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Preparation of Pt/polypyrrole–para toluene sulfonate hydrogen peroxide sensitive electrode for the utilizing as a biosensor

Servet Çete[1] & Özgür Bal[2]

[1]*Department of Chemistry, Faculty of Sciences, Gazi University, Ankara, Turkey, and* [2]*Department of Chemistry, Institute of Sciences, Gazi University, Ankara, Turkey*

Abstract

A film electrode with electropolymerization of pyrrole (Py) and para-toluene sulfonate (pTS) as a anionic dopant is prepared and its sensitivity to hydrogen peroxide is investigated. The polypyrrole is deposited on a 0.5 cm^2 Pt plate an electrochemically prepared pTS ion-doped polypyrrole film by scanning the electrode potential between − 0.8 and + 0.8 V at a scan rate of 20 mV/s. The electrode's sensitivity to hydrogen peroxide is investigated at room temperature using 0.1 M phosphate buffer at pH 7.5. The working potential is found as a 0.3 V. The concentrations of pyrrole and pTS are 50mM M and 25 mM. Polypyrrole was coated on the electrode surface within 10 cycles. İmmobilization of glucose oxidase carried out on Pt/polypyrrole–para toluene sulfonate (Pt/PPy-pTS) film by cross-linking with glutaraldehyde. The morphology of electrodes was characterized by SEM and AFM. Moreover, contact angle measurements were made with 1 μL water of polymer film and enzyme electrode. It has shown that enzyme electrode is very sensitive against to glucose.

Keywords: anionic dopant, biosensor, glucose, para toluene sulfonate, polypyrrole

Introduction

The determination of hydrogen peroxide is very important in chemical, biological, clinical, and many other fields. Likewise content in various commercial products such as contact lenses solutions, cosmetics, and pharmaceutical formulations (Choi and Yiu 2004, Arslan et al. 2000).

Hydrogen peroxide is a product formed in many reactions catalyzed by oxidase enzyme. The amount of substrate determined by the biosensors prepared with oxidase enzyme is based upon the oxidation of hydrogen peroxide product at a certain potential (Choi and Yiu 2004, Pei and Li 2000, Laudet et al. 2003, Arai et al. 1996). Electrochemically polymerized conducting polymers have attracted over the last two decades. The considerable switching capability of conducting polymers between the conducting oxidized (doped) and the insulating reduced (undoped) state is the basis of many applications (Rajesh and Takashima 2004).

A number of conducting polymers, such as polyaniline, polythiophene, polyphenylene, and polypyrrole (PPy) have used for assembling of biosensors for application in clinical diagnostics. Conducting polymer-based biosensors have been mentioned for the determination of some clinically important metabolites, such as glucose, lactate, urea, galactose, and cholesterol etc (Cosnier 1999, Malhotra et al. 2006, Pratima et al. 2007).

PPy has been extensively investigated on account of its facile polymerization,ease of membrane formation, high conductivity, and chemical stability. Several approaches to hybridize PPy with other materials have been tried so as to endow the parent PPy with increased mechanical strength for desired applications such as functional electrodes, electrochromic devices, and sensors etc. (Chaubey et al. 2000, Palmisano et al. 1997, Ramanathan et al. 1995).

The dopant anion in PPy plays a critical role in determining the physical and chemical properties of these conducting polymers (Thompson et al. 2011).

The dopant is used to improve the electrical, mechanical, or other physical properties of the polymer (Thompson et al. 2011, 2006a, 2006b, Richardson et al. 2007, Evans et al. 2009, Miller et al. 1987, Xiao et al. 2007).

It has shown that incorporation of large sized dopant anion, p-toluene sulfonate (pTS) into PPy during electropolymerization results in growing porosity. It has been expressed that flexible pTS-doped PPy films can be used for increased loading of glucose oxidase and hence are possibly to provide durable electrode response to substrate concentration (Pratima et al. 2007, Ramanathan et al. 1996).

There are the advantages of the use of glutaraldehyde for immobilization of enzyme or protein.

Using of glutaraldehyde as crosslinker for clinical use is important (Chang 1971).

