyclic voltammograms were obtained and increasing thickness indicates that the polymer is formed on the surface of the electrode (Fig. 2). Electrical conductivity measurements were carried out by using standard four-probe technique. The conductivities of p(DTP-Ph-NH$_2$) and p(DTP-Ph-Ph-NH$_2$) were measured as 1.17 and 1.85 S/cm, respectively, after synthesizing of these polymer under optimum conditions. Conductivities of p(DTP-Ph-NH$_2$)/Au NP/GOx and p(DTP-Ph-Ph-NH$_2$)/Au NP/GOx structures were also measured, after getting of these structures under optimum conditions. Conductivity values of these structures were found as 3.68 and 6.45 S/cm for p(DTP-Ph-NH$_2$)/AuNP/GOx and p(DTP-Ph-Ph-NH$_2$)/AuNP/GOx structures respectively.

3.3. Preparation and Optimization of the Biosensors

For p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* biosensor after 100 cycles of monomer, and for p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* after 60 cycles of *DTP-Ph-Ph-NH$_2$* on gold electrode surface, mercaptoethane sulfonic acid and p-aminothiophenol functionalized gold nanoparticles and aniline modified GOx were added to the medium. In 0.1 M phosphate buffer (pH = 7.4), between −0.1 V and +1.1 V, with 100 mV/s scan rate, immobilization of the enzyme in polymer matrix with gold nanoparticles was achieved. In Fig. 3. Cyclic voltammogram of the biosensor can be followed.

Modified gold nanoparticle has the affinity for aniline modified GOx, therefore the immobilization quality directly related with the ratio between AuNP and GOx added to the biosensor. Glucose responses were checked for 0.5 mM glucose and optimum ratio was obtained when the biosensor was prepared 1.1:1 [AuNP]/[GOx] ratio for p*DTP-Ph-Ph-NH$_2$/AuNP/GOx* biosensor and 1.5:1 [AuNP]/[GOx] ratio for p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* biosensor as seen in Fig. 4. Above these values due to high covalent binding of AuNPs to GOx, conductivity of the AuNP may be affected, and this may cause decrease in glucose biosensing.

