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Amperometric catechol biosensor based on polyaniline–polyphenol oxidase

Yongyan Tan, Xiaoxia Guo, Jinghui Zhang, Jinqing Kan*

School of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, PR China

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ABSTRACT

A novel catechol biosensor was described based on the immobilization of polyphenol oxidase (PPO) into polyaniline (PANI), which was easily constructed by direct electropolymerization of aniline in a solution containing ionic liquid, 1-ethyl-3-methylimidazolium ethyl sulfate (EMIES). The developed biosensor for the detection of catechol has a linear range of 1.25–150 μmol dm^{-3}. The maximum response current (I_{max}) and the Michaelis–Menten constant (k'_m) are 0.62 μA and 146 μmol dm^{-3}, respectively. The activation energy (E_a) of the PPO catalytic reaction is 31.1 kJ mol^{-1} in the B–R buffer. The biosensor shows good reproducibility (a relative standard deviation of 3.1% was obtained) and remarkable long-term stability (it retains 75% of the original activity after four months). The effects of potential and pH on the response current of the biosensor are also described.

1. Introduction

Most of phenolic compounds show harmful effects on human health, animals and plants, which results in an acute environmental problem. So it is very important to develop the quick and effective methods for the quantitative detection and control of the phenolic compounds. Some methods have been available for phenolic compound detection, including spectrophotometry (Espin et al., 1997), capillary electrophoresis (Schoning et al., 2005), Voltammetry method (Zen and Chen, 1998; Liu et al., 2002; Yu et al., 2009) and chromatography (Castillo et al., 1997; Cui et al., 1999). Nevertheless, complex sample pre-treatment procedures, low sensitivities and time-consuming manipulations limit their practical applications. Recent years, amperometric polyphenol oxidase (PPO/tyrosinase) biosensors have been extensively reported in the literature for the detection of various phenolic compounds due to its high sensitivity (Gomes et al., 2004), fast response (Rosatto et al., 1999), simplicity, and easy miniaturization. The materials (biocompatibility, porosity and electron-transfer efficiency, etc.) and methods of immobilizing enzyme have important influence on the activity and the stability and other performance of a biosensor (Mu, 1994; Singh et al., 2006). To date, various materials such as magnetic nanoparticles (Wang et al., 2008), self-gelatinizable graft copolymer of poly(vinyl alcohol) with 4-vinylpyridine (Zhang et al., 1999), sol–gels (Li et al., 1998; Wang et al., 2000; Zejli et al., 2008), magnetic nanoparticles-coated carbon nanotubes (CNTs) nanocomposite (Cheng et al., 2009), clays (Fan et al., 2007), inorganic nanomaterials (Li et al., 2006; Shan et al., 2007), polymers (Dempsey et al., 2004; Vedrine et al., 2003), conducting copolymers (Kiralp et al., 2003) and self-assembled monolayers (SAMS) (Campuzano et al., 2003) have been reported to immobilize PPO. However, some materials are either complicated or/and time-consuming for preparation, or bad biocompatibility, or low capacity for immobilization of polyphenol oxidase, leading to inefficient performance for the detection of phenolic compounds. Therefore, it is of great significance in both theory and practice to explore a simple and efficient technology, and biocompatible supports with high capacity for immobilizing PPO.

Conducting polymers show very interesting chemical and physical properties owing to their unique conjugated π-electron system. Among conducting polymers, polyaniline (PANI) (Palaniappan, 2001) has been attracted considerable attention because of its many advantages: the monomer (aniline) is relatively inexpensive, polymerization method is simple. In particular, PANI has high conductivity, good redox reversibility and stability in both aqueous solutions and air for its applications in biosensors (Wang and Mu, 1999). Good environmental stability is considered important for the long-term stability of a biosensor. Nevertheless, PANI synthesized in proton acid solution lost its redox activity (Huang et al., 1986) and conductivity (Focke et al., 1987) at pH > 4, which may be adverse to electron-transfer reactions of the biosensor. Fortunately, PANI prepared in the ionic liquid, 1-ethyl-3-methylimidazolium ethyl sulfate (EMIES), still maintains a good electroactivity and conductivity at high pH (up to 12). This is because the buffer capacity and porosity of ionic liquid-doped PANI chain is improved, which is favorable for adjusting pH in the microenvironment of the PANI film and also facilitates mass/charge transfers during the redox

* Corresponding author. Tel.: +86 514 87975590 9415; fax: +86 514 87975590 8410.
E-mail address: jqkan@yzu.edu.cn (J. Kan).

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process (Mu, 2007; Li et al., 2007; Musameh et al., 2002). Wang et al. (2009) in our group have confirmed that PANI prepared in EMIES "tends to agglomerate into interconnected networks with higher porosity. Such network-like structure increases effective surface area of the substrate electrode to load large amount of enzyme".

