GLUCOSE OXIDASE-POLYPYRROLE ELECTRODES SYNTHESIZED IN *p*-TOLUENESULFONIC ACID AND SODIUM *p*-TOLUENESULFONATE
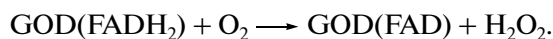
© 2011   G. Ozyilmaz, A. T. Ozyilmaz, F. Can

University of Mustafa Kemal, Faculty of Arts and Sciences, Department of Chemistry, 31040 Hatay-Turkey
*e-mail: gozyilmaz@gmail.com*
Received May 20, 2010

Amperometric glucose biosensors have been developed based on entrapment on platinum (Pt) electrode using cyclic voltammetry technique in glucose oxidase (GOD) and pyrrole containing *p*-toluenesulfonic acid (pTSA) or sodium *p*-toluenesulfonate (NapTS) as supporting electrolyte solutions. Both of electrolyte solutions were suitable media for the formation and deposition of polypyrrole-GOD (PPy-GOD) layers on Pt substrate. Pt/PPy-GOD electrodes brought about in different morphological properties as well as different electrochemical and biochemical response. The highest responses obtained in pTSA and NapTS electrolytes were observed at pH of 4.5 and 7.0 for Pt/PPy-GOD electrodes, respectively. While linearity was observed between 0.0–1.0 mM glucose substrate for both electrodes, I_{max} value of Pt/PPy-GOD$_{NapTS}$ electrode was approximately twice as high as that of Pt/PPy-GOD$_{pTSA}$ electrode as 25.4 and 14.2 µA, respectively. Five commercial drinks were tested with enzyme electrodes and compared with results obtained spectrophotometrically using glucose kit. Results revealed that Pt/PPy-GOD$_{NapTS}$ electrode exhibited better biosensor response.

Detection of glucose is rather a common practise both in medicine and also in food nutrient industry. There are several ways to measure glucose level based on chemical methods using Folin-Wu, Fehling, Benedict, *o*-toluidine reagent, etc. [1, 2]; enzymatic methods such as glucose oxidase [3, 4] in addition to electrochemical methods [5, 6]. Enzymatic methods are widely used due to specific features of the substrate and absence of any side effects. Detection of glucose level with glucose biosensor is an intensively investigated research area on account of its several advantages, e.g. ease of application, rapidity, simple pretreatment and time saving. Glucose oxidase (EC 1.1.3.4), which is the first enzyme used to construct enzyme electrode, catalyses the oxidation of glucose and produces hydrogen peroxide according to the reaction given below:

$$Glucose + GOD(FAD) \longrightarrow gluconolactone + GOD(FADH_2)$$

$$GOD(FADH_2) + O_2 \longrightarrow GOD(FAD) + H_2O_2.$$

Since hydrogen peroxide concentration is proportional to glucose concentration, the latter can be easily determined by current response detected by the amperometric method from hydrogen peroxide oxidation reaction:

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-.$$
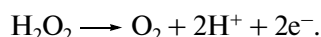
There is a considerable demand for the immobilization of GOD onto electrode surfaces and preparation of biosensors based on electrocatalytic systems of enzymes [7–11]. Conducting polymer immobilization matrices are conceptually attractive as a means of providing a three-dimensional 'conducting' network, where charge transfer can occur between a redox species and the polymer within the pores of the matrix [12]. In particular, immobilization of enzymes on electrode surfaces via various conductive polymers such as polypyrrole [9, 13–15], polyaniline [8, 10, 16], poly(3-methylthiophene-co-thiophene-3-acetic acid) [17], poly(phenylendiamine) [7, 18, 19], Poly(3,4-ethylenedioxythiophene) [20], poly(*o*-anisidine) [16, 21] has been extensively studied because of its high conductivity, stability and ease in preparation. Since polypyrrole is compatible with most enzymes and can be easily synthesized from pyrrole monomer in either aqueous or organic solution, the polypyrrole/glucose oxidase (PPy/GOD) biosensor is the most attractive topic of research in biosensor area [22]. When entrapment method is used to prepare the GOD electrode, biosensor properties such as porosity of conductive polymer, diffusion rate of glucose and O_2 into enzyme microenvironment, the catalytic efficiency of GOD and charge transfer rate are affected by electrosynthesis technique, electrolyte solution and its pH, potential range and scan rate. Choosing the electropolymerization conditions for PPy/GOD system in terms of electrolyte solution is crucial because both anions and cations influence the polymer morphology and its electrochemical properties. There are several studies related with PPy/GOD electrode preparation in different electrolyte systems, namely KCl solution [22–24], phosphate buffer [25], NaCl [26], osmium complex containing tetramethylammoniumperchlorate [14].

It is well known that when acidic electrolyte solution is used, synthesized polymer becomes more conductive, an important parameters for biosensors [27]. However, partial denaturation of the enzymes can be observed when entrapment method is used as an immobilization way. Therefore, it was found worthy to investigate the effect of acidity of electrolyte used in establishing the glucose bio-

sensor while different anion was absent in the electrolyte solution.

In this study, GOD was entrapped in PPy which synthesized by cyclic voltametry technique onto Pt to constitute PPy/GOD electrode. Synthesis was achieved in acidic and neutral supporting media as paratoluensulfonic acid (pTSA) and sodium paratoluenesulfonate (NapTS). Accordingly, the effects of pH and glucose concentration on biosensor response were studied. Operational stability for 50 uses depending on glucose concentration as well as storage stability were investigated. Glucose concentration of five different beverage samples was determined by Pt/PPy-GOD electrodes prepared in pTSA and NapTS electrolytes.

MATERIALS AND METHODS

All the chemicals used were of analytical grade. Glucose oxidase (GOD) from *Aspergillus niger* (Sigma). Pyrrole was freshly distilled and stored in the dark. The solutions were prepared with double distilled water and all experiments were carried out at room temperature open to atmosphere. In this study, all the electrochemical experiments were performed in a single compartment cell with three electrode configurations. Reference electrode was an Ag/AgCl (3 M, KCl) electrode and counter electrode was a platinum sheet with a surface area of $2.0\ cm^2$. A CHI 660b model digitally controlled electrochemical analyzer (serial number: A1420) was used in electrochemical experiments. All of the potential values were referred to the Ag/AgCl (3 M, KCl) electrode. The working electrode was a platinum sheet with a surface area of $0.18\ cm^2$. Before electropolymerization, rinsed in 1 : 1 ethanol acetone mixture, washed with bi-distilled water and dried.