Optimization of polymer thickness which affects the biosensor response was performed for the monomers of *DTP-Ph-NH$_2$* and *DTP-Ph-Ph-NH$_2$*. For p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* biosensor monomer of *DTP-Ph-NH$_2$* was polymerized between 20–120 cycles and the biosensors
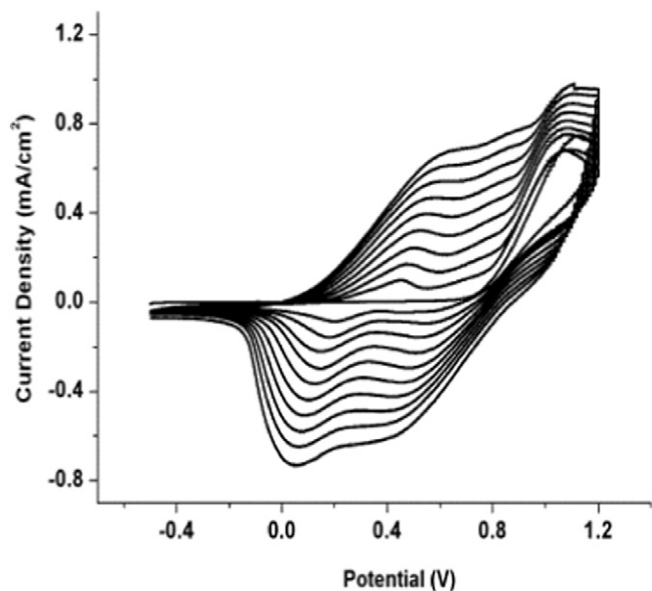
Fig. 2. Cyclic voltammograms of *DTP-Ph-Ph-NH₂* in TBAPF₆/Dichloromethane/acetonitrile medium with a scan rate of 100 mV/S.
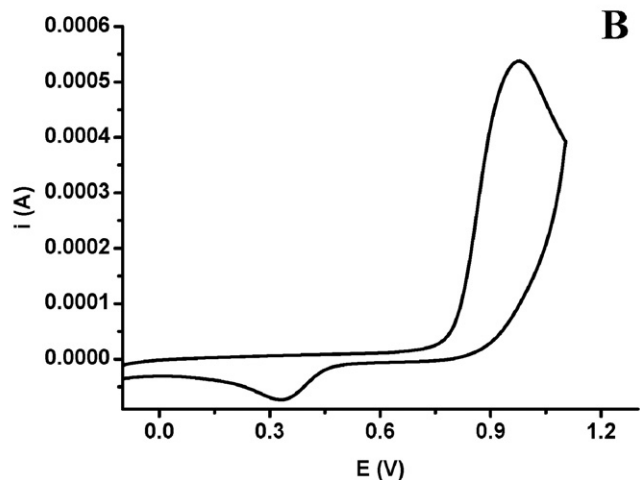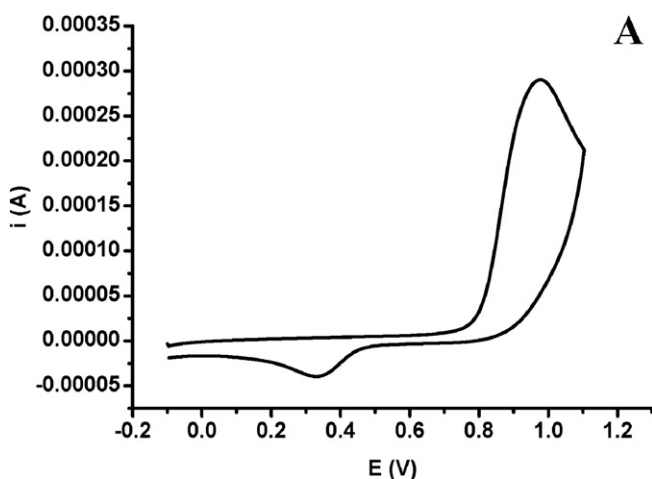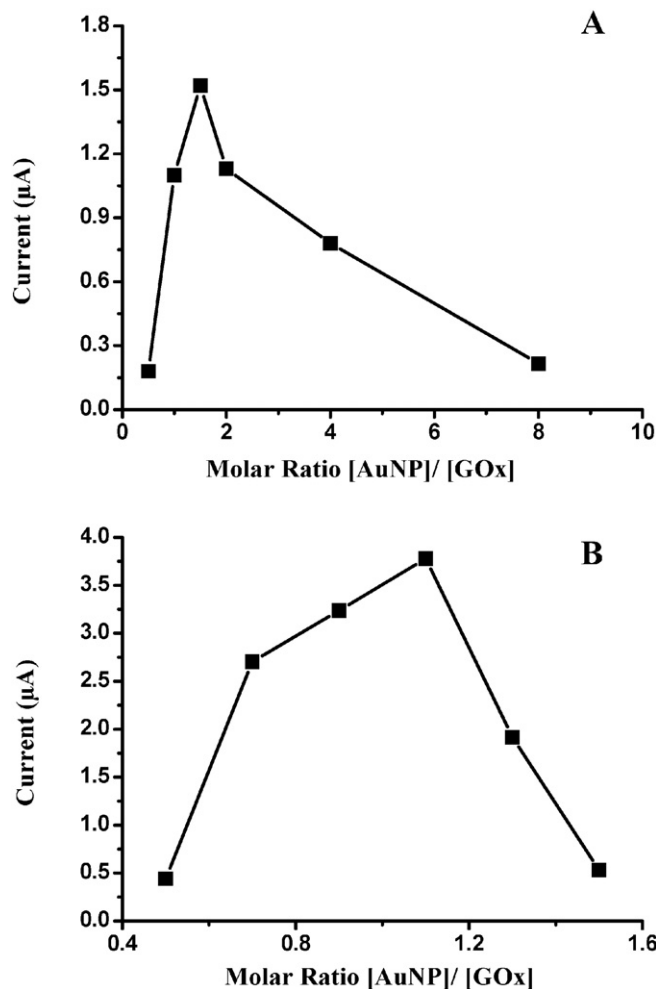

Fig. 4. Optimization of molar ratio for *AuNP/GOx* towards 0.5 mM glucose. A) P(*DTP-Ph-NH₂*)/*AuNP/GOx* biosensor. B) p(*DTP-Ph-Ph-NH₂*)/*AuNP/GOx* biosensor.

were tested towards 0.5 mM glucose. The optimum polymer thickness was obtained with 100 cycles. For p(*DTP-Ph-Ph-NH₂*)/*AuNP/GOx* biosensor between 20–90 scans of *DTP-Ph-Ph-NH₂*, different biosensors were prepared and their glucose response was followed towards 0.5 mM glucose. 60 scans of the monomer gave the highest response and after this amount the thickness of the electrode increases and immobilization cannot be obtained in a good way. For further experiments 60 scans of *DTP-Ph-Ph-NH₂* were used as shown in Fig. 5.