So far, the immobilizing techniques of enzyme employed frequently by using polyaniline fall into four categories, one-step process (Bartlett et al., 1996; Shinohara et al., 1988), two-step process (Mu et al., 1991), template process (Kan et al., 2004) and cross-linking process (Wang et al., 2009). Each of them has its own advantages and disadvantages. Early one-step process for immobilizing enzymes using polyaniline was almost carried out in neutral buffer (Shinohara et al., 1988), which results in low capacity for enzyme and poor electron-transfer efficiency due to the polyaniline film with compact structure (Huang and Richard, 2004; MacDiarmid and Epstein, 1995; Sengupta et al., 2008) and poor conductivity (Focke et al., 1987). Although, one-step process has fallen out of favor in preparation of enzyme biosensor for a period of time, it also has advantages over others, such as simplicity and low cost. If the above-mentioned deficiencies can be overcome, one-step process will be a promising method for immobilizing enzymes using polyaniline.

In this study, a meliorative PANI–PPO biosensor has been prepared by direct electropolymerization, i.e. one-step process, in a buffer containing EMIES. The process is unrelated to PPO's isoelectric point, which is different from two-step process (Mu et al., 1991). Moreover, it is easy to control the thickness of enzyme film by adjusting the time of polymerization. At the same time, catechol was selected as the model polyphenol in our reaction systems, because the PPO biosensors for catechol exhibit higher sensitivity, wider linear range and lower detection limit (Fan et al., 2007; Liu et al., 2000; Kochana et al., 2008; Shan et al., 2007; Zejli et al., 2008). The experiment results show that the biosensor can maintain good electrochemical activity and long-term stability.

2. Experimental

2.1. Materials

Polyphenol oxidase (PPO) (EC 1.14.18.1, 1050 U mg^{-1}) was purchased from Sigma. Catechol was supplied by Bio Basic Inc. and used as received. Aniline (reagent grade) was distilled under vacuum, obtaining a colorless liquid. All other reagents were of analytical grade and used as received without further purification. Britton–Robinson buffer (B–R buffer) was prepared by adding 0.04 mol dm^{-3} acetic acid and 0.04 mol dm^{-3} boric acid in a solution of 0.04 mol dm^{-3} orthophosphoric acid and its pH was adjusted with sodium hydroxide solution (Navalon et al., 2002). Solutions were all prepared with double-distilled water.

2.2. Measurements and apparatus

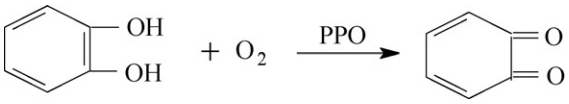
A CMBP-1 bipotentiostat/galvanostat was used for PANI–PPO film synthesis. YD-1 precision potentiostat was employed for the determination of the response current. All electrochemical studies were performed with a conventional three-electrode system. A saturated calomel electrode (SCE) and two platinum sheets (diameter 5 mm) were used as reference electrode, working electrode and counter electrode, respectively. The pH values of the solutions were determined using a PHS-25A pH meter. AC impedance measurements were conducted using an Autolab/PGSTAT30 (Eco Chemie, The Netherlands) with a three-electrode system. Cyclic voltammetry (CV) was performed by using a CHI660B electrochemical workstation (Shanghai Chenhua Instruments, Shanghai, China). The surface morphologies of the electrodes were observed by HITACHI S-4800 scanning electron microscopy (SEM).

2.3. Preparation of the biosensor

The preparation of the biosensor with one-step method (as shown in Fig. 1A) was carried out in a solution consisting of 0.2 mol dm^{-3} aniline, 20% EMIES (v/v), 0.2 g dm^{-3} PPO, 0.3 g dm^{-3} SDS and adequate amount of hydrochloric acid (HCl) with pH 5 at room temperature at 0.7 V (vs. SCE). The solution was stirred with a magnetic stirrer. The PPO was directly entrapped into the polyaniline film during polymerization of aniline. The thickness of the film was controlled through adjusting polymerization time. The PANI–PPO film was rinsed with double-distilled water. When not in use, the biosensor was preserved at 4 °C in a dry state.

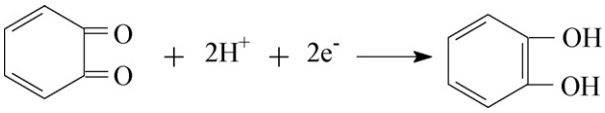
2.4. Principle and method of the measurement

PPO catalytic reaction is as follows:



(1)

Formation of *o*-quinone is detected by the amperometric current method during reduction at the electrode:



(2)

The general scheme for amperometric detection of phenolic compounds is shown in Fig. 1B.

