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Glucose-Sensitive Biosensor Design by Zinc Ferrite ($ZnFe_2O_4$) Nanoparticle-Modified Poly (o-toluidine) Film

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Abstract

Glucose oxidase (GOD) immobilized poly(o-toluidine) (POT) coated Pt electrode was designed for glucose-sensitive biosensor. Since POT film structure affects enzyme activity, parameters of enzyme immobilization and POT synthesis conditions were optimized. Optimal monomer concentration for POT film synthesis was determined as 40 mM and the scanning rate was determined as 50 mV/s. As for the immobilization process results, GOD, glutaraldehyde (GAL) and chitosan (Chi) concentrations were decided as 2 mg/ml 0.10%, and 0.5% for the Pt/POT electrode. Zinc ferrite nanoparticle ($ZnFe_2O_4$NP) was immobilized together with POT film in the presence of GOD enzyme. It was revealed that ZnFe2O4NP increased the current responses and stability of the Pt/POT electrode.

Keywords:

Poly (o-toluidine), glucose oxidase, amperometric biosensor, zinc ferrite nanoparticle, conductive polymer

Article history:

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Introduction

Glucose oxidase (GOD)-based enzyme electrodes are commonly used in biosensor design for glucose determination (Uang et al., 2003; Guo et al., 2011; Salimi et al., 2011; Khun et al., 2012; Zhang et al., 2013). An enzyme-containing biosensor is formed as a result of the immobilization of the protein molecule onto the substrate surface. This is necessary for the creation of glucose biosensor. In order to obtain the GOD biosensor, there are various strategies on immobilization

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techniques, such as incorporation or entrapment into the polymer structure during electropolymerization synthesis (Eftekhari 2004; Yu et al., 2005; Ozyilmaz et al., 2011), application to the electrode surface with gel substrate (Pauliukaite et al., 2006; Hui et al., 2005; Huang et al. 2011; Haighi et al., 2012 ; Wang et al.,2013), crosslinking with glutaraldehyde (Ghica et al., 2009), by covalent bonding (Shervedani et al., 2007; Abu-Rabeah et al., 2009), or adsorbed/ on solid surface (Jiang et al., 2012; Salimi et al., 2011 ; De Jesus et al., 2013;). Chitosan, known as a natural cationic polymer, attracts the attention of researchers due to its good adhesion, permeability and its film-forming ability. Therefore, chitosan is added to the gel structure for enzyme immobilization to glutaraldehyde or other reagents (Li et al., 2012). However, the structurally low conductivity of chitosan negatively affects the performance of enzyme-based biosensors such as GOD. (Zhang et al 2013). Research is being carried out to solve this problem caused by chitosan by adding components such as nanoparticles and nanocomposites to its structure (Li et al 2012; Chen et al., 2011; Khun et al., 2012; Wang et al., 2013), ionic liquid (Zhang et al 2013) or modifying the interface between chitosan and the substrate surface via conductive polymers (Chen et al., 2011), by Prussian Blue (Zhan et al., 2013), by nanoparticles (Guo et al., 2011; Chen et al., 2011,). Various conductive polymers were utilized to obtain glucose biosensor like polyaniline (PANI) (Ozyilmaz et al., 2023; Ozdemir et al 2010), polypyrrole (Raicopol et al 2013; Ozyilmaz et al., 2011; Ozyilmaz et al., 2018), poly(o-anisidine) (POA) (Ozyilmaz et al., 2017; Savale et. al 2009). Recent studies have determined that dimensionally reduced nanostructured metal oxides have unique advantages in immobilizing enzymes. At the same time, their large surface area shows that they have high sensitivity due to their desired microstructure and contribution to the electron transfer between active sites of enzyme and electrode. ZnO is among the most used nanoparticles for this purpose (Jung and Lim 2013; Wang et al., 2011).

This study discusses the design of glucose biosensor electrodes using poly (o-toluidine) (POT), a conductive polymer. The effect of POT synthesis parameters and zinc ferrite ($ZnFe_2O_4$) nanoparticle concentration on the structure of biosensor was determined by obtaining current response at different glucose concentrations. Also, kinetic parameters and operational stability of enzyme electrode were obtained.

