CuO-Doped Mesoporous Silica Hybrid for Rapid and Sensitive Amperometric Detection of Phenolic Compounds

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Abstract
This work constructed an amperometric biosensing platform using CuO doped mesoporous silica hybrid (CuO/SBA-15) as a carrier. The CuO/SBA-15 showed a pair of redox peaks of $Cu^{2+/0}$. Upon immobilization of tyrosinase on the hybrid, the resulting biosensor exhibited a rapid (<0.5 s) and sensitive amperometric response to phenolic compounds under the optimized conditions. The linear response to catechol ranged from 1.2×10^{-9} to 3.0×10^{-5} M. The activation energy for enzymatic reaction was calculated to be 26.6 kJ mol^{-1}. The apparent Michaelis–Menten constants of the enzyme electrode were estimated to be 54.6, 145, 17.0, 74.8 and 633 μM for catechol, phenol, *p*-cresol, *m*-cresol and dopamine hydrochloride, respectively. The metal oxide doped mesoporous silica hybrid exhibited excellent performance for construction of new biosensors.

Keywords: Amperometry, Biosensors, CuO doped mesoporous silica, Hybrid nanoparticles, Phenolic compounds

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1. Introduction

Phenolic compounds have been widely used in wood preservatives, textiles, herbicides and pesticides. However, many of these compounds and their derivatives are extremely harmful to human health due to the strong carcinogenic effect. Therefore, it is urgent to develop some sensitive methods for rapid monitoring of those pollutants. Electrochemical techniques are most suitable for in situ monitoring of phenolic compounds due to their high sensitivity, rapid response, and convenient operation. Particularly, amperometric biosensor based on tyrosinase (Tyr) has been considered as a promising tool for the detection of phenolic compounds [1–3], in which the electrochemical signal comes from the reduction of *o*-quinones produced in the enzymatic oxidation of phenolic compounds by oxygen in presence of Tyr [4,5]. Thus it is crucial to explore new materials for effective immobilization of the enzyme and enhancement of the electrocatalytic reduction of *o*-quinones.

Various materials, such as polymers, clays and nanomaterials, have been used for immobilization of Tyr on electrode surface [6–11]. However, most of the resulting biosensors suffer from bad biocompatibility and low sensitivity for detection of phenolic compounds. Therefore, exploiting novel biocompatible support with high loading capacity of Tyr is of considerable interest for the construction of biosensors for phenols. As excellent enzyme immobilization matrices, mesoporous silica (MPS) sieves have attracted growing interest [12–14] due to their mesoporous structure and excellent biocompatibility. For example, the direct electron transfer between hemoglobin and an electrode has been realized by immobilizing the protein on well-ordered hexagonal mesoporous silica structures (SBA-15) [15], and a bienzyme-channeling sensor has also been constructed for glucose detection with a wide concentration range by entrapping both glucose oxidase and horseradish peroxidase in mesopores of SBA-15 [16]. However, MPS itself has no catalytic activity, and much effort has been devoted to modify MPS with active components for extensive application [17].

Copper oxide doped mesoporous silica material has been prepared as a novel catalyst with excellent catalytic activity in many heterogeneous oxidation reactions such as the oxidation of phenol and benzene [18,19]. This work constructed an amperometric biosensor for phenolic compounds using CuO doped mesoporous silica SBA-15 with the aid of colloidal Au. The mesopores could enhance the loading of tyrosinase (Tyr) molecules on the electrode, and simultaneously avoid aggregation of proteins. Based on the high catalytic activity of CuO, the *o*-quinone could be rapidly generated in the Tyr entrapped mesopores, leading to a fast and sensitive amperometric response. The proposed biosensor for phenolic compounds showed a good performance with a wide concentration range, a low detection limit, a short analytical time and acceptable stability. The metal oxide doped mesoporous silica hybrid provides a promising platform for

enzyme immobilization and enhancing the sensitivity and response rate of amperometric biosensors in analytical applications.

