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Direct electron transfer from glucose oxidase immobilized on polyphenanthroline-modified glassy carbon electrode

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ABSTRACT

This study reports direct electron transfer (DET) from immobilized glucose oxidase (GOx) *via* grafted and electropolymerized 1,10-phenanthroline monohydrate (PMH). The layer of poly-1,10-phenanthroline (PPMH) was gained *via* electrochemical deposition, which was used to create the PPMH-modified GC-electrode (PPMH/GC-electrode). Further, the GOx was immobilized on the PPMH/GC-electrode. The effect of surface-modification by the PPMH on the electron-transfer between enzyme and electrode-surface and some other electrochemical/analytical-parameters of newly designed enzymatic-electrode were evaluated. The PPMH/GC-electrode showed superior DET to/from flavine adenine dinucleotide cofactor of GOx, while some redox-compounds including ferrocene and $K_3[Fe(CN)_6]$ were completely electrochemically inactive on the PPMH/GC-electrode. It was also found that the resulting GOx/PPMH/GC-electrode functioned as a "direct response type" glucose-biosensor. The biosensor showed excellent selectivity towards glucose and demonstrated good operational-stability. According to our best knowledge, this study is the first scientific report on electrochemical-polymerization of PMH on the GC-electrode in non-aqueous media followed by its application in the design of glucose-biosensor.

1. Introduction

The DET is an unique feature, which is useful for development of new bioelectronics-based devices including self powered sensors (Kakehi et al., 2007). The DET could be achieved between enzymes and conducting polymer- (Ramanavicius et al., 1999), gold- (Ikeda et al., 1991) and carbon-based (Ramanavicius et al., 2008a,b) electrodes. Carbon-based materials seem especially promising for biosensor design (Gorton, 1995) due to a high variety of allotropic forms and easy modification by organic materials including deposition of conducting polymers. Glassy-carbon (GC) is one of the most used forms of carbon for the formation of the electrodes and recently, the GC-electrodes become widely used in electroanalytical applications (Solak et al., 2003; Ustundag and Solak, 2009) and in studies related to kinetics of electrode-reactions. In addition to this, modification of electrode-surface on the molecular scale allows efficient electron-transfer between some redox proteins and the electrode; this can be considered to be the basis for understanding the redox properties of protein and for development of basic biosensors suitable for bioanalytical purposes (Wang et al., 2009a,b; Shan et al., 2009; Ramanavicius et al., 2008a,b).

Among the number of FAD-dependent-enzymes the glucose oxidase (GOx) is the most frequently used model enzyme due to its high bioelectrocatalytic activity and biochemical stability (Wang and Musameh, 2003; Ramanavicius et al., 2006; Endo et al., 2009; Hanashi et al., 2009; Kausaite et al., 2009). It contains FAD redox center, which catalyzes the electron transfer from glucose to glucolactone. Owing to the deep embedding of the redox center, the electron transfer rate from the active site of GOx to the GC-electrode is practically not detectable. Therefore, the redox mediators are needed for the driving of bioelectrocatalytic processes in order to increase the electron-transfer-rate between the enzyme and GC-electrode. Until now, the sol–gel-matrixes, polymer-films, carbon-nanotubes, ordered-mesoporous-silica and organic-guest-molecules have been used in order to facilitate electron-transfer from GOx to electrode (Shan et al., 2009; Kausaite et al., 2009; Ramanaviciene et al., 2006; Tsai et al., 2009; Zhao et al., 2009). Majority of electrochemists have realized the advanced bioelectrochemical routes, which are based on application of various organic compounds as redox-mediators, including ferrocene-derivatives, quinones, ferrocyanide and some metal-complexes with inorganic-

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ligands including some phenanthroline-derivatives (PDs) (Zawada and Bukowska, 2000; Jasimuddin et al., 2005; Lozano-Camargo et al., 2007; Ding et al., 2008; Li et al., 2007; Oztekin, 2008; Oztekin and Yazicigil, 2009; Ryabov et al., 1999). In addition, our groups have tested a number of PDs adsorbed on the graphite-electrodes as possible redox-mediators for the GOx and it was found that some PDs show excellent electron-transfer capabilities (Oztekin et al., 2010a). The grafting of PDs on the GC-electrode was reported by our group (Oztekin and Yazicigil, 2009) and electrochemical-polymerization was reported by the other authors (Lozano-Camargo et al., 2007; Cobos-Murcia et al., 2005; Faridbod et al., 2008; Chen, 1996; Mora et al., 2004; Venkatanarayanan et al., 2008; Ramirez-Silva et al., 2004). However, according to our best knowledge, none of the scientific papers related to the application of electro-polymerized PDs and/or PDs that are not involved as ligands in larger organometallic-complexes were reported until now. All mentioned facts open a new avenue for technological break-through in the application of PDs-based electrochemically generated polymers for biosensorics.

