

Preparation of a Polypyrrole-Polyvinylsulphonate Composite Film Biosensor for Determination of Phenol Based on Entrapment of Polyphenol Oxidase

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Abstract: In this paper, a novel amperometric phenol biosensor with immobilization of polyphenol oxidase (tyrosinase) on electrochemically polymerized polypyrrole-polyvinylsulphonate (PPy-PVS) film has been accomplished via the entrapment technique on the surface of a platinum electrode. The amperometric determination is based on the electrochemical reduction of quinon generated in the enzymatic reaction of phenol. The effects of pH and temperature were investigated and optimum parameters were found to be 8.0 and 30 °C, respectively. The linear working range of the electrode was 1.0×10^{-7} – 5.0×10^{-6} M. The storage stability and operation stability of the enzyme electrode were also studied.

Keywords: phenol biosensor; amperometry; polypyrrole (PPy); polyvinylsulphonate (PVS); entrapment; interference effect

INTRODUCTION

Phenolic compounds are released into the environment by industrial wastes [1]. Phenols exist as pollutants of air, water, and soil. Phenol is easily adsorbed by humans, regardless of the type of exposure. High levels of phenol cause damage to animal health. Prolonged oral or subcutaneous exposure causes damage to the lungs, liver, kidney, and genito-urinary tract. In view of major concern regarding toxicity of these compounds, there is a growing need for innovative devices to monitor phenols in complex environments, food, pharmaceutical and industrial matrices. Determinations of phenol compounds have an important role in the field of biomedicine and environmental science. There are many methods for phenol determination, such as gas chromatography and spectrophotometric analysis [2, 3]. However, these methods suffer from complicated pretreatments. An amperometric biosensor based on an enzyme, tyrosinase, has been considered to be a promising method because of its effectiveness and simplicity [4–7].

For phenolic compounds' determinations, polyphenol oxidase (also known as tyrosinase, EC 1.14.18.1), which is a copper-containing enzyme, is used [8]. This enzyme catalyzes phenol's oxidation and *o*-quinone takes form as a product of the enzymatic reaction. This is accomplished in two reaction steps. In the first step, tyrosinase oxidizes phenol into the corresponding catechol. In the second step, this catechol is reoxidized into *o*-quinone. Amperometric

reduction of the generated quinone is then used as the quantification method [9,10].

A variety of methods for the immobilization of tyrosinase with an electrochemical transducer have been reported, such as cross-linking on the surface of electrodes [11–13], incorporation within a carbon paste matrix [14, 15], and entrapment in polymer films [8, 16].

In this paper we report the immobilization of polyphenol oxidase onto PPy–PVS film via an entrapment procedure for determination of phenol. The parameters affecting the performance of the biosensor (substrate concentrations, the pH, and temperature) were investigated.

MATERIAL AND METHODS

Instrumentation and Reagents

All electrochemical experiments were carried out using an CHI 660B Electrochemical Workstation conventional three-electrode system equipped with a Pt plate (0.5 cm²) as the working electrode, an Ag/AgCl electrode (3 M KCl) as the reference electrode, and a platinum wire (diameter and length, 1 mm, 4 cm, respectively) for the counter electrode and with an activity of 0.12 mg mL⁻¹, and polyphenol oxidase was purchased from Sigma. Pyrrole and sodium polyvinylsulphonate were supplied by Fluka. All other chemicals were obtained from Sigma. All the solutions were prepared using distilled water.

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Preparation of Pt/PPy-PVS Film Electrode

The surface of the Pt plate electrode was cleaned [17] and covered with a conducting polypyrrole in polyvinylsulphonate media [18]. The electrode was immersed in an 0.1 M pyrrole monomer in aqueous solution of the sodium salt of polyvinylsulphonate (25%). The solution was purged with argon in order to remove the oxygen. The electropolymerization of pyrrole upon the electrode surface was performed by the cyclic voltammetry between -1.0 and $+2.0$ V at a scan rate of 100 mV/s and 4 cycles (Figure 1). The electrode was washed with buffer solution after the coating process.