2. Experimental

2.1. Chemicals

Tyrosinase (EC 1.14.18.1, 5370 U mg^{-1} from mushroom), pluronic P123 triblock copolymer surfactant ($EO_{20}PO_{70}EO_{20}$, Mav: 5800), poly(diallyldimethylammonium chloride) (PDDA, 20%, *w*/*w* in water, MW: 200000–350000) , dopamine hydrochloride (DA) were obtained from Sigma (USA). Dopamine hydrochloride injection (10 mg/mL, 52.8 mM) was obtained from Harvest Pharmaceutical CO., LTD (China). Other reagents were of analytical reagent grade. All aqueous solutions were prepared with twice-distilled water. 0.1 M phosphate buffer saline (PBS) was prepared by mixing K_2HPO_4 and KH_2PO_4 solutions. Phenolic solutions were prepared in 0.1 M pH 6.0 PBS daily. Following the procedure mentioned in the literature [20], the colloidal gold nanoparticles of 13 nm diameter were prepared and stored at 4 °C.

SBA-15 was synthesized according to the previous protocol [21]. In brief, 4.06 g of pluronic P123 was dissolved in 150 mL of 1.6 M HCl. Then, 8.5 g of tetraethyl *ortho*-silicate was added. The resulting mixture was stirred for 5 min and kept at 308 K for 20 h. The solid product was filtered, washed with water and ethanol, and dried in an oven for 4 h at 413 K. To completely remove the surfactant, the as-synthesized product was stirred in ethanol-HCl mixture (1:1, V/V) for 30 min, filtered, washed with water, dried in an oven at 413 K, and subsequently calcined at 823 K in air for 4 h to form SBA-15.

The CuO/SBA-15 hybrid was prepared with a wet impregnation method [22]. Exactly, 4.0 g SBA-15 was dispersed in 50 mL of 0.14 M $Cu(NO_3)_2$ solution at 333 K. Under stirring, 50 mL of 0.16 M Na_2CO_3 solution was added dropwise to the suspension. The mixture was filtered after aging at 333 K for 2 h. The solid product was washed, dried at 393 K overnight, and calcined at 723 K for 4 h to obtain CuO/SBA-15 hybrid powder, which was stored in a silica gel desiccator.

2.2. Apparatus and Measurements

Electrochemical measurements were performed on a CHI 812B electrochemical workstation (CH Instruments Inc., USA) with a conventional three-electrode system. Glassy carbon electrode (GCE, diameter 3.0 mm), saturated calomel electrode and a platinum wire were used as working, reference and auxiliary electrodes, respectively. Scanning electron microscopic (SEM) images of CuO/SBA-15 film on indium tin oxide slides and energy dispersive spectrum (EDS) of CuO/SBA-15 on carbon adhesive tape were obtained on a Hitachi S-4800 scanning electron microscope (Japan). Transmission electron microscopic (TEM) images were obtained using a JEM-2100 TEM (Japan). All the amperometric responses were obtained at constant potential, and the error bars were obtained with three measurements.

2.3. Preparation of Tyr/CuO/SBA-15/Au Modified GCE

GCE was polished to a mirror finish with 0.1 and 0.05 μm alumina slurry followed by rinsing thoroughly with doubly distilled water and successive sonication in 1:1 nitric acid, acetone and doubly distilled water, then allowed to dry at room temperature. 5.0 μL mixture of CuO/SBA-15 (10 μg in 0.1 M PBS), gold nanoparticles (1.0 μL of the as-prepared colloid) and Tyr (15 μg in 0.1 M PBS) was dropped on the GCE and dried at room temperature to obtain the CuO/SBA-15/Au/Tyr modified GCE. To maintain its stability, a drop of 4.0 μL 0.05% PDDA solution was cast on the membrane and dried at room temperature. For comparison, SBA-15/Tyr and CuO/SBA-15/Tyr modified GCEs were prepared with the similar procedure. All enzyme electrodes were stored in 0.1 M pH 6.0 PBS at 4 °C when not in use.

3. Results and Discussion

3.1. Characterization of CuO/SBA-15 Hybrid

The SEM image of CuO/SBA-15 film displayed a one-dimensional structure with the outer diameter from 260 to 320 nm (Figure 1A). After amplification, the well-dispersed mesopores can be observed on the surface (Figure 1B), which provided a significant increase of effective area for protein loading.