The aim of the present report is to study electrochemical polymerization of the PMH on the GC-electrode in a non-aqueous media followed by the application of this PPMH-modified GC-electrode (PPMH/GC) in the design of glucose-biosensor. The effect of surface-modification by the PPMH on the electron-transfer between enzyme and electrode-surface and some other electrochemical/analytical parameters of newly designed enzymatic-electrode was evaluated. According to our best knowledge, this study is the first scientific report on the application of the PPMH/GC-electrode as a glucose-biosensor.

2. Experimental

2.1. Chemicals and materials

All chemicals were of analytical grade. The GOx from *Aspergillus niger* (E.C.1.1.3.4.) with catalytic-activity of 295 U/mg, and glutaraldehyde were purchased from AppliChem GmbH (Darmstadt, Germany). The D-(+)-glucose was obtained from Carl Roth GmbH&Co. (Karlsruhe, Germany).

The solution of 1.0 mM PMH was prepared in the HPLC grade acetonitrile (CH_3CN) JT Baker (Ecatepec, Mexico) that included 100 mM of tetrabutylammoniumtetrafluoroborate (TBATFB) Sigma–Aldrich (Zurich, Switzerland). In addition to this, 1.0 M solution of glucose was prepared in distilled water at least 24 h before use to allow glucose to mutarotate and to reach the equilibrium between α- and β-forms. The buffer solution is prepared as a mixed solution of 0.05 M sodium acetate and 0.05 M sodium phosphate buffer with 0.1 M KCl, pH 6.0 (A-PBS).

2.2. Instrumentation

Electrochemical modification of GC-electrodes and electrochemical-characterization by cyclic voltammetry (CV) was performed using a potentiostat/galvanostat from Gamry Instruments (Warminster, USA) equipped with three-electrode-cell. The amperometric-measurements were performed using an Autolab PGSTAT 30 Potentiostat/Galvanostat (Utrecht, Netherlands) that is operated by the GPES software Eco Chemie (Utrecht, Netherlands). All experiments were carried out inside a Faraday-cage at ambient temperature (at 25 °C). All solutions used for the electrochemical measurements were deaerated with nitrogen for at least 5 min. The GC-electrode with the electrochemically active area of 0.071 cm^2 and Pt-electrode with the electrochemically active-area of 0.3 cm^2 were used as a working- and a counter-electrode, respectively. An Ag/AgCl-electrode in saturated KCl ($Ag/AgCl/KCl_{sat}$) was used as a reference-electrode in an aqueous-media and an Ag/Ag^+ (10 mM $AgNO_3$) was used as a reference-electrode in a non-aqueous-media.

In order to obtain a cleaned/renewed electrode-surface, GC-electrodes were polished and cleaned prior to the electrochemical experiments according to the procedures described previously (Oztekin and Yazicigil, 2009; Oztekin et al., 2010b,c).

2.3. Electrode modification by PMH

The modification of the GC-electrode was performed accordingly to previously described procedure (Oztekin, 2008; Oztekin and Yazicigil, 2009). Polished and cleaned GC-electrodes were modified by CV in 1.0 mM PMH and 100 mM TBATFB dissolved in CH_3CN. The cyclic voltammograms (CVs) were registered at the scan rate of 100 mV/s, the potential was swept from +1200 mV to +2700 mV *vs.* Ag/Ag^+ (in 10 mM $AgNO_3$). The PMH-modified GC-electrodes (PPMH/GC) were characterized by the chronoamperometry (CA) and CV. Electrochemical characterization was performed with the same electrochemical devices and software as it was used for the modification of GC-electrode. In order to clean the PPMH/GC-electrodes they were washed and sonicated before each treatment step.