Immobilization of Polyphenol Oxidase on Pt/PPy-PVS Film Electrode

A Pt/PPy-PVS-Polyphenol oxidase film electrode was used against the Pt electrode, which was used as the counter electrode. The reference electrode was composed of Ag/AgCl. Immobilization of polyphenol oxidase (PPO) was carried out by the physical entrapment approach. The electrode was immersed in 0.1 M pyrrole monomer in aqueous solution of the sodium salt of polyvinylsulphonate (25%) and 2 mL of PPO enzyme (0.12 mg/mL) was added into the solution. The solution was then purged by argon for the removal of oxygen before electropolymerization. Electropolymerization was performed in the mode of cyclic voltammetry to immobilize polyphenol oxidase onto the electrode. The scanning voltage range was from -1.0 to $+2.0$ V with a scanning rate of 100 mV/s. After the fabrication of polyphenol oxidase entrapped Polypyrrole film onto the PPy-PVS working electrode was finished, the electrode was rinsed with deionized water to remove the unreacted pyrrole monomer and free polyphenol oxidase.

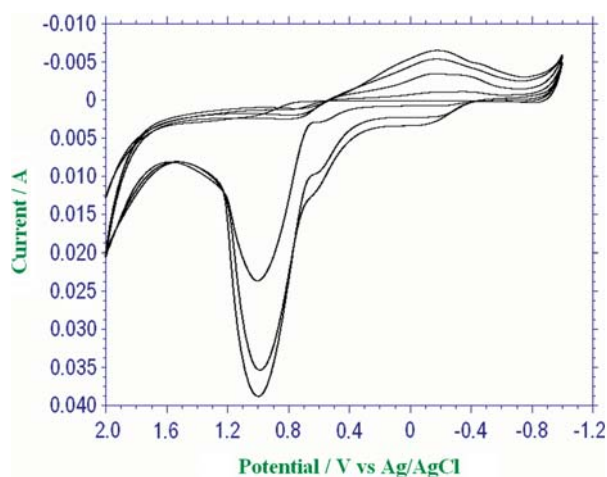


Figure 1. Growth of PPy-PVS films in aqueous solution of 0.1 M pyrrole and 25% Na-PVS at a scan rate of 100 mV s $^{-1}$ versus Ag/AgCl (saturated).

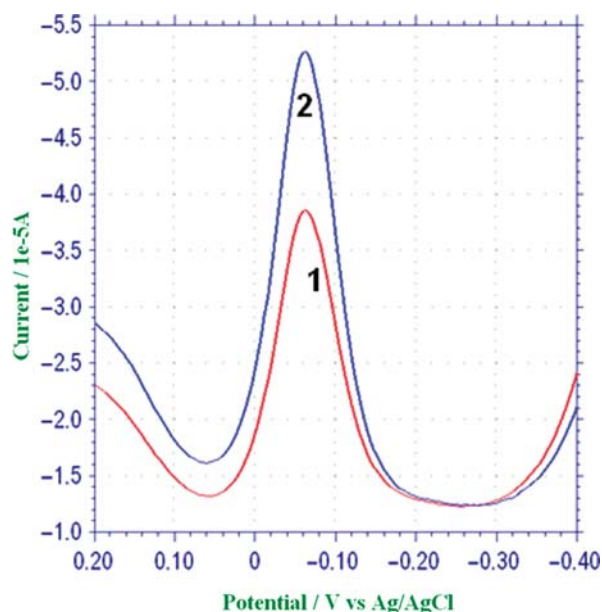


Figure 2. Square wave voltammogram of quinone in phosphate buffer (pH 8.0). 1. 3.0×10^{-5} M quinone, 2. 5.8×10^{-5} M quinone.

Immobilized enzyme electrode was kept in a refrigerator at 4°C in phosphate buffer when it was not in use.

Amperometric Biosensor Measurements

Amperometric response studies were carried out in phosphate buffer. Operational stability, storage stability, pH, and temperature were determined via application of -0.09 V (versus Ag/AgCl) by detecting the reduction current of quinon. After the background current reached a stable value, phenol solution was added to the cell using a micropipette and stirred for 10 min. Then the resulting current

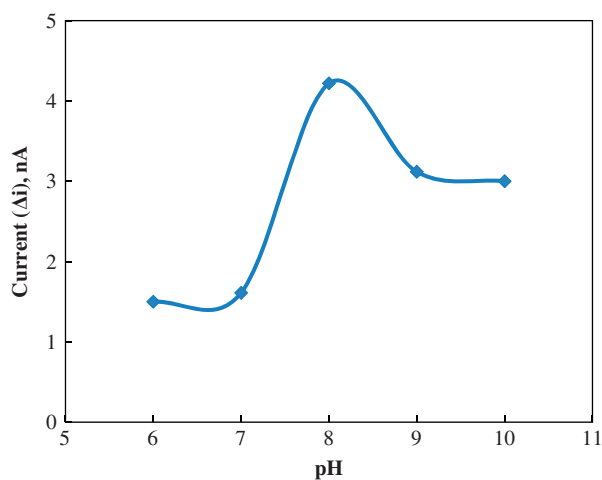


Figure 3. The effect of pH on the response of the biosensor (in the phosphate buffer containing 1.0×10^{-6} M phenol, operating potential is -0.09 V, 30°C).

