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Amperometric biosensor based on tyrosinase immobilized onto multiwalled carbon nanotubes-cobalt phthalocyanine-silk fibroin film and its application to determine bisphenol A

Huanshun Yin[a,b], Yunlei Zhou[a], Jing Xu[a], Shiyun Ai[a,*], Lin Cui[a], Lusheng Zhu[b,*]

[a] College of Chemistry and Material Science, Shandong Agricultural University, Taian 271018, Shandong, China
[b] College of Resources and Environment, Shandong Agricultural University, Taian 271018, Shandong, China

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ABSTRACT

An amperometric bisphenol A (BPA) biosensor was fabricated by immobilizing tyrosinase on multiwalled carbon nanotubes (MWNTs)-cobalt phthalocyanine (CoPc)-silk fibroin (SF) composite modified glassy carbon electrode (GCE). In MWNTs-CoPc-SF composite film, SF provided a biocompatible microenvironment for the tyrosinase to retain its bioactivity, MWNTs possessed excellent inherent conductivity to enhance the electron transfer rate and CoPc showed good electrocatalytic activity to electrooxidation of BPA. The cyclic voltammogram of BPA at this biosensor exhibited a well defined anodic peak at 0.625 V. Compared with bare GCE, the oxidation signal of BPA significantly increased; therefore, this oxidation signal was used to determine BPA. The effect factors were optimized and the electrochemical parameters were calculated. The possible oxidation mechanism was also discussed. Under optimum conditions, the oxidation current was proportional to BPA concentration in the range from 5.0×10^{-8} to 3.0×10^{-6} M with correlation coefficient of 0.9979 and detection limit of 3.0×10^{-8} M (S/N = 3). The proposed method was successfully applied to determine BPA in plastic products and the recovery was in the range from 95.36% to 104.39%.

1. Introduction

At present, pollutants and related diseases are of great concern, and bisphenol A (BPA, 2,2′-bis(4-hydroxyphenyl)propane) is among these pollutants. It has been demonstrated that BPA exhibits estrogenic activity, which mimics the action of the hormone estrogen [1]. Its blood levels in men and women are associated with reproduction dysfunctions, endometrial hyperplasia, recurrent miscarriages, abnormal karyotypes and polycystic ovarian syndrome [2]. However, BPA is a major component in the production of polycarbonate (PC) and epoxy resin (EP) which are widely used as plastic food containers, inner surface coating of food, beverage cans, water bottles, baby bottles, and sealants in dentistry. Because of the incomplete reaction, the unreacted BPA will be present in plastic products. For example, the residual levels of BPA in PC baby bottles ranged from 1 to 599 ppm, with average levels from 9.9 to 177 ppm [3]. Nevertheless, what is more serious is that the migration of BPA from these materials into food has been reported [4–7], which could threaten the health of humans because humans may routinely ingest trace amounts of BPA. In addition, BPA can also be released into the environment during the manufacturing process or the degradation products of plastics [8]. A recent report has indicated that health risks can result from exposure to doses much lower than the limit of 0.05 mg kg^{-1} body weight day^{-1} [9]. In order to evaluate actual human health risks caused by BPA exposure, it is necessary to achieve accurate data on their levels in the environment, even at very low levels. Therefore, it is desirable to develop an easy and sensitive analytical method for the determination of trace amounts of BPA.

Until now, the widely used methods for the determination of BPA are liquid chromatography (LC) [10] and gas chromatography (GC) [11]. These techniques have the high sensitivity and low detection limit. However, they also require time-consuming pre-treatment steps, and thus do not allow for rapid processing of multiple samples. Moreover, these instrumentations are rather complicated, expensive, and are hardly employed for on-site measurement. Hence, there is a demand for new analytical technique to determine the low concentration of BPA.

In recent years, electrochemical biosensors based on tyrosinase have attracted many interests for the advantages of good reliability, fast response, inexpensive instrument, low energy consumption, simple operation, time saving and high sensitivity. Thus, they are widely investigated on determining some phenolic environmental pollutants, such as phenol [12,13], catechol [14,15], 17β-estradiol

* Corresponding author. Tel.: +86 538 8247660; fax: +86 538 8242251.
E-mail addresses: ashy@sdau.edu.cn (S. Ai), lushzhu@sdau.edu.cn (L. Zhu).