2.4. Immobilization of GOx on PPMH/GC-electrode

The basic method for rapid and simple preparation of enzyme-modified electrodes has been successfully applied in screening for new redox mediators (Ramanavicius et al., 2006, 2008a,b; Kausaite et al., 2009). In order to get the GC-electrode subsequently modified by the PPMH and the GOx (GOx/PPMH/GC-electrode), three drops of 3 μL of 10 mg/mL GOx solution were deposited on the PPMH/GC-electrode. After deposition of each drop the electrodes were dried at room temperature. Each next drop was added after drying of previously added drop at the room temperature. In this study the method based on the cross-linking by glutaraldehyde was performed by storing for 20 h of the PPMH/GC-electrode with deposited GOx in the vapor of glutaraldehyde at +4 °C in a closed-vessel as it was previously described (Ramanavicius, 2007; Ramanavicius et al., 2008a,b; Ramanaviciene et al., 2006). Prior to the electrochemical-measurements, the electrodes were thoroughly washed with distilled-water to remove non-crosslinked enzyme. Prepared electrodes were kept in a closed-vessel over A-PBS, pH 6.0, at +4 °C until they were used in experiments. During these experiments GOx/PPMH/GC-electrodes were used as working-electrodes, $Ag/AgCl/KCl_{sat}$ was used as a reference-electrode and the Pt wire was used as a counter-electrode.

2.5. Characterization of GOx/PPMH/GC-electrode

Modified electrodes were characterized by CV, chronoamperometry (CA) and compared with the results of the bare GC-electrode and the GC-electrode with immobilized GOx (GOx/GC). In order to check the reproducibility of GOx/PPMH/GC-electrodes, they were investigated by detecting 0.5 mM of glucose successfully for ten times. Moreover, the stability of GOx/PPMH/GC-electrode was also checked and for this aim two different control experiments were performed: in the first group of these experiments, the GOx/PPMH/GC-electrodes were stored in A-PBS, pH 6.0, at +4 °C in refrigerator for 5, 10, 20 and 30 days, then CVs were registered in A-PBS, pH 6.0. In the second group, the GOx/PPMH/GC-electrode was examined by continuous potential-cycling (20 cycles in A-PBS, pH 6.0, within −100 and −800 mV *vs.* $Ag/AgCl/KCl_{sat}$) were performed.

3. Results and discussion

3.1. Electrochemical characterization of GC-electrode

In our previous study, which was related to the grafting of the PMH to the GC-electrode, it was reported that the irreversible-peak current has decreased with an increasing number of the potential-cycles and after some cycles it has reached the steady-state conditions (Oztekin and Yazicigil, 2009). This gradual decrease in the peak current by potential cycling could have been attributed to the formation of the PPMH layer, which at some extent is grafted to the GC-surface (Oztekin and Yazicigil, 2009).

Experimental results indicated that the surface-concentration and thickness of the deposited PPMH-based polymeric layer has increased gradually by the number of potential cycles. Ellipsometric measurements confirmed that after 30 potential cycles the PPMH layer was in the range of 22.4 ± 1.7 Å (Oztekin and Yazicigil, 2009). Some more results on electrochemical characterization of GC-electrode modified by PMH are addressed in our previous paper (Oztekin and Yazicigil, 2009). However in previous study we expected just formation of the PMH monolayer grafted to the carbon-surface, while by present study we confirm formation of the PPMH layer, which was predicted by the other authors (Lozano-Camargo et al., 2007; Cobos-Murcia et al., 2005; Ramirez-Silva et al., 2004). The characteristic PMH oxidation peaks disappeared by formation of the PPMH-layer because well-conjugated system with well distributed electron density was formed by electropolymerization. For this reasons it is the first report on electrochemical polymerization of PDs in non-aqueous media followed by the application of this electrode in design of glucose biosensor.