Material and Methods

Aspergillus niger origin GOD (EC 1.1.3.4, …U/mg), o-toluidine (OT), chitosan (Chi), zinc ferrite nanoparticles ($ZnFe_2O_4$NP, <100 nm), glucose anhydrous, glutaraldehyde (GAL) were purchased from Sigma. OT was used after distillation, stored in dark. All other reagents were of analytical grade and used without further purification. Electrochemical analyzers, Chi606 was used to synthesis polymer film and measuring current values and Chi660b was used to impedance studies. All electrochemical experiments were performed in a single compartment cell with three electrode configurations. The reference electrode was an Ag/AgCl (3M KCl) electrode and counter electrode was a platinum plate with surface area of 0.25 cm2.

Preparation of Enzyme Electrode

Preparation of the enzyme electrode was carried out in three steps. Firstly, POT films were obtained on the Pt surface. Secondly, the obtained Pt/POT was placed in chitosan and GOD containing solution for 3 seconds and this electrode was dried for 2 hours in an open environment. Finally, the Pt/POT/GOD-Chi electrode was kept in glutaraldehyde solution for 10 seconds to ensure cross-linking between GOD amine groups and Chi to prevent the escape of the GOD component. The formation of the Pt/POT-GOD electrode by ZnFe2O4 nanoparticle was carried out with adding the nanoparticles to Chi+GOD containing solution and homogenizing it using a sonicator. When not in use, the electrode was stored at 4°C.

Polymer Film Synthesis

Poly(o-toluidine) was synthesized on Pt electrode in neutral medium of 0.20 M sodium oxalate solution (NaOX) and o-toluidine as monomer. The POT films were obtained by optimize the parameters of synthesis as scan rate, segment number and monomer concentration after comprehensive preliminary studies. POT homopolymer films were obtained by applying cyclic voltammetry technique in NaOX + o-toluidine monomer solution, at the potential range of 0.2 to 2.0 V. Structure, conductivity and porosity of polymer films play an important role in response current of enzyme electrodes. So, electro polymerization parameters were determined in detail of scan rate, segment number and monomer concentration.

Biochemical Characterization of Enzyme Electrodes

For all electrodes obtained in the study, current values were obtained depending on glucose concentrations between 0.1 to 3.0 mM at constant potential. The glucose solution was constantly stirred during measurements. The current value gets using glucose free buffer solution was subtracted from that of glucose + buffer solution. Net current value as ųA was accepted as current response. Optimal pH values for each electrode were determined by using glucose solution prepared with buffer solutions at different pHs. KM and Imax values were calculated from the Lineweaver-Burk Graph obtained from the current values read depending on different glucose concentration at optimum pH value. Reuse stability was investigated by using the electrodes 20 successive times in 5 mM glucose.

Results and Discussion

Optimization of POT Synthesis Parameters

The synthesis parameters of POT films were obtained for scan rate, segment number and monomer concentration by monitoring current response depending on glucose concentration. Monomer concentrations were changed between 0.04% - 0.07% for POT homopolymer. Since the structure of the poly(o-toluidine) film is important for the immobilization of the GOD molecule, in order to obtain a proper POT film, POT films were first synthesized on Pt electrodes at o-toluidine monomer concentrations between 40 and 70 mM. Concentration ranges used for o-toluidine monomer were

decided by preliminary studies. The concentrations of Chi, GOD and GAL and scan rate, segment number were kept constant while GOD electrodes were fabricated. Current responses were obtained at different glucose concentrations using the prepared Pt electrodes. Figure 1, given as a percentage of the maximum current value, shows the measured current values of the Pt/POT electrode depending on different glucose concentrations. In Figure 1, both highest current responses and linearity were obtained for the Pt/POT electrode with the POT film synthesized using 40 mM o-toluidine monomer.