The cell used to determine the response current consisted of the PANI–PPO biosensor, a platinum electrode (auxiliary electrode), a saturated calomel electrode (SCE) and the B–R buffer without and with the substrate. YD-1 precision potentiostat, which is accu-

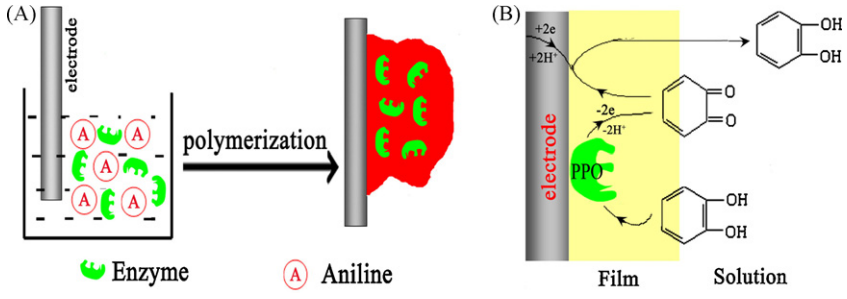


Fig. 1. (A) Scheme of one-step process. (B) Scheme of the bioelectrocatalytic cycle in the biosensor for the detection of catechol as substrate

rate within 1 nA, was used to determine the response current. The method was similar to that described in previous work (Mu et al., 1991). Firstly, the background current (I_0) of the PANI–PPO biosensor in buffer without substrate was measured. Then the current (I_S) of the PANI–PPO biosensor in buffer with substrate was measured. It is known that in amperometry the respond current of biosensor firstly increases with the increasing time, and then appears a plateau or decreases, the plateau or peak current is recorded as I_S. Finally, the response current was taken as the measured value ($I = I_S - I_0$) in the following experiments. All data were measured repeatedly at least three times, and the relative error is less than 3%. All potentials given here are referred to an SCE.

3. Results and discussion

3.1. Impedance plots

Electrochemical impedance spectrum was investigated (shown in Fig. 2) to understand the interface properties of modified electrode. In general, there are two frequency regions in an electrochemical impedance spectrum (EIS). At a high-frequency region, the semicircle portion corresponds to the charge-transfer kinetics of the redox couple at the electrode interface, while at a low-frequency region, the straight line is formed by the diffusion control of the reactant species. Fig. 2 shows the Nyquist plots of bare platinum electrode (a), PANI (b) and PANI–PPO (c) films in B–R buffer (pH 6.5) containing 5 mmol dm^{-3} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ at 50 mV and their frequency range is from 0.1 to 10^5 Hz. The lowest R_{ct} (89 Ω) on the bare platinum electrode (curve a) indicates the fastest electron transfer rate between the bare platinum electrode and $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. And the impedance of the electrode increases (R_{ct} = 202 Ω) in the presence of PANI film (curve b), which obstructs the electron transfer of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Moreover, On the PANI–PPO film (curve c), the R_{ct} increases (R_{ct} = 900 Ω), owing to PPO insetting into the interspace of the PANI film and incorporating with the PANI film. The macromolecular structure of non-conductive PPO influences the charge transfer. The result indicates that PPO is immobilized into PANI film. In addition, cyclic voltammograms and scanning electron microscopy images (figures are omitted here) also show that PPO was immobilized successfully.

Sabatani et al. (1993) suggested that the change of the charge-transfer resistance is related to the apparent electrode coverage (θ), which is as follows:

$$\theta = 1 - \frac{R_{ct}^{o}}{R_{ct}} \quad (3)$$

where R_{ct}^{o} and R_{ct} are the charge-transfer resistances of a bare electrode and its electrode modified by thiophenol (TP), respectively.

Similarly, for polyaniline electrode and its electrode modified by PPO,

$$\theta' = 1 - \frac{R_{ct}^{PANI}}{R_{ct}^{PANI-PPO}} \quad (4)$$

where R_{ct}^{PANI} and $R_{ct}^{PANI-PPO}$ are the charge-transfer resistances of the PANI electrode and its electrode modified by PPO, respectively. The value of θ' is about 77.5% according to Eq. (4). This also indicates PPO is immobilized into the PANI film successfully. More studies are needed to better understand the relationship between the charge-transfer resistance and the amount of immobilized PPO.

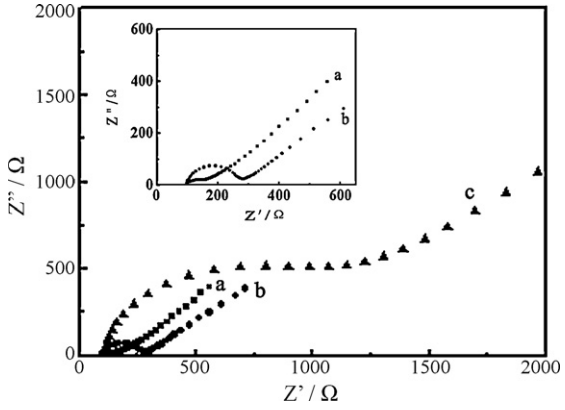


Fig. 2. Nyquist plots of the electrochemical impedance spectroscopy (EIS) for bear platinum (a), PANI (b) and PANI–PPO (c) films in B–R buffer (pH 6.5) containing 5 mmol dm^{-3} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (the inset is the enlarged drawing of curves (a) and (b) of the figure).

