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*-RESEARCH ARTICLE-*

# Poly(N-Methylpyrrole)-Chitosan layers for Glucose Oxidase Immobilization for Amperometric Glucose Biosensor Design

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**Abstract**

In this study, Pt electrode was coated by poly(N-methypyrrol) (PNMP) film, then Glucose Oxidase (GOD) was immobilized onto PNMP layer with thin chitosan (Chi) gel and finally, electrode was reacted with glutaraldehyde (GAL) to form crosslinking between $–NH_2$ groups of Chi and GOD to prevent enzyme leakage from Chi. GOD-based electrode was used to measure current response depending on glucose concentration by chronoamperometric method. Due to preparation of electrode conditions have significant effect on current values which were measured and optimized in presence of glucose, polymer synthesis and GOD immobilization conditions detailed. Therefore, the effect of N-methylpyrrole monomer concentration, scan rate, Chi concentration, GOD concentration and GAL concentration on biosensor response was investigated by classical method. In sight of obtained data, optimal monomer concentration and scan rates for PNMP synthesis were determined as 50 mM and 20 mV/s, respectively. Optimal Chi, GOD and GAL concentrations were found as 1,00%, 4 mg/mL and 0.025 %, respectively. SEM images of Pt, PNMP coated Pt and GOD immobilized Pt electrodes were obtained; Imax and $K_M$ values were calculated using Lineweaver-Burk plot. After 20 successive uses of same enzyme electrode in 5 mM glucose solution, it kept still its 91.3 % of initial activity.

**Keywords:**

Glucose, poly(N-methylpyrrole), amperometric biosensor, glucose oxidase, Chitosan

**Article history:**

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## Introduction

Determination of glucose level in biological samples such as in blood, urine *etc.* and also in several foods such as fruit juice, beverages *etc.* is very common procedure. Up to date, amperometric, potentiometric, coulometric, and impedimetric glucose biosensors have been developed. Amongs them, the amperometric glucose biosensors are poised to play a leading role in blood glucose monitoring owing to its simplicity and easy-to-use methodology. The GOD based glucose biosensors rely on the biocatalytic reaction involving the reduction of the flavin group (flavin adenine dinucleotide, FAD) of GOD by glucose. The reduced form of the flavin group ($FADH_2$) then reacts with molecular oxygen present in sample to regenerate the oxidized form of GOD (FAD). And subsequent measurements of hydrogen peroxide provide an indirect means for the quantification of glucose concentration (Deng et al. 2014). Princple of GOD based amperometric biosensor was given in Figure 1.

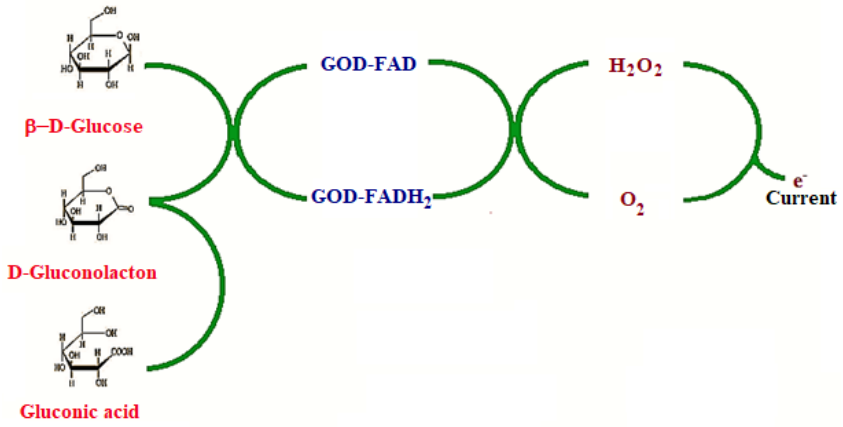


Figure 1. Principle of GOD based amperometric glucose biosensor

Construction of the enzyme containing biosensor is required immobilization of protein molecule onto/into the electrode which plays an important role in the research of glucose biosensor. There are several strategies on immobilization techniques to get GOD biosensor such as crosslinking with glutaraldehyde (Kadam *et al.*(all the et al should be in italic form) 2011, Shkotova et al. 2016), applying on an electrode with a gel matrix (Huang et al. 2012, Pauliukaite et al. 2010, Haighi et al. 2012), entrapment or incorporation in polymer matrix during electropolymerization (Ozyilmaz et al. 2011, Uang *et al.*2003, Eftekhari 2004), by covalent attachment (Abu-Rabeah et al. 2009, Ekiz et al. 2011, Shervedani et al. 2007) or adsorbed on electrode surface (Guadalupe de Jesus et al. 2013, Salimi et al. 2011, Jiang et al. 2012). Chitosan is a type of natural cationic polymer, which has shown attractive characteristics such as film–forming ability, permeability, and good adhesion. Therefore, chitosan can be used as a gel matrix for enzyme immobilization through GAL or another reagent (Li et al. 2012). Several electropolymers were used to fabricate the glucose biosensor such as polypyrrole (PPy) (Ozyilmaz et al. 2011, Raicopol et al 2013, Olea et al 2008), polyaniline (PANI) (Ozdemir et al 2010, Yao et al. 2015, Tang et al 2015), poly(o-anisidine) (POA) (Savale & Shirsat 2009, Borole et al. 2007), poly(o-phenylenediamine) (Rothwell et al 2010), polythophen derivative (Abasiyanik et al. 2010).

