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Synthesis and Characterization of Some N-substituted Polypyrrole Derivatives: Towards Glucose Sensing Electrodes

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Synthesis and chemical oxidative polymerization of some N-substituted pyrroles ((N-Py)s) were carried out using Clauson-Kaas method and $FeCl_3$ in $CHCl_3$ medium, respectively. The produced polymers, N-(p-benzoic acid)polypyrrole (NpbPPy); N-(o-aminophenyl)polypyrrole (NoaPPy); N-(m-nitrophenyl) polypyrrole (NmnPPy), were characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) analysis. An enzymatic glucose biosensor is fabricated through immobilizing glucose oxidase (GOX) into N-substitued polypyrrole matrixes. The parameters affecting the fabrication and experimental conditions of biosensors were optimized. The best biosensor results were obtained for NpbPPy matrix. The sensitivity of the proposed biosensor permitted the determination of glucose in the concentration range of 0–9.2 M with a detection limit of 1×10^{-6}. The apparent Michaelis–Menten constant (K_M^{app}) for the sensor was found to be 25.95 mM.

Keywords: Polypyrroles, N-substituted pyrrole, glucose, sensors, enzymes

1 Introduction

The intrinsically conducting polymers are a relatively new class of polymeric materials and are sensitive to changes in the polymeric chain environment (1). Among conducting polymers, many polypyrrole (PPy) and derivatives have been synthesized due to their high electrical conductivity and applications such as fabrication of actuators, sensors and electrochromic devices (2–7). However, the poor mechanical properties of PPy, together with its naturally poor solubility in common solvents, cause some handicaps to extend the application areas (8, 9). The modification of the monomer structure could be an effective method to improve the solubility and processibility of polypyrrole (10). Furthermore, various studies have been carried out on the synthesis and polymerization of derivatives of pyrrole (11).

3-Substituted and N-substituted pyrroles are the most used derivatives to increase the solubility and fusibility of polymers (12–15). However, substitution of pyrrole ring has decreased electroactivity and electrical conductivity of the oxidized polymer due to a lack of ring planarity (16, 17). 3-Substituted pyrroles are asymmetrical molecules, as opposed to N-substituted pyrroles are fundamentally symmetrical. Thus, polymerization of N-substituted pyrrole is resulting in an increased order and a planarity of the polymer backbone. Consequently, the N-substituted pyrrole derivatives are more desirable forms than their 3-substituted counterparts.

Biosensors are currently receiving great interest because of the possibilities they offer to accurately measure the wide variety of substrates. Especially, the development of glucose biosensor has received considerable attention because determination of glucose concentration is very important in clinical applications (18–24). During the past several years, considerable attention has been focused on conducting polymers (25–28) which provide a great potential for the immobilization of biomolecules.

Although the preparation of the enzyme-immobilized electrode by the electropolymerization of pyrrole in the presence of the enzyme has some advantageous properties, it has a problem in electrode stability (29, 30). For this reason, a variety of experiments have been performed with derivatives of pyrrole to improve the enzyme electrode durability. The authors reported that the amperometric glucose sensors were prepared by the electropolymerization of 3-(1-pyrrolyl)propionic acid (PPA) in the presence of the enzyme, following the treatment with water-soluble carbodiimide(1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, EDC). EDC has been used to provide the covalent

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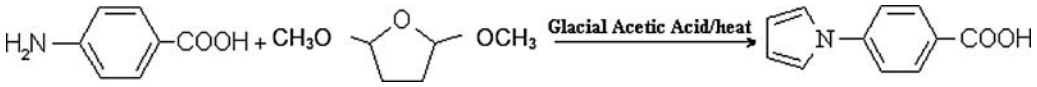


Fig. 1. The synthesis procedure of NpbPy.

bonding between glucose oxidase (GOX) and polypyrrole derivatives (31). The copolymer of pyrrole (PPy) and 1-(2-carboxyethyl)pyrrole (PPy-COOH) was employed as a novel conducting support-material for fabricating GOX-immobilized electrodes (32).

In this work, we aimed the synthesis and chemical oxidative polymerization of N-(p-benzoic acid) pyrrole (NpbPy), N-(o-aminophenyl) pyrrole (NoaPy) and N-(m-nitrophenyl) pyrrole (NmnPy) using $FeCl_3$ as an oxidant in $CHCl_3$ medium. The polymers were characterized by SEM and FTIR measurements. The glucose biosensor properties of the N-substitued polypyrroles ((N-PPy)s) were investigated using amperometric method and a novel glucose biosensor was developed.