Correspondence: Servet Çete, Department of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey. Tel: 0090-312-2021137. Fax: + 0090-312-2122279. E-mail: scete@gazi.edu.tr

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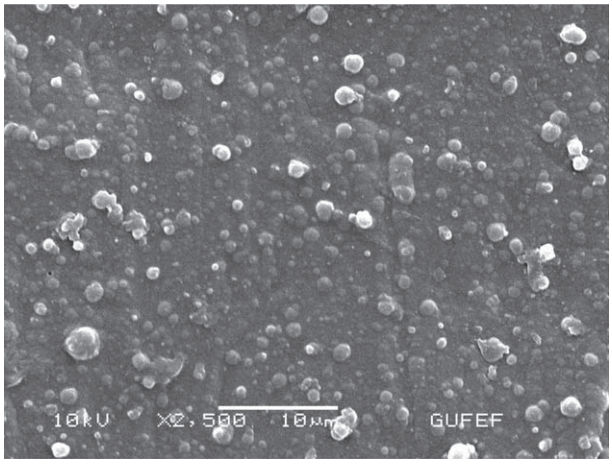

Figure 1. The SEM of Pt/PPy-pTS electrode.

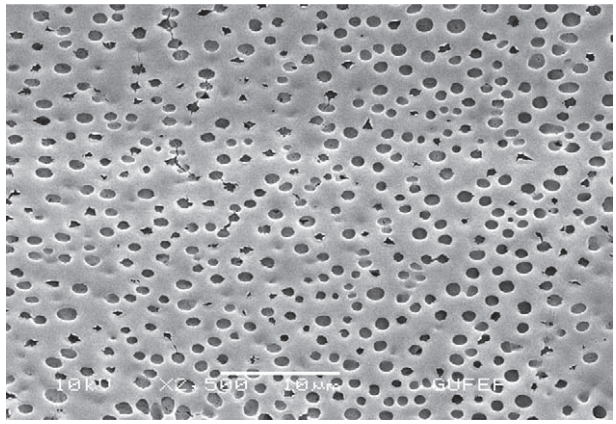

Figure 2. The SEM of Pt/PPy-pTS-Glucose oxidase-Glutaraldehyde-Albumine.

In this study, a platinum/polypyrrole–p-toluene sulfonate (PPy-pTS) electrode sensitive to hydrogen peroxide was prepared for the use as a biosensor. pTS was added to the cell as anionic dopant. The sensitivity of preparing film electrode was investigated. The effects of working potential, number of cycles, concentrations of monomer, and anionic dopant were studied. The effect of enzyme electrode was investigated against to glucose.

Materials and methods

Reagents

pTS was obtained from Merck, the supporting electrolyte sodium perchlorate and pyrrole were supplied by Aldrich. All the other chemicals were purchased from Sigma. All the solutions were prepared by the use of twice-distilled water.

Equipments

All the electrochemical experiments were performed with BAS 100 B/W electrochemical analyzer. The pH of the buffer solution was carried out using ORION Model 720A pH/ionometer. The electrochemical experiments were carried out in a three-electrode cell. Aconventional three-electrode system was equipped with a Pt plate (0.5 cm^2) as the working electrode, an Ag/AgCl electrode (3 M KCl) as the reference electrode, and a platinum wire (diameter and length, 1 mm and 4 cm, respectively) for the counter electrode.

The Electropolymerization of PPy–pTS film

The surface of the Pt plate electrode cleaned according to (Cochet et al. 2001, Yıldırımoğlu et al. 2009) was covered by theelectropolymerization of pyrrole and pTS (Thompson et al. 2011). The electrode was immersed in a 10 mL solution of 50 mM pyrrole and 25 mM pTS. The solution was purged with nitrogen in order to remove the oxygen. The electropolymerization of pyrrole upon the electrode surface was performed by the cyclic voltammetric scans between − 0.8 and + 0.8 V at a scan rate of 20 mV/s. The electrode was washed with buffer solution after the coating process.

The sensitivity of PPy–pTS film electrode to hydrogen peroxide

The response current of electrode covered with PPY was determined as follows.

An buffer solution of pH 7.5 (10 ml, 25°C) was added to the cell. The solution included 0.1M phosphate to adjust pH and 0.1 M sodium perchlorate as a supporting electrolyte. The electrodes were brought to maintaning by keep in at 0.3 V for approximately 4 h, and steady current (*i*a) was saved. The different concentration of hydrogen peroxide was added to the cell. The currents acquired at 0.3 V were recorded every 200 sec. The difference in current values were plotted against the hydrogen peroxide concentration. Linear region of current values was determined.