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[16], etc., and some satisfactory results have been obtained. In the presence of dissolved oxygen, tyrosinase could catalyze the oxidation of phenolic compounds to o-diphenols. The products of the first catalytic process could be further catalytically oxidized to the corresponding o-quinones, which will be reduced at the electrode surface, reforming the original o-diphenols, thus forming a bioelectrocatalytic amplification cycle [17]. The reduction signal of o-quinone is normally used to determine phenolic compound for the purpose of preventing interference [12,14,16,18–20]. However, the content of dissolved oxygen will influence the catalytic oxidation of phenolic compounds to o-quinones by tyrosinase, and this will further influence the reduction of o-quinones. In order to decrease or eliminate this influence factor, the base solution is frequently saturated with air or pure oxygen, but it will increase the operation complexity. Therefore, based on the oxidation signal of phenolic compounds at tyrosinase-based biosensor for determination of phenolic compounds would be a promising alternative method.

However, to the best of our knowledge, determination of phenolic compounds using the oxidation signal at tyrosinase-based biosensor has not yet been reported. In order to achieve it, in this work, tyrosinase was immobilized onto the composite film of silk fibroin (SF), multiwalled carbon nanotubes (MWNTs) and cobalt phthalocyanine (CoPc) at glassy carbon electrode. MWNTs and CoPc have been widely used to fabricate sensors and biosensors. Recently, immobilization of unsubstituted metal phthalocyanine (MPc) at the surface of carbon nanotubes (CNTs) has been achieved by means of noncovalent adsorption [21]. The resulting MPc-CNTs complexes possess the catalytic properties of MPc without any destruction of electronic properties and structures of CNTs [22,23]. SF is a natural protein with carboxyl and amino groups. It is a kind of excellent matrix for enzyme immobilization with attractive properties, including excellent biocompatibility, thermal stability, nontoxicity, hygroscopicity, low cost [24]. With the synergistic effect of SF-MWNTs-CoPc incorporated with tyrosinase modification materials, the oxidation response of BPA enhanced greatly at Tyr-SF-MWNTs-CoPc/GCE in contrast to those at Tyr/GCE, Tyr-SF/GCE, CoPc/GCE, MWNTs/GCE, MWNTs-CoPc/GCE and Tyr-MWNTs-CoPc/GCE. Accordingly, a novel biosensor based on the oxidation signal of BPA was fabricated for the first time. In order to assess the selectivity of the proposed biosensor, various main interferents were investigated for their effects on the assay of BPA. The effect factors were optimized and the kinetic parameters were calculated. As a preliminary application in environmental monitoring, the new biosensor was used to evaluate the levels of BPA in plastic products.

2. Experiments

2.1. Reagents

Tyrosinase (EC 1.14.18.1, 5370 unit mg^{-1} from mushroom) was purchased from Sigma (USA). CoPc was obtained from Tokyo Chemical Industry Co., Ltd. (Japan). MWNTs were from Nachen S&T Ltd. (China) and treated according to the reported procedure [25]. BPA was obtained from Beijing Chemical Technology Co., Ltd. (China). Silkworm cocoon was obtained from a local market and treated as reported to obtain SF (3%, w/w) [26,27]. Phosphate buffer solution (PBS) was prepared by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4. All of the chemicals were of analytical reagent grade and all the solutions were prepared with redistilled deionized water from quartz.

2.2. Apparatus

All electrochemical measurements were performed with CHI 660C electrochemical analyzer (Shanghai Chenhua Co., China) with a conventional three-electrode cell. The working electrode was bare GCE (CHI104, d = 3mm) or modified GCE. A Pt wire and a saturated calomel electrode (SCE) were used as the counter and the reference electrode, respectively. A PHS-3C Exact Digital pH meter (Shanghai KangYi Co., Ltd., China) was used for preparing the buffer solution, which was calibrated with standard pH buffer solutions. High-pressure liquid chromatography (HPLC) was performed on Waters model 510 system (USA) comprising a Kromasil 100-5C18 (250 mm × 4.6 mm) column (Sweden) equipped with a Waters 2487 dual λ absorbance detector (USA) using a mobile phase consisting of 20 mM PBS (pH 7)-acetonitrle (65:35, v/v) at a flow rate of 0.8 mL min^{-1}. All the reported potential values were quoted versus SCE.

2.3. Preparation of Tyr-SF-MWNTs-CoPc modified glassy carbon electrode

Before modification, the bare glassy carbon electrode was polished to a mirror-like with 0.3 and 0.05 μm alumina slurry on micro-cloth pads, rinsed thoroughly with redistilled deionized water between each polishing step, then washed successively with redistilled deionized water, anhydrous alcohol and redistilled deionized water in an ultrasonic bath and dried in air before use.