3.2. Effect of potential on the response current

The effect of applied potential on the response current of the PANI–PPO biosensor in the B–R buffer containing 40 μmol dm^{-3} catechol (pH 6.5) is shown in Fig. 3A. It can be seen that the response current of the PANI–PPO biosensor increases gradually as the potential decreases from 175 to 50 mV and then increases rapidly in the range of 50 to −50 mV, which is due to the increased driving force for fast reduction of *o*-quinone at low potential. The response current of PPO/silica sol–gel (Wang et al., 2000) increases rapidly and then approaches a plateau in the range of 156 to −244 mV. While the response current of PPO/polyaniline–ionic liquid–carbon nanofiber (Zhang et al., 2009) increases rapidly from −200 to −50 mV, and then decreases as the potential more negative. It can be seen that the effect of applied potential on PPO biosensor is correlated to immobilizing materials and/or methods. For PANI–PPO biosensor in our work, the applied potential was set at 50 mV to exactly obtain the response current value in the following experiments. The interference from other electroactive species (such as ascorbic acid, dopamine or some metal ions) could also be minimized at this potential.

3.3. Effect of catechol concentration on the response current

Fig. 3B represents the relationship between the response current of the PANI–PPO biosensor and the catechol concentration in B–R buffer (pH 6.5) at 50 mV. The response current increases linearly with increasing concentration of catechol in the range of 1.25–150 μmol dm^{-3} (inset in Fig. 3B), so the enzyme catalytic reaction of catechol is the first-order reaction. Then, with further increasing the concentration of catechol, the response current increases slowly, i.e. the enzyme reaction shows a transition from first to zero-order. Based on Integrated Wastewater Discharge Standard (GB 8978, 1996), the concentration of catechol should be in the range of 2.75–9.17 μmol dm^{-3}. So the biosensor can satisfy the demand of practical applications.

Since the electrode responses are kinetic, the apparent Michaelis–Menten constant (k'_m) can be calculated for the immobilized PPO by an amperometric method as suggested by Shu and Wilson (1976). Based on the data in Fig. 3B, plot of I^{-1} against $[s]^{-1}$ is got as shown in inset II of Fig. 3B. The maximum current response (I_{max}) and apparent Michaelis–Menten constant (k'_m) can be calculated from the intercept and slope, respectively. The I_{max} and k'_m are 0.62 μA and 146 μmol dm^{-3}, respectively. The value of k'_m here is lower than that reported for the free PPO (280 μmol dm^{-3}) using catechol as the substrate (Brown et al., 1994). k'_m is the equilibrium constant for the dissociation of the enzyme–substrate complex and is inversely related to the "affinity" of the enzyme for the substrate

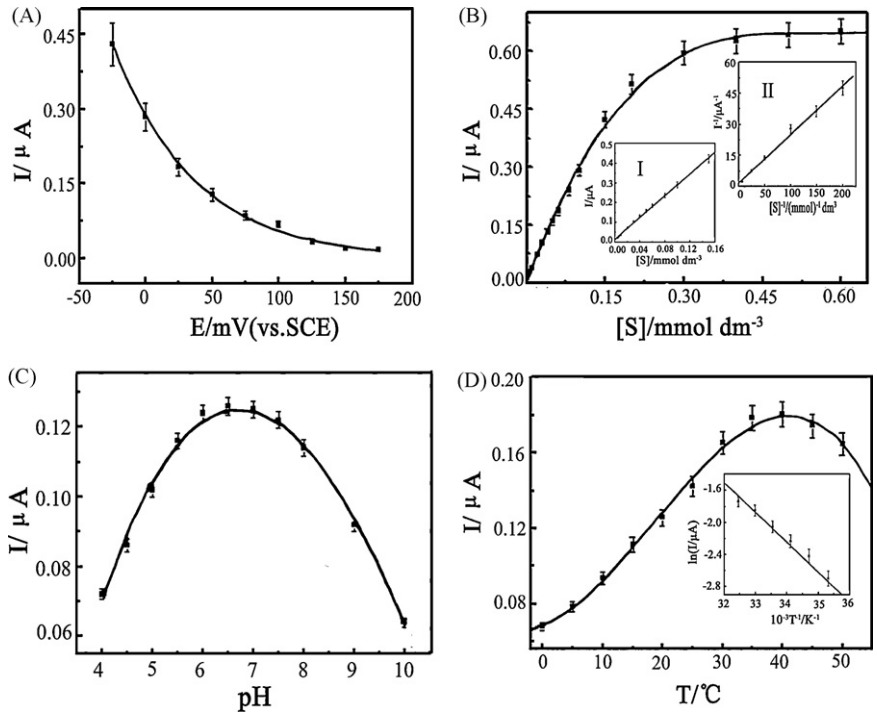


Fig. 3. (A) The relationship between response current and potential. 40 μmol dm^{-3} catechol, pH 6.5, 25 °C. (B) The relationship between response current and catechol concentration. pH 6.5, 50 mV, 25 °C. (C) The relationship between response current and pH. 40 μmol dm^{-3} catechol, 50 mV, 25 °C. (D) The relationship between response current and temperature. 40 μmol dm^{-3} catechol, pH 6.5, 50 mV.