3.2. Electrochemical characterization of GOx/PPMH/GC-electrode

The electrochemical interaction of enzymes with redox mediators is highly important in creation of enzymatic-biosensors, because only in very rare cases DET between enzymes and electrodes is possible. Many electron-transfer mediators have been used in biosensor design, e.g., modification of the GC-electrodes phenyl–C_nH_{2n}–COOH moieties by electrochemical reduction of aryldiazonium salts bearing *in situ* generated carboxylic acid groups has been reported (Pellissier et al., 2008). GOx-based electrodes were prepared by electrochemical copolymerization of thiophene, thiophene-3-acetic acid and dicyclopentadienyl iron-1,4-dienylmethyl-2-(thiophen-3-yl) acetate on the GC-electrode (Abasiyanik and Senel, 2010). The GC-electrode modified by polymerized brilliant cresyl blue suitable for glucose detection in the range of 31 μM was reported (Ghica and Brett, 2009). Direct-electrochemistry of the GOx immobilized on a composite-matrix based on chitosan and $NdPO_4$ nanoparticles underlying on GC-electrode resulted limit-of-detection (LOD) of 0.08 mM (Sheng et al., 2009). Glucose-biosensor based on the GC-electrode modified with ordered mesoporous silica-SBA-15 and Nafion with the LOD in the range of 0.07 mM was reported (Wang et al., 2009c). Moreover, recently, numerous methods have been utilized to immobilize enzymes in order to improve the electron-transfer process (Wang et al., 2009a; Shan et al., 2009; Ramanavicius et al., 2008a,b; Tsai et al., 2009; Zhao et al., 2009).

PMH-derivatives involved in complexes with heavy-metal ions were used in the design of amperometric-biosensors, while in present study we have applied the PMH, which was not involved into metal complexes. In order to investigate the effect of the PMH on the GOx/PPMH/GC-electrode in DET between enzyme and electrode, the CVs were registered under different conditions. Fig. 1 shows CVs of the bare GC, PPMH/GC and GOx/PPMH/GC-electrodes in the A-PBS, pH 6.0, at the scan rate of 100 mV/s under N_2-saturated conditions. No reduction or oxidation peaks in the

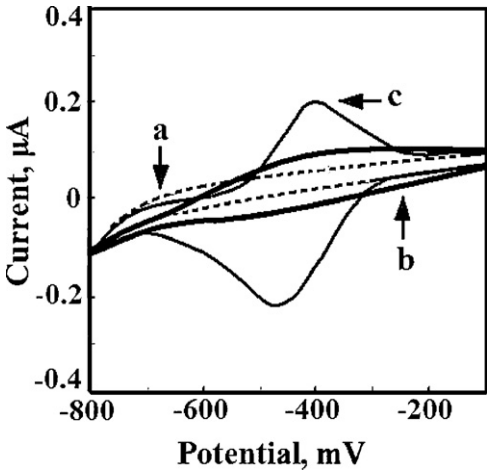

Fig. 1. Cyclic voltammograms of bare GC (a) PPMH/GC (b) and GOx/PPMH/GC (c) electrodes in 0.1 M A-PBS, pH 6.0, at scan rate 100 mV/s. Potentials are referred *vs.* Ag/AgCl/KCl_{sat}.

CVs of bare GC and PPMH/GC were observed (Fig. 1, curves a and b) in the potential range between −100 mV and −800 mV, what can be attributed to a good conjugation of the PPMH polymeric backbone and formation integrated valent-electron-bound. Further evidence towards this kind of conjugation is appearance of a pair of well-defined and fundamentally symmetric redox-peaks for the GOx/PPMH/GC-electrode, which indicates that FAD from the GOx can undergo a reversible direct electrochemical oxidation/reduction on the GOx/PPMH/GC-electrode (Fig. 1; curve c). The anodic- and cathodic-peak potentials belong to the FAD/$FADH_2$ of GOx, which is involved in GOx/PPMH/GC, are located in potential range between −414 mV and −480 mV *vs.* Ag/AgCl/KCl_{sat}. The formal potential of the GOx/PPMH/GC-electrode, calculated from the average value of anodic- and cathodic-peak potentials (E^0) was −447 mV. This value is very close to the standard potential FAD/$FADH_2$, which is −460 mV in pH 7.0 at 25.8 °C. It supports the suggestions that the GOx after immobilization preserved native structure (Tinoco et al., 1978). For the reasons above, it can be predicted that the reversible two-electron redox reaction takes place at the GOx/PPMH/GC-electrode. This reaction is attributed to FAD, which is tightly bounded to active site of the GOx, and/or to FAD, which is loosely bounded to active site of the GOx or is even diffused for the GOx active site. But as it is demonstrated below the PPMH layer significantly increases electron transfer rate from GOx what is very important for evaluated bioanalytical system.