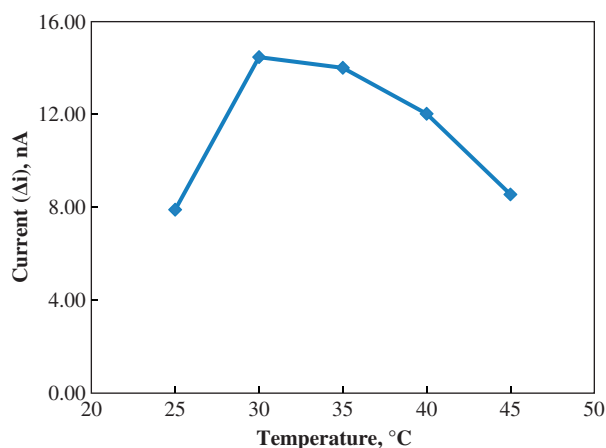


Figure 4. The effect of temperature on the response of the biosensor (in the phosphate buffer pH = 8.0) containing 1.0×10^{-6} M phenol operating potential is -0.09 V.

difference was recorded. Research on operational and storage stability as well as effects of pH and temperature was carried out using 1.0×10^{-6} M concentration of phenol.

RESULT AND DISCUSSION

In this study, we prepared a new phenol biosensor with the entrapment of polyphenol oxidase (PPO) on poly(pyrrole)-polyvinylsulphonate (PPy-PVS) composite film. The parameters affecting the performance of the biosensor were examined.

The Working Potential

In 0.1 M pH = 8.0 phosphate buffer solution, electrochemical behavior of quinone was investigated with

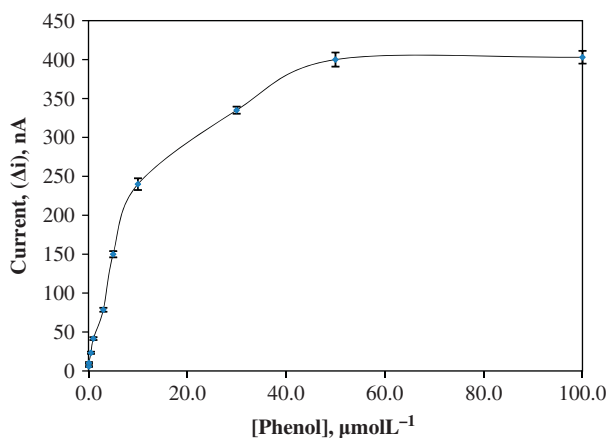


Figure 5. The effect of phenol concentration upon the amperometric response of the biosensor (Michealis-Menten plot, in the phosphate buffer (pH 8.0), operating potential is -0.09 V, 30 °C).

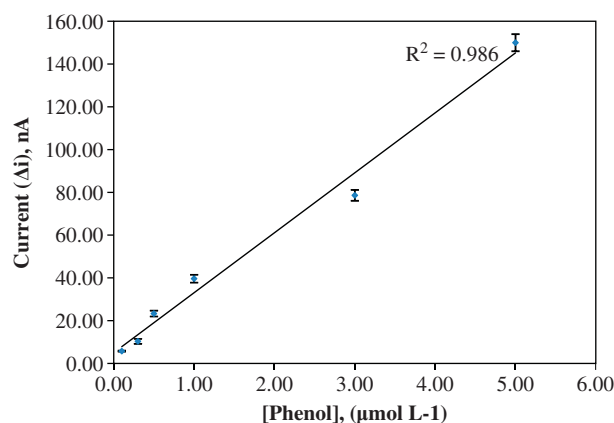


Figure 6. Calibration curve for phenol biosensor in the phosphate buffer (pH 8.0), operating potential is -0.09 V, 30 °C).

square wave voltammetry. The reduction peak potential of quinone was found to be -0.09 V (Figure 2).

Effects of pH and Temperature

Immobilized polyphenol oxidase enzyme showed maximum activity at pH 8.0 and 30 °C. The buffers at various pH values were tested to investigate the effect of pH. The pH values of the buffers were varied between 6.0 and 10.0. The measurements were performed at a constant phenol concentration of 1.0×10^{-6} M. Figure 3 shows that the maximum response was obtained at pH 8.0. For phenol biosensor, pH values different from 8.0 were employed in literature (pH 7.5, 7.0) [16, 19]. This was attributed to the fact that the used polymer and the type of immobilization were different.