(Carr and Bowers, 1980). So the lower value of the k'_m indicates the affinity between PPO and catechol is stronger than that of the free PPO. It can be seen from Table 1 that the value of k'_m is markedly dependent on immobilizing materials and methods. We do not know why the values of k'_m for the PPO/polyaniline–ionic liquid–carbon nanofiber is so small (Zhang et al., 2009) and that for the PPO/nano-$CaCO_3$ is so large (Shan et al., 2007), because there is no relevant explanation in their reports.

To evaluate the selectivity of the biosensor, the effects of some possible interfering substances on response current were examined in B–R (pH 6.5) containing 40 μmol dm^{-3} catechol with and without 4.0 mmol dm^{-3} ascorbic acid, 4.0 mmol dm^{-3} dopamine, 40 mmol dm^{-3} Cu^{2+} or 40 mmol dm^{-3} $[Fe(CN)_6]^{3-}$. The response current of the PANI–PPO biosensor exhibited no significant changes (relative standard deviation less than 3.0%).

3.4. Effect of pH on the response current

The effect of pH on response current in the buffer containing 40 μmol dm^{-3} catechol at 50 mV is shown in Fig. 3C. It can be seen that the response current increases with increasing pH from 4.0 to 6.5, which may be attributed to the activity of enzyme increases with increasing pH. On the contrary, at the range from 6.5 to 10, the response current decreases with increasing pH. The decrease of response current of the PANI–PPO biosensor cannot be ascribed to the decrease of conductivity of the PANI with increasing pH, and which is caused by the decreasing of the activity of enzyme. Because the PANI synthesized in an acidic solution containing EMIES has good electroactivity and high conductivity at high pH (Mu, 2007). The optimum pH of the immobilized PPO obtains at about 6.5, which is close to that of the free PPO from mushroom (pH 6.2) (Bru et al., 1989). According to Table 1, it is found that the optimum pHs for PPO/Fe_3O_4 nanoparticles–chitosan nanocomposite (Wang et al., 2008), PPO/laponite clay–chitosan nanocomposite (Fan et al., 2007), PPO/nano-$CaCO_3$ film (Shan et al., 2007) and PPO/polyacrylamide microgels (Perez et al., 2006) are also close to that of the free PPO. However, optimum pHs for some immobilized PPO are quite different from that of free PPO. Kiralp et al. (2003) reported MM/PPy/PPO with the optimum pH of 9. The reason is that negative groups of the matrix protons are concentrated around the

Table 1
Comparison of PPO biosensors using different matrix and method.

PPO (tyrosinase) biosensor based on different immobilization matrix and method	k'_m (μmol dm^{-3})	Optimum pH	Optimum temperature (°C)	E_a (kJ mol^{-1})	Reference
PPO immobilized into PANI film	146	6.5	40	31.1	This work
PPO entrapped in Fe_3O_4 nanoparticles–chitosan nanocomposite	96.9	6.5	35	Not reported	Wang et al. (2008)
Tyrosinase entrapped in laponite clay–chitosan nanocomposite matrix	160	6.5	40	34.5	Fan et al. (2007)
Tyrosinase immobilized in ZnO/chitosan matrix	40	4.5	50	Not reported	Li et al. (2006)
PPO entrapped into nano-$CaCO_3$ film	256	6	45	24.6	Shan et al. (2007)
PPO in conducting copolymers of thiophene functionalized methyl monomer (MM) with pyrrole	200	9	60	Not reported	Kiralp et al. (2003)
Tyrosinase immobilized in polyaniline–ionic liquid–carbon nanofiber composite	1.44	7	35	38.8	Zhang et al. (2009)
PPO entrapped in Polyacrylamide microgels	Not reported	6	25	53.78	Perez et al. (2006)

Table 2
Comparison of different analytical methods for the detection of catechol.