Preparation of the enzyme electrodes. Enzyme modified electrodes were prepared by incorporating enzyme into the polypyrrole (PPy) during the polymerization of pyrrole (Py) on Pt electrode. To this end, thin films of PPy were synthesized electrochemically on platinum electrode using cyclic voltammetric technique, in presence of GOD. In order to use similar electrochemical conditions, the electropolymerization of Py was carried out in the 0.20 M pTSA or 0.20 M NapTS as supporting electrolyte containing 0.10 M Py and 2.50 mg/ml GOD. PPy films on Pt electrode were deposited by applying sequential linear potential scan rate of 50 mV/s between 0.10 to 1.00 V for NapTS solution and 0.10 to 0.75 V for pTSA solution in 50 cycles. Enzyme modified PPy films were obtained with 50 cycles for electropolymerization. Having been deposited, the films were washed with bi-distilled water and dried. Enzyme electrodes obtained in NapTS and pTSA electrolytes were referred to as Pt/PPy-GOD$_{NapTS}$ and Pt/PPy-GOD$_{pTSA}$, respectively.

Electrochemical characterization of the enzyme electrodes. Lineer sweep voltametry technique was applied to Pt/PPy-GOD$_{NapTS}$ and Pt/PPy-GOD$_{pTSA}$ electrodes at potential range between 0.10 and 0.75 V by 1 mV s^{-1} scan rate. Cyclic voltammetry was also applied at the same po-

tential ranges using 5.0 mV s^{-1} scan rates. The characterization curves of enzyme modified PPy films were obtained with 10 cycles.

Biochemical characterization of the enzyme electrodes. Both electrodes were biochemically characterized by monitoring the current values using glucose solutions at various conditions such as different potential, pH and substrate concentration rates. Operational stabilities in 5, 10 and 20 mM glucose solutions by 50 repeatedly uses and storage stabilities at 10°C were also investigated. Five different beverages such as malt drink, fizzy drink, wine, honey and grape juice were tested by Pt/PPy-GOD$_{NapTS}$ and Pt/PPy-GOD$_{pTSA}$ electrodes for determination of glucose concentration and compared with results obtained using commercial glucose kit.

RESULTS AND DISCUSSION

Synthesis. Figure 1 shows the first and fiftieth scans of the cyclic voltammograms recorded for Pt electrode in monomer free electrolyte (*1*), 0.10 M Py and electrolyte (*2*), and GOD and Py containing electrolyte (*3*) solutions in supporting electrolytes as 0.20 M NapTS (a) and in 0.20 M pTSA (b), respectively.

All measurements were taken at scan rate of 50 mV s^{-1}. In absence of monomer and GOD, anodic and cathodic current values obtained for NapTS medium remained almost constantly close to zero up to 0.75 V. With the aim of better quality PPy film, PPy film in NapTS solution was deposited on Pt electrode by applying sequential linear potential scan rate 50 mV/s between 0.10 to 1.00 V potential range. In Py containing NapTS solution, potentiodynamic behaviours were significantly different from that of monomer free solution. While current values remained constant close to zero up to 0.34 V, the rapid anodic current increase after approx. 0.58 V was related to the oxidation of pyrrole. It must be noted that the current values decreased dramatically with increasing cycle numbers. In every case, these current values recorded for NapTS and Py containing solution after 0.34 V were significantly higher when compared with that of pure NapTS solution. During first reverse scan, cathodic peaks after approx. 0.36 V was attributed to the reduction of previously produced thin PPy film on platinum electrode. Cathodic current values were found to decrease regularly with increasing scanning numbers. In presence of GOD containing Py and NapTS solution, first cyclic voltammogram was significantly different from those recorded in monomer containing aqueous NapTS and only NapTS solution. Yet, behaviours similar to those of GOD free medium were obtained up to 0.74 V in presence of GOD with negligible small current values. Then, anodic current wave, which shifted to cathodic potential during every scan, was attributed to GOD oxidation process. During this potential scanning, the monomer oxidation process was observed as an increase in current. These anodic current values rose in increasing scan number. Yet, these values recorded for monomer oxidation in GOD containing
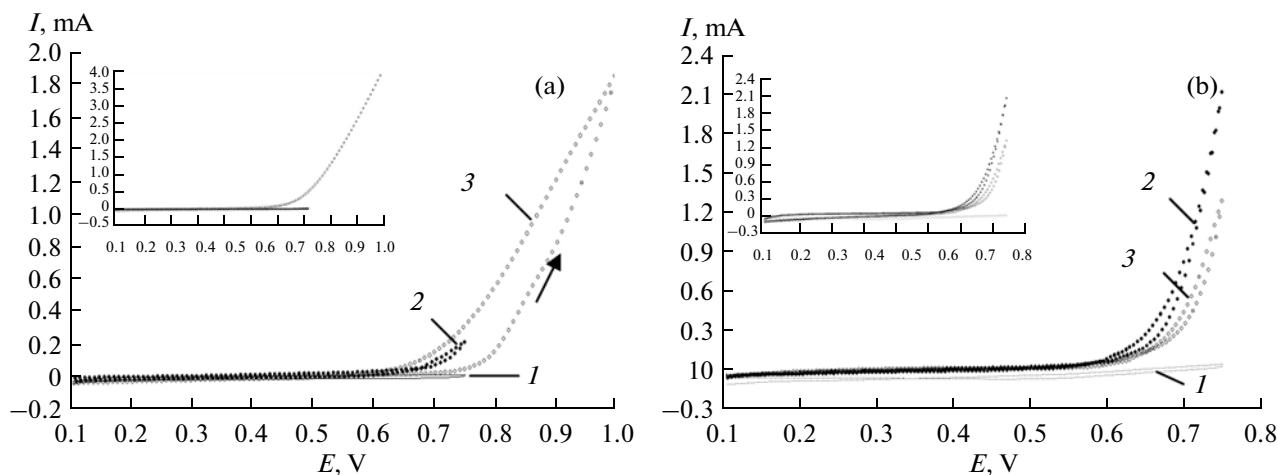
Fig. 1. First cyclic voltammograms recorded for Pt electrode in free monomer electrolyte (*1*), Py and electrolyte (*2*) and GOD, Py and electrolyte (*3*) solutions, in 0.20 M NapTS (a) and in 0.20 M pTSA (b) such as electrolyte solution, respectively. Scan rate: 50 mV s^{-1}. Inset graphs are fiftieth CVs.