The present study deals with the fabrication of a glucose biosensor based on redox polymer as PNMP. Construction of biosensor was optimized in terms of polymer film synthesis as well as

enzyme immobilization parameters. Enzyme electrodes were characterized by SEM images, kinetic parameters and operational stabilities.

## Materials and Methods

### *Chemicals*

*Aspergillus niger* origin GOD (EC 1.1.3.4), N-methlypyrrole (NMPy), chitosan (Chi), glucose anhydrous, glutaraldehyde (GAL) were purchased from Sigma. All other reagents were of analytical grade and used without further purification.

### *Preparation of enzyme electrodes*

Enzyme electrode was prepared including three steps. Firstly, PNMP film were synthesized on Pt electrode. Secondly, PNMP/Pt electrode was immersed in GOD contained Chi solution for 6 seconds (GOD-Chi/PNMP/Pt) and electrode was dried for 2 hour open to atmosphere. Lastly, GOD-Chi/PNMP/Pt electrode was interacted with GAL solution for 10 sec for crosslinking between amine groups of enzyme and Chi to hinder GOD leakage. Electrodes were stored at 4°C when they were not used.

### *Synthesis of Homopolymer films*

PNMP film was achieved in a single compartment cell with three electrode configurations. The reference electrode was an Ag/AgCl (3 M KCl) electrode and the counter electrode was a platinum plate with a surface area of 0.25 $cm^2$. CHI 660b model electrochemical analyzer (serial number: A1420) was employed in electrochemical experiments. All of the potential values were referred to the Ag/AgCl (3 M KCl) electrode. PNMP film was synthesized onto Pt electrode with 0.25 $cm^2$ surface area by cyclic voltammetry technique in monomer containing 0.15 M sodium oxalate electrolyte solution applying potential range between -0.5 and +1.5V. Potential range was chosen after preliminary studies.

Biosensor electrode was optimized in terms of NMP monomer concentration, scan rate, GOD concentration, Chi concentration and GAL concentration.

### *Electrochemical measurements*

Electrochemical experiments were performed in a single compartment cell with three electrode configurations. The reference electrode was an Ag/AgCl (3 M KCl) electrode and the counter electrode was a platinum plate with a surface area of 0.25 $cm^2$. CHI 660b model electrochemical analyzer (serial number: A1420) was employed in electrochemical experiments. All of the potential values were referred to the Ag/AgCl (3 M KCl) electrode. The enzyme activity on the biosensor response was monitored chronoamperometrically at 0.60 V. The chronoamperometric measurements were performed at room temperature in steady state conditions in potassium phosphate buffer (50 mM, pH 7.0) solution while each measurement was lasted 120 s.

### *Characterization of Enzyme Electrode*

After determining the optimal construction parameters of enzyme electrode, SEM images at different preparation stages were obtained. Then, by using current values depending on glucose concentration, Michaelis-Menten and Lineweaver-Burk plots were obtained and Imax and $K_M$

kinetic parameters were calculated. Operational stability of enzyme electrode was investigated by 20 successive use in 5 mM of glucose solution.

**Results**

***The effect of monomer concentration on enzyme electrode efficiency***

PNMP synthesis was carried out using N-methylpyrrole (NMP) containing 0.15 M of sodium oxalate ($Na_2C_2O_4$) electrolyte solution. PNMP film was achieved by cyclic voltammetry technique applying potential range between -0.5 and +1.5V. To choose potential range, several preliminary studies were carried out. Firstly, the effect of NMP monomer concentration at PNMP synthesis on current response was investigated. Therefore, NMP concentration was changed as 10, 25, 50 and 75 mM while scan rate was kept constant at 50 mV/s by applying 10 segments. Then, PNMP coated electrodes were immersed into 2 mg/ml GOD containing 0.5% Chi solution for 6 s GOD-Chi and finally double layered electrodes were kept in %0.1 GAL solution for 10 s. After 20 hours storage at 4 °C, current response was measured by chronoamperometric method at 0.6 V constant potential. Glucose solution was continuously stirred during measurement. The net current value that was calculated by subtracting current value of glucose-free buffer solution from that of glucose-containing solution. All electrodes were compared according to current values depending on glucose concentration. Figure 2 was obtained by proportion the current values to the highest current value obtained in the study. As seen in Figure 2, except enzyme electrode which was prepared using 75 mM NMP, all electrodes showed linear current response with glucose concentration. The highest efficiency and linearity was observed for enzyme electrode which was prepared by 50 mM NMP monomer concentration. Thus, PNMP film was synthesized using 50 mM NMP monomer at the subsequent studies.