Analytical method	Experimental conditions	Linear range (mol dm^{-3})	Stability	Reference
PPO biosensors				
Polyphenol oxidase (PPO) immobilized into PANI film	B–R buffer (pH 6.5), 25 °C, 50 mV	1.25×10^{-6} to 1.5×10^{-4}	Maintained 90% of its initial activity after 30 days and retained 75% activity after 120 days	This work
PPO encapsulated in silica sol–gel composite film	0.05 mol dm^{-3} (M) PBS (pH 7), 25 °C, −44 mV	1.0×10^{-7} to 1.0×10^{-4}	Maintained 73% of initial activity after intermittent use for 21 days	Wang et al. (2000)
PPO encapsulated in alumina sol–gel matrix	0.2 M PBS (pH 7), 30 °C, −34 mV	1.0×10^{-7} to 3.0×10^{-5}	Lost 100% activity of initial activity after 21 days	Zejli et al. (2008)
PPO entrapped in laponite clay–chitosan	0.1 M PBS (pH 6.5), 25 °C, −50 mV	5.3×10^{-9} to 4.0×10^{-5}	Retained 88% of its initial activity after 60 days and decreased to 55% after 75 days	Fan et al. (2007)
PPO entrapped into nano-$CaCO_3$ film	0.1 M PBS (pH 6.5), 25 °C, −200 mV	6.0×10^{-9} to 2.0×10^{-5}	Remained 70% of its original response after 56 days	Shan et al. (2007)
Tyrosinase entrapped in polythiophene film	0.1 M sodium phosphate buffer (pH 6.5) and 0.1 M KCl, 25 °C, −200 mV	-2.5×10^{-5}	Retained about 30% of its initial activity after 12 days	Vedrine et al. (2003)
Tyrosinase immobilized on a self-assembled monolayer	0.05 M PBS buffer (pH 7.0), 25 °C, −144 mV	2.0×10^{-7} to 1.0×10^{-4}	Not reported	Campuzano et al. (2003)
Tyrosinase immobilized in polyaniline–ionic liquid–carbon nanofiber composite	0.1 M PBS buffer (pH 7.0), 25 °C, −50 mV	4.0×10^{-10} to 2.1×10^{-6}	Retained 94% of its initial activity after 25 days and decreased to 70% after 40 days	Zhang et al. (2009)
High-performance liquid chromatography (Gradient I)	1×10^{-2} M luminol and 5.0×10^{-7} M $K_3Fe(CN)_6$, luminol (pH 13.3)	4.58×10^{-7} to 4.58×10^{-5}	Not reported	Cui et al. (1999)
Capillary electrophoresis	20 mM MES buffer (pH 6.1), 1.5×10^6 mV	2×10^{-5} to 1.0×10^{-4}	Not reported	Schoning et al. (2005)
Voltammetry method	PBS buffer (pH 7.4), 1.76×10^3 mV	7×10^{-7} to 1.5×10^{-5}	Not reported	Zen and Chen (1998)
Pulse voltammetry	NaAc–HAc buffer (pH 4.6)	1.0×10^{-7} to 6.8×10^{-6}	Not reported	Liu et al. (2002)
Simultaneous voltammetry determination	0.10 M PBS buffer (pH 7.0)	5×10^{-7} to 3.5×10^{-5}	The peak currents remain more than 94% of their initial values for two weeks	Yu et al. (2009)

PBS: phosphate buffer solution. MES: 2-(4-morpholino) ethanesulfonic acid hydrate.

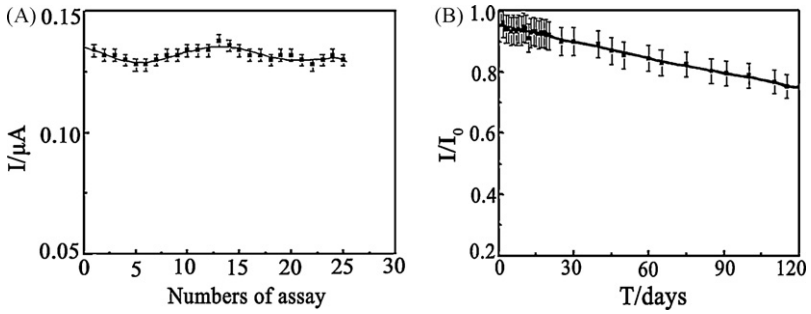


Fig. 4. The stability of the biosensor. 40 μmol dm^{-3} catechol, pH 6.5, 50 mV, 25 °C. (A) The operational stability of the biosensor. (B) The long-term stability of the biosensor.

enzymes to protect them against the high concentration of OH^-. Li et al. (2006) reported that the optimum pH of PPO/ZnO/chitosan was 4.5, which reason was not mentioned in the literature. Therefore, it seems that the optimum pH of the immobilized PPO is closely related to immobilization matrix and method.

3.5. Effect of temperature on the response current

The effect of temperature on response current in the buffer (pH 6.5) containing 40 μmol dm^{-3} catechol at 50 mV is presented in Fig. 3D. It can be seen that the response current increases with increasing temperature in the range of 0–40 °C and then decreases as the temperature increases further. At higher temperatures, the response current decreases owing to the partial denaturation of the enzyme. The optimum temperature for the immobilizing PPO is 40 °C. It is close to that for the PPO immobilized using laponite clay–chitosan nanocomposite (Fan et al., 2007) and is greater than those for the PPO immobilized using other materials, including poly-amphipilic pyrrole (Stanca and Popescu, 2004), polyacrylamide microgels (Perez et al., 2006), polythiophene film (Vedrine et al., 2003), polythiophene alginate gel beads, poly(acrylamide-co-acrylic acid) hydrogels (Yahsi et al., 2005) and polyaniline–ionic liquid–carbon nanofiber composite (Zhang et al., 2009), namely 21, 25, 25, 35, and 35 °C, respectively. These results indicate that the thermal stability of biosensors is obviously influenced by the immobilization material and method. The higher thermal stability of the biosensor in this work may be due to the fact that the appropriate porosity structure of PANI restricts PPO mobility and prevents unfolding (Hanefeld et al., 2009). Considering most enzymes could be easily denatured at high temperature and the practical application, so 25 °C was selected in the study unless otherwise specified.