Further, TEM image of the CuO/SBA-15 sample showed a well-organized ordered pore structure (Figure 1C). After mixed with colloidal gold solution, the gold nanoparticles with the diameter of about 13 nm were conglutinated on the surface of the pore structure (Figure 1D), which verified the effective hybrid of CuO/SBA-15 and gold nanoparticles.

3.2. Electrochemical Response at Different Modified GCEs

The cyclic voltammogram of SBA-15 modified electrode did not show any obvious response in 0.1 M pH 6.0 PBS, while that of CuO/SBA-15 modified GCE showed a pair of redox peaks at −0.061 and −0.135 V (Figure 2A, curves a and b). Moreover, the cyclic voltammogram of SBA-15 modified GCE in pH 6.0 PBS in the presence of Cu^{2+} showed a couple of redox peaks at −0.055 and −0.135 V, which was consistent with that of CuO/SBA-15 modified GCE. Thus the redox couple should be attributed to $Cu^{2+/0}$ redox couple [23,24]. Although CuO could be reduced during cathodic scanning, only 9.6×10^{-12} mol of CuO was reduced to Cu in each cycle according to the cyclic voltammogram (Figure 2A, curve b). The content of CuO at CuO/SBA-15 modified GCE was analyzed to be 15 wt% by EDS technique (Figure 1D, Inset), corre-

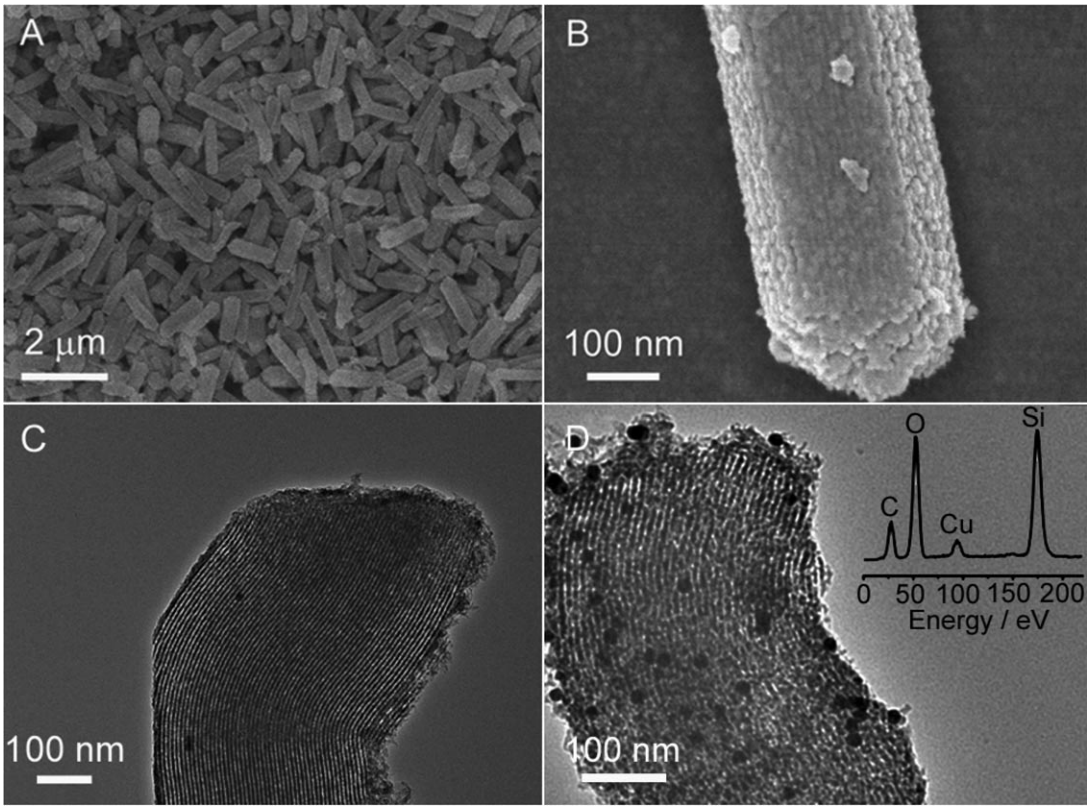

Fig. 1. SEM images of CuO/SBA-15 film (A) and single hybrid nanoparticle (B), and TEM images of CuO/SBA-15 before (C) and after (D) mixed with colloidal gold solution. Inset in (D): EDS spectrum of CuO/SBA-15 on carbon adhesive tape.