For preparation of the enzyme electrode, 2 mg mL^{-1} tyrosinase solution was first prepared with 0.1 M pH 7.0 PBS and then mixed with SF solution (1:1, v/v). MWNTs were functionalized with CoPc (represented as MWNTs-CoPc) by mixing a slurry of CoPc (2 mg) in redistilled deionized water (1 mL) with a 2 mg mL^{-1} of MWNTs, followed by ultrasonication for 30 min [23]. 10 μL of the suspension of functionalized MWNTs-CoPc was dropped on the fresh prepared GCE surface. After the solvent was evaporated, the electrode surface was thoroughly rinsed with redistilled deionized water and dried in air (noted as MWNTs-CoPc/GCE). Then, 10 μL mixed solution of SF and tyrosinase was dropped on the surface of MWNTs-CoPc/GCE. After dried under ambient condition for 2 h, the tyrosinase modified electrode was wasted with redistilled deionized water to remove the unimmobilized mixture (noted as Tyr-SF-MWNTs-CoPc/GCE). For comparison, Tyr/GCE, Tyr-SF/GCE, CoPc/GCE, MWNTs/GCE, MWNTs-CoPc/GCE and Tyr-MWNTs-CoPc/GCE were fabricated with the similar procedures. All of the modified electrodes were stored at 4 °C in a refrigerator before use.

2.4. Preparation of BPA solution

Five kinds of plastic products were purchased from a local supermarket. These samples were cut into pieces by scissors and washed twice with redistilled deionized water. Then the plastic products were treated as the following process to obtain sample solutions. 1.00 g plastic product pieces and 30 mL redistilled deionized water were added into a beaker, sealed with fresh-keeping film and stood for 5 days. After filtrated, the liquid phase was collected in a 50 mL volumetric flask and diluted to volume with redistilled deionized water. Otherwise, the spiked sample solution was prepared as the same method after adding the known-amount of BPA standard solution.

3. Results and discussion

3.1. Cyclic voltammetric behaviors of BPA

Fig. 1 showed cyclic voltammograms of BPA at GCE (a), Tyr/GCE (b), Tyr-SF/GCE (c), MWNTs-CoPc/GCE (d), Tyr-MWNTs-CoPc/GCE (e) and Tyr-SF-MWNTs-CoPc/GCE (f). Compared with other electrodes, the oxidation peak current of BPA was small at GCE, Tyr/GCE and Tyr-SF/GCE, indicating a weak oxidation of BPA. Among them,

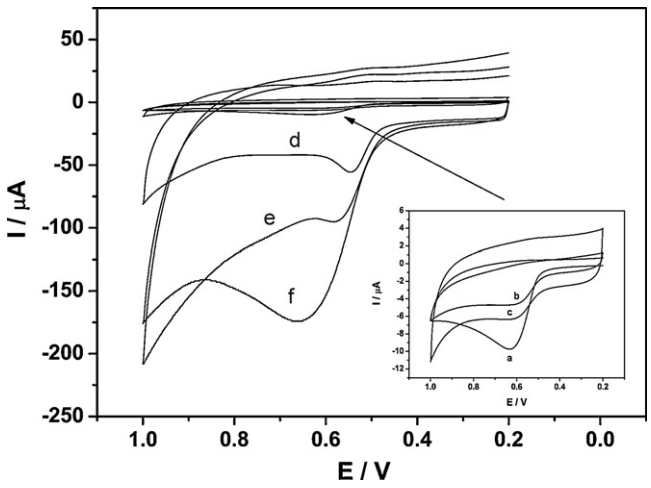


Fig. 1. Cyclic voltammograms of 2.5 mM BPA at GCE (a), Tyr/GCE (b), Tyr-SF/GCE (c), MWNTs-CoPc/GCE (d), Tyr-MWNTs-CoPc/GCE (e) and Tyr-SF-MWNTs-CoPc/GCE (f) in 0.1 M PBS (pH 7.4). Scan rate: 100 mV s^{-1}.