Py and NapTS solution were fairly higher than those of Py and NapTS solution. This could be due to higher conductivity of PPy film with GOD freshly synthesized in presence of GOD including solution than in GOD free conditions, in NapTS solution. During the fiftieth scan, current values recorded for GOD containing Py and NapTS solution were significantly higher when compared with that of monomer containing aqueous NapTS and pure NapTS solution (Fig. 1a, insert). The position of the GOD oxidation wave shifted to negative direction such as approx. 0.57 V, during the fiftieth scan. The Pt surface in GOD containing Py and NapTS condition was covered with a greenish, uniform film.

In order to determine different processes occurring at Pt electrode surfaces, Pt electrode was coated with PPy in aqueous pTSA and GOD solutions. The first and the fiftieth cyclic voltammogram curves with and without GOD in monomer containing and monomer free pTSA medium were given in Fig. 1b. In presence of pTSA solution, behaviours similar to that of NapTS medium were observed as almost constantly close to zero from 0.10 to 0.75 V. In Py containing pTSA solution, while current values remained almost zero in a wide potential range up to approx. 0.60 V, current increases after this potential value were attributed to the oxidation of monomer. In presence of GOD containing Py and pTSA solution, although first cyclic voltammogram was significantly similar to those recorded in monomer containing aqueous pTSA solution, current values obtained for GOD containing Py and pTSA solution were relatively lower when compared with those of monomer containing aqueous pTSA and pure pTSA solution. This could be due to higher conductivity of PPy film synthesized in presence of GOD free acidic solution when compared with that of GOD containing Py and pTSA conditions. During the fiftieth scan, also the current values recorded for Py and pTSA solution were significantly higher when compared with those of GOD

containing Py and pTSA solution and pure pTSA solution (Fig. 1b, insert). The position of the GOD oxidation wave remained at almost the same potential, during the fiftieth scan. The Pt surface in GOD containing Py and pTSA condition was covered with a black uniform film.

SEM images of Pt/PPy electrodes prepared in pTSA and NapTS with GOD and GOD free, which are referred to here as Pt/PPy-GOD$_{pTSA}$, Pt/PPy-GOD$_{NapTS}$, Pt/PPy$_{pTSA}$ and Pt/PPy$_{NapTS}$, respectively were given in Fig. 2. The morphology of PPy film can be influenced by the dopant anion, composition of the electrolyte, current or potential for polymerization and electrode configuration [28]. PPy films on Pt/PPy electrodes obtained in NapTS and pTSA solution has different morphologies as seen in Figs. 2a and 2c, respectively. This may be due to different hydrogen ions concentration which can affect the PPy charge. It was also seen from the Figs. 2b and 2d that SEM images of Pt/PPy-GOD$_{NapTS}$ and Pt/PPy-GOD$_{pTSA}$ electrodes were different from their enzyme free counterpart. As the polymerization conditions such as monomer and enzyme concentration, applied potential and electrode configuration were the same in this study, the different morphology might be related to interactions between enzyme molecules and the conducting polymer [28].

Electrochemical characterization of enzyme electrodes. GOD enzyme entrapped polypyrrole (PPy) electrodes were obtained for glucose oxidation. Linear sweep voltammograms recorded for glucose oxidation currents of GOD immobilized PPy coated platinum (Pt/PPy-GOD$_{NapTS}$ and Pt/PPy-GOD$_{pTSA}$) electrodes in solutions with and without different glucose concentration are given in Fig. 3. On GOD modified PPy electrodes, electron transfer between enzymes and the electrode surface through PPy film was obtained with higher current values. Higher oxidation currents for 1, 10 and 20 mM glucose solution above 0.52 V versus Ag/AgCl were testified to the
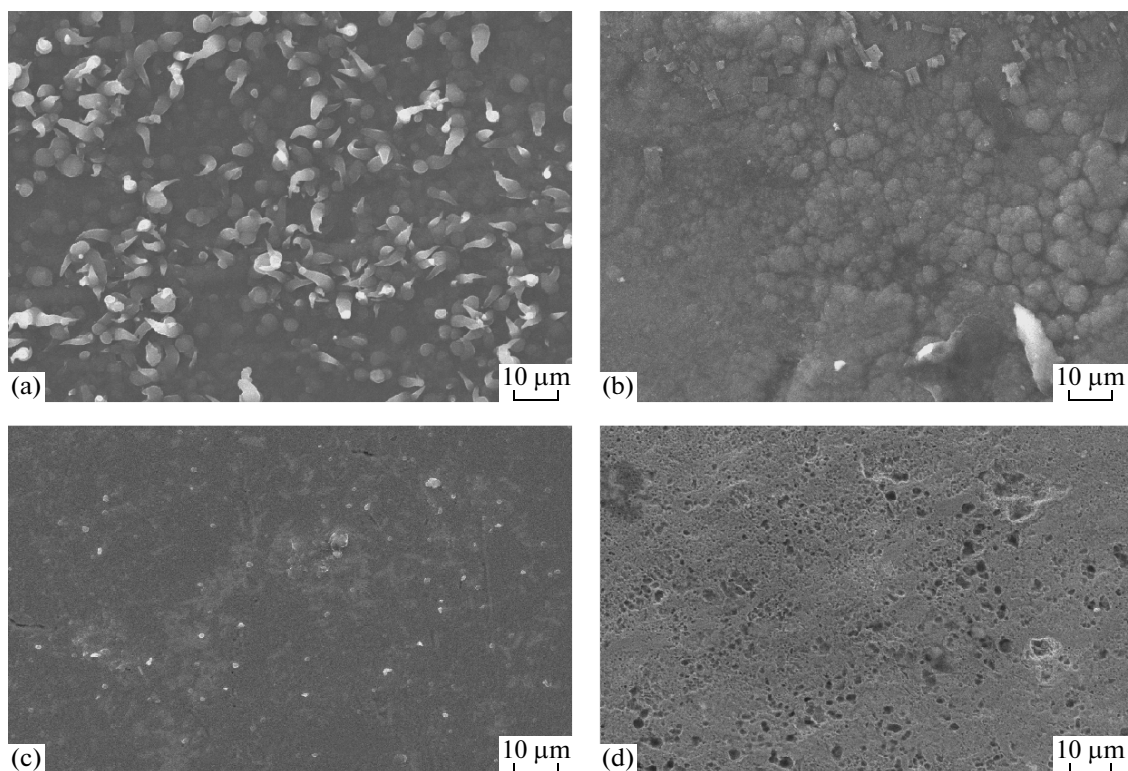
Fig. 2. SEM images of Pt/PPy$_{NapTS}$ (a), Pt/PPy-GOD$_{NapTS}$ (b), Pt/PPy$_{pTSA}$ (c), Pt/PPy-GOD$_{pTSA}$ (d) electrodes.
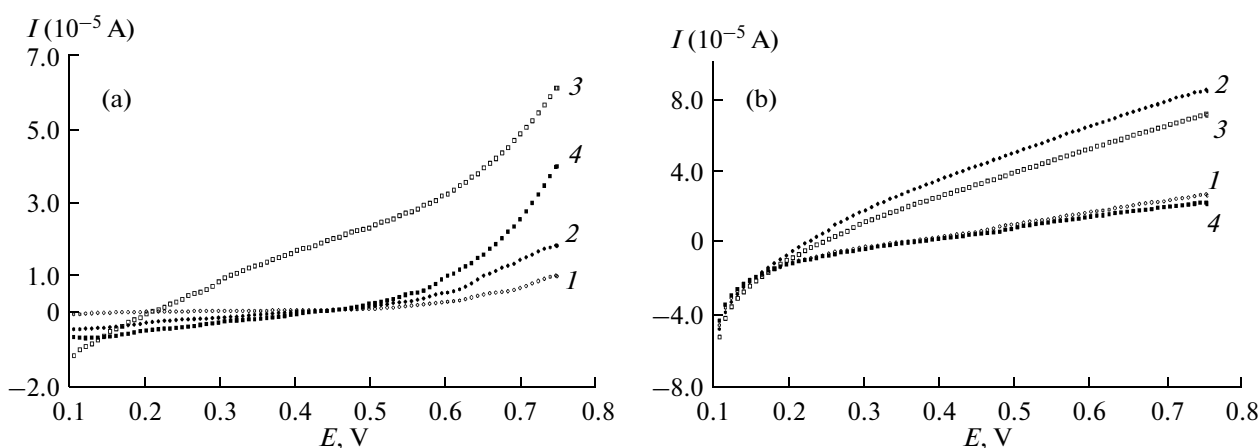