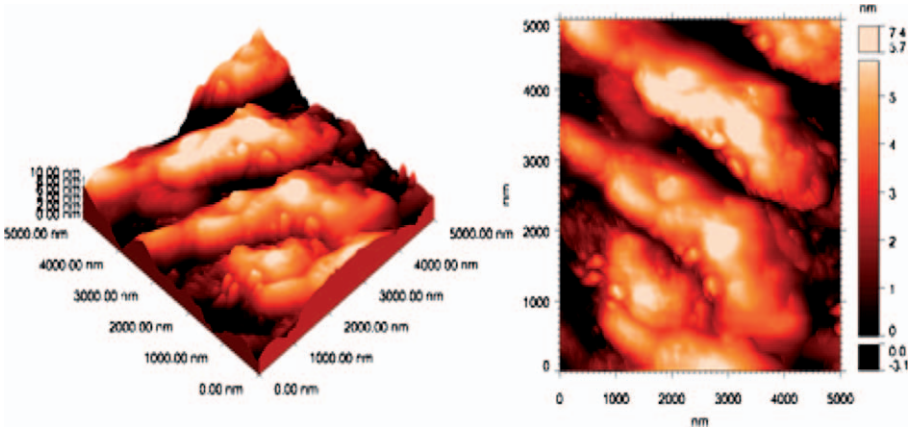

Figure 3. The AFM of Pt/PPy-pTS electrode.

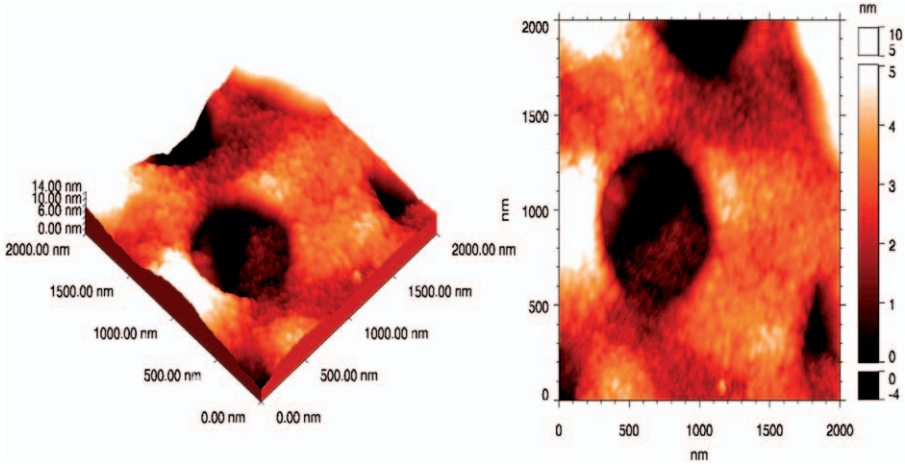

Figure 4. The AFM of Pt/PPy-pTS-Glucose oxidase-Glutaraldehyde-Albumine.

Immobilizaton of glucose oxidase on Pt/PPy-pTS film electrode

Immobilization of glucose oxidase was carried out by the approach cross-linking. The concentrations of pyrrole and paratoluensulphonate were 50 mM and 25 mM, respectively. Solution of amount 100 µL occurring from 37.5 µL of 2.5% glutaraldehyde solution, 50 µL of the stock solution of GOD (10 U/mL) prepared in 0.1 M phosphate buffer (pH 7.5), and 1 mg of bovine serum albumin were adsorbed onto the surface of PPy–PVS films. Then left 2 hours and washed 2–3 times with phosphate buffer. After the fabrication of glucose oxidase onto the PPy-pTS working electrode was finished, the electrode was rinsed with deionized water to remove the unreacted pyrrole monomer and free glucose oxidase. Immobilized enzyme electrode was kept in a refrigerator at the in phosphate buffer when it was not in use (Karyakin 2001).

The sensitivity of PPy–pTS-glucose oxidase-glutaraldehyde-albumin enzyme electrode to hydrogen peroxide

The response currents of enzyme electrode were determined as follows.