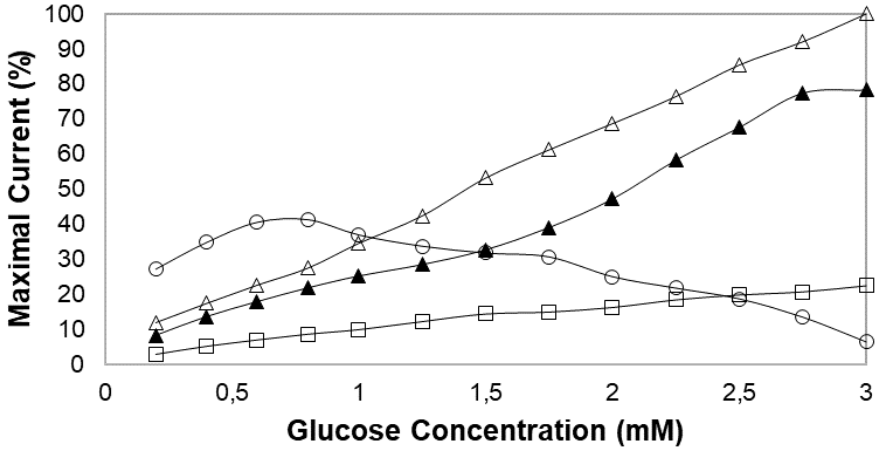


Figure 2. The effect of NMP monomer concentration on the efficiency of enzyme electrode. □: 10 mM; ▲:25 mM △:50 mM; O: 75 mM NMP

***The effect of scan rate on enzyme electrode efficiency***

In this stage of study, PNMP film was synthesized at 50 mM monomer concentration applying three different scan rate as 20, 50 and 100 mV/s and maximal current % values depending on glucose concentration was given in Figure 3. The highest results were observed when PNMP film

was synthesized by applying 20 mV/s scan rate in Figure 3. Also, current responses decreased by increasing scan rate for applying PNMP film synthesis.

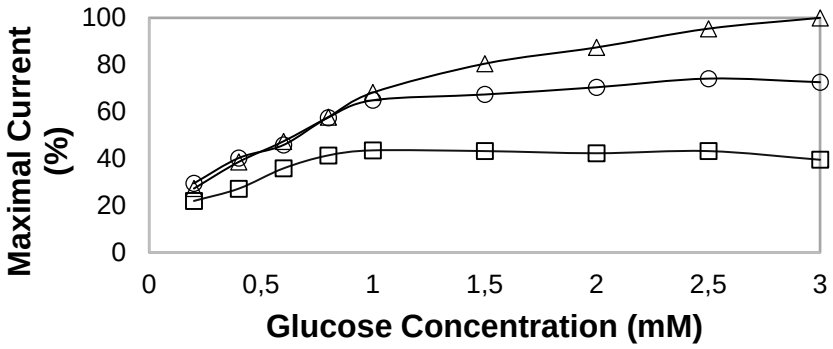


Figure 3. The effect of NMP monomer concentration on the efficiency of enzyme electrode. Scan rate: △:20 mV/s; O: 50 mV/s; □:100 mV/s

PNMP film growth curves was seen in 15 mM sodium oxalate and 50 mM NMP monomer mix solution and applying 20 mV/s scan rate and 10 segment in Figure 4. As seen in Figure 4, current values between -0.50 V and 0.50 V were approximately zero.