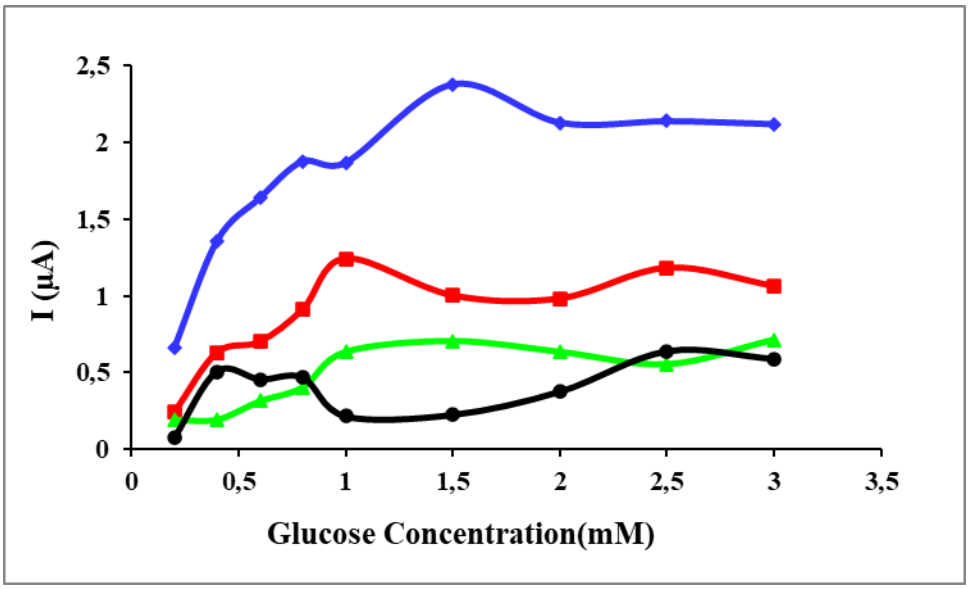

Figure 1. Current responses obtained for glucose concentration of Pt/POT-GOD electrodes fabricated by POT film synthesized for different concentrations of o-toluidine such as 40 mM (♦); 50 mM (■); 60 mM (▲) and 70 mM (•).

As the scan rate increases too much, defects and irregularities in the structure of the synthesized conductive polymer films increase. Therefore, when creating biosensor electrodes, it is important to create a more regular and densely stacked polymer film with appropriate scan rate. Since conductivity of polymer film is affected by scan rate depending on the structure of the conductive polymer, it will also affect the electron transfer rate, which is important for measurements of biosensor electrodes. For this reason, Pt electrodes were coated by POT homopolymer film applying scan rates of 100, 50 and 20 mV/s. POT homopolymer films were synthesized by applying proper scan rate at the potential range of 0.20 to 2.0 V in 0.150 M NaOX solution + the optimum monomer concentration. Therefore, to subject the POT film synthesis to the same duration, 10, 26 and 50 cycles were applied to the scan rates of 20, 50 and 100 mV/s, respectively. Figure 2 shows the current response of the Pt/POT-GOD electrodes obtained with the POT polymer film synthesized with different scan rates. As seen in Figure 2, the current values obtained by applying a scan rate of 50 mV/s for the Pt/POT-GOD electrode were the highest for all glucose concentrations. Additionally, 50 mV/s for POT synthesis was chosen as the optimal

scan rate, as Pt electrodes exhibited a more linear current response for current measurements against different glucose concentrations.