According to the Arrhenius formula:

$$\ln k = \ln A - \frac{E_a}{RT}$$

where k is the rate constant and E_a is the apparent activation energy. When the electrode surface area, the quantity of the enzyme, pH value and concentration of substrate are constant, the response current is proportional to the rate constant k (Yokoyama et al., 1989), so $\ln k$ can be replaced with $\ln I$ in the Arrhenius formula. The relationship of $\ln I$ versus $1/T$ is a straight line as shown in the insert of Fig. 3D. The value of E_a in the B–R buffer is 31.1 kJ mol^{-1} (calculated from the slop of the straight line), which is higher than that of the free PPO (9 kJ/mol) (Wang et al., 1997). It clearly shows that the value of E_a is affected by the immobilization matrix and method (Table 1).

3.6. The stability

Stability is an important parameter to be considered for a biosensor. The operational stability of the biosensor was examined in the solution containing 40 μmol dm^{-3} catechol (Fig. 4A). A relative standard deviation value of 3.1% is obtained from 25 successive determinations, which indicates a good repeatability of the biosensor to catechol. The long-term stability of the biosensor is also investigated in the same solution. The response current of the biosensor decreases gradually and retains about 75% of its initial value after 120 days, which measured at intervals of 5–10 days (Fig. 4B). It still remains good performance till now (over 240 days). It can be seen that PPO biosensor in our work has better long-term stability than others (Table 2). The reason is that PANI prepared in ionic liquid has strong adhesion and good biocompatibility. Compared with other analytical methods in Table 2, the PANI–PPO biosensor in our work has relatively wider linear range, and is easy to manipulate, timesaving and not requiring sample pre-treatment.

4. Conclusions

The catechol biosensor is constructed easily and successfully by direct electropolymerization of aniline in a solution consisting of aniline, EMIES, PPO, HCl and SDS at 0.7 V. The results of CV, SEM, and EIS show that PPO is immobilized into PANI film. The smaller value of the Michaelis–Menten constant k'_m indicates that the affinity between immobilized PPO and catechol is stronger than that between free PPO and catechol. The PANI prepared with EMIES has higher porosity and electrochemical activity compared to that prepared without EMIES at the same conditions, which will helpful to improve load capacity of enzyme and electron-transfer efficiency. The good biocompatibility of PANI provides a good microenvironment for the protection of the immobilized PPO. Therefore, the biosensor exhibits a higher thermal stability, operational stability, long-term stability and relatively wider linear range. All of these advantages are suitable for commercial and practical applications.

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References

Bartlett, P.N., Birkin, P.R., Palmisano, F., Benedetto, G.D., 1996. J. Chem. Soc. Faraday Trans. 92, 3123–3130.
Brown, R.S., Male, K.B., Luong, J.H.T., 1994. Anal. Biochem. 222, 131–139.
Bru, R., Sanchez-Ferrer, A., Garcia-Carmona, F., 1989. Biotechnol. Bioeng. 34 (3), 304–308.
Carr, P.W., Bowers, L.D., 1980. Enzyme Kinetics in: Immobilized Enzymes in Analytical and Clinical Chemistry. John Wiley & Sons, Inc., New York, pp. 26–60.
Campuzano, S., Serra, B., Pedrero, M., Manuel de Villena, F.J., Pingarron, J.M., 2003. Anal. Chim. Acta 494, 187–197.
Cheng, Y.X., Liu, Y.J., Huang, J.J., Li, K., Xian, Y.Z., Zhang, W., Jin, L.T., 2009. Chim. Acta 54, 2588–2594.
Castillo, M., Domingues, R., Alpendurada, M.F., Barcelo, D., 1997. Anal. Chim. Acta 353 (1), 133–142.
Cui, H., He, C.X., Zhao, G.W., 1999. J. Chromatogr. A 855, 171–179.
Dempsey, E., Diamond, D., Collier, A., 2004. Biosens. Bioelectron. 20, 367–377.