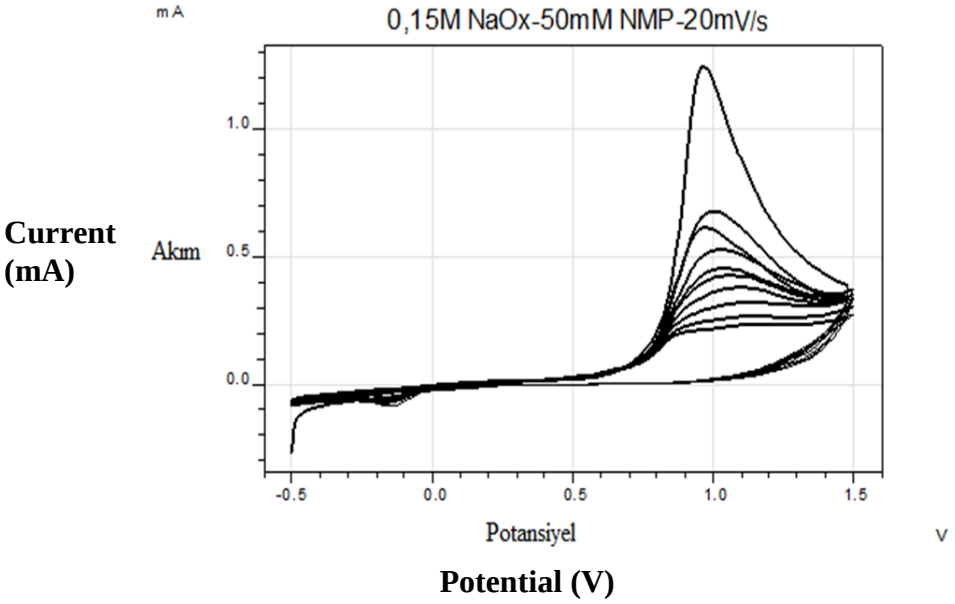


Figure 4. PNMP film growth curves (50 mM NMP in 0.15 sodium oxalate, 20 mV/s scan rate, 10 segment)

The current increase started from 0.5 V to approximately 1.0 V which was corresponding to monomer oxidation. Current value decreased during film growth depending on segment number due to steadily decreasing in conductivity of PNMP film at each cycle.

***The effect of chitosan concentration on enzyme electrode efficiency***

PNMP film was synthesized at optimum conditions and enzyme electodes were constructed using chitosan solutions at different concentrations between 0.1-1.0 % with 2 mg/ml GOD. The current response depending on glucose concentration was given in Figure 5.

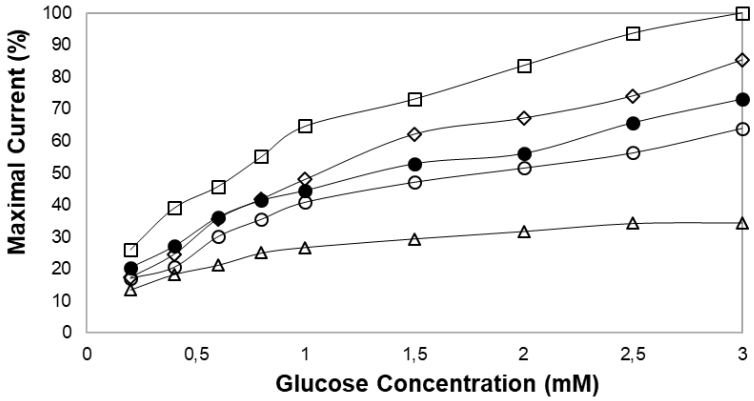


Figure 5. The effect of chitosan concentration on the efficiency of enzyme electrode. (△: 0.10%; O: 0.25%; ●: 0.50%, ◇: 0.75 %and □: 1.0 % Chi)

Figure 5 shows that current response depending on glucose concentration increased by increase in Chi concentration which was used at biosensor construction. The highest values were obtained for 1.0 % Chi concentration.

***The effect of GOD concentration on enzyme electrode efficiency***

Enzyme electrodes were prepared at optimal conditions by chancing GOD concentration between1 and 4 mg/ml in %1 Chi gel. Current responses were given in Figure 6. As seen, the highest values were get when enzyme electrode was prepared using 4 mg/ml GOD concentration.

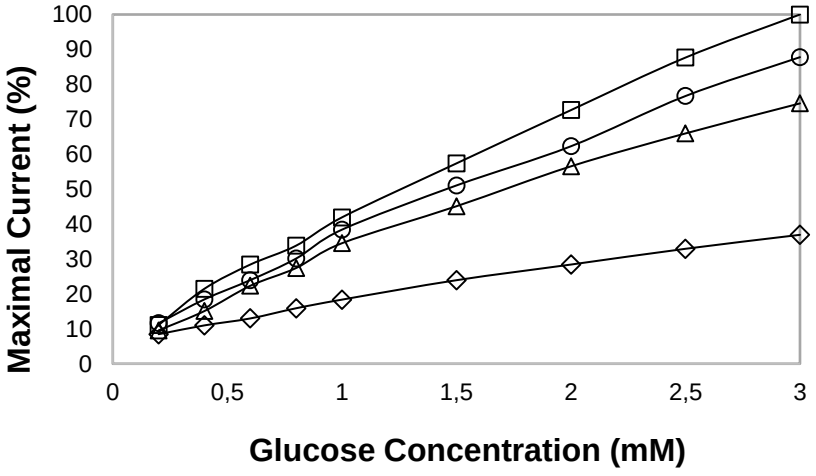


Figure 6. The effect of GOD concentration on the efficiency of enzyme electrode (◇: 1 mg/ml, △: 2 mg/ml; O: 3 mg/ml ; and □: 4 mg/ml)

***The effect of GAL concentration on enzyme electrode efficiency***

Enzyme electrodes which were constructed by 1% Chi and 4 mg/ml GOD were modified using GAL at different concentrations ranging between 0.025-0.10 %. Figure 7 shows the current response depending on GAL concentrations.