2 Experimental

2.1 Materials

p-Aminobenzoic acid, 1,2-diaminobenzene, m-nitroaniline (Across) and 2,5-dimethoxytetrahidrofuran (Sigma) used without further purification. All of the solvents were analytical reagents. All of the following reactions were carried out under nitrogen atmosphere. Reagent ferric chloride, $FeCl_3$, obtained from Riedel de Haen, was used as an oxidizing agent for chemical polymerization of monomers. Chloroform ($CHCl_3$) (Riedel de Haen) and ethanol (EtOH) (Merck) were used without further purification. Glucose oxidase (GOX, EC 1.1.3.4, 179,000 units/g, type VII-S from Aspergillusniger, Sigma), and D-(+)-Glucose anhydrous (Fluka) were used to design the biosensor. 25% Glutaraldehyde (GA) water solution (Aldrich) was used as crosslinking agent. The buffer solution was prepared using $NaH_2PO_4.2H_2O$ (Riedel De Haen) and NaOH (Riedel De Haen) and used as supporting electrolyte. Alumina polishing suspension agglomerate (0,05cr micron) (Baikowski) was used as electrode polisher. Double-distilled water was used for preparation of the buffer solution.

2.2 Synthesis of Monomers

N-(p-benzoic acid) pyrrole, N-(o-aminophenyl)pyrrole and N-(m-nitrophenyl)pyrrole were synthesized according to Clauson-Kaas method (33, 34).

2.2.1. *Synthesis of N-(p-benzoic acid) pyrrole (NpbPy)*

0.059 mol of p-aminobenzoic acid in 25 mL of acetic acid was mixed in 100 mL flask on the magnetic stirrer. 0.059 mol of 2,5-dimethoxytetrahidrofuran was added to the mixture during 10-15 min. Then, acetic acid was removed from solution using with micro destillation setup. The dark colored precipitate was crystallized and NpbPy was observed (Fig. 1).

IR (KBr) cm^{-1}: 1505 (C=C); 1300 (C-N); 1695 (C=O); 3450 (O—H); **^{1}H-NMR (400 MHz, CDCl$_3$)**: δ (ppm) = 7.92 (dd, J_1 = 8.1 Hz, J_2 = 1.7 Hz, 2H), 7.64(dd, J_1 = 8.1 Hz, J_2 = 2.4 Hz, 2H), 6.98 (t, J = 2.2 Hz, 2H), 6.23 (t, J = 2.2 Hz, 2H), 10.25 (s, 1H).

2.2.2. *N-(o-aminophenyl) pyrrole (NoaPy)*

The synthetic procedure was similar to that of N-(p-benzoic acid) pyrrole. But it was synthesized by a coupling reaction of 1,2-diaminobenzen with 2,5-dimethoxytetrahidrofuran. At the and of the procedure NoaPy was crystallized and purified. The synthesis reaction was shown in Figure 2.

IR (KBr) cm^{-1}: 1510 (C=C); 1320 (C-N); 1270 (C-N) (amino group); 3375 (N-H) **^{1}H NMR (400 MHz, CDCl$_3$): δ(ppm)** = 7.25 (m, 1H), 7.18 (m, 1H), 6.88 (t, J = 2.4 Hz, 2H), 6.73 (m, 1H), 6.59 (m, 1H), 6.28 (t, J = 2.4 Hz 2H), 3.68 (NH_2 peak, s, 2H).

2.2.3. *N-(m-nitrophenyl) pyrrole (NmnPy)*

The same procedure was applied for the synthesis of N-(m-nitrophenyl) pyrrole. 0.059 mol of m-aniline was added to 25 mL of acetic acid. 2,5-Dimethoxytetrahidrofuran was added to this mixture and refluxed for 1 h. After removing the acetic acid, the dark colored precipitate was observed NmnPy (Fig. 3).

IR (KBr) cm^{-1}: 1530 (C=C); 1295 (C-N); 1348 (specific C-NO_2) **^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm)** = 7.98 (m, 1H), 7.7 (m, 1H), 7.53 (m,1H), 6.92 (t, J = 2.2 Hz, 2H), 7.07 (m, 1H), 6.21 (t, J = 2.2 Hz, 2H).