To evaluate the reversible electron transfer phenomenon in more detail, the CVs at different scan rates were recorded in A-PBS, pH 6.0, under N_2-saturated conditions (Fig. 2A). The evaluation of CVs was registered at the different scan rates and it illustrates that by increasing of scan rate the anodic peak potentials are shifted to a more positive direction, while the cathodic peaks are proportionally shifted towards more negative potentials, while keeping almost the same formal potential. The ratio of cathodic and anodic peak currents for the GOx/PPMH/GC-electrode is nearly identical. Peak currents are linearly dependent on the scan rate, (Fig. 2B), it indicates "surface-controlled" electrochemical-oxidation/reduction of the FAD/$FADH_2$ involved in the GOx-structure. Fig. 3 presents the Laviron plot of the dependency of peak potentials on logarithm of scan rate for CVs presented in Fig. 2A. It can be seen from the plot that the peak potentials are independent on the scan rate if it is relatively low scan rates and are proportional to the "logarithm" of scan rate as predicted by Laviron's theory, for scan rates above 200 mV/s. The electron-exchange rate constant (k_s) can be calculated according to the model of Laviron for a surface-controlled

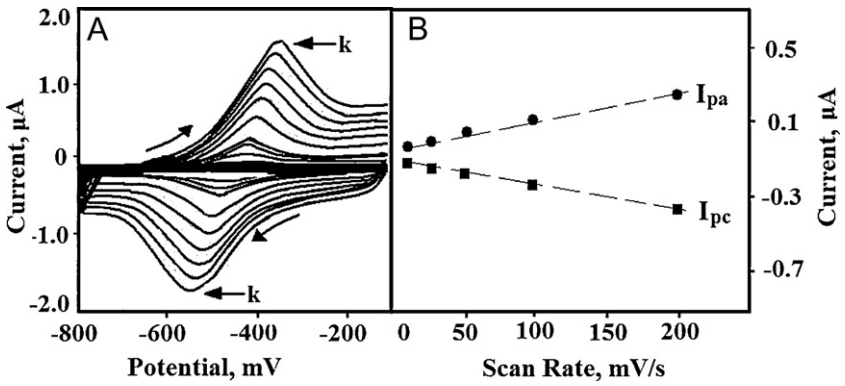

Fig. 2. (A) Cyclic voltammograms of GOx/PPMH/GC electrode in 0.1 M A-PBS, pH 6.0, at different scan rates: 10 (a), 25 (b), 50 (c), 100 (d), 200 (e), 300 (f), 400 (g), 500 (h), 600 (i), 700 (j) and 800 (k) mV/s. Potentials are referred *vs.* $Ag/AgCl/KCl_{sat}$. (B) Cathodic and anodic peak currents *vs.* scan rate.

electrode process, in which the charge transfer coefficient (α) is calculated as 0.52 (Laviron, 1979a). Although in our study calculated charge-transfer-coefficient is higher than it was calculated for the GOx immobilized over boron-doped carbon nanotubes deposited on the GC-electrode (Deng et al., 2008) and for the GOx immobilized on the GC-electrode modified with Nafion and mesoporous carbon FDU-15 (Wang et al., 2009b) it is almost 0.5 what is very close to α values calculated in our study. The electron transfer rate constant (k_s) was determined to be $1.32\,cm\,s^{-1}$ and this value is higher than it was reported for the GC-electrode modified by carbon nanotubes ($1.16\,cm\,s^{-1}$) (Deng et al., 2008) and this indicates that the PPMH facilitate the electron-transfer between the GOx and the electrode. According to Laviron's equation (Laviron, 1979b):

$$I_p = n^2F^2\frac{VA\Gamma}{4RT} \quad (1)$$

where A is the electrode-area; n is the number of electrons; R is an ideal gas constant, T is the temperature in Kelvin; F is Faraday's constant; V is the scan rate and Γ is the surface-coverage of the GOx on the electrode-surface that is estimated to be $8.50 \times 10^{-11}\,mol/cm^{-2}$. This value is higher than the theoretical value ($2.86 \times 10^{-12}\,mol/cm^{-2}$) for the GOx monolayer deposited on the bare-electrode suggesting that the PPMH provide a larger effective surface-area for enzyme immobilization. This value is also in the same range with the value of $8.77 \times 10^{-11}\,mol/cm^{-2}$ for the GOx/CdTe/CNTs/Nafion-electrode and it was reported that it is a two-electron transfer reaction (Liu et al., 2007).