Temperature has a great effect on enzyme activity and it is important to investigate the dependence of the response of the enzyme electrode to the temperature. The

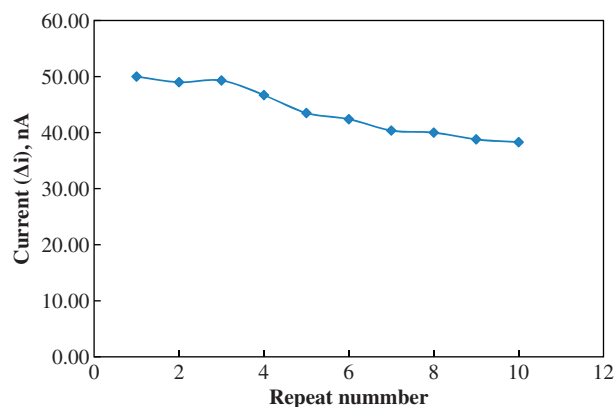


Figure 7. Measure number of the biosensor (in the phosphate buffer (pH 8.0), operating potential is -0.09 V, 30 °C).

temperature influence on the response of phenol enzyme electrode was tested between 25°C and 45°C at pH 8.0 using constant phenol concentration of 1.0×10^{-6} M (Figure 4). As seen from the figure, the electrode response increases with temperature up to 30°C and decreases afterwards. The highest electrode response was obtained at 30°C. For phenol biosensor, temperature values different from 30°C were employed in literature (40°C, 21°C) [11, 19]. This was attributed to the fact that the used polymer and the type of immobilization were different.

Effect of Substrate Concentration on Biosensor

The value of the Michaelis–Menten kinetic parameter (K_m), which shows the enzyme–substrate kinetics, was determined by the analysis of the graph of enzymatic reaction. V_{max} is the maximum rate for enzymatic reaction. The effect of the substrate concentration on the reaction rate, catalyzed by immobilized PPO, was studied by using different concentrations (1.0×10^{-7} – 1.0×10^{-4} M) of phenol substrate (Figure 5). With the increase in substrate concentration, there was an increase in amperometric current signal. There were linear parts ranging between 1.0×10^{-7} – 5.0×10^{-6} M (R^2 : 0.99). The graph for the calibration curve is given in Figure 6. It has been shown that the linearity of the graph was highly satisfactory and it can be used for the quantitative determination of phenol. The detection limit of the biosensor was 1.0×10^{-8} M and the response time of the biosensor was 300 s. Kinetic parameters $K_{m(app)}$ and I_{max} for the enzyme biosensor were detected at constant temperature (30 °C) and pH (pH 8.0) in varying substrate concentrations. $K_{m(app)}$ and I_{max} were calculated as 2.5 μ M, 0.125 μ A/min, respectively, using $1/[phenol]$ - $1/\Delta i$ graph (Lineweaver-Burk). K_m values for immobilized PPO presented in the literature are 100.0 mM, 70 μ M [16,19].

Operational Stability and Storage Stability

The biosensor was used at optimum conditions for 10 activity assays in one day to determine the operational stability. Storage stability of the biosensor was determined by performing activity assays within 45 days. The operational stability was studied by applying activity assay (under optimum conditions) 10 times in the same day at constant temperature, pH, and substrate concentration (1.0×10^{-6}). At the end of the 10 measurements, the biosensor showed 77 % of its initial activity (Figure 7). The activity assay was applied within 45 days to display the storage stability of immobilized enzyme. It was observed that 57% of initial activity was lost on the forty-fifth day.

Interference Effect

A few cations found in wastewater, such as Cd^{2+} , Pb^{2+} , Sn^{2+} , As^{3+} , As^{5+} , Cr^{3+} , Cr^{6+} , Sb^{3+} , Mn^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , were studied for any interfering effect on the analysis of phenol. 1.0×10^{-2} M – 1.0×10^{-5} M concentrations of cations were added. It was observed that Cd^{2+} , Pb^{2+} , Sn^{2+} , As^{3+} , As^{5+} , Cr^{3+} , Cr^{6+} , Sb^{3+} , Mn^{2+} , Co^{2+} , and Ni^{2+} had no interfering effects on the analysis of phenol. However, the interfering effect of copper (1.0×10^{-2} – 1.0×10^{-5} M) on the analysis of phenol was observed.

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

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