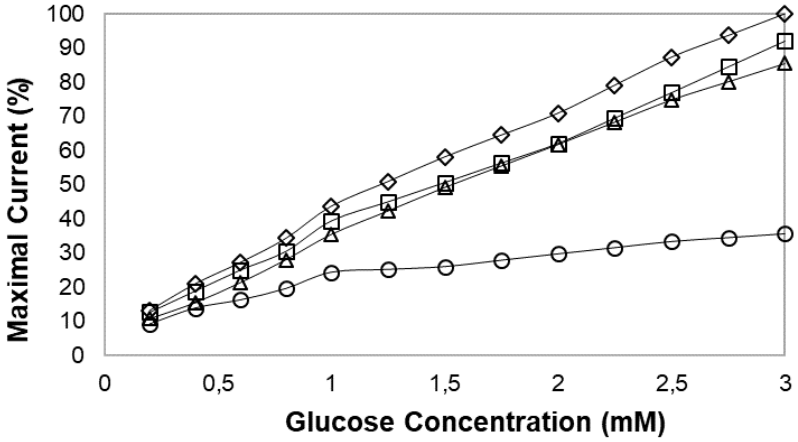


Figure 7. The effect of GAL concentration on the efficiency of enzyme electrode (O: 0.025 %; □: 0.050%; △: 0.075 % and ◇: 0.10 % GAL)

As seen in Figure 7, the lowest and highest current values were observed for 0.025 and 0.10 % GAL concentrations, respectively, while results obtained for those of 0.05 and 0.075 % GAL concentrations were very similar.

***Characterization of enzyme electrodes***

Enzyme electrode was prepared at optimal conditions. Firstly, PNMP film was synthesized using 0.15 M sodium oxalate electrolyte solution containing 50 mM NMP monomer by cyclic voltammetry technique applying 25 mV/s scan rate with 10 segment potential range between -0.5-1.5 V. Then, PNMP coated electrode was immersed into 4 mg/ml GOD containing 1.0% Chi solution to form thin enzyme layer onto PNMP film. Finally, enzyme modified electrode was kept in 0.10 % GAL solution to generate crosslink between enzyme molecule and Chi matrix prevent enzyme leakage. Bare Pt, PNMP coted (PNMP/Pt) and also GOD-Chi-GAL layered Pt (GAL/Chi-GOD/PNMP/Pt) electrodes were characterized firstly by SEM images which were given in Figure 8.

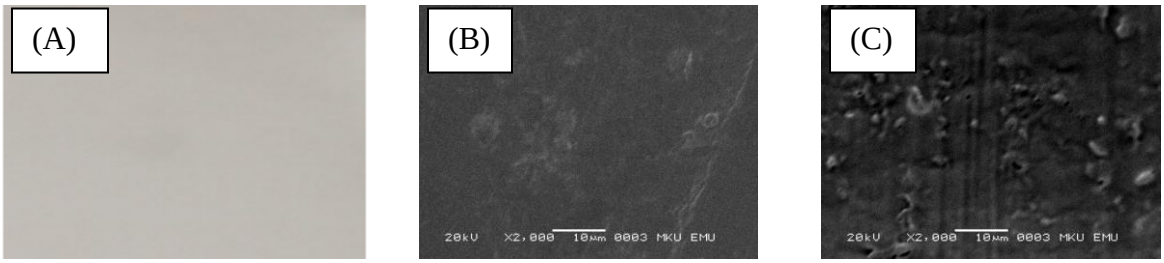


Figure 8. SEM images of bare Pt (A), PNMP/Pt (B) and GAL/Chi-GOD/PNMP/Pt (C) electrodes.

It was clearly seen in Figure 8 that, modification made on Pt electrode changed the surface morphology.

Current responses depending on glucose concentration measured using enzyme electrode which was get applying optimal parameters to get Michaelis-Menten (Figure 9-A) and Lineveawer-Burk (Figure 9-B) graphs. Kinetic parameters, Imax and KM values were calculated from the line equation as 28.33 μA and 2.88 mM, respectively.