the oxidation peak current of BPA at Tyr/GCE was smaller than that at GCE, which could be attributed to the nonconductivity film of tyrosinase with low bioactivity on GCE surface, leading to decrease the oxidbillity to BPA. The similar phenomenon was also obtained at Tyr-SF/GCE, but the oxidation peak current of BPA at Tyr-SF/GCE was a little bigger than that at Tyr/GCE, demonstrating that SF could retain the bioactivity of tyrosinase to a certain extent. However, this oxidation current was still lower than that at GCE due to the insulation of the SF layer, because only the molecules which diffused through the nano-pores could contribute to the voltammetric response [28]. When MWNTs-CoPc composite was immobilized on Tyr/GCE and Tyr-SF/GCE, respectively, the remarkably enhanced oxidation signals were obtained, which could be attributed to the synergy effect of MWNTs-CoPc composite to effectively catalyze BPA oxidation and promote the electron transfer rate [23]. In order to testify it, the oxidation of BPA was also carried out at CoPc/GCE and MWNTs/GCE, respectively. Both the oxidation currents at the above two electrodes were lower than that at MWNTs-CoPc/GCE (data not shown), which further confirmed the synergy catalytic effect of MWNTs-CoPc composite towards BPA oxidation. Comparing the oxidation peak current obtained at Tyr-MWNTs-CoPc/GCE and Tyr-SF-MWNTs-CoPc/GCE, one can see that SF played an important role in retaining the bioactivity of tyrosinase and enhancing the enzyme catalytic sites accessible to BPA, which is in accordance with the results obtained from the comparison of Tyr/GCE and Tyr-SF/GCE. Moreover, SF can also increase the immobilization stability of MWNTs-CoPc composite on GCE surface due to its excellent film forming ability. Furthermore, when comparing the oxidation response of BPA at MWNTs-CoPc/GCE, Tyr-MWNTs-CoPc/GCE and Tyr-SF-MWNTs-CoPc/GCE, we are surprised to find that the oxidation current of BPA at Tyr-SF-MWNTs-CoPc/GCE was significantly higher than that at MWNTs-CoPc/GCE and Tyr-MWNTs-CoPc/GCE. The reason might be attributed to the synergy effect of tyrosinase, SF and MWNTs-CoPc composite, in which Tyr possesses good catalytic activity and bioactivity to improve BPA oxidation, SF possesses excellent biocompatibility and film forming ability to maintain the biocatalytic activity of tyrosinase [29], MWNTs-CoPc composite possesses excellent catalytic activity to faciliate the electrochemical oxidation of BPA and increase the electrontransfer rate. Integrating the above descriptions, one can conclude that the electrochemical oxidation response of BPA cab be significantly increased in the coexistence of Tyr-SF-MWNTs-CoPc, which could be applied to determine trace amount of BPA. However, the oxidation potential of Tyr-SF-MWNTs-CoPc/GCE (0.625 V) was a little bigger than that of Tyr-MWNTs-CoPc/GCE (0.582 V), which might be attributed to the resistance of the insulating SF layer, a natural polymeric fibrous protein. It blocked any direct mass transport and electron transfer of BPA to the GCE [28,30]. The similar phenomenon was also obtained with the studies of electrochemical oxidation of morin and interaction with DNA [31] and cyclic voltammetric studies of serotonin at sodium dodecyl sulfate modified carbon paste electrode [32]. Although many researchers have successfully applied biosensors based on tyrosinase to determine phenolic compounds by the reduction current of quinone [12,14,16,18–20], in this work, the oxidation signal was used. Considering the determination sensitivity and electrode stability, Tyr-SF-MWNTs-CoPc/GCE was chosen as the working electrode in the following research.

Many researchers have demonstrated that the electrode response of BPA was a typical of totally irreversible electrode reaction [33–37] due to the contamination of electrode surface by the oxidation product of BPA, an extraordinarily unconspicuous reduction peak (at about 0.48 V) was observed in the reverse scan at MWNTs-CoPc/GCE, Tyr-MWNTs-CoPc/GCE and Tyr-SF-MWNTs-CoPc/GCE (Fig. 1), which might be attributed to the reduction of the oxidation product of BPA. However, this unconspicuous reduction peak was not obtained at GCE, Tyr/GCE and Tyr-SF/GCE (insert of Fig. 1), which suggested that MWNTs-CoPc composite could resist the oxidation product of BPA to contaminate electrode surface, but the resistance capability was extremely finite according to the very weak reduction peak. Because the reduction peak is extraordinarily unconspicuous, we think that the oxidation of BPA is a primary electrode process in this work, and this electrode reaction could be considered to be an irreversible electrode process.

3.2. Optimization of experimental parameters

The effect of enzyme loading on the biosensor from 0.5 to 3.5 mg mL^{-1} was investigated. As shown in Fig. 2a, the current response increased with increasing amount of tyrosinase immobilized on the electrode and reached the maximum at 2 mg mL^{-1}, then decreased significantly when the amount of tyrosinase increased further, which could be attributed to the increase of film thickness, leading to an increase of interface electron transfer resistance, making the electron transfer more difficult, and concentration-dependent denaturation at the interface [38]. Therefore, 2 mg mL^{-1} tyrosinase immobilized on the electrode was chosen for all subsequent experiments.

The effect of pH value on the current response of the biosensor to 2.5 mM BPA in 0.1 M PBS was investigated with the pH range from 5.8 to 8.6 and the results were shown in Fig. 2b. It can be seen that the oxidation peak current gradually increased with an increasing pH value from 5.8 to 7.4. However, the oxidation peak current conversely decreased when the pH further increased to 8.6. At low pH range, the increase in the current response with an increasing pH could be attributed to the activity increase of tyrosinase. The optimum biosensor response was obtained at pH 7.4, which was in accordance with the optimum pH range of 5–8 reported for free tyrosinase [39]. This indicated that the immobilization procedure has not altered the activity of tyrosinase. Therefore, a pH of 7.4 was chosen in this work.