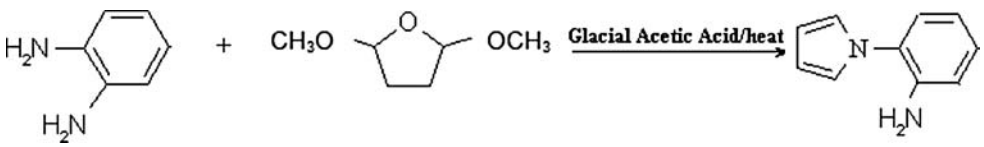


Fig. 2. The synthesis procedure of NoaPy.

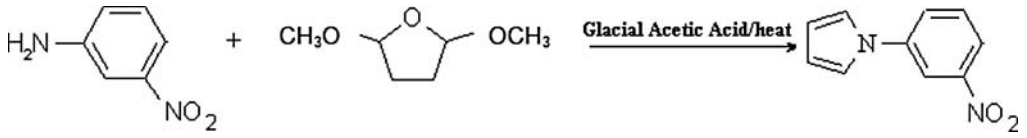


Fig. 3. The synthesis procedure of NmnPy.

2.3 Synthesis of Polymers

2.3.1. *Synthesis of N-(p-benzoic acid) polypyrrole (NpbPPy)*

NpbPy (2.0×10^{-4}mol, 0.0374 g) was dissolved in 10 ml $CHCl_3$. This solution was treated with an ultrasonic bath for 5 min to obtain the best dispersion of NpbPy. The solution was maintained in an inert N_2 atmosphere and under magnetic stirring, whereas $FeCl_3$ (5.0×10^{-4} mol, 0.0812 g) in 10 ml of $CHCl_3$ were slowly dropped to the monomer solution during 10 min. n_{ox}/n_{mon} ratio was taken as 2.5 for all synthesis. After polymerization time 24 h, precipitated polymer was filtered and washed with firstly $CHCl_3$ than ethanol until the filtrate was colorless. Finally, the polymer was dried at 50°C for 24 h. under vacuum environment.

The same procedure were applied for polymerization of N-(o-aminophenyl) pyrrole (NoaPy) and N-(m-nitrophenyl) pyrrole (NmnPy). Polymerization reactions of monomers was schematized in Figure 4(a–c).

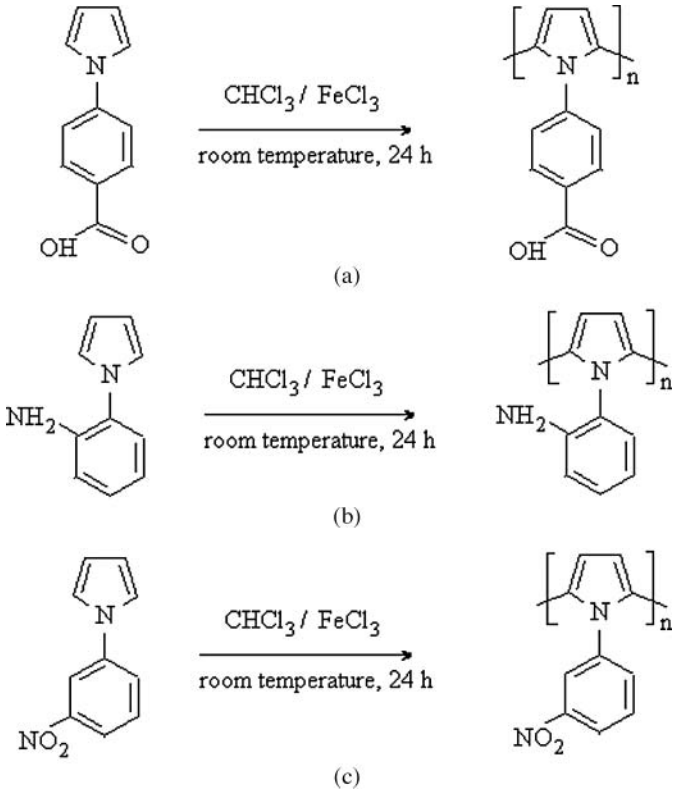


Fig. 4. Polymerization reactions of (a) N-(p-benzoic acid) pyrrole (NpbPy), (b) N-(o-aminophenyl) pyrrole (NoaPy) and (c) N-(m-nitrophenyl) pyrrole (NmnPy).