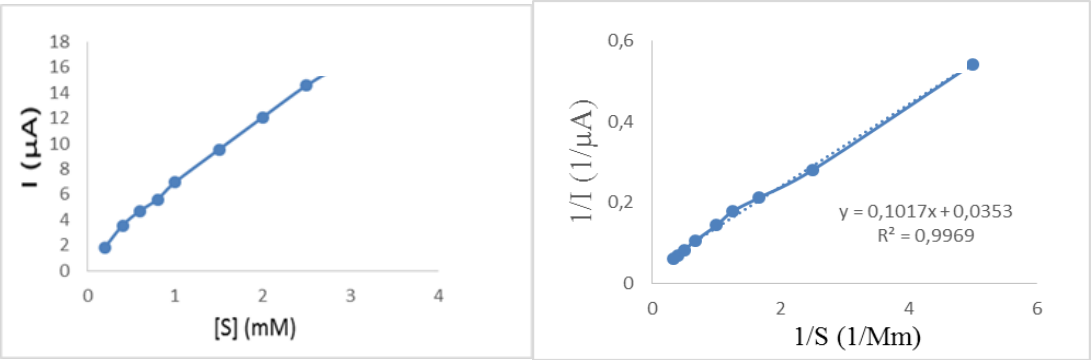


Figure 9. Michaelis-Menten (A) and Lineveawer-Burk (B) graphs of enzyme electrode

Operational stability of enzyme electrode was investigated by 20 successive uses in 5 mM glucose solution and each net current value was proportioned to net current value of first cycle to calculate remaining activity %. Remaining activities depending on reuse numbers were given in Figure 10.

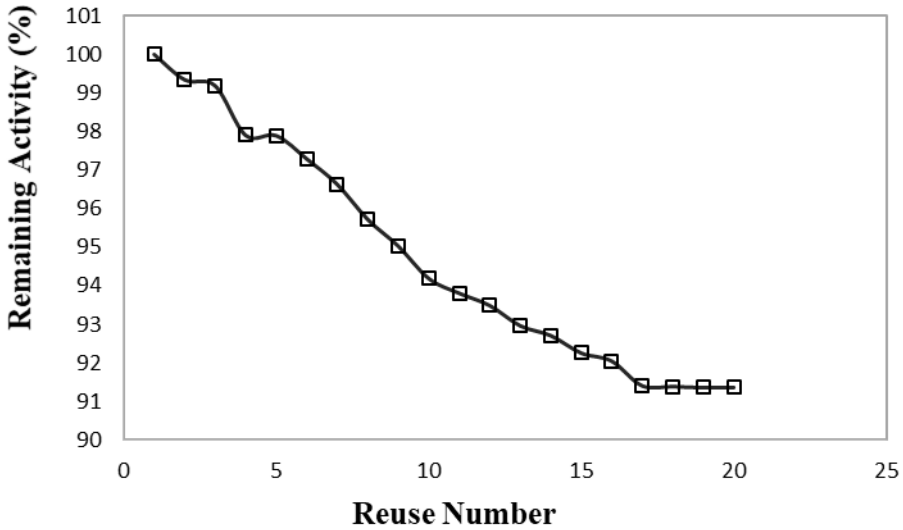


Figure 10. Operational stability of enzyme electrode

As seen in Figure 10, decrease in activity was less than 10 % of initial activity at the end of the 20th cycle. Remaining activity was almost constant as approximately 91.4% at 17th and following cycles.

**Discussion**

In this study it was aimed the construction of glucose sensitive enzyme electrode. To this, Pt electrode coated by PNMP by cyclic voltammetry technique in sodium oxalate electrolyte solution containing NMP monomer. Then GOD was immobilized onto PNMP film as in thin Chi layer. When enzyme electrode was immersed into glucose solution, firstly a biochemical reaction occurs between glucose and GOD. Before this, glucose molecule should diffuse from bulk solution to Chi matrix to be able to react enzyme molecule. So Chi, GOD and GAL concentrations affect the diffusion rate consequently $H_2O_2$ production. Formed $H_2O_2$ molecules are oxidized due to applied constant potential (0.6V) and electrons are transfered to Pt electrode via PNMP layer. Thus, conductivity and physical property of PNMP coating important for current value measured which is proportional with glucose concentration. Therefore, not PNMP synthesis conditions such as monomer concentration and scan rate but also GOD immobilization conditions such as Chi, GOD and GAL concentrations affect the current response. In this sight, biosensor construtions parameters were optimized and results were given in Table 1.