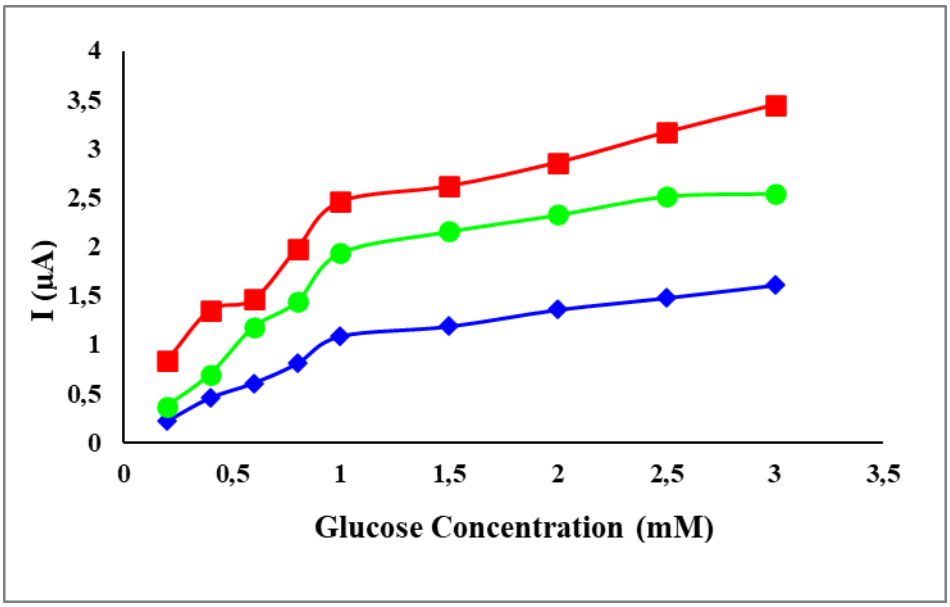

Figure 2. Current responses obtained for glucose concentration of Pt/POT-GOD electrodes fabricated by POT film synthesized for different scan rates; (♦: 20 mV/s-2 segment, ■:50 mV/s-4 segment, ●:100 mV/s-8 segment).

POT homopolymer films were obtained on the Pt electrode by 2, 4 and 6 segment number. The synthesis of POT film was carried out by proper scan number between 0.20 to 2.0 V in 0.150 M NaOX + o-toluidine by optimal monomer concentration. Figure 3 shows the current responses of Pt/POT-GOD electrodes were obtained applying different segment number. In Figure 3, the current responses of the electrodes fabricated by applying 2 segments were highest at low glucose concentration. On the other hand, it was determined that the current values of Pt/POT-GOD electrodes obtained by POT film synthesized by applying 4 segments increased depending on the increasing glucose concentration. At the same time, it was observed that the linearity of the curve increased.

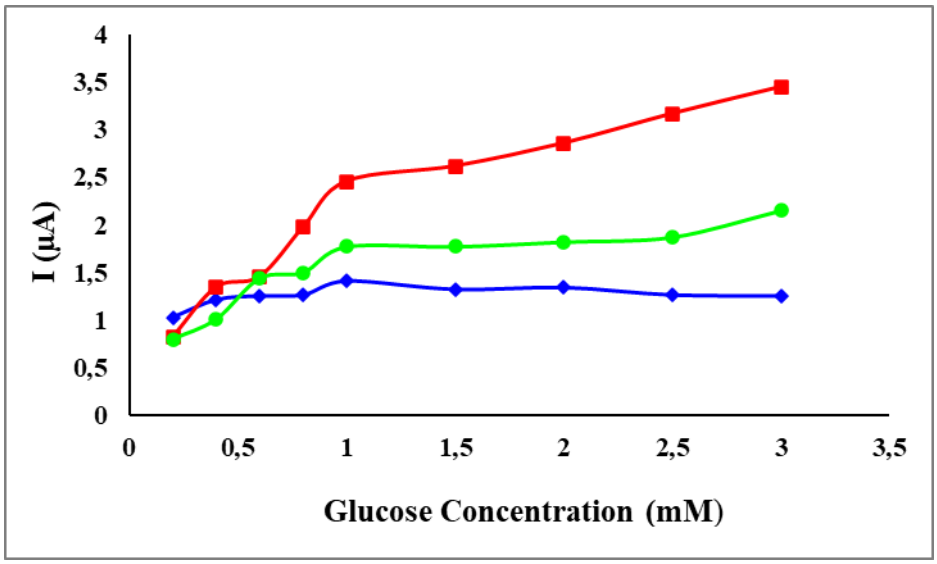

Figure 3. Current responses obtained for glucose concentration of Pt/POT-GOD electrodes fabricated by POT film synthesized for different segments (♦: 2 segments, ■:4 segment, ●:6 segment).