An buffer solution of pH 7.5 (10 ml, 25°C) was added to the cell. The solution included 0.1 M phosphate to adjust pH and 0.1 M sodium perchlorate as a supporting electrolyte. The enzyme electrodes were brought to maintaning by keep in at 0.3 V for approximately 5 h, and steady current (*i*a) was saved. The different concentration of glucose was added to the cell. The currents acquired at 0.3 V were recorded every 200 sec. The difference in current values were plotted against the glucose concentration.

Results and discussion

In this study, we prepared a new PPy-pTS film hydrogen peroxide sensitive electrode for the utilizing as a biosensor. The parameters effecting to the performance of film electrode were examined. The immobilization of glucose oxidase upon of PPy-pTS film was carried out. It is shown that enzyme electrode is very sensitive against to glucose.

SEM (scanning electron microscope) studies of PPy–pTS film

In these experiments, scanning electron microscope JEOL Model 6060 LV instrument was used. The morphology of electrode was characterized by SEM (Figures 1 and 2). The SEM picture of PPy-pTS are shown in Figure 1. There is SEM related with Au/PPy-pTS) film in literature (Thompson et al. 2011). The SEM picture (Figure 1) acquired for the Pt/PPy-pTS electrode demonstrates fine micro-spheres resulting in the porous surface (Pratima et al. 2007). The diameter of these microspheres is approximately in the range of 0.3–1.5 µm. The SEM image of Pt/PPy-pTS-Glucose oxidase-glutaraldehyde-Albumin is given in Figure 2.

AFM (atomic force microscope) studies of PPy–pTS film

In these experiments, atomic force microscope JEOL Model 6060 LV instrument was used. The morphology of electrode was characterized by AFM (Figures 3 and 4). The AFM picture of PPy-pTS are shown in Figure 3 The AFM picture (Figure 3) acquired for the Pt/PPy-pTS electrode demonstrates fine micro-spheres resulting in the porous surface (Pratima et al. 2007). The AFM image of Pt/PPy-pTS-Glucose oxidase-Glutaraldehyde- Albumin is given in Figure 4.

Figure 5. The Contact Angle of Pt/PPy-pTS electrode.

Figure 6. The Contact Angle of Pt/PPy-pTS-Glucose oxidase-Glutaraldehyde-Albumine.

Contact angle studies of PPy–pTS film

In these experiments, contact angle krüsse model DSA 100 instrument was used. The hydrophilicty of electrode was characterized by contact angle (Figures 5 and 6). The contact angle picture of PPy-pTS are shown in Figure 5. The contact angle picture (Figure 5) acquired for the Pt/PPy-pTS electrode demonstrates to be hydrophobic of surface. The contact angle of Pt/PPy-pTS-Glucose oxidase-Glutaraldehyde-Albumin is given in Figure 6. It has shown that enzyme electrode is hydrophilic more than free enzyme electrode. This state can be attributed hydrophilic groups of protein.

Amperometric response measurements of Pt/PPy–pTS electrode

Since the PPy-pTS-coated electrode prepared was designed to be utilized as a biosensor, the determination of its sensitivity to hydrogen peroxide consisted as a result of enzymatic reactions. The results of amperometric response currents detected for Pt/PPy–pTS electrodes were shown in Figure 7. The figure shows that the PPy-pTS electrode gives a linear response.

The determination of working potential

After preparing Pt/PPy-pTS electrodes, oxidation of the hydrogen peroxide was tried at a different potential (Figure 8). For this purpose, five different potentials (0.3, 0.4, 0.5, 0.6, and 0.7 V) were studied. The biggest current value was achieved at 0.70 V. At high potentials, interferences caused by exogenous substances present in body fluids (e.g., ascorbic acid) (Yıldırımoğlu et al. 2009, Zhang et al. 2007). Therefore 0.3 V was selected as working potential.

The studies of pyrrole concentration

The electrodes' sensitivity to hydrogen peroxide was studied in order to explain the important of pyrrole concentration (Figure 9). For this reason several pyrrole concentrations (25, 50, 100, and 150 mM) were tried. The lowest correlation coefficient of electrode was found at 25 mM pyrrole concentration (Figure 9). The best correlation coefficient was seen in 50 mM pyrrole. It was shown that the high pyrrole concentration was not suitable for polymer film. Surface of these electrodes prepared by 50 mM pyrrole was more smooth and strong than the other electrodes. Therefore, the pyrrole concentration was used as a 50 mM in the next trying.