Table 1. Optimum prameters for biosensor construction

| **Biosensor Construction Parameter** | **Optimum Value** |
|---|---|
| Monomer Concentration (mM) | 50 |
| Scan Rate (mV/s) | 20 |
| Chi Concentration (%) | 1.0 |
| GOD Concentration (mg/ml) | 4.0 |
| GAL Concentration (%) | 0.10 |

When PNMP synthesized using different concentrations of NMP, current values firstly increase upto 50 mM NMP, and then decrease at higher monomer concentration. Changing in monomer concentration affects the pore size synthesized polymer film. When monomer concentration is low, polymer film will form with high pore size to which GOD and Chi layer adsorb weakly. However, in case of high monomer concentration, polymer film with heterogen structure forms on the electrode surface and at this occasion GOD and Chi layer will be readily desorbed. Yet, if a proper monomer concentration is used, the polymer film will be suitable for adherently adsorbtion of GOD and Chi layer and easy in electrons transfer which are formed from $H_2O_2$ decomposition to Pt electrode. Scan rate also has great effect on porosity and conductivity of polymer film. As the scan rate increases, a porous and irregular polymer forms which causes difficulty for adsorption GOD onto polymer film. When applying several scan rates for PNMP as 20, 50 and 100 mV/s, the highest current responses were observed for 20 mV/s. This result may be due to less porous polymer film formation in case of low scan rate (Özyılmaz et al. 2006). Chi concentration is so important not only having effect on adsorption of GOD molecules onto polymer film but also glucose diffusion rate from the bulk solution. GAL prevents the lekage of GOD from Chi gel matrix by crosslinking between amin groups of Chi and GOD molecules. If GAL

concentration was low, crosslinking is not enough to prevent leakage. Because current value depending on glucose molecule can be measured by $H_2O_2$ produced enzymatically, increase in GOD concentration affects the measured current value.

Imax value is the highest current value that able to measure whereas, KM shows the substrate interest to enzyme molecule. High Imax value and low KM value indicate the high efficiency for enzyme molecule. In this study, Imax and KM values were calculated as 28.33 μA and 2.88 mM, respectively. Kadam et al. (2011) constructed an amperometric biosensor by coating poly(pyrrole-co-N-methypyrrole) onto Pt electrode galvanostatically and determined Imax and KM values as 16.66 μA and 3.2 mM, respectively. Arslan et al. (2011) immobilized GOD via polyaniline-polyvinylsulfonate on Pt electrode to construct amperometric glucose biosensor and they reported Imax and KM values as 0.122 μA and 0.186 mM, respectively. They also investigated operational stability of the electrode and they found remaining activity as 75% at the end of 20 reuses. In our study, after 20 reuses, measured current was 91.3% of first current.

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**References**

Abasiyanik, M. F & Senel, M. (2010). Immobilization of glucose oxidase on reagentless ferrocene-containing polythiophene derivative and its glucose sensing application, Journal of *Electroanalytical Chemistry*, 639, 21–26

Abu-Rabeah, K., Niţă, I.I., Tencaliec, A.M., Marks, R.S. (2009). New approach of constructing biosensing matrices by physical and chemical crosslinking of biotin-alginate with alginate-pyrrole, *Electrochimica Acta,* 54, 4359-4364

Arslan F., Ustabaş S., Arslan H. (2011). An Amperometric Biosensor for Glucose Determination Prepared from Glucose Oxidase Immobilized in Polyaniline-Polyvinylsulfonate Film. *Sensors*, 11, 8152-8163

Borole D. D., Kapadi U. R., Mahulikar P. P., Hundiwale, D. G. (2007). Glucose oxidase electrodes of polyaniline, poly(o-anisidine) their co-polymer as a biosensor: a comparative study, *Journal of Material Science*, 42,4947–4953

Deng, H., Teo, A.K.L., Gao, Z. (2014). An interference-free glucose biosensor based on a novel low potentialredox polymer mediator. *Sensors and Actuators B,* 191, 522– 528

Eftekhari, A. (2004). Electropolymerization of aniline onto passivated substrate and its application for preparation of enzyme-modified electrode, *Synthetic Metals,* 145, 211-216

Ekiz, F., Oğuzkaya F., Akin M., Tanyeli C., Toppare L. (2011). Synthesis and application of poly-SNS-anchored carboxylic acid: a novel functional matrix for biomolecule conjugation, *Journal of Materials Chemistry*, 21, 12337-12343

Guadalupe de Jesus, C., Lima, D., Santos, V., Wohnrath, K., Pessoa, C.A. (2013), Glucose biosensor based on the highly efficient immobilization of glucose oxidase on layer-by-layer films of silsesquioxane polyelectrolyte, *Sensors and Actuators B,* 186, 44-51

Haighi, B., Nazari, L., Sajjadi, S.M., (2012). Fabrication and application of a sensitive and highly stable copper hexacyanoferrate modified carbon ionic liquid paste electrode for hydrogen peroxide and glucose detection, *Electroanalysis*, 24, 2165-2175.