Fig. 3. Lineer sweep voltammograms for glucose oxidation of 0 mM (*1*), 1 mM (*2*), 10 mM (*3*) and 20 mM (*4*) for Pt/PPY-GOD$_{NapTS}$ (a) and Pt/PPY-GOD$_{pTSA}$ (b) electrodes, scan rate: 1 mV s^{-1}, 0.10 M phosphate buffer solution.

presence of glucose in the solutions. For buffer solution without glucose, dramatically low oxidation current values were observed due to deterioration of polypyrrole film when potential was moved to more positive potential than 0.46 V. But, it must be noted that this degradation process was not significant in terms of electron transfer of polymer film.

For Pt/PPY-GOD$_{NapTS}$ electrode, glucose oxidation was performed with glucose concentration up to 20 mM.

Maximum oxidation current was observed with glucose concentration of 10 mM. However, glucose oxidation current values increased in cathodic direction with the increasing glucose concentration when the potential was lower than approx. 0.46 V, except 10 mM glucose solution. Oxidation current values for 20 mM were significantly lower than that of 10 mM. Similar behavior was reported by Yu et al. [28] who argued that this case could be explained by sensitivity to glucose concentration up to 20 mM. For Pt/PPY-GOD$_{pTSA}$ electrode, irregular oscil-
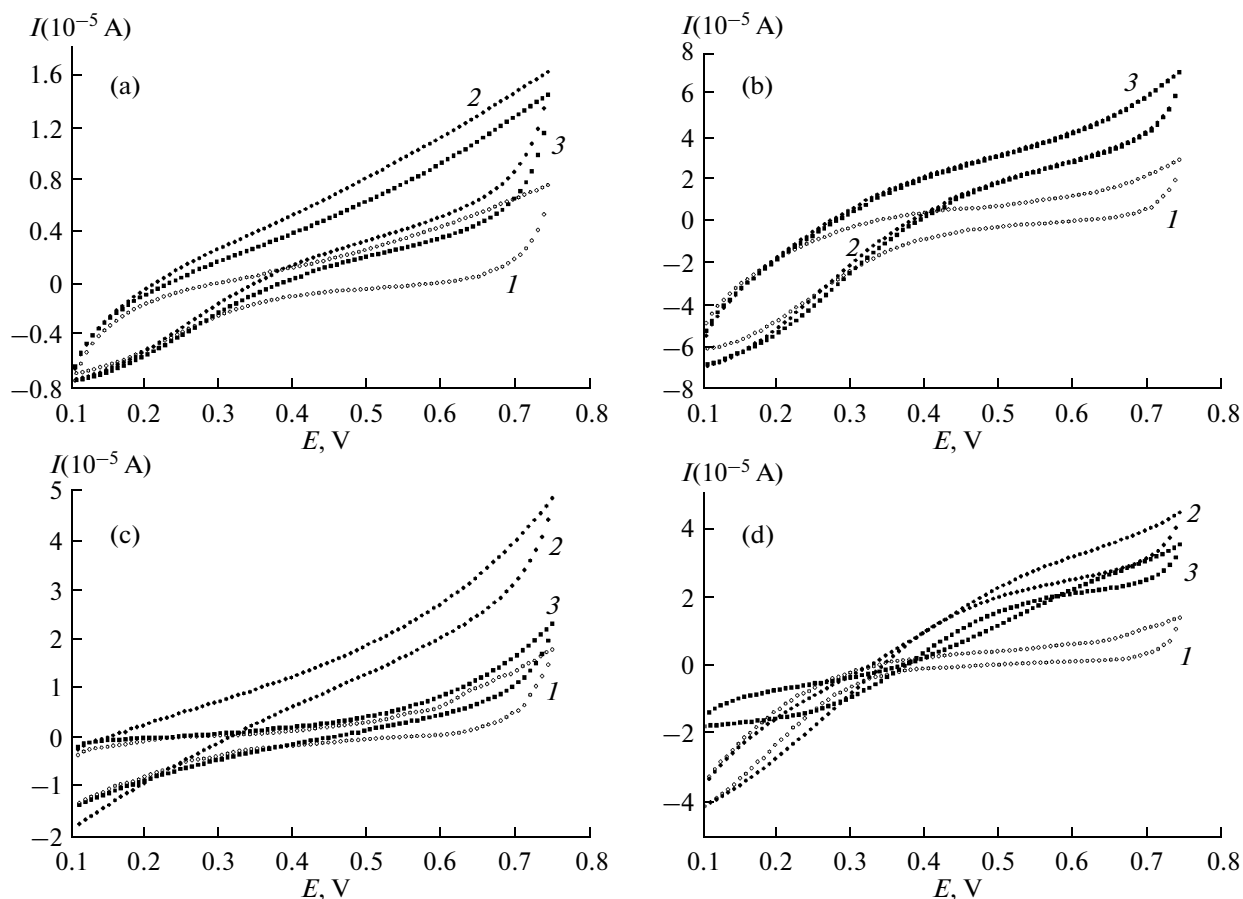
Fig. 4. First (a, c) and tenth (b, d) cyclic voltammograms for glucose oxidation of 0 mM (*1*), 10 mM (*2*) and 20 mM (*3*) on Pt/PPY-GOD$_{pTSA}$ (a, b) and Pt/PPY-GOD$_{NapTS}$ (c, d) electrodes, scan rate: 5 mV s^{-1}, 0.10 M phosphate buffer solution.