Previous studies have shown that the pH of solution is important for the electrochemical behavior of redox-enzymes including GOx (Sheng et al., 2009). In order to determine the effect of pH on the GOx/PPMH/GC-electrode, the CVs were recorded in A-PBS at six different pH values, at the scan rate of 100 mV/s under N_2 saturated conditions. The pH values were selected between 5.0 and 9.0, corresponding CVs are presented in Fig. 4A. For each different pH, pair of stable and well-defined redox-peaks that belong to the $FAD/FADH_2$ forms of cofactor of enzyme were observed (Fig. 4A). The CVs at different pH-values show that by increasing of pH from 5.0 to 9.0, the peak-potentials in all CVs become more negative what is in good agreement with other published results (Wang et al., 2009b). According to some other studies, the maximal-responses for GOx-based biosensors have been registered in PBS at: pH 6.0 (Ramanavicius et al. 2006; Ivnitski et al., 2006), pH 6.98 (Deng et al., 2008), pH 7.1 (Wang et al., 2009c). Fig. 4B represents the plot of pH *vs.* formal-potential and it illustrates that the formal-potential has a linear-relationship at different pH values. The slope of the line in this plot was found as −62.5 mV/pH, which is close to the theoretical value of −58.5 mV/pH for a reversible two-proton/two-electron transfer based electrochemical redox process (Ivnitski et al., 2006), which was observed due to the redox reaction of the $FAD/FADH_2$:

$$GOx - FADH_2 \leftrightarrow GOx - FAD + 2e^- + 2H^+ \quad (2)$$

Since it is a two-electron transfer process, which is coupled with two-proton reaction, the peak-potentials should be pH-dependent.

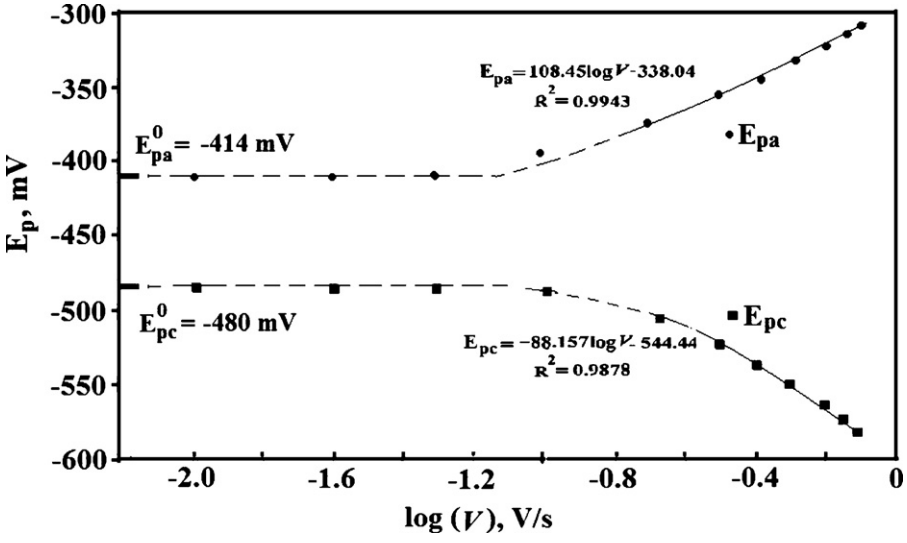

Fig. 3. Laviron's plot for the dependence of peak potentials on logarithm of scan rate for cyclic voltammograms presented in Fig. 2.