2.4 Characterization of Samples

NMR measurements of monomers were performed on a Bruker Avance model spectrometer (DPX-400 MHz FT-NMR) in $CDCl_3$ media. FTIR spectra of the monomers and polymers were recorded on a Perkin-Elmer model spectrometer (Beaconsfield, Beuckinghamsshire, HP91QA, England) between 400–4000 cm^{-1} with a resolution of 4 cm^{-1} in the transmission mode, at room temperature. Scanning electron microscopy (SEM) analysis of the polymers were performed using a Phillips XL-30S FEG model scanning electron microscope. Glucose enzyme electrode studies were carried out in the three-electrode cell equipped with a Gamry 300 potentiostat (Gamry Instruments, US). The platinium bare and Ns-PPy/GOX modified platinium electrodes were used as working electrodes. The platinum wire and Ag/AgCl electrodes were used as counter and reference electrodes, respectively.

2.5 Fabrication of Glucose Sensor

3 mg of (N-PPy)s were dispersed in 100 μL acetonitrile in an ultrasonic bath for 10 min. Pt working electrode was modified with 15 μL of this dispersion by using the dropping and drying method. 500 μL of phosphate buffer solution (0.1 mol/L, pH 6.9) was used to prepare GOX (2.5 mg) enzyme solution. An aliquot of 2 μL 5% GA solution and 10 μL of the enzyme solution were mixed completely. After that, 5 μL of this mixture solution was dropped on to N-substitued polypyrrole modified Pt electrodes. Subsequently, a crosslinking procedure was continued during 30 min at room temperature. When not used, glucose sensor was stored at 4°C.

2.6 Apparatus and Measurements for Glucose Detection

Glucose detection applications of modified Pt electrodes were achieved in a three-electrode cell with separate compartments for the reference electrode (Ag/AgCl) and for the counter electrode (Pt wire). Oxygen was introduced above the solution at a constant flow rate to keep it saturated with oxygen during the measurements. Polymer modified electrodes were prepotentiostated at +0.6 V/Ag/AgCl in a 0.1 mol l^{-1} phosphate buffer in order to allow back ground current to diminish to a constant value. The steady-state anodic current was also measured amperometrically due to the electrooxidation of H_2O_2 produced enzymatically at +0.6 V/ Ag/AgCl in a 0.1M O_2 saturated phosphate buffer in which known amounts of glucose solution were added.