The amount of the cycles to modify the surface of conducting polymer with [AuNP]/[GOx], directly affects the amount of GOx immobilized. For p(*DTP-Ph-NH₂*)/*AuNP/GOx* biosensor, using 1.5/1 [AuNP]/[GOx] amount, different cycles of [AuNP]/[GOx] were performed between 10 and 100. As the amount of cycles increases the amount of GOX immobilized also increases up to 80 scans. Above 80 scans, despite the increase in GOx amount the glucose response did not increase which may be due to increase also in AuNP may causes aggregations and decrease in electron transfer rate to the electrode. When 80 cycles of [AuNP/GOx] were performed, highest response toward 0.5 mM glucose was obtained and this was used for further experiments (Fig. 6A). For p(*DTP-Ph-Ph-NH₂*)/*AuNP/GOx* biosensor with different cycle amounts between 5–50 cycles, using 1.1/1 [AuNP]/[GOx] amount, glucose biosensing towards 0.5 mM glucose was tested. When the biosensor was prepared using 20 cycles of [AuNP]/[GOx] the highest response in current was obtained. After 20 cycles, amperometric responses decrease due to difficulties in electron transfer to the electrode due to high GOx loading (Fig. 6B).
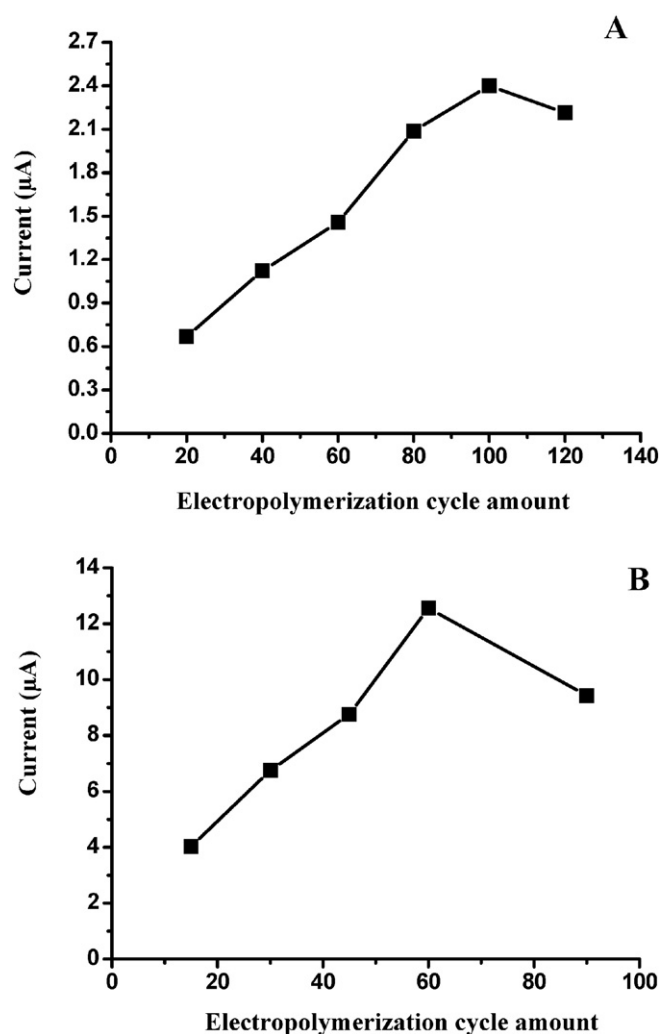

Fig. 3. Cyclic voltammogram of A) p(*DTP-Ph-NH₂*)//*AuNP/GOx* biosensor and B) p(*DTP-Ph-Ph-NH₂*)//*AuNP/GOx* biosensor in phosphate buffer, pH 7.4 with a scan rate of 500 mV/s.

Fig. 5. Electropolymerization cycle amount optimization for A) p(*DTP-Ph-NH$_2$*)//*AuNP/GOx* biosensor and B) p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* biosensor.

Fig. 6. Optimization of cycles amount for *AuNP/GOx* towards 0.5 mM glucose. A) p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* biosensor. B) p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* biosensor.

Under optimized conditions, glucose biosensing was analytically characterized between 0.1 mM and 2.5 mM glucose concentration for p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* biosensor and 0.05 mM–1 mM glucose concentration for p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* biosensor, respectively (Fig. 7). LOD value was obtained for p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* as 5.00×10^{-2} mM and for p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* as 9.86×10^{-5} mM using S/N ratio.