lation in current values depending on glucose concentration was observed. Maximum current was detected with glucose concentration of 1 mM. The declining current values with increasing glucose concentration were related to poor electron communication between enzyme active centre and electrode. This was attributed to poor amount of GOD entrapped in polypyrrole matrix, which had low current value passed for the electropolymerization in pTSA solution when compared with that of NapTS solution (Figs. 1a and 1b). Besides, the partial denaturation of GOD in 0.20 M pTSA solution, which is at low pH value was another reason for the limited GOD entrapped and moved into polymer matrix.

Figure 4 shows the cyclic voltammograms for the glucose oxidation on the Pt electrode of GOD entrapped PPy film formed in pTSA solution, which is referred to here as Pt/PPY-GOD$_{pTSA}$. In presence of glucose containing buffer solution, oxidation current values for Pt/PPY-GOD$_{pTSA}$ electrode were fairly higher when compared with that of glucose free buffer solution during first scan (Fig. 4a, *1*). Maximum oxidation current values were obtained with glucose concentration of 10 mM. Yet, the current values recorded for 20 mM were significantly lower than that of 10 mM glucose concentration. This

case suggested that Pt/PPY-GOD$_{pTSA}$ electrode had sensitivity to glucose concentration up to 10 mM. With both glucose concentrations, oxidation current values decreased proportional to an increase in scan numbers as seen in Fig. 4b, *1* which showed the results of tenth scan number. Current values during every scan were highest in 10 mM glucose concentration solution. Buffer solution with and without glucose, higher anodic current values were obtained in case of glucose, indicating the oxidation of glucose together with PPy while the oxidation/reduction redox behaviours of PPy film were observed during the first and the tenth anodic and cathodic scans of cyclic voltammograms. Yet, oxidation current values recorded for Pt/PPY-GOD$_{pTSA}$ electrode were rather higher when compared with that of Pt/PPY-GOD$_{NapTS}$ electrode in presence of glucose.

Figures 4c, 4d shows cyclic voltammograms for the glucose oxidation on the Pt electrode of GOD entrapped PPy film formed in NapTS solution, which is referred to here as Pt/PPY-GOD$_{NapTS}$. In case of glucose free buffer solution, current values over 0.28 V remained almost constant in vicinity of zero up to 0.51 V, while small current values at approx. 0.28 V were observed during first anodic and cathodic scans of cyclic voltammograms. These redox

Relative activity, %
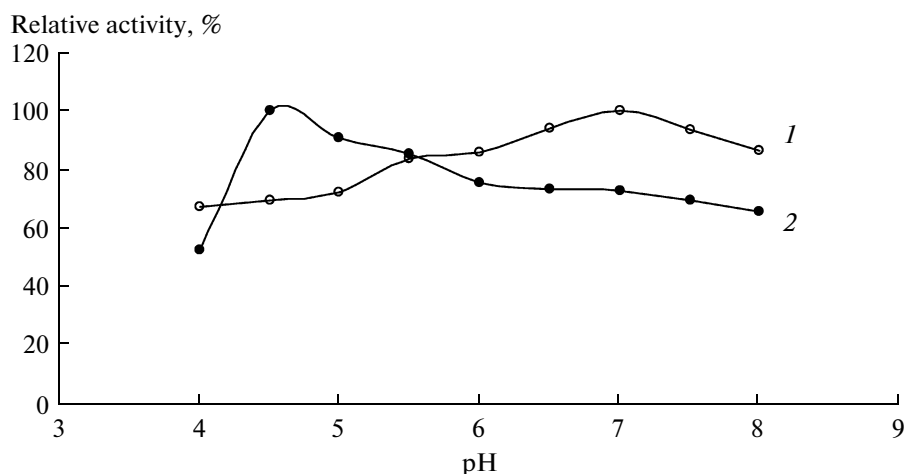
Fig. 5. The effect of pH on current responses of Pt/PPY-GOD$_{NapTS}$ (*1*) and Pt/PPY-GOD$_{pTSA}$ (*2*) electrodes (at 10 mM of glucose solution).

peaks were due to oxidation/reduction of PPY film. The oxidation current wave which increased rapidly after 0.51 V was attributed to the deterioration of PPy film. In presence of glucose containing buffer solution, oxidation current values for Pt/PPY-GOD$_{NapTS}$ electrode were higher when compared with that of glucose free buffer solution. These values decreased with increasing glucose concentration. Besides, the oxidation current values recorded for 10 mM glucose concentration solution started to increase at former potential values. This proved that the electron transfer between the enzyme active centre and the electrode was rather vigorous. In presence of buffer solution with and without glucose (Figs. 4c, 4d), cyclic voltammograms of the tenth scan for Pt/PPY-GOD$_{NapTS}$ electrode were significantly different from those recorded in glucose containing buffer and buffer solution. In glucose containing buffer solutions, current values decreased due to activity loss of Pt/PPY-GOD$_{NapTS}$ electrode, while an increament was observed for glucose free buffer solution due to the detriment of PPy, during the tenth scan. Moreover, similar behaviours to that of 10 mM glucose medium were obtained up to 0.13 V, in presence of 20 mM glucose solution with negligible small current values. Then, anodic current increases for 10 mM glucose solution were observed after approx. 0.13 V due to glucose oxidation process.

Biochemical characterization of enzyme electrode. The biochemical characterization of the enzyme electrodes was carried out by measuring current response at varied conditions. The principle of the determination of the current response is based on the decomposition of hydrogen peroxide which was produced during the enzyme-catalyzed reaction. Catalytic activities of most enzymes are affected from their ambient pH values. The pH value is an important variable that affects enzyme activity and therefore current response of the enzyme electrodes. Therefore, generally determined is the pH value known as optimal pH, at which enzyme exhibits the maximum reaction

rate. So, current values were measured using 10 mM glucose solutions at varied pH between 4.0 and 8.0 for Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes at first. Figure 5 represents the effect of pH on the response of Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes.