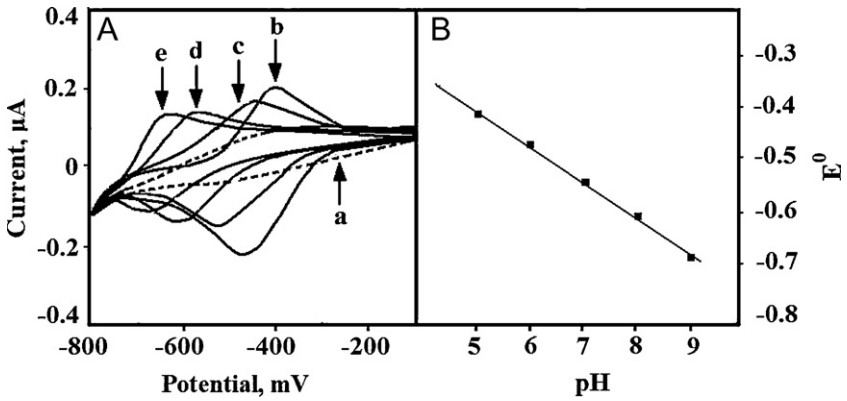

Fig. 4. (A) Cyclic voltammograms of GOx/PPMH/GC electrode at different pH values of 0.1 M A-PBS: (a) 5.0, (b) 6.0, (c) 7.0, (d) 8.0 and (e) 9.0 at the scan rate of 100 mV/s. Potentials are referred *vs.* Ag/AgCl/KCl_{sat}. (B) Formal potential *vs.* pH values.

The idea of the reversible reaction coupled with two-electron thought as a result of the equality of current peaks was supported by this indication. In the other studies this slope was calculated as: −61.5 mV/pH for electrode modified by boron-doped-carbon-nanotubes (Deng et al., 2008), −55.64 mV/pH for Nafion and mesoporous carbon FDU-15 modified GC-electrode (Wang et al., 2009b), −52.1 mV/pH for the $NdPO_4$ nanoparticles/chitosan composite film modified GC-electrode (Sheng et al., 2009).

3.3. Study of electrocatalytic activity of GOx

Previous section indicated that the electron-transfer at the GOx/PPMH/GC-electrode was registered, while this section is presenting some electrocatalytic-experiments, which were performed in order to prove the enzymatic-activity of the GOx within GOx/PPMH/GC-electrode. For this purpose electrocatalysis of the GOx was investigated by monitoring of changes in the redox-peaks in the presence of different glucose concentrations at the potential-range from −100 mV to −800 mV. The CVs of the GOx immobilized on the PPMH/GC-electrode in A-PBS, pH 6.0, under oxygen-free conditions were registered at several different concentrations of glucose as it is presented in Fig. 5A. With the addition of glucose into A-PBS, pH 6.0, the anodic-peak increased what is clear indication of the electrocatalytic properties of the GOx activity. However at the same time the cathodic-peak current increased as well, this effect could have been attributed to the effect of some FAD-moieties, which are loosely bounded to apo-enzyme and/or is diffused from the GOx, because such FAD-moieties at some extent are usually present in the GOx-samples. The increase of the anodic peak current of the GOx/PPMH/GC-electrode reached the steady-state at 4.0 mM of glucose (Fig. 5B) and the LOD for glucose was found as 0.05 mM, while some other authors also reported comparable LODs for GOx-based amperometric-biosensors, e.g., 0.030 mM (Zhu et al., 2007), 0.1 mM (Wu et al., 2007a,b), 0.08 mM (Sheng et al., 2009), and 0.09 mM (Wang et al., 2009b).

As mentioned below, when the glucose concentration exceeds 4.0 mM, the differences in the peak currents (ΔI) reaches the steady-state value (Fig. 5B) showing a typical hyperbolic dependence of ΔI *vs.* glucose concentration, which could be well described by Michalis–Menten kinetics. The apparent Michalis–Menten's constant (K_m^{app}), as an indicator of enzyme–substrate reaction kinetics, was calculated to evaluate the biological activity of the immobilized enzyme and it was calculated using the Lineweaver–Burk plot (Kausaite-Minkstimiene et al., 2010). The evaluation of corresponding plot yielded K_m^{app} of 0.64 mM. The K_m^{app} value is significantly smaller than K_m reported for the dissolved GOx, which is 27.0 mM (Rogers and Brandt, 1971). Here observed decrease of K_m^{app} is in line with that reported in other GOx-based biosensors, e.g., 5.1 mM (Huang et al., 2005), 10.5 mM (Wu et al., 2007a,b); 8.5 mM (Liu et al., 2008); 3.12 mM (Qiu et al., 2009), 0.91 mM (Salimi et al., 2009), 0.2 mM (Deng et al., 2008), 0.03 mM (Zhilei et al., 2010). The smaller K_m^{app} value calculated in our study indicates that the GOx immobilized on the PPMH/GC possesses higher enzymatic-activity and higher affinity towards glucose if compared with native GOx.