3.4. Sample Application

Glucose amount in spiked human blood samples was tested and recovery analyses were performed to test the accuracy of the designed biosensors. Using p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* biosensor 96.5% recovery was obtained with RSD% value of 0.93% whereas using p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* biosensor 99.4% recovery was obtained with RSD% value of 0.87%.

4. Conclusion

In conclusion, in this research a novel approach for constructing different glucose biosensors using conducting polymers of 4-(4H-dithyinol[3,2-b:2′,3′-d]pyroll-4-yl)aniline and 4-(4H-dithynol[3,2-b:2′,3′-d]pyroll-4-yl)-[1,1′-biphenyl]-4-amine was performed. The surface of gold electrode was modified with mercaptoethane sulfonic acid and p-aminothiophenol functionalized gold nanoparticles and aniline
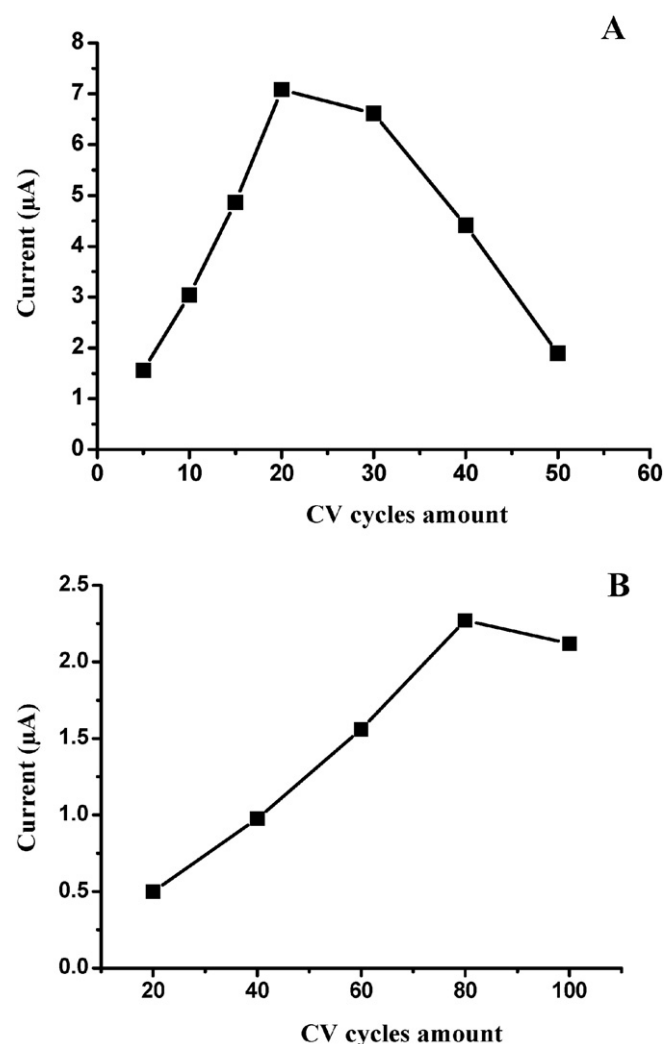
modified GOx. Electrochemical measurements were carried out by following the consumed oxygen due to the enzymatic reaction of glucose oxidase. p(*DTP-Ph-NH$_2$*)/*AuNP/GOx* and p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* biosensors showed a very good linearity between 0.1 and 2.5 mM, and 0.05 and 1 mM for glucose, respectively. LOD value was obtained for p(*DTP-Ph-NH$_2$*)/*GOx/AuNP* as 5.00×10^{-2} mM and for p(*DTP-Ph-Ph-NH$_2$*)/as 9.86×10^{-5} mM using S/N ratio. p(*DTP-Ph-Ph-NH$_2$*)/*AuNP/GOx* was better in performance compared to p(*DTP-Ph-NH$_2$*)/*AuNP/GOx*. The resulted biosensors were compared with other conducting polymer based biosensors and given in Table 1.

It can be concluded that, increasing the conjugation by Ph substitution to the conducting polymer, the glucose biosensing was increased. Optimization of molar ratio amount of *AuNP/GOx*, cycles amount to immobilize *AuNP/GOx*, conducting polymer thickness were examined. The designed biosensors were used to determine glucose in spiked blood samples in terms of recovery%. Both biosensors showed reliable recovery results. These modification strategies can be light to future researches and could be used in glucose or other important analytes.