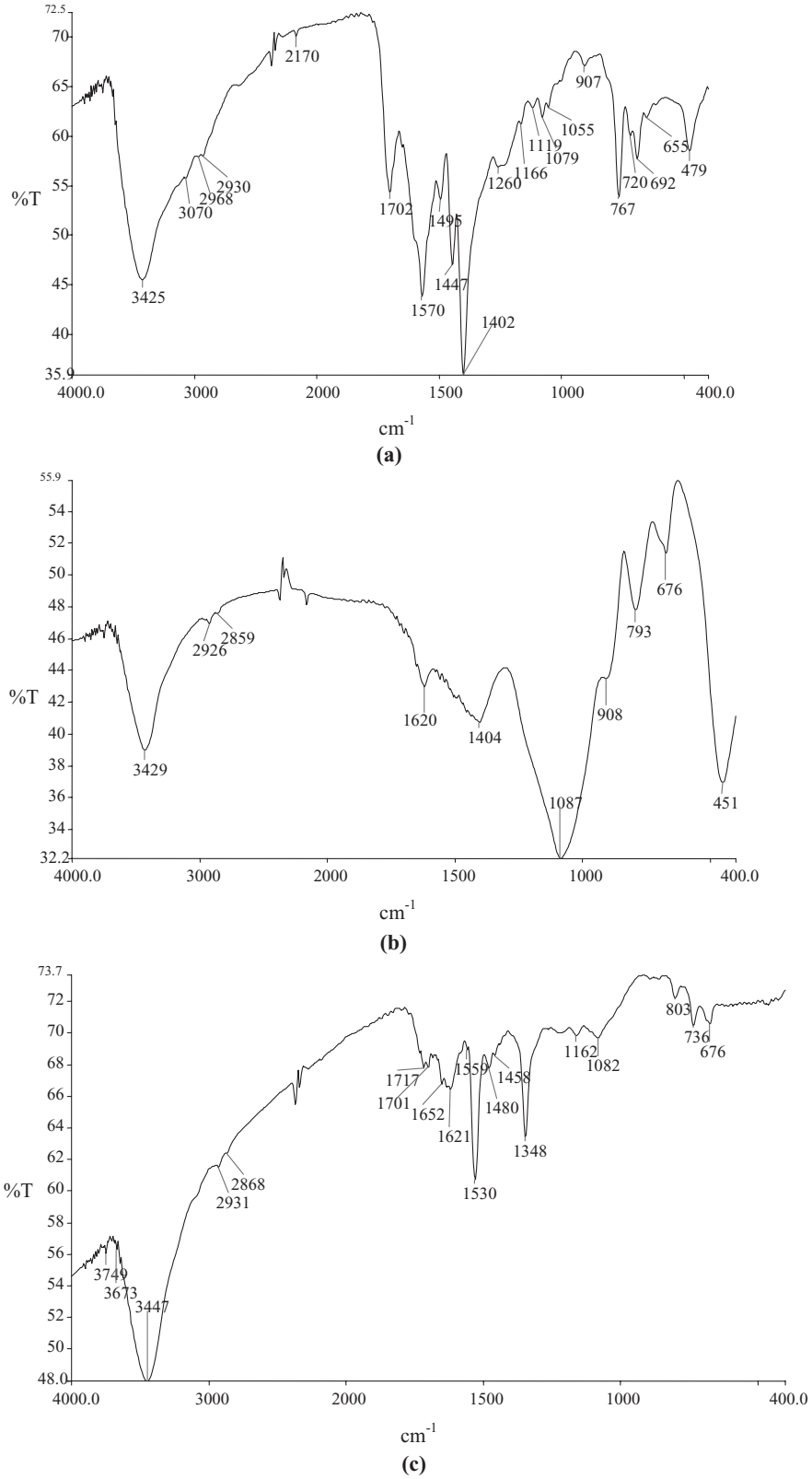


Fig. 5. FTIR spectra of (a) N-(p-benzoic acid) polypyrrole (NpbPPy), (b) N-(o-aminophenyl) polypyrrole (NoaPPy) and (c) N-(m-nitrophenyl) polypyrrole (NmnPPy).

3 Results and Discussion

3.1 FTIR Results of N-Substituted Polypyrroles

FTIR spectra of (N-PPy)s were shown in Figure 5(a–c). The sharp peak at 1570 cm^{-1} is one of the characteristic bands of pyrrole ring. Whereas the peaks around 1000 cm^{-1} are out of plane =CH vibrations, absorption band at 1447 cm^{-1} is attributed to the in plane =CH stretching vibrations of pyrrole and phenyl rings (35) (Fig. 5a). The peaks appeared in the range of 1260 and 1055 cm^{-1} are the characteristic peaks for pyrrole rings (36). The bands, which are belongs to either pyrrole or phenyl ring were observed from the FTIR spectra of (N-PPy)s (Fig. 5a–c). The COOH groups in the structure of NpbPPy was verified via the absorption peak at 1702 cm^{-1} belongs to C=O stretching vibration (32) (Fig. 5a).

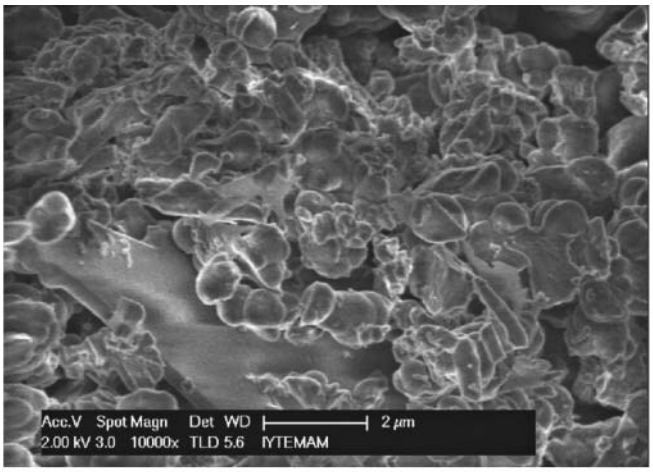
The characteristic bands at about 1559, 1530 ve 1348 cm^{-1} were assigned to the stretching vibration of the nitro group in the FTIR spectrum which belongs to NmnPPy (Fig. 5c). The 1162 and 1082 cm^{-1} bands of the NmnPPy confirm the presence of benzene ring in the structure. The peaks at around 803, 736 and 655 cm^{-1} in the spectra of (N-PPy)s were assigned to the out-of-plane vibration of three adjacent carbo–hydrogen bonds in the spectrum reflected the substituted benzene ring (38). It shows that, the intensity of these peaks in the spectrum of NpbPPy was higher than that of other polymers. As a result of this, it can be proposed that the NpbPPy (Fig. 5a) has the highest polymerization according to the other polymers.