Espin, J.C., Morales, M., Garcia-Ruiz, P.A., Tudela, J., Garcia-Canovas, F., 1997. J. Agric. Food Chem. 45, 1084–1090.
Fan, Q., Shan, D., Xue, H.G., He, Y.Y., Cosnier, S., 2007. Biosens. Bioelectron. 22, 816–821.
Focke, W.W., Wnek, G.E., Wei, Y., 1987. J. Phys. Chem. 91, 5813–5818.
GB8978, 1996. Integrated Wastewater Discharge Standard by the State Bureau of Technical Supervision (SBTS) of China, 1996.
Gomes, S.A.S.S., Nogueira, J.M.F., Rebelo, M.J.F., 2004. Biosens. Bioelectron. 20, 1211–1216.
Hanefeld, U., Gardossi, L., Margner, E., 2009. Chem. Soc. Rev. 38, 453–468.
Huang, J.X., Richard, B.K., 2004. J. Am. Chem. Soc. 126, 851–855.
Huang, W.S., Humphrey, B.D., MacDiarmid, A.G., 1986. J. Chem. Soc. Faraday Trans. 82, 2385–2400.
Kan, J.Q., Pan, X.H., Chen, C., 2004. Biosens. Bioelectron. 19, 1635–1640.
Kiralp, S., Toppare, L., Yagci, Y., 2003. Int. J. Biol. Macromol. 33, 37–41.
Kochana, J., Gala, A., Parczewski, A., Adamski, J., 2008. Anal. Bioanal. Chem. 391, 1275–1281.
Li, J., Chia, L.S., Goh, N.K., Tan, S.N., 1998. Anal. Chim. Acta 362, 203–211.
Li, J., Xu, Y.H., Wei, H., Huo, T., Wang, E.K., 2007. Anal. Chem. 79, 5439–5443.
Li, Y.F., Liu, Z.M., Liu, Y.L., Yang, Y.H., Shen, G.L., Yu, R.Q., 2006. Anal. Biochem. 349, 33–40.
Liu, J., Bi, S.P., Yang, L., Gu, X.D., Ma, P.J., Gan, N., Wang, X.L., Long, X.F., Zhang, F.P., 2002. Analyst 127, 1657–1665.
Liu, Z., Liu, B., Kong, J., Deng, J., 2000. Anal. Chem. 72, 4707–4712.
MacDiarmid, A.G., Epstein, A.J., 1995. Synth. Met. 69, 85–92.
Mu, S.L., 1994. J. Electroanal. Chem. 370 (1–2), 135–139.
Mu, S.L., 2007. Electrochim. Acta 52, 7827–7834.
Mu, S.L., Xue, H.G., Qian, B.D., 1991. J. Electroanal. Chem. 304, 7–16.
Musameh, M., Wang, J., Merkoci, A., Lin, Y.H., 2002. Electrochem. Commun. 4, 743–746.
Navalon, A., Blanc, R., Reyes, L., Navas, N., Vilchez, J.L., 2002. Anal. Chim. Acta 454, 83–91.
Palaniappan, S., 2001. Eur. Polym. J. 37, 975–981.
Perez, J.P.H., Lopez, M.S-P., Lopez-Cabarcos, E., Lopez-Ruiz, B., 2006. Biosens. Bioelectron. 22, 429–439.
Rosatto, S.S., Kubota, L.T., Neto, G.O., 1999. Anal. Chim. Acta 390, 65–72.
Sabatani, E., Cohen-Boulakia, J., Bruening, M., Rubinstein, I., 1993. Langmuir 9, 2914–2981.
Schoning, M.J., Jacobs, M., Muck, A., Knobbe, D.T., Wang, J., Chatrathiand, M., Spillmann, S., 2005. Sens. Actuators 108, 688–694.
Sengupta, P.P., Kar, P., Adhikari, B., 2008. React. Funct. Polym. 68, 1103–1112.
Shan, D., Zhu, M.J., Han, E., Xue, H.G., Cosnier, S., 2007. Biosens. Bioelectron. 23, 648–654.
Shinohara, H., Chiba, T., Aizawa, M., 1988. Sens. Actuators 13 (1), 79–86.
Singh, S., Solanki, P.R., Pandey, M.K., Malhotra, B.D., 2006. Sens. Actuators B 115, 534–541.
Shu, F.R., Wilson, G.S., 1976. Anal. Chem. 48, 1679–1686.
Stanca, S.E., Popescu, I.C., 2004. Bioelectrochemistry 64, 47–52.
Wang, B., Zhang, J., Dong, S., 2000. Biosens. Bioelectron. 15, 397–402.
Wang, H.Y., Mu, S.L., 1999. Sens. Actuators B 56, 22–30.
Wang, J., Liu, J., Cepra, G., 1997. Anal. Chem. 69, 3124–3127.
Wang, P., Li, S.Q., Kan, J.Q., 2009. Sens. Actuators B 137, 662–668.
Wang, S.F., Tan, Y.M., Zhao, D.M., Liu, G.D., 2008. Biosens. Bioelectron. 23, 1781–1787.
Vedrine, C., Fabiano, S., Tran-Minh, C., 2003. Talanta 59, 535–544.
Yahsi, A., Sahin, F., Demirel, G., Tumturk, H., 2005. Int. J. Biol. Macromol. 36, 253–258.
Yokoyama, K., Tamiya, E., Karube, I., 1989. J. Electroanal. Chem. 273, 107–117.
Yu, J.J., Du, W., Zhao, F.Q., Zeng, B.Z., 2009. Electrochim. Acta 54, 984–988.
Zejli, H., Cisneros, H.-H. de J.L., Naranjo-Rodriguez, I., Liu, B.H., Temsamani, K.R., Marty, J.L., 2008. Anal. Chim. Acta 612, 198–203.
Zen, J.M., Chen, P.J., 1998. Electroanalysis 10, 12–15.
Zhang, J., Lei, J.P., Liu, Y.Y., Zhao, J.W., Ju, H.X., 2009. Biosens. Bioelectron. 24, 1858–1863.
Zhang, J.Z., Li, B., Xu, G.B., Cheng, G.J., Dong, S.J., 1999. Analyst 124, 699–703.