The effect of pTS concentration

In this study, pTS was used as an anionic dopant. The sensitivities of electrodes to hydrogen peroxide were determined in order to clarify the role of pTS concentration. Four different pTS concentrations (10, 25, 50, 100 mM) were used (Figure 10). Figure 10 shows that correlation coefficients of film electrodes covered in 10, 50, and 100 mM pTS were lower than 25 mM. The best linear response was appeared with the use of 25 mM para-toluene sulfonate. All of the experiments were carried out with Pt/PPy–pTS electrodes prepared by maintaining the pTS concentration at 25 mM.

The effect of thickness of PPy film

The effects of film thickness on the sensitivity of the electrodes against hydrogene peroxide concentration were studied for different cycles (Figure 11). Figure 11 indicates that that the electrodes prepared by 6, 8, 10, 14, and 20 cycles have high linearity. The highest response

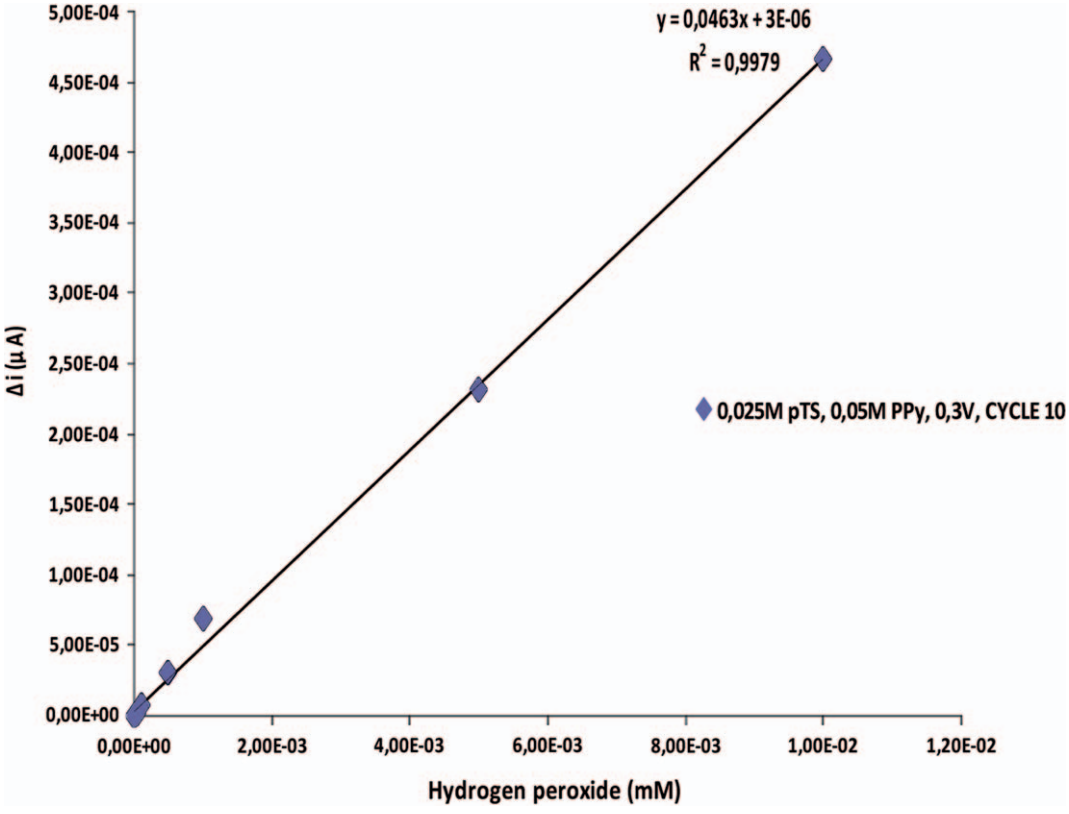

Figure 7. Amperometric response measurements of Pt/PPy–pTS Electrode.