The effect of chitosan, glucose oxidase and glutaraldehyde concentrations on enzyme immobilization conditions were studied for enzyme electrode activity and the current values obtained for 3 mM glucose were given in Figure 4 in A, B and C, respectively. The GOD-based Pt/POT electrode fabricated using 0.5% Chi solution gave the highest current response (Figure 4A). In Figure 4 (B) and (C), the highest current values were determined when Pt/POT-GOD electrodes were obtained by adding 0.10% glutaraldehyde and 2 mg/ml glucose oxidase for enzyme-based Pt/POT electrodes. So, parameters of enzyme immobilization were determined as 0.5% chitosan, 2 mg/ml glucose oxidase and 0.10% glutaraldehyde for POT based electrodes in the continue studies. Since GOD enzyme activity is affected by enzyme immobilization conditions, it is expected that hydrogen peroxide amount that will emerge and the current values obtained will also be affected.

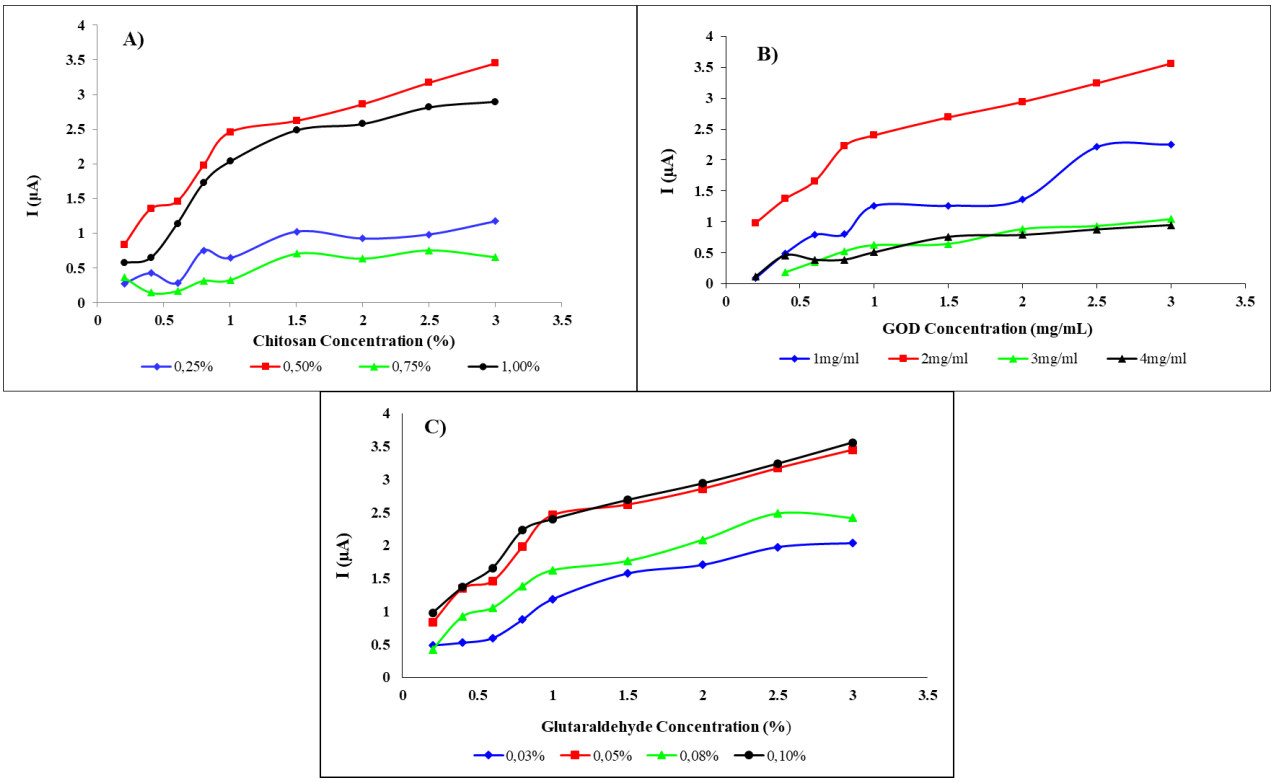

Figure 4. Current responses of POT based electrodes prepared by different immobilization conditions such as concentrations of Chi (A), GOD (B), GAL(C).