3.2 SEM Results

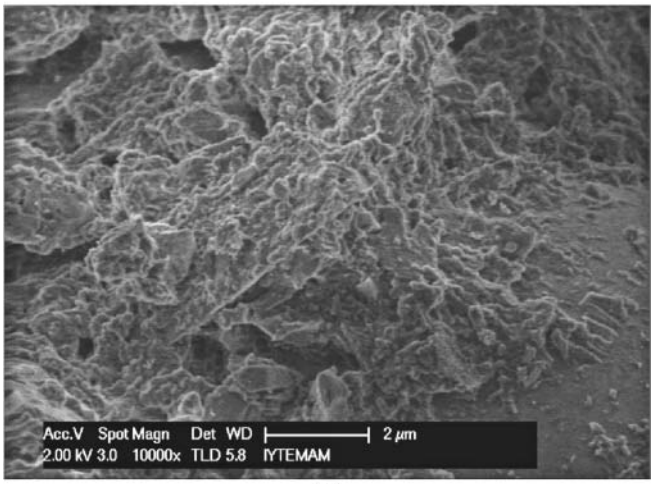
Scanning electron microscopy (SEM) was performed in order to examine the morphologies of (N-PPy)s since images give information whether substitue group is effective on morphology or not. NpbPPy has a cauliflower-like structure which is similar to polypyrrole (39) (Fig. 6a). The morphology of NpbPPy with three-dimensional (3D) structure has a large number of gaps and pores, which are beneficial to incorporate the enzyme (40). NoaPPy and NmnPPy have more flat morphology according to NpbPPy (Fig. 6b, c). Glucose sensor studies confirmed the morphology results.

3.3 Glucose Detection

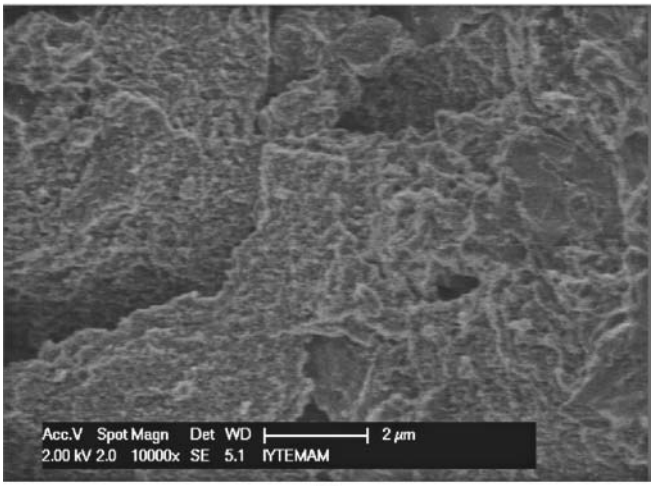
GOX catalyses, in the presence of molecular oxygen, the oxidation of β-D-glucose into gluconic acid and hydrogen peroxide. As a consequence, the amperometric detection of glucose was assayed by potentiostating the NpbPPy/GOX electrode at 0.6 V to oxidize the enzymically generated hydrogen peroxide. At a constant amount of GOX (0,021 g), NpbPPy, NoaPPy and NmnPPy modified Pt electrodes were used for the detection of glucose. Among (N-PPy)s, only the NpbPPy modified electrode has response to glucose addition. By the condensation reaction with −COOH groups on NpbPPy, GOX can be immobilized either with effect of glutaraldehyde or covalently through amide linkage on the polymer film (32).



(a)



(b)



(c)

Fig. 6. SEM images of (N-PPy)s (a) NpbPPy, (b) NoaPPy, (c) NmnPPy.

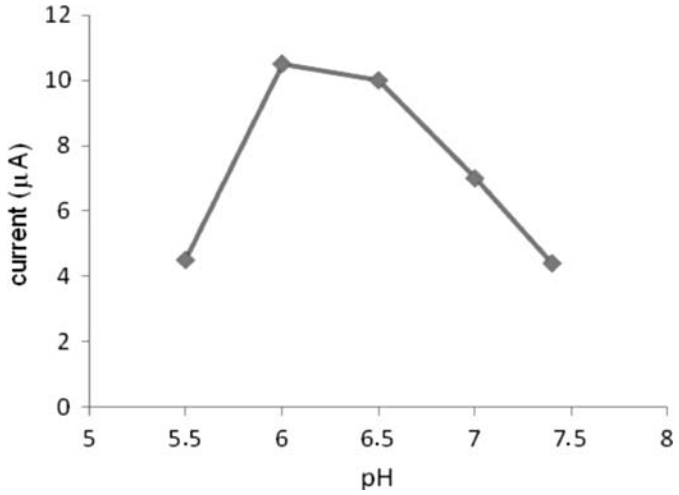


Fig. 7. Effect of the pH on the response of Pt/NbpPPy.