The relationship between the oxidation peak potential (E_{pa}) and pH was shown in Fig. 2c. A linear shift of E_{pa} towards negative potential with an increasing pH indicated that protons were directly involved in the oxidation of BPA. The slope of the plot of E_{pa} versus pH is −54 mV pH^{-1}, which is approximately close to the theoretical value of −57.6 mV pH^{-1} at 291.15 K, indicating that the electron transfer was accompanied by an equal number of proton in electrode reaction [40].

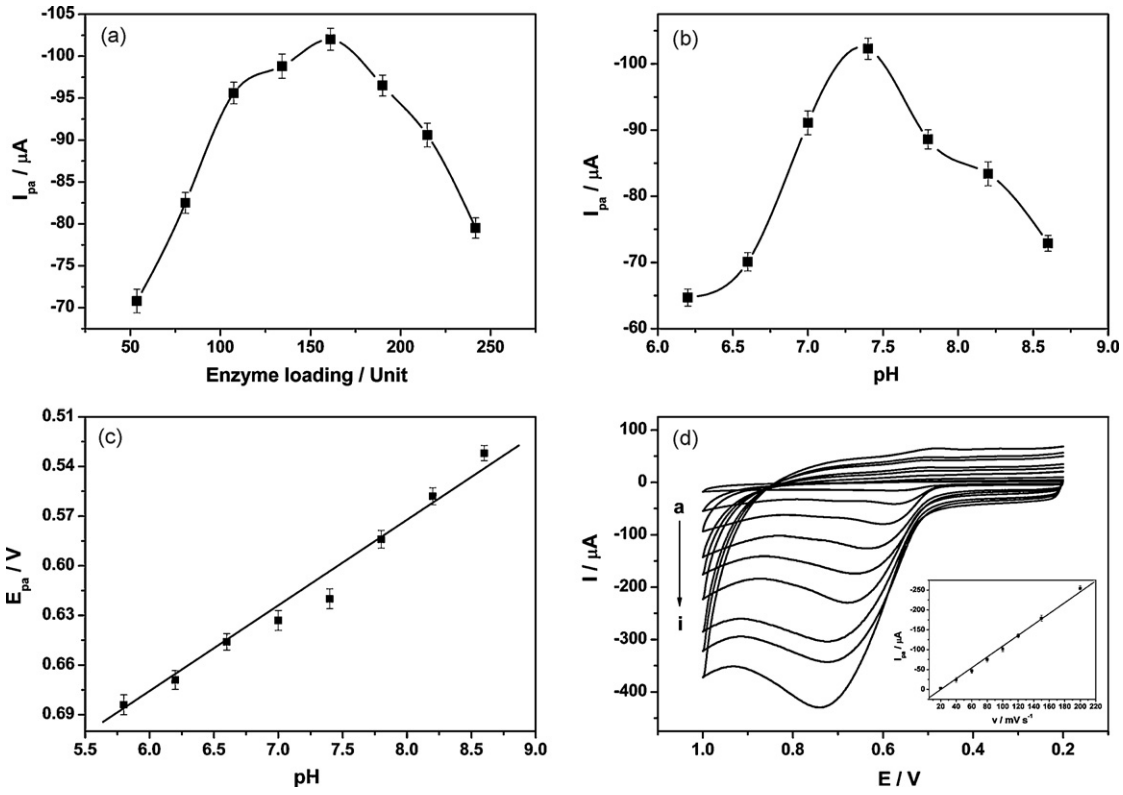
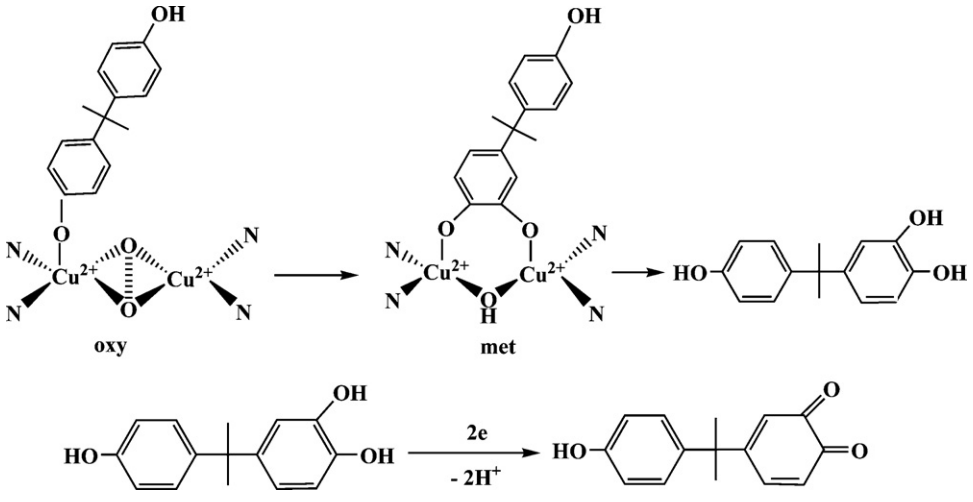