spond to 1.9×10^{-8} mol of CuO at every proposed electrode. These results indicated that CuO, as an essential component of the electrode, had almost no change during the whole detection process. Owing to the excellent conductance and large surface area, the gold nanoparticles accelerated the electron transfer between CuO and electrode and increased the peak currents (Figure 2A, curve c). After entrapment of Tyr, the resulting CuO/SBA-15/Au/Tyr modified electrode showed much lower response of $Cu^{2+/0}$ due to the decrease of the conductance (Figure 2A, curve d), but could achieve highly sensitive response to phenolic compounds (Figure 2A, curves e and f). In addition, the CuO/SBA-15/Au modified GCE did not show any response to phenolic compounds, indicating

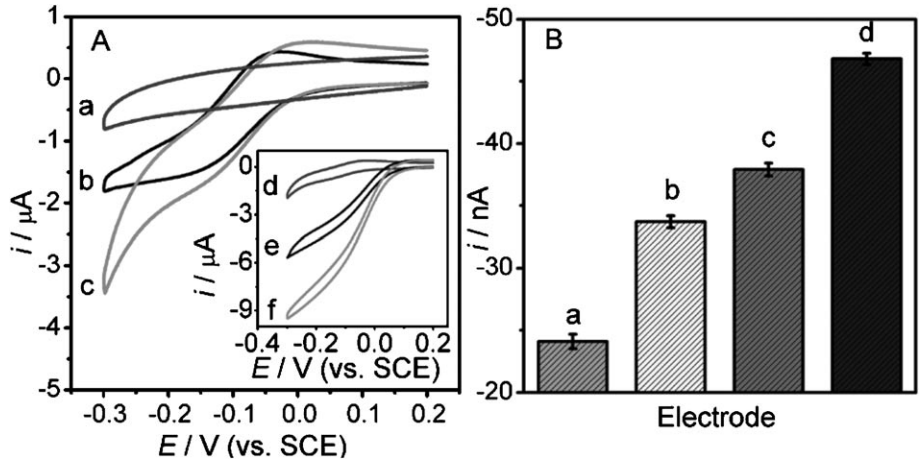

Fig. 2. (A) Cyclic voltammograms of SBA-15 (a), CuO/SBA-15 (b), CuO/SBA-15/Au (c) modified GCEs in 0.1 M pH 6.0 PBS. Inset: Cyclic voltammograms of CuO/SBA-15/Au/Tyr modified GCE 0.1 M pH 6.0 PBS in absence (d) and presence of 4 (e) and 8 μM (f) catechol. Scan rate: 100 mV s^{-1}. (B) Amperometric responses of SBA-15/Tyr (a), SBA-15/Au/Tyr (b), CuO/SBA-15/Tyr (c), CuO/SBA-15/Au/Tyr (d) modified electrodes to 50 nM catechol in 0.1 M pH 6.0 PBS at −0.2 V.

that the enhanced signal originated from the reduction of *o*-quinone produced by the enzymatic oxidation of catechol.

The amperometric response of SBA-15/Au/Tyr modified electrode to 50 nM catechol increased by 39.8% compared with SBA-15/Tyr modified electrode (Figure 2B, histograms a and b), indicating that a positive effect of Au nanoparticles on the sensor response due to the promoted electron transfer by gold nanoparticles. Moreover, attributed to the excellent catalytic activity of CuO [18,19], the CuO/SBA-15/Tyr modified electrode showed a 57.6% enhanced amperometric response (Figure 2B, histogram c). In comparison with these electrodes, the CuO/SBA-15/Au/Tyr modified electrode had the best performance for the detection of phenolic compounds (Figure 2B, histogram d).