3.3.1. *Effect of the pH on the Response of NpbPPy Modified Pt Electrode*

The pH value is an efficient factor on the current response of the enzyme electrodes. It also prevents the loss of the enzyme activity under polymerization conditions (41). For this purpose, the pH dependence of the response of the NpbPPy modified Pt electrode (Pt/NpbPPy) has been investigated using 0.1M phosphate buffer (Fig. 7). The maximum current of enzyme electrode increased from pH 5.5 to 6.0 and then decreased as pH increases further. The pH 6.0 is the best value to see the maximum performance of the enzyme electrode where it is in agreement with the biosensor which was developed for glucose detection by using with a polypyrrole/polyacrylamide (PPy/PA) microparticles by incorporating the enzyme (42).

3.3.2. *Effect of the Glucose Concentration*

Figure 8 reveals a typical current-time curve of the Pt/NpbPPy at 0.60V to the successive addition of glucose in a stirred solution. The response current increases with addition of glucose and finally reaches to a steady-state value. An essential time to reach the steady state value was roughly 50 s. The response time of developed enzyme electrode can be compared with literature (42–46).

The linearity range of the sensor can be estimated from the glucose calibration curve in Figure 9. The response current of the Pt/NpbPPy (Fig. 9) was linear with glucose concentration to 9.2 mM with a detection limit of 1×10^{-6} M. The maximum sensing current was 8 μA. Film kinetics were characterized using the simplest and most common approach which consists in using the Michaelis–Menten equation (Eq. (3)). That equation is the most important