Fig. 2. Effect of enzyme loading (a), pH (b and c) and scan rate (d) on the oxidation response of 2.5 mM BPA in 0.1 M pH 7.4 PBS. (b) Effect of pH on oxidation current. (c) Effect of pH on oxidation potential. (d) Curve *a*–*i* is obtained at 20, 40, 60, 80, 100, 120, 150, 200 and 300 mV s^{-1}, respectively. The inset graph of Fig. d is the plot for the dependence of peak current on the scan rate.

As shown in Fig. 2d, the effect of scan rate (v) on the oxidation of BPA was investigated by cyclic voltammetry. A linear relationship of peak current versus scan rate was obtained in the range from 20 to 300 mV s^{-1} (inset of Fig. 2d), which indicated that the electrode reaction was a typical absorption-controlled process. In addition, with the increase of scan rate, E_p shifted positively, and the linear relationship could be expressed as $E_{pa} = 0.03 \ln v + 0.54$ ($R = 0.9854$, E_{pa} in V, v in mV s^{-1}). For an adsorption-controlled and irreversible electrode oxidation process, according to Laviron equation [41], αn (α is charge transfer coefficient, n is electron transfer number) was calculated to be 0.84 from the slope of E_{pa} versus $\ln v$. Generally, α is assumed to be 0.5 in the totally irreversible electrode process. So, n is 2. Because the number of electrons and protons involved in the oxidation process was equal in this work, the electrooxidation of BPA at the biosensor is a two-electron and two-proton process.

It is well known that tyrosinase is a copper protein, which shows two catalytic functions, that is, ortho-hydroxylation of monophenols to o-diphenols in the presence of molecular oxygen (cresolase activity) and a two-electron oxidation of o-diphenols into o-quinones (catecholase activity) [42]. BPA is monophenol compound, which could bind to the axial position of one of the coppers of the oxy tyrosinase site during the cresolase cycle to generate o-diphenolate. When tyrosinase was immobilized on the SF-MWNTs-CoPc/GCE surface, the oxidation peak current of



Scheme 1. The possible mechanism for BPA oxidation at Tyr-SF-MWNTs-CoPc/GCE biosensor.

Table 1
Performance comparison of the proposal biosensor for BPA detection with other sensors.

Sensor	Linear range (μM)	Detection limit (μM)	References
Tyr-SF-MWNTs-CoPc/GCE	0.05–3	0.03	This work
Tyrosinase/poly(thionine)/GCE	–	23	[45]
Tyrosinase/boron-doped diamond electrode	–	1	[16]
Tyrosinase-MWCN paste electrode	1–16	1	[46]
Tyrosinase-CPE	1–20	0.15	[18]
Tyrosinase-CPE	0.1–15	0.1	[46]
MCM-41/CPE	0.088–0.22	0.038	[47]
Tyrosinase-SWCN paste electrode	0.1–12	0.02	[46]
CoPc-CPE	0.0875–12.5	0.01	[37]
dsDNA/Au	0.01–1	0.01	[48]
CPE	0.025–1	0.0075	[35]

CPE: carbon paste electrode; SWCN: single wall carbon nanotubes; MWNT: multiwall carbon nanotubes.

BPA was much higher than that at SF-MWNTs-CoPc/GCE, which might be attributed the electrooxidation of o-diphenolate to o-quinone after the enzyme catalytic oxidation of BPA to generate o-diphenolate. In the light of our results and precious reports of electrochemical reaction mechanisms of phenolic compounds on tyrosinase-based biosensor [18], the mechanism for electrocatalytic oxidation of BPA on the fabricated biosensor could be expressed as follows (Scheme 1).

3.3. Electrochemical effective surface area

The electrochemical effective surface area for bare GCE and Tyr-SF-MWNTs-CoPc/GCE can be calculated according to the slope of the plot of Q versus $t^{1/2}$ obtained by chronocoulometry using 0.1 mM $K_3[Fe(CN)_6]$ as model complex (the diffusion coefficient D of 0.1 mM $K_3[Fe(CN)_6]$ is 7.6×10^{-6} cm^2 s^{-1} [43]) based on Anson equation [44]. In this work, the slopes of the curves of $Q - t^{1/2}$ were 6.857×10^{-6} (GCE) and 1.862×10^{-4} (Tyr-SF-MWNTs-CoPc/GCE), respectively. Therefore, A was calculated to be 0.233 cm^2 and 6.32 cm^2 for GCE and Tyr-SF-MWNTs-CoPc/GCE, respectively. The results indicated that the electrode effective surface area was increased obviously after modification of GCE with Tyr-SF-MWNTs-CoPc, which would increase the oxidation site, enhance the oxidation current of BPA, and thus the determination sensitivity would be increased and the detection limit would be lowed.