3.3. Optimization of Variables

When optimizing one variable, other variables such as the amount of Tyr, solution pH, potential and temperature were at their optimal values. Figure 3A shows the effect of the amount of Tyr on the biosensor response to 50 nM catechol. With the increasing amount of enzyme loading, the amperometric response significantly increases and trends to an maximum value when the amount of Tyr is above 15 μg. This indicates that the catalytic current is controlled by the enzyme activity in the hybrid film. However, the excess Tyr is not beneficial for an electrode response due to the diffusion barrier. Therefore, the configuration with 15 μg of Tyr was chosen for biosensor preparation.

The effect of pH on the biosensor response was investigated over a pH range of 5.0–8.0 (Figure 3B). The maximum response was obtained at pH 6.0, which was in good accordance with other results for Tyr-based biosensors [2,3,5,8] and close to that of the free Tyr from mushroom (pH 6.2) [25]. This indicated that the immobilization procedure did not alter the optimum pH of Tyr. Therefore, 0.1 M pH 6.0 PBS was used as the electrolyte in subsequent experiments.

The dependence of the proposed biosensor on the applied potential was displayed in Figure 3C. The amperometric response increased rapidly with the decreasing potential from 0 to −0.2 V. Afterward, a small decrease of the response was observed, which might be attributed to an increase in the direct reduction of oxygen at the electrode surface, leading to the oxygen depletion within the coating and hence to a decrease in the enzymatic rate [8]. Thus, −0.2 V was selected as the applied potential.

The amperometric response was related to the detection temperature. As shown in Figure 3D, the response increased with the increasing temperature from 5 to 30 °C. The maximum response was achieved at 30 °C. According to the Arrhenius equation, the apparent activation energy of the enzymatic reaction could be calculated to be 26.6 kJ mol^{-1}, which was smaller than 38.8 kJ mol^{-1} at polyaniline-ionic liquid-carbon nanofiber-Tyr modified electrode [7]. For a simple experimental procedure and long lifetime of the biosensor, further measurements were performed at room temperature (25 °C).

3.4. Amperometric Sensing of Phenolic Compounds

Figure 4 shows a typical current-time plot for the amperometric biosensor upon the successive additions of different amounts of catechol to 10 mL PBS at −0.20 V. Under optimal conditions, the proposed biosensor showed a linear amperometric response over the concentration range of catechol from 1.2×10^{-9} to 3.0×10^{-5} M with a

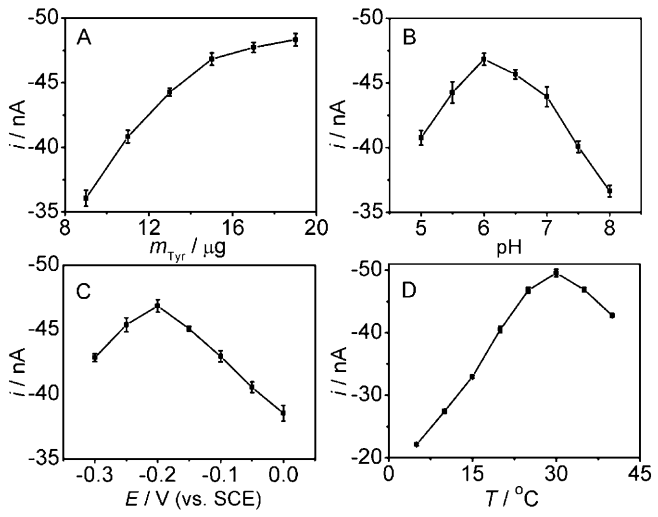

Fig. 3. Effects of loading amount of tyrosinase (A), pH (B), applied potential (C) and temperature (D) on amperometric response of CuO/SBA-15/Au/Tyr modified GCE to 50 nM catechol in 0.1 M PBS at other optimal conditions.