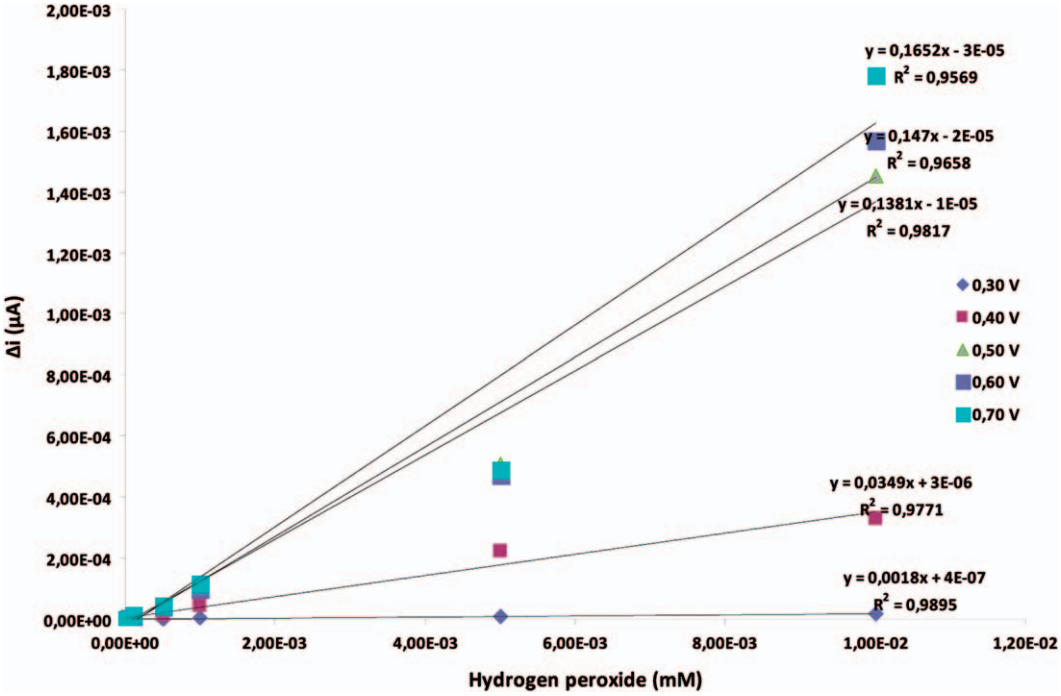

Figure 8. The effects of different potentials on amperometric response measurements.

currents were obtained for electrodes prepared by 10 cycles. Moreover, this electrode have much more mechanical strength and reproducibility. The observed higher value of amperometric response current may be attributed to the thin film. Pt surface was too thick for the higher cycles. Therefore, amperometric response currents were poor. For the electrodes prepared by 10 cycles, the surface of the electrode was more smooth and durable than the others.

Amperometric response measurements of Pt/PPy–pTS-glucose oxidase-glutaraldehyde-albumin

Since the PPy-pTS-coated electrode prepared was designed to be utilized as a biosensor, the determination of its sensitivity to hydrogen peroxide consisted as a result of enzymatic reactions. The results of amperometric response currents detected for Pt/PPy–pTS-Glucose oxidase electrodes were shown in Figure 12. The figure shows that the enzyme electrode was very sensitive against to glucose. This enzyme