The Effect of $ZnFe_2O_4$ Nanoparticle Modification on Current Response

Biosensor enzyme electrode was developed by $ZnFe_2O_4$NP using different four concentrations such as 0.1; 0.5 ; 2.0 and 4.0 mg for per ml of chitosan solution. It can be seen in Figure 5 that adding $ZnFe_2O_4$NP to the polymer film increased the biosensor efficiency. The Pt/POT-GOD electrode obtained when 2 mg $ZnFe_2O_4$NP/ml was added to the GOD + Chi solution showed the highest current values. The current values obtained for other amounts of nanoparticle were lower than those of ZnFe2O4NP-free Pt/POT electrode, on the other hand, the efficiency of electrode enhanced when 2.0 mg $ZnFe_2O_4$NP/ml was used. Nanoparticles were used to increase glucose oxidase electrode in the literature.

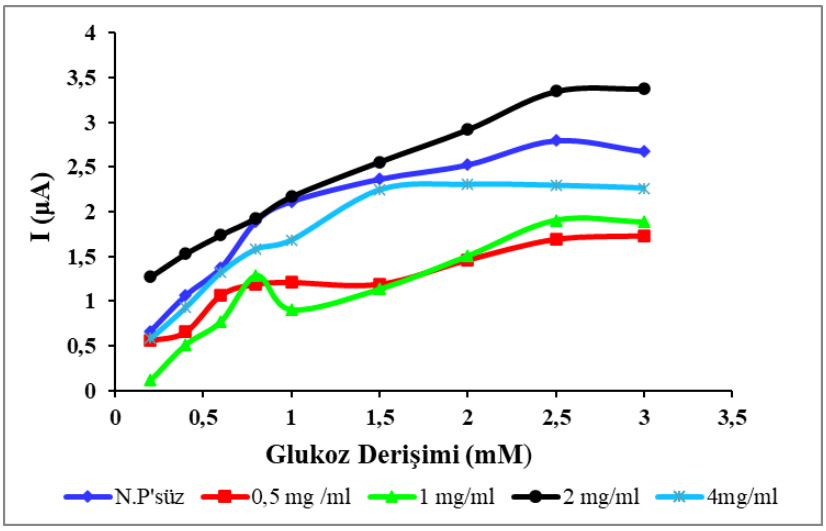

Figure 5. Current response of POT based biosensors modified with $ZnFe_2O_4NP$. : $ZnFe_2O_4NP$-free; :0.5; :1.0 ; : 2.0 and : 4.0 mg/ml.

Biochemical Characterization of Enzyme Electrode

Kinetic parameters, operational stability and optimal pH values were also studied for biochemical characterization with the Pt/POT-GOD electrode obtained using optimal data. The current responses of the electrodes were obtained for different pH values in 3 mM glucose solution. Both Pt/POT/Chi-GOD-$ZnFe_2O_4NP$ and Pt/POT/Chi-GOD electrodes showed the highest current responses at pH 6.0 given in Table 1. KM and Imax values of both of Pt/POT-GOD electrodes given in the Table 1 were estimated by Lineweaver-Burk curve using current responses measured for glucose concentrations range from 0.10 to 10 mM. As seen in Table 1, I_{max} value of Pt/POT/Chi-GOD electrode was considerably lower than that of Pt/POT/Chi-GOD-$ZnFe_2O_4NP$ electrode. With this result, $ZnFe_2O_4NP$ in the enzyme-containing polymer film contributed to the increase of Imax value. KM value of Pt/POT enzyme electrode that was modified by $ZnFe_2O_4NP$ was slightly lower than that of this nanoparticle free counterpart.