Acknowledgments
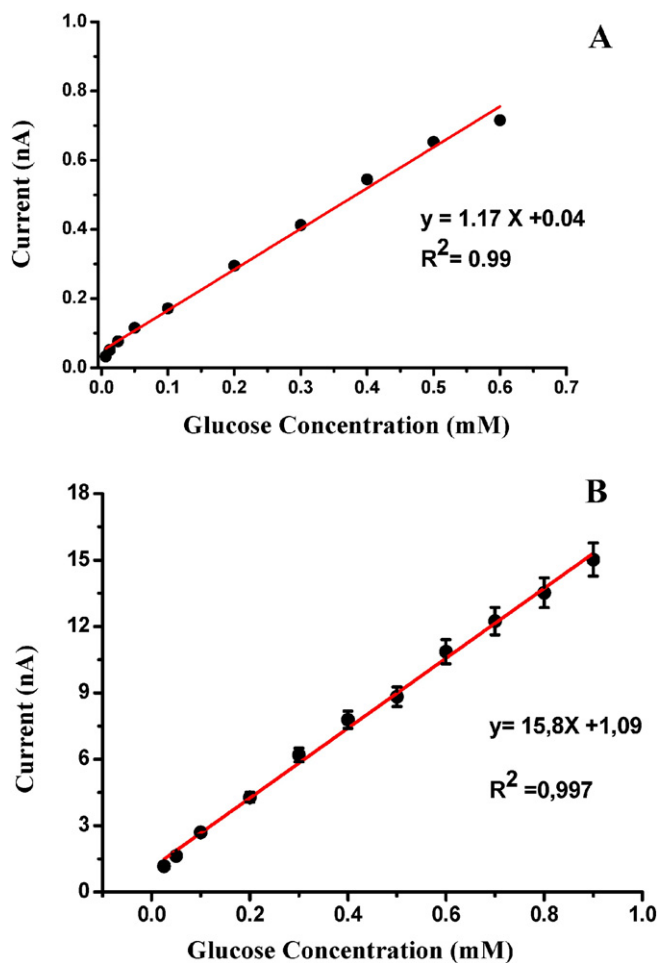
Fig. 7. Calibration graphs for A) p(*DTP-Ph-NH₂*)/*AuNP*/*GOx* biosensor and B) p(*DTP-Ph-Ph-NH₂*)/*AuNP*/*GOx* biosensor.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jelechem.2016.03.034.

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Table 1
Some features of conducting polymer-based biosensors in the literature.

Electrode	Conducting polymer	Principle of detection (working potential)	Linearity for glucose	LOD	Ref
GE	TBT	CA (-0.7 V)	0.1–2.5 mM	29.3 μM	[38]
GE	SNS-NH₂/AuNP	CA (-0.7 V)	0.002–5.0 mM	2.1 μM	[39]
GE	SNS-NH₂/SNS NH₂Fe/GOx	CA ($+0.45$ V)	0.5–5.0 mM	0.18 mM	[40]
GE	DTP-Aryl-NH₂	CA (-0.7 V)	0.05–1.0 mM	5 μM	[41]
PGE	DTP-Alkyl-NH₂	CA (-0.7 V)	0.3–25 mM	0.074 μM	[42]
Pt E	GOx/PtNP/PA	CA ($+0.56$ V)	0.01 to 8 mM	0.7 μM	[43]
Pt E	Al₂O₃/PPy/GOx	CA ($+0.4$ V)	0.5–10 mM	30 μM	[44]
Gold E	DTP-Ph-NH₂/GOx/AuNP	CA (-0.7 V)	0.1–2.5 mM	5.00×10^{-2} mM	This Work
Gold E	DTP-Ph-Ph-NH₂/AuNP/GOx	CA (-0.7 V)	0.05–1.0 mM	9.86×10^{-2} μM	This Work

Abbreviations: CA: Chronoamperometry; GE: Graphite electrode; PGE: Pencil graphite electrode; TBT: 2-Dodecyl-4,7-di(thiophen-2-yl)-2H-benzo[d][1,2,3]triazole; SNS-NH₂: 4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl)benzenamine; AuNP: Gold nanoparticle; PBDT: Poly(4,7-di(2,3)-dihydrothienol[3,4-b][1,4]dioxin-5-yl-benzo[1,2,5]thiadiazole); DTP-NH₂: N-functionalized dithienopyrroles; Ferrocene (Fe). PtNP: Platinum nanoparticles; PA: polyaniline; PPy: polypyrole.

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