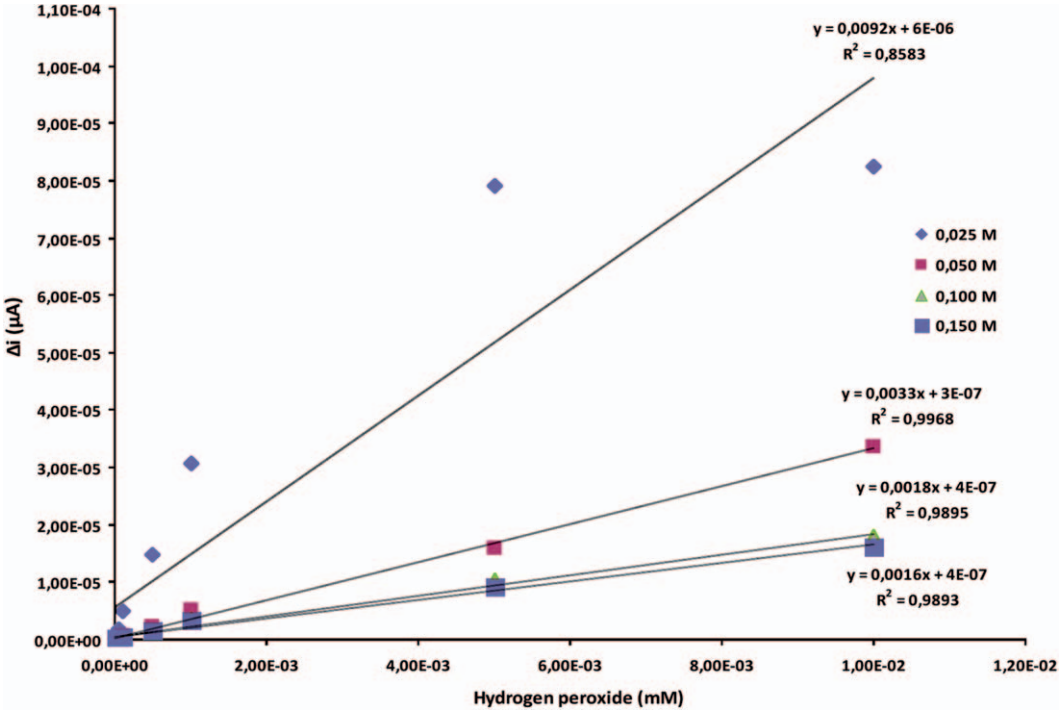

Figure 9. The effects of different pyrrole concentrations on amperometric response measurements.

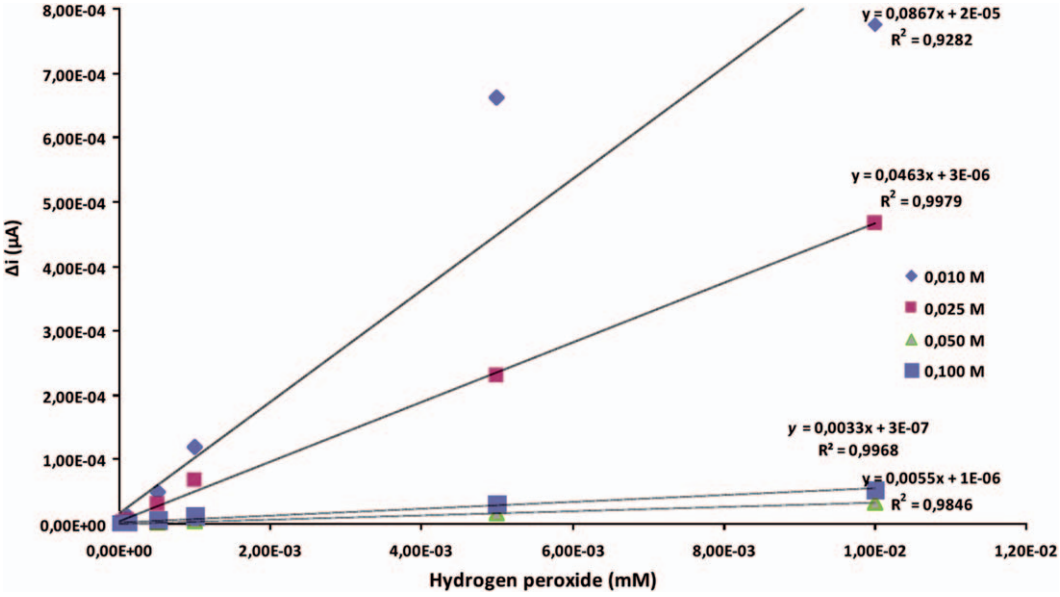

Figure 10. The effects of different concentrations of para-toluene sulfonate on amperometric response measurements.

electrode can be used for determination of glucose in biological fluids.

Conclusion

In this study, a PPy-pTS electrode sensitive to hydrogen peroxide was prepared for the use as a biosensor. The optimum conditions for the enzyme electrode were found to be: the pyrrole concentration, 50 mM; the pTS concentration, 25 mM; the working potential, 0.3 V; the number of cycles, 10. On account of the increased pore size of the pTS-doped PPy film which is suitable for the immobilization of enzymes. It is shown that enzyme electrode was very sensitive against to glucose. This enzyme electrode can be used for determination of glucose in blood and urine.