Table 1. Optimal pH values and kinetic parameters of Pt/POT-GOD electrodes with and without $ZnFe_2O_4NP$.

	pH	**K_M (mM)**	**I_{max}(µA)**
Pt/POT/Chi-GOD/GAL	6	0.43	3.01
Pt/POT/Chi-GOD-$ZnFe_2O_4$/GAL	6	0.59	3.23

Operational stabilities of the Pt/POT electrode with and without $ZnFe_2O_4NP$ were also investigated by 20 successive in the 5 mM glucose solution. The results of measurements repeated five times were similar. For all 20 cycles, obtained current values as the percentage of initial current

were calculated and Fig.6 shows the results. It was observed that the initial current activities of both Pt/POT/Chi-GOD and Pt/POT/Chi-GOD-$ZnFe_2O_4$NP electrodes were almost the same. At the end of 20th cycles, the current values of Pt/POT/Chi-GOD and Pt/POT/Chi-GOD-$ZnFe_2O_4$NP electrodes were observed to be a little of increase with 101.0% and 107.0% according to the first measured current values. This may occur due to changes in the POT film and GOD structure, which will increase enzyme activity and electron transfer. These results show that the enzyme electrodes are quite stable for reuse.

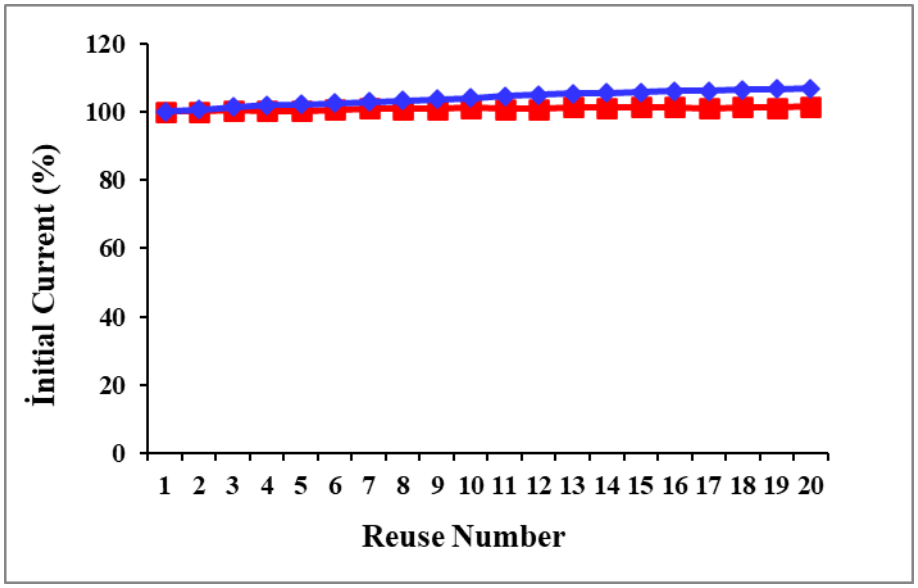

Figure 6. Operational stabilities of Pt/POT/Chi-GOD and Pt/POT/Chi-GOD-$ZnFe_2O_4$NP electrodes.

In conclusion, to fabricate a glucose-sensitive enzyme electrodes, double-layer POT polymer films with and without nanoparticle were synthesized on the Pt surface. The efficiency of the obtained electrodes was determined by measuring current values at different glucose concentrations. Accordingly, it has been observed that since synthesis conditions such as monomer concentration, scan rate and segment number change the surface structure of the electrodes, they also change the current responses of the biosensor electrodes. In addition, the current responses of biosensor electrodes are affected by the amounts of Chi, Gal, GOD and $ZnFe_2O_4$NP in the POT polymer film structure. The stability and current values were higher for Pt/POT/Chi-GOD-$ZnFe_2O_4$NP electrode than that of Pt/POT/Chi-GOD electrode. Therefore, it is thought that the reason for the increase in the current responses of the Zn-containing enzyme electrode is that the nanoparticle contributes to the increase in the conductivity of the polymer film and the electron transfer between the metal and enzyme interfaces. So, the necessity of determining biosensor design parameters in order to increase the efficiency of biosensor electrodes was revealed in this study.

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

All authors' contributions are equal for the preparation of research in the manuscript.

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