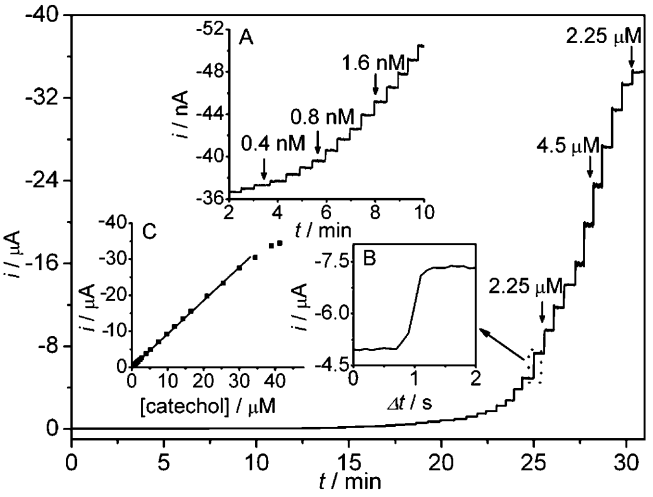

Fig. 4. Typical current–time response curve of the biosensor upon successive additions of catechol in 0.1 M pH 6.0 PBS. Inset: amplified time–current curves (A and B) and linear calibration curve (C). Applied potential: −0.2 V. The amperometric response is the difference of currents measured before and after addition of analyte.

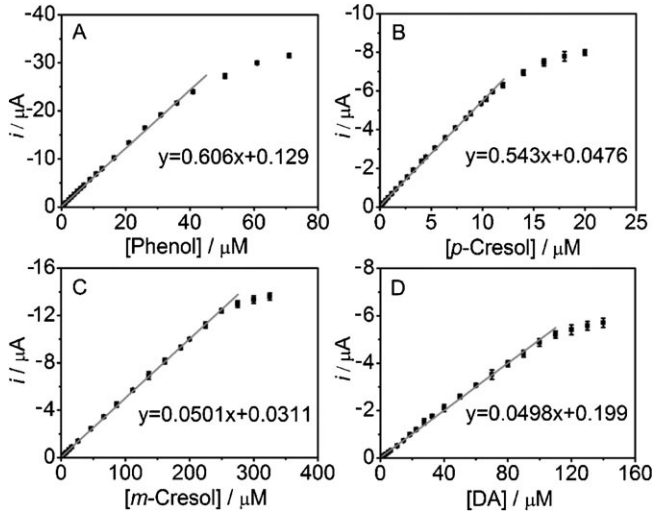

Fig. 5. Calibration curves for different phenolic compounds in 0.1 M pH 6.0 PBS. Applied potential: −0.2 V.

correlation coefficient of 0.9998 (Figure 4C). The detection limit was 4×10^{-10} M, at which the response and noise were 4.0 and 1.3 nA, respectively. The response time (defined as the time to reach 95% of the steady-state current) was less than 0.5 s, which was much shorter than that of 10 s in our previous report [7]. Such a rapid response indicated fast diffusion of the substrate across the film and fast electron exchange between Tyr and its substrate.

The modified electrode exhibited highly sensitive amperometric responses to the analogs of phenolic compounds, such as catechol, phenol, *p*-cresol, *m*-cresol and DA. The calibration curves for these compounds were showed in Figure 5, and the response characteristics were listed in Table 1. The sensitivity of the proposed biosensor to catechol was 13.1 ± 0.1 A M^{-1} cm^{-2}, which was higher than a majority of biosensors based on Tyr immobilized in other matrices reported previously (Table 2). Although a biosensor based on calcium phosphate cement showed a higher sensitivity to catechol (46.6 A M^{-1} cm^{-2}) [3], the response decreased gradually down to 40% after 9 days of storage in the buffer solution, indicating worse stability than the proposed biosensor, which was discussed in next section. The value of apparent Michaelis–Menten constant (K_M^{app}) for catechol was obviously lower than those reported for other biosensors such as chitosan/layered double hydroxides (LDH) (130 μM) [8] and Tyr/polycrystalline bismuth oxide (BiOx) (113 μM) [2], indicating that the Tyr immobilized in CuO/SBA-15/Au film had higher affinity to phenolic compounds. Although the response of catechol was much faster and stronger than those of monophenols, it showed larger K_M^{app} than that of *p*-cresol. The good response to catechol was due to the fact that catechol is a diphenol, it could be quickly oxidized by a one-step enzymatic reaction to *o*-quinone [5]. The better affinity of *p*-cresol resulted from stronger interaction between the composite matrix and enzyme substrate.