This film electrode can be used for preparing of enzyme electrodes to determine of the hydrogen peroxide.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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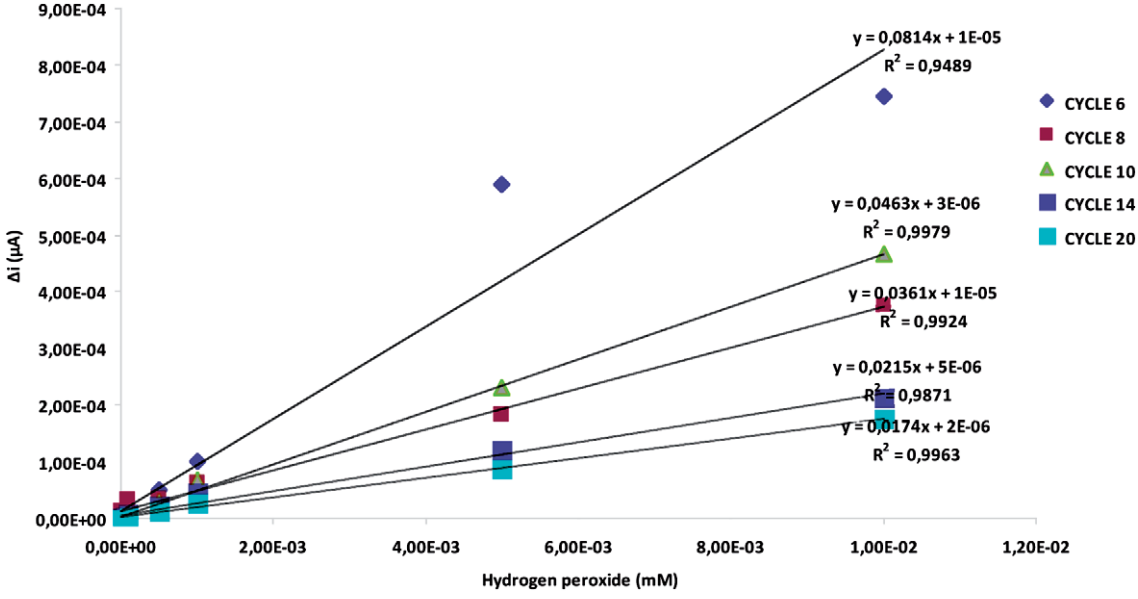

Figure 11. The effects of thickness of polypyrrole film on amperometric response measurements.

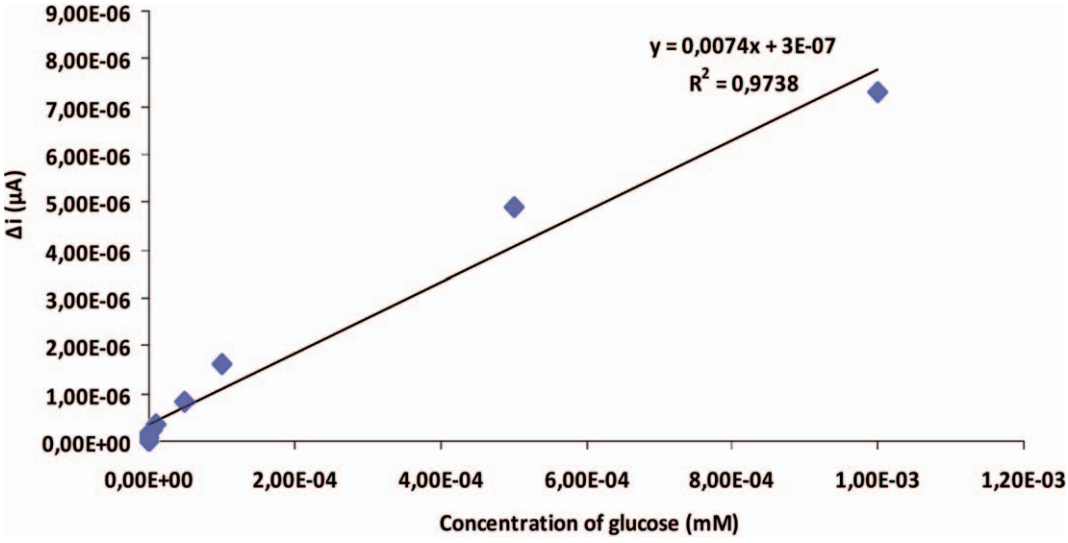

Figure 12. Amperometric response measurements of Pt/PPy–pTS-Glucoseoxidase-glutaraldehyde-albumin electrode.

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