Amperometric investigation of here presented glucose-biosensor demonstrated that the linear-range reaches 2.0 mM of glucose at electrode potential of +200 mV (Fig. 6). The reproducibility of this glucose-biosensor was also investigated by detecting 0.5 mM of glucose successfully for ten times. The calculated

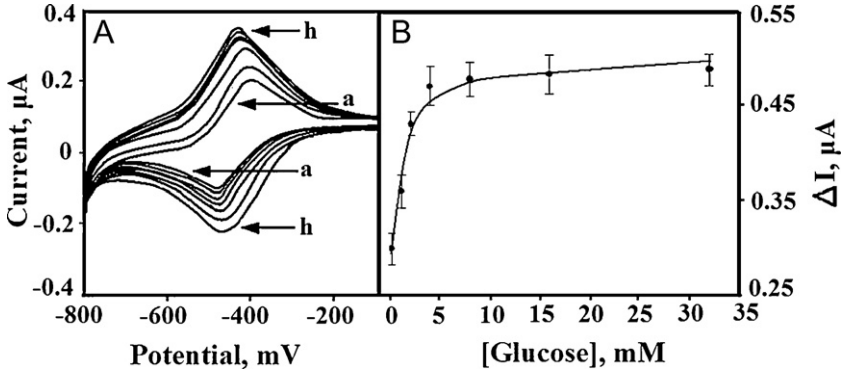

Fig. 5. (A) Cyclic voltammograms of GOx/PPMH/GC electrode in 0.1 M A-PBS, pH 6.0, at the scan rate of 100 mV/s in the absence of glucose (a) and the presence of glucose: 0.05 mM (b), 1.0 mM (c), 2.0 mM (d), 4.0 mM (e), 8.0 mM (f), 16 mM (g), 32 mM (h). Potentials are referred *vs.* Ag/AgCl/KCl_{sat}. (B) The difference of catalytic-current (ΔI) *vs.* glucose concentration.

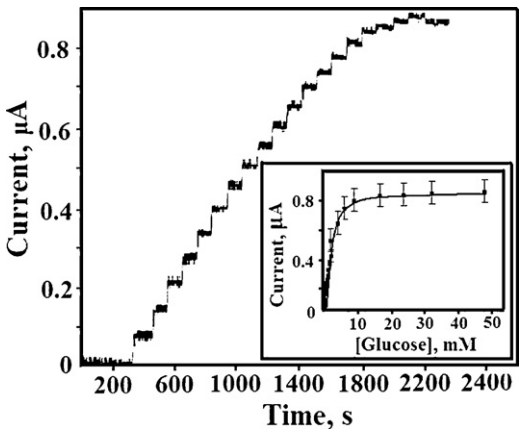

Fig. 6. Amperograms of GOx/PPMH/GC electrode at +200 mV *vs.* Ag/AgCl/KCl_{sat} upon successive addition of 0.05–48 mM glucose to 0.05 M A-PBS, pH 6.0. Inset: amperometric responses *vs.* glucose concentration at +200 mV *vs.* Ag/AgCl/KCl_{sat}.

standard deviation (1.17%) illustrates that the prepared electrode has a good reproducibility and this electrode exhibits better reproducibility than that reported by Shan et al. (2009) (3.2%), Deng et al. (2008) (3.5%), and Liu et al. (2007) (3.3%). The variation coefficient for 10 measurements was calculated as 2.91%.

4. Conclusions and future developments

The PMH was electrochemically grafted and polymerized on the GC-electrode by potential cycling in non-aqueous media *vs.* Ag/Ag^+ (10 mM $AgNO_3$). After this modification the GOx was immobilized on the PPMH/GC-electrode. The effect of the surface-modification by the PPMH on the electron-transfer between enzyme and electrode and some other electrochemical/analytical parameters of newly designed enzymatic-electrode were evaluated. The GOx immobilized on the PPMH/GC-electrode realized a reversible electrochemical oxidation/reduction, based on the exchange of two electrons and two protons. The PPMH was very efficiently involved in the DET between the enzyme and the electrode. The GOx/PPMH/GC-electrode showed a very high sensitivity to glucose at lower potential range and with lower K_m^{app} value than it was reported before. According to our best knowledge, this is the first report on evaluation of PPMH as a redox mediator and/or DET material suitable for the GOx. The presented and discussed data show that the PPMH is promising DET material for the development of amperometric-biosensors, biofuel cells and other bioelectronic devices.

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