As seen in Fig 5, the biosensor response increased with increasing pH value from 4.0 to 7.0 for Pt/PPy-GOD$_{NapTS}$ electrode, and from 4.0 to 4.5 for Pt/PPy-GOD$_{pTSA}$ electrode, then decreased as pH increased further. Maximum responses were observed at 4.5 and 7.0 pH values for Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes, respectively. Ren et al. [29], PPy synthesized onto Pt electrode in pTSA containing almost neutral (pH 6.86) electrolyte solution, on which GOD was adsorbed to get glucose biosensor. When they investigated the effect of pH on the response of the PPy/GOD electrode, they observed the maximum response at pH value of 6.86.

The effect of substrate concentration on Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes was investigated using glucose solution with concentration varying between 0.10 and 30.0 mM at optimal pH values for each electrode types. Current value of glucose free solution was subtracted from current values of glucose solutions to calculate the response of electrode depending on substrate concentration. As seen in Figs. 6a, 6b, current values increase in parallel with the rise in glucose concentration at Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes, respectively.

Electrodes (current values were measured by 10 mM glucose solution in optimal pH buffers) linearity was observed upto 1.0 mM while current values were not proportional with glucose concentration at upper concentration values.

I_{max} values were calculated as 25.4 and 14.2 µA whereas K_M^{app} values were 5.2 and 1.7 mM for Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes, respectively by using Lineweaver-Burk plots. It was clearly seen that, response
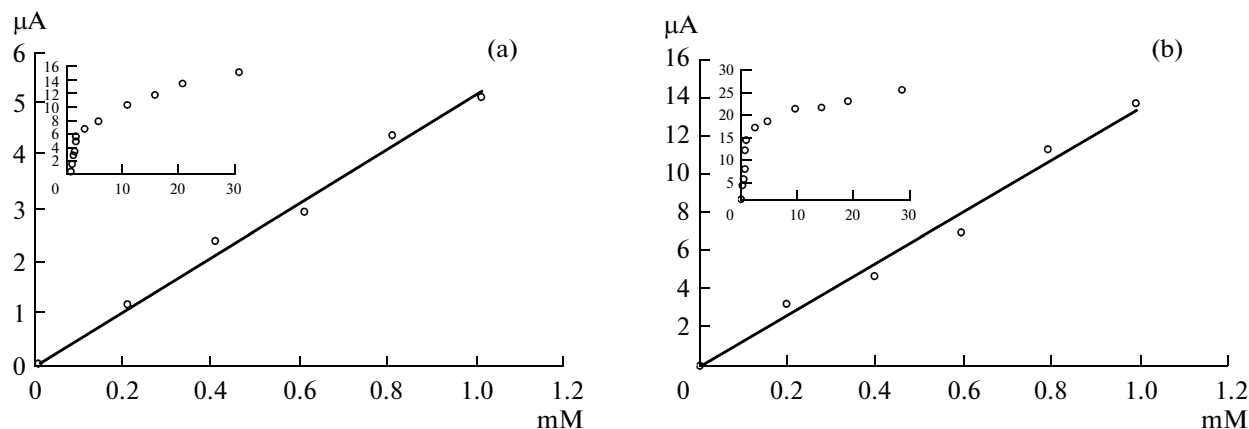
Fig. 6. The effect of glucose concentration (mM) on current responses (μA) of Pt/PPY-GOD$_{pTSA}$ electrode (at pH 4.5, a) and Pt/PPY-GOD$_{NapTS}$ electrode (at pH 7.0, b).
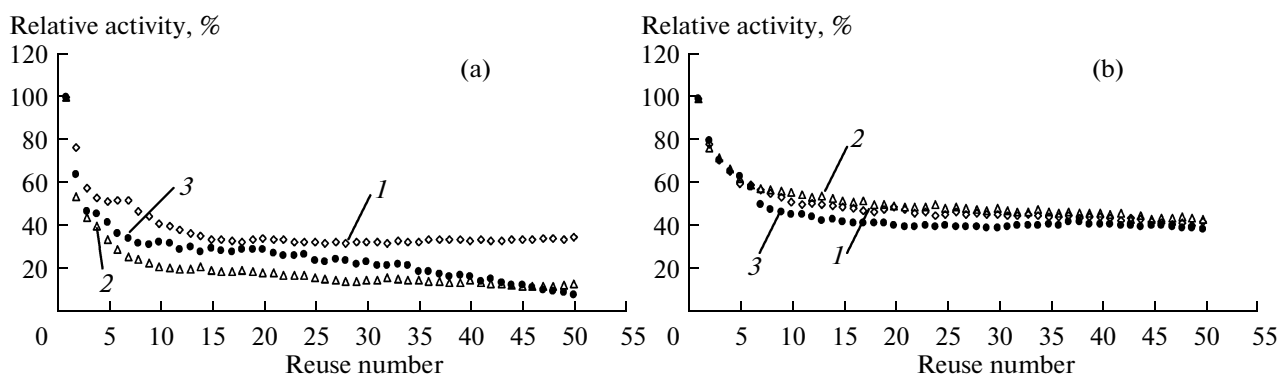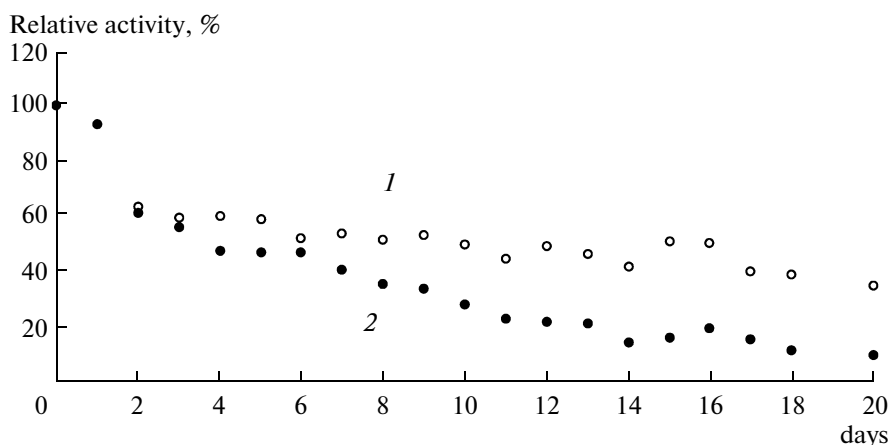

Fig. 7. Reusability of Pt/PPY-GOD$_{pTSA}$ (a) and Pt/PPY-GOD$_{NapTS}$ (b) electrodes in glucose solution at different concentration 5 (*1*), 10 (*2*), 20 mM (*3*).