3.4. Amperometric response

Since amperometry under stirred conditions has a much higher current sensitivity than voltammetry, it was used to estimate the detection limit of BPA. Fig. 3a showed a typical amperometric current–time response of the biosensor under the optimum experimental conditions after successive addition of BPA to PBS under stirring every 50 s. With the increase of BPA concentration, the biosensor could achieve the steady state current within less than 6 s, which indicated a fast electron transfer process. The apparent Michaelis–Menten constant (K_M^{app}) for immobilized tyrosinase was calculated to be 0.012 mM from the data obtained from the amperometric response, which was lower than that of 0.222 mM at tyrosinase/poly(thionine)/GCE [45], indicating that tyrosinase immobilized on SF-MWNTs-CoPc composite matrix strongly adsorbed on bulk GCE surface and increased affinity to BPA. As shown in Fig. 3b, the linear range spanned the concentration of BPA from 5.0×10^{-8} to 3.0×10^{-6} M and the regression equation can be expressed as $I = -0.71c - 1.1$ ($R = 0.9979$). The detection limit was estimated to be 3.0×10^{-8} M (S/N = 3). The BPA detection performances of the proposed biosensor were compared with other sensors. The results were shown in Table 1. It can be seen that the Tyr-SF-MWNTs-CoPc/GCE offered reasonable linear range for BPA detection and the detection limit was lower than some of previous reports. These indicate that Tyr-SF-MWNTs-CoPc/GCE is an excellent platform for the detection of BPA.

To further demonstrate the practicality of the proposed method, the biosensor was used to determine BPA in five kinds of plastic samples (PVC mineral water bottle, EP beverage can, PVC synthetic leather, PC compacting disk, PC water container). Under the optimized conditions, a certain volume of sample solution was added into pH 7.4 PBS, and then analyzed according to the above-described procedure. The amperometric response of BPA in the PVC

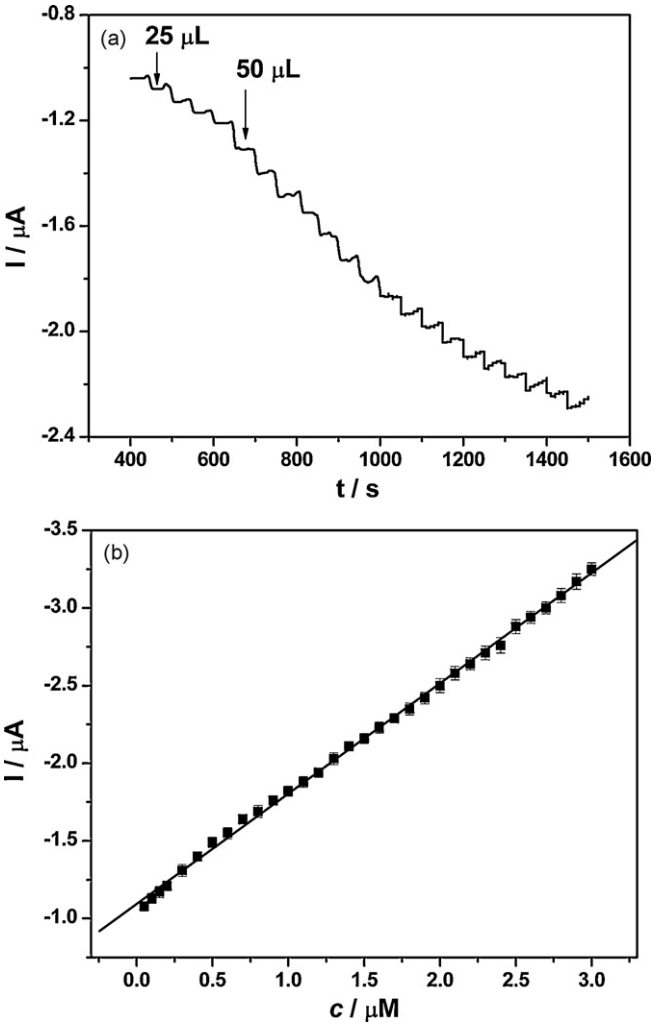


Fig. 3. (a) Amperometric response of the biosensor for successive additions of 0.05 μM and 0.1 μM BPA into stirring PBS (pH 7.4). Applied potential: 0.6 V versus SCE. (b) Calibration curve.