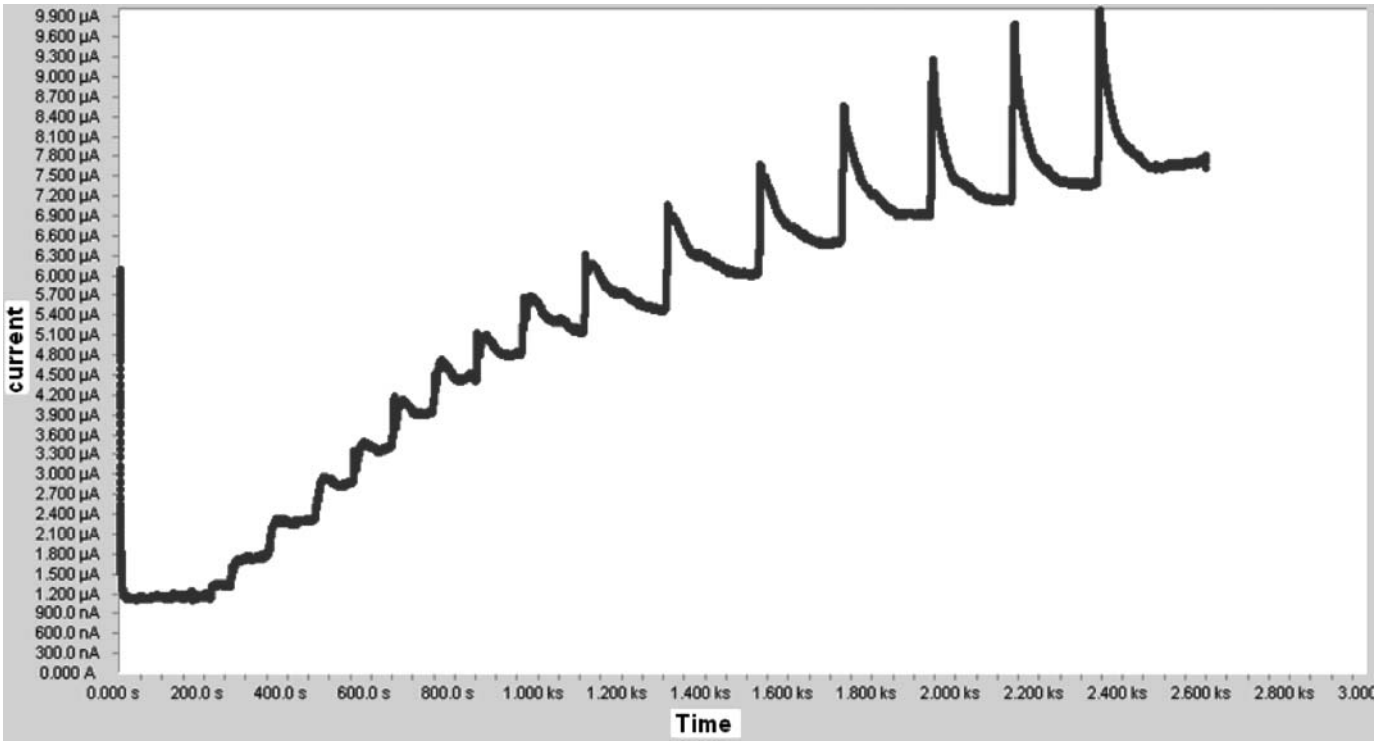


Fig. 8. Typical amperometric response of Pt/NpbPPy enzyme electrode applying a potential step to +0.60V vs. Ag/AgCl for glucose in 0.10M phosphate buffer solution (pH 6.0) at room temperature.

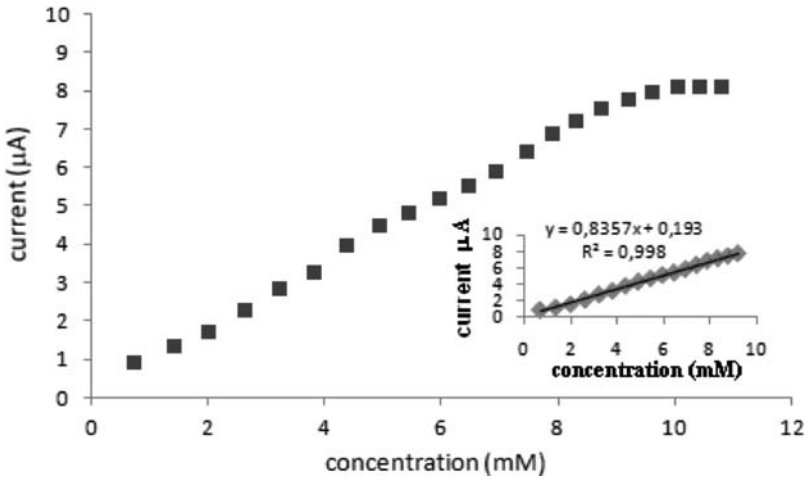


Fig. 9. Changes in the response of the Pt/NpbPPy enzyme electrode with the glucose concentration in 0.10M phosphate buffer solution (pH 6.0).

chemical reaction for the catalysis of biological chemical reactions with apparent kinetic constants:

$$I = \frac{I_M^{app} + [Glu]}{K_M^{app} + [Glu]} \quad (1)$$

where [Glu] is the glucose concentration, I_M^{app} is the apparent maximum current measured in excess of glucose and K_M^{app} is the apparent Michaelis Menten constant.

The enzyme electrode confirms Michaelis–Menten kinetics, and the value of the apparent Michaelis constant is a function of oxygen concentration in solution, as has been reported previously (47).

The apparent Michaelis–Menten constant (K_M^{app}) was calculated from the corresponding Lineweaver–Burk plots (curve was ignored here). (K_M^{app}) value for Pt/NpbPPy enzyme electrode was calculated as 25.95 mM. This apparent K_M value was higher than that (23.53 mM) of the soluble GOX from A. niger. K_M values obtained from both the systems indicate no diffusional limitations in the cross linked state and the non-denaturating character of the procedure of enzyme anchoring (48). Conformably, the character of Michaelis–Menten constant (K_M), the stronger will be the affinity between enzyme and substrate, whereas smaller the value of K_M. The K_M constant obtained in this study is lower than that of previous studies (49–51).

4 Conclusions

Some novel N-substitued pyrroles were successfully synthesized, polymerized and characterized by FTIR and SEM results. An enzyme electrode based on the immobilization of GOX in N-(p-benzoic acid) polypyrrole (NpbPPy) modified electrode was developed. The amount of immobilized GOX was improved with the presence of COOH units in substituted polypyrrole, NpbPPy film. It revealed that the enzyme immobilization procedure has no influence on denaturation of the enzyme structure when enzyme electrode was prepared. The excellent characteristics and performance of the proposed biosensor, such as low detection limit, fast response time, show that this NpbPPy modified electrode is suitable for enzyme immobilization and biosensor construction.

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