of Pt/PPY-GOD$_{NapTS}$ electrode was as two much as higher than that of Pt/PPY-GOD$_{pTSA}$ electrode. Moreover, K_M^{app} value of Pt/PPY-GOD$_{pTSA}$ electrode was higher than that of Pt/PPY-GOD$_{NapTS}$ electrode. It may be probably due to acidic medium of pTSA electrolyte solution used for preparation of Pt/PPY-GOD$_{pTSA}$ electrode which played a role in partial denaturation of GOD protein causing a decline in affinity to glucose substrate. It was observed that PPy film was synthesized as more conductive when pTSA electrolyte was used as electrolyte as seen from Fig. 4 and Fig. 5. However, when current value of glucose free solution was subtracted from glucose solution at certain concentration, it was clearly seen that Pt/PPY-GOD$_{NapTS}$ electrode showed better biosensor response. So, these results indicated that biosensor response was more affected from GOD efficiency. Ma et al. [30] prepared GOD/PPy film on to Pt electrode by adsorption of GOD on PPy polymer, and they found linearity ranging between 0 and 17 mM at a potential of 0.40 V [30]. Ekanayake and Preethichandra [31] established an amperometric glucose biosensor by adsorbing GOD onto platinum-coated nonporous alumina electrode and they

reported the K_M^{app} and I_{max} values as 7.01 mM and 120 μA, respectively.

Operational stability of Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes was investigated by 50 repeated uses in three glucose solutions at 5, 10 and 20 mM. Results were given in terms of relative activity percentage depending on reuse number in Figs. 7a and 7b for Pt/PPY-GOD$_{pTSA}$ and Pt/PPY-GOD$_{NapTS}$ electrodes, respectively.

As observed usually in enzymatic studies in the literature, response sharply declined in the first 5 uses and then decreased slowly in subsequent uses for all measurements. Operational stability of Pt/PPY-GOD$_{NapTS}$ electrode was not affected from glucose concentration as seen in Fig. 7b. Operational stabilities which realized as approximately 40% at the end of 50 uses were similar for Pt/PPY-GOD$_{NapTS}$ electrode used with 5, 10 and 20 mM glucose substrates. However, in the case of Pt/PPY-GOD$_{pTSA}$ electrode, while similar results were obtained for 10 and 20 mM glucose (approximately 10%), more stability (35.2%) was observed for 5 mM glucose solution at the end of 50 uses. Pt/PPY-GOD$_{NapTS}$ electrode showed better operational

Fig. 8. Storage stability of Pt/PPY-GOD$_{NapTS}$ (*1*) and Pt/PPY-GOD$_{pTSA}$ (*2*) electrodes (current values were measured by 10 mM glucose solution in optimal pH buffers).

stability than that of Pt/PPY-GOD$_{pTSA}$ electrode. Immobilized enzymes generally lose activity depending on the number of uses [32, 33]. In the case of GOD, two more factors namely production of H$_2$O$_2$ and gluconic acid cause decrease in activity by repeated use. H$_2$O$_2$ inactivate GOD [34, 35] and decrease pH by gluconic acid accumulation around the microenvironment of GOD [32] and then causing denaturation of the enzyme. It was expected that if the amounts of accumulated H$_2$O$_2$ and gluconic acid varied, the decrease in activity would change too. But, when Pt/PPY-GOD$_{NapTS}$ electrode was used, the tendencies of 3 curves were almost the same. It may be glucose concentrations were higher than K_M values of 1.7 mM, which caused a similar activity close to V_{max}. On the other hand, H$_2$O$_2$ did not accumulate due to its electrochemical oxidation, so it did not cause an inactivation. However, in the case of Pt/PPY-GOD$_{pTSA}$ electrode whose K_M value was 5.2 mM, it was supposed that, GOD activity in consequence of gluconic acid accumulation increased by increasing glucose concentration. Therefore, stability decreased by increasing the glucose concentration upto 40th uses, as seen in Fig. 7a. Better operational stability was observed with Pt/PPY-GOD$_{NapTS}$ electrode probably due to partial neutralization of gluconic acid by reaction solution

with pH 7.0, which was higher than that of Pt/PPY-GOD$_{pTSA}$ electrode (pH 4.5).

Storage stabilities of Pt/PPY-GOD$_{NapTS}$ and Pt/PPY-GOD$_{pTSA}$ electrodes were investigated for 20 days and results were given in Fig. 8. As seen, Pt/PPY-GOD$_{NapTS}$ electrode was more stable than that of Pt/PPY-GOD$_{pTSA}$ electrode. Pt/PPY-GOD$_{NapTS}$ and Pt/PPY-GOD$_{pTSA}$ electrodes showed 34.6 and 9.32% of their initial activity, respectively. It should be noted that electrodes were repeatedly used while investigating storage stability. So, results exhibited not only storage stability, but also operational stability. Another reason for decline in the response of both Pt/PPy-GOD electrodes might be the leaching out GOD from the PPy during glucose sensing.

Pt/PPY-GOD$_{NapTS}$ and Pt/PPY-GOD$_{pTSA}$ electrodes were also used to determine the glucose concentrations of varied beverages such as malt drink, fizzy drink, wine, fresh grape juice and honey. Measurements were done by properly diluting each sample. Glucose concentrations were also determined spectrophotometrically by glucose kit. As seen in table, glucose levels of five beverages determined by Pt/PPY-GOD$_{NapTS}$ electrode was more similar to UV results. Although glucose concentrations of malt drink, fizzy drink and honey were similar to glucose values obtained spectrophotometrically, results of fresh grape

Compared of Pt/PPY-GOD$_{NapTS}$ and Pt/PPY-GOD$_{pTSA}$ electrodes according to glucose level measurements of varied beverages

Samples	Glucose level determined by glucose kit, w/v %	Glucose level by Pt/PPy-GOD electrodes, w/v %	
		Pt/PPY-GOD$_{pTSA}$	Pt/PPY-GOD$_{NapTS}$
Malt Drink	3.47	3.61	3.37
Fizzy Drink	4.29	4.27	4.34
Fresh Grape Juice	5.68	2.35	5.36
Honey	28.48	28.70	27.2
Wine	0.05	0.09	0.04

juice and wine were not satisfying when Pt/PPY-GOD$_{pTSA}$ electrode was used.

There are several studies about GOD entrapment in PPy to establish the glucose biosensor in electrolyte solutions at different pH such as pH 7.0 [14, 36], pH 5.0. Uang and Chou [22] prepared glucose biosensor by GOD entrapping in PPy on Pt rod in KCl electrolyte with different pH between 2.8 and 11.5 by galvanostatic method, and they reported that the best results were obtained in terms of response when they constructed the biosensor at the neutral pH. In this study we firstly focused on pH effect of electrolyte solution which was used for enzyme electrode preperation. As anions were the same as *p*-toluenesulphonate in both media, only acidities were different.