Table 2
Determination of BPA in plastic samples.

Sample[a]	Determined by modified GCE						Determined by HPLC (μM)[b]
	Measured (μM)[b]	Added (μM)	Predicted (μM)	Found (μM)[b]	RSD (%)	Recovery (%)	
PVC mineral water bottle	0.605	0.20	0.805	0.824	4.54	102.36	0.818
EP beverage can	0.898	0.20	1.098	1.047	4.91	95.36	1.051
PVC synthetic leather	1.024	1.00	2.024	1.954	2.37	96.54	2.011
PC compacting disk	0.235	0.20	0.435	0.454	4.13	104.36	0.416
PC water container	0.105	0.10	0.205	0.214	3.48	104.39	0.198

[a] All the plastic products were made in China.
[b] Each sample was determined under the same conditions for three times.

mineral water bottle was illustrated in Fig. 4. To study the accuracy of the proposed method, recovery experiments were carried out by standard addition method. The percentage of the recovery values was calculated by comparing the concentration obtained from the samples with the totally actual and added concentrations. The results obtained are listed in Table 2. The recoveries were in the range 95.36% to 104.39%, indicating that this method had good accuracy. For comparison, the BPA concentrations were also detected with HPLC and the results were also shown in Table 2. The analytical results obtained by both methods showed a good agreement. Thus, the biosensor may be satisfactorily applied to the determination of BPA levels in practical samples.

3.5. Reproducibility, stability and interference

The fabrication reproducibility of the biosensor was investigated by determining the response of 1.5 μM BPA at eight different electrodes prepared independently. The RSD was found to be 4.8%, indicating acceptable fabrication reproducibility. The stability of the biosensor was estimated by measuring the response of 1.5 μM BPA every 10 days. Between measurements, the electrodes were stored at 4 °C in PBS (pH 7.4). The current response of the electrode decreased to 92% after 10 days, while 85% of the original response retained after 20 days. The electrode still retained 75% of its original response even after 30 days. The acceptable stability of the modified electrode could attribute to the stability of SF-MWNTs-CoPc composite matrix material.

To evaluate the selectivity of the biosensor, the influence of some possible interfering substances was examined in PBS (pH 7.4) containing 1 μM BPA. The results suggested that 100-fold concentration of phenol, hydroquinone, pyrocatechol, hydroxyphenol, 4-nitrophenol, 2,4-dinitrophenol, 2,6-ditertbutylphenol and dioctyl phthalate had no influence on the signals of BPA with deviations below 5%. Otherwise, some ions, such as 100-fold concentration of K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Cu^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-} and NO_3^- had no influence on BPA determination.

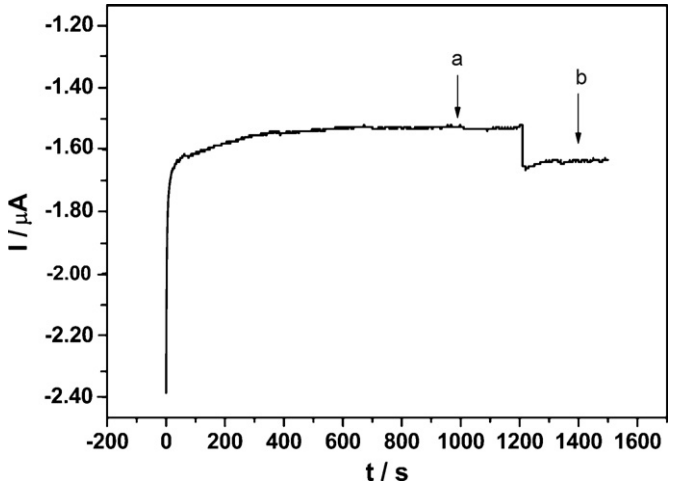


Fig. 4. Illustration for the determination of BPA by the biosensor in sample of PVC mineral water bottle before (a) and after (b) addition of 0.2 μM BPA standard solution.

4. Conclusion

An amperometric BPA biosensor was fabricated based on tyrosinase immobilized onto SF-MWNTs-CoPc composite matrix and a novel sensitive and reliable electrochemical method was developed to determine trace amounts of BPA in plastic products using the oxidation signal. With the synergistic effect of the Tyr-SF-MWNTs-CoPc, the oxidation peak current increased remarkably compared with Tyr/GCE, Tyr-SF/GCE, CoPc/GCE, MWNTs/GCE, MWNTs-CoPc/GCE and Tyr-MWNTs-CoPc/GCE. Moreover, this is the first time to report the determination of BPA at the tyrosinase-based biosensor utilizing the oxidation signal of BPA. In addition, the fabricated biosensor exhibited high sensitivity, good reproducibility and acceptable stability.

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