Huang, Y., Qin X., Li Z., Fu, Y., Qin C., Wu, F., Su, Z., Ma, M., Xie, Q., Yao, S.H., Hu, J. (2012). Fabrication of a chitosan/glucose oxidase–poly(anilineboronic acid)–Aunano/Au-plated Au electrode for biosensor and biofuel cell. *Biosensors and Bioelectronics*, 31, 357-362

Jiang, Y., Zhang, Q., Li, F., Niu, L. (2012). Glucose oxidase and graphene bionanocomposite bridged by ionic liquid unit for glucose biosensing application, *Sensors and Actuators B*, 161, 728-733

Kadam, S.B., Datta K., Ghosh P. & Shirsat, M.D (2011). Poly(Pyrrole)-Poly(N-Methylpyrrole) Composite Matrix for Amperometric Biosensor Design, *International Journal of Polymeric Materials*, 60,233–243

Li, J., Yuan, R., Chai, Y., Che, X., Li, W. (2012). Construction of an amperometric glucose biosensor based on the immobilization of glucose oxidase onto electrodeposited Pt nanoparticles-chitosan composite film, *Bioprocess Biosystem Engineering*, 35, 1089-1095

Olea, D., Viratelle, O., Faure, C. (2008), Polypyrrole-glucose oxidase biosensor. Effect of enzyme encapsulation in multilamellar vesicles on analytical properties, *Biosensors and Bioelectronics*, 23, 788-794

Ozdemir, C., Yeni, F., Odaci, D., Timur, S. (2010) Electrochemical glucose biosensing by pyranose oxidase immobilized in gold nanoparticle-polyaniline/AgCl/gelatin nanocomposite matrix, *Food Chemistry*, 119, 380-385

Ozyilmaz G., Ozyilmaz A.T., Can F. (2011). Glucose Oxidase- Polypyrrole Electrodes Synthesized In P-Toluenesulfonic Acid And Sodium p-Toluenesulfonate, *Applied Biochemistry and Microbiology*, 47,217-225

Özyılmaz A.T., Erbil, M., Yazıcı, B. (2006). The electrochemical synthesis of polyaniline on stainless steel and its corrosion performance, *Current Applied Physics*, 6, 1-9

Pauliukaite, R., Ghica, M.E., Fatibello-Filho, O., Brett, C.M.A. (2010). Electrochemical impedance studies of chitosan-modified electrodes for application in electrochemical sensors and biosensors. *Electrochimica Acta*, 55, 6239-6247

Raicopol, M., Prună, A., Damian, C., Pilan, L. (2013). Functionalized single-walled carbon nanotubes/polypyrrole composites for amperometric glucose biosensors, *Nanoscale Research Letters*, 8, 1-8

Rothwell, S.A., Killoran S.J.,O'Neill, R.D. (2010). Enzyme Immobilization Strategies and Electropolymerization Conditions to Control Sensitivity and Selectivity Parameters of a Polymer-Enzyme Composite Glucose Biosensor, *Sensors*, 10, 6439-6462

Salimi, A. Noorbakhsh, A (2011). Layer by layer assembly of glucose oxidase and thiourea onto glassy carbon electrode: fabrication of glucose biosensor, *Electrochimica Acta*, 56, 6097-6105

Savale P. A. & Shirsat M. D., (2009). Synthesis of Poly(o-anisidine)/H2SO4 Film for the Development of Glucose Biosensor, *Applied Biochemistry and Biotechnology*, 159,299–309

Shervedani, R.K., Hatefi-Mehrjardi, A. (2007). Electrochemical characterization of directly immobilized glucose oxidase on gold mercaptosuccinic anhydride self-assembled monolayer, Sensors and Actuators, B: *Chemical*, 126, 415-423

Shkotova L.V., Piechniakova N.Y., Kukla O.L., Dzyadevych S.V. (2016). Thin-film amperometric multisensor for simulraneous determination of lactate and glucose in wine, *Food Chemistry*, 197, 972-978

Tang, W., Li L., Zeng X., (2015). A glucose biosensor based on the synergistic action of nanometer-sized TiO2 and polyaniline, *Talanta*, 131, 417–423

Uang, Y.M., Chou, T.S., 2003. Fabrication of glucose oxidase/polypyrrole biosensor by galvanostatic method in various pH aqueous solutions. *Biosensors and Bioelectronics*,19: 141-147

Yao, Y., Wu, S.G., Xu, H.H., Wang, L.W. (2015). High-Sensitive Glucose Biosensor Based on Ionic Liquid DopedPolyaniline/Prussian Blue Composite Film 2015 C*hınese Journal of Chemıcal Physıcs,* 28, 755-761