***

Pt/PPY-GOD$_{NapTS}$ and Pt/PPY-GOD$_{pTSA}$ electrodes were prepared with cyclic voltametry technique in two different electrolyte solutions with NapTS and pTSA electrolytes which were neutral and acidic solutions, respectively. Entrapment of GOD into PPy was achieved in both electrolyte systems as explained in electrochemical and biochemical characterization studies. While acidic medium is preferred to enhance conductivity of polypyrrole, it also causes the partial denaturation of GOD enzyme. So, it was concluded that NapTS proved to be better medium to construct enzyme electrode in terms of response, operational stability, storage stability and analytical applications.

ACKNOWLEDGEMENTS

REFERENCES

1. Yebra, M.C., Gallego, M., and Valcarcel, M., *Anal. Chim. Acta*, 1993, vol. 276, pp. 385−391.

2. Undeen, A.H., Vander, and Meer R.K., *J. Eukaryot. Microbiol.*, 2007, vol. 41, pp. 129−132.

3. Hall, M.B. and Keuler, N.S., *J. Aoac Int.*, 2009, vol. 92, pp. 50−60.

4. Yuen, V.G. and McNeill, J.H., *J. Pharmacol. Toxicol.*, 2000, vol. 44, pp. 543−546.

5. Lee, J. and Park, S.M., *Anal. Chim. Acta*, 2005, vol. 545, pp. 27−32.

6. Voloshin, A.G., Teterin, G.A., Kolesnikova, I.P., Metelichenko, B.L., Kapenasyuk, N.S., Kalyuzhnaya, E.A., Muntyan, G.I., Seidguseinov, A.A., Kalikhman, V.L., *Ukrainskii Khimicheskii Zhurnal*, 1985, vol. 51, pp. 845−848.

7. Garjonyte, R., Malinauskas A., *Sensor. Actuat. B-Chem.*, 1999, vol. 56, pp. 85−92.

8. Eftekhari, A., *Synthetic Met.*, 2004, vol. 14, pp. 211−216.

9. Rubio Retema, J., Lopez Cabarcos, E., Mecerreyes, D., and Lopez-Ruiz, B., *Biosens. Bioelectron.*, 2004, vol. 20, pp. 1111−1117.

10. Gerard, M. and Malhotra, B.D., *Curr. Appl. Phys.*, 2005, vol. 5, pp. 174−177.

11. Shan, D., Wang, S., He, Y., and Xue, H., *Mater. Sci. Eng. C*, 2008, vol. 28, pp. 213−217.

12. Skinner, N.G. and Hall, E.A.H., *J. Electroanal. Chem.*, 1997, vol. 420, pp. 179−188.

13. Liu, X., Neoh, K.G., Cen, L., and Kang, E.T., *Biosens. Bioelectron.*, 2004, vol. 19, pp. 823−834.

14. Reiter, S., Habermüller, K., and Schuhmann, W., *Sens. Actuat. B-Chem.*, 2001, vol. 79, pp. 150−156.

15. Shirsat, M.D., Too, C.O., and Wallace, G.G., *Electroanal.*, 2008, vol. 20, pp. 150−156.

16. Borole, D.D., Kapadi, U.R., Mahulikar, P.P., and Hundiwale, D.G., *J. Mater. Sci.*, 2007, vol. 42, pp. 4947−4953.

17. Liu, C.J., Kuwahara, T., Yamazaki, and R., Shimomura, M., *Eur. Polym. J.*, 2007, vol. 43, pp. 3264−3276.

18. Ekinci, E., Karagozler, A.A., and Karagozler, A.E., *Synthetic Met.*, 1996, vol. 79, pp. 57−61.

19. Jing-Juan, X. and Hong-Yuan, C., *Anal. Biochem.*, 2000, vol. 280, pp. 221−226.

20. Nien, P.C., Tung, T.S., and Ho, K.C., *Electroanal.*, 2006, vol. 18, pp. 1408−1415.

21. Angaleeswari, B., Dura Amirtham, R.M., Jeevithaa, T., Vaishnavi, V., Eevera, T., Berchmans, S., and Yegnaraman, V., *Sensors Actuat. B-Chem.*, 2008, vol. 129, pp. 558−565.

22. Uang, Y.M. and Chou, T., *Biosens. Bioelectron.* 2003, vol. 19, pp. 141−147.

23. Fortier, G., Brassard, E., and Belanger, D., *Biosens. Bioelectron.*, 1990, vol. 5, pp. 473−490.

24. Shin, M.C. and Kim, H.S., *Biosens. Bioelectron.*, 1996, vol. 11, pp. 161−169.

25. Wang, J.J., Myung, N.V., Yun, M.H., and Monbouquette, H.G., *J. Electroanal. Chem.*, 2005, vol. 575, pp. 139−146.

26. Chen, C., Jiang, Y., and Kan, J., *Biosens. Bioelectron.*, 2006, vol. 22, pp. 639−643.

27. Saidman, S.B., *Electrochim. Acta*, 2003, vol. 48, pp. 1719−1726.

28. Yu, E.H. and Sundmacher, K., *Process Saf. Environ.*, 2007, vol. 85, pp. 489−493.

29. Ren, X., Jiang, Y., Liang, X., Liu, J., and Zhang P., *Bioinformatics and Biomedical Engineering*, The 2nd Int. Conference on 16−18 May, Shanghai, 2008, pp. 1617−1620.

30. Ma, M., Qu, L., and Shi, G., *J. Appl. Polym. Sci.*, 2005, vol. 98, pp. 2550−2554.

31. Mala Ekanayake, E.M.I., and Preethichandra, D.M.G., *IEEE Instrum. Meas.*, 2008, vol. 57, pp. 1621−1626.

32. Ozyilmaz, G. and Tukel, S.S., *Appl. Biochem. Microbiol.*, 2007, vol. 43, pp. 29−35.

33. Ozyilmaz, G. and Tukel, S.S., *J. Microbiol. Biotechnol.*, 2007, vol. 17, pp. 960−967.

34. Kleppe, K., *Biochemistry*, 1966, vol. 5, pp. 139−143.

35. Greenfield, P.F., Kittrell, J.R., and Laurence, R.L., *Anal. Biochem.*, 1975, vol. 65, pp. 109−124.

36. Olea, D., Viratelle, O., and Faure, C., *Biosens. Bioelectron.*, 2008, vol. 23, pp. 788−794.