3.5. Repeatability, Reproducibility and Stability of the Biosensor

The continuous cyclic voltammogram of CuO/SBA-15/Au/Tyr modified GCE in pH 6.0 PBS showed a 4% decrease of the response of $Cu^{2+/0}$ couple at the twentieth cycle compared with that at the first cycle, indicating the good stability of the electrode. The good repeatability of the same CuO/SBA-15/Au/Tyr modified electrode was

Table 1. Response characteristics of the proposed biosensor to various phenolic compounds (average values ± *SD* were obtained with three biosensors).

Phenol compound	Linear range (M)	R^2	Sensitivity (A $M^{-1}cm^{-2}$)	Detection limit (nM)	K_M^{app} (μM)
Catechol	1.2×10^{-9} to 3.0×10^{-5}	0.9998	13.1 ± 0.1	0.4	54.6 ± 0.6
Phenol	3.0×10^{-9} to 4.1×10^{-5}	0.9985	8.6 ± 0.1	1.0	145 ± 1
p-Cresol	4.0×10^{-9} to 1.1×10^{-5}	0.9992	7.7 ± 0.1	1.0	17.0 ± 0.2
m-Cresol	4.0×10^{-8} to 2.5×10^{-4}	0.9998	0.71 ± 0.02	10	74.8 ± 0.2
DA	2.0×10^{-7} to 1.0×10^{-4}	0.9976	0.70 ± 0.01	80	633 ± 4

Table 2. Amperometric performances of different biosensors based on Tyr for catechol detection.

Materials	Sensitivity (A M−1cm−2)	Linear range (M)	Detection limit (M)	Reference
ZnO	2.4	1.5×10^{-7} to 4.0×10^{-5}	8.0×10^{-8}	[1]
BiOx	11.3	4.0×10^{-9} to 1.5×10^{-5}	1.0×10^{-9}	[2]
$CaHPO_4$	46.6	1.0×10^{-9} to 3.0×10^{-6}	1.0×10^{-9}	[3]
Au- polypyrrole	7.0	5.0×10^{-8} to 7.0×10^{-5}	3.0×10^{-8}	[10]
$CaCO_3$	6.8	6.0×10^{-9} to 2.0×10^{-5}	4.0×10^{-10}	[11]
Chitosan/Fe_3O_4	0.514	8.3×10^{-8} to 7.0×10^{-5}	2.5×10^{-8}	[6]
PANI–IL–CNF	296	4.0×10^{-10} to 2.0×10^{-6}	1.0×10^{-10}	[7]
Chitosan/LDH	2.75	3.6×10^{-9} to 4.0×10^{-5}	3.6×10^{-10}	[8]
Au–graphite–Teflon	10.6	1.0×10^{-8} to 8.0×10^{-6}	3.0×10^{-9}	[9]
CuO/SBA-15/Au	13.1	1.2×10^{-9} to 3.0×10^{-5}	4.0×10^{-10}	This work

Table 3. Determination of dopamine in different dilutions of iatric injection (I: original injection, II: 5 times dilution, III: 25 times dilution) (average values ± *SD* were obtained with three biosensors).

Sample	Concentration (μM)		Error
	M (added)	M (found)	
I	26.4	25.2 ± 0.6	4.5 %
	42.4	40.4 ± 0.5	4.7 %
II	4.24	4.04 ± 0.04	4.9 %
	10.6	10.1 ± 0.2	4.7 %
III	1.06	1.08 ± 0.02	1.9 %
	2.12	2.15 ± 0.02	1.4 %

verified at the catechol concentration of 50 nM, and the relative standard deviation (*RSD*) for six determinations was 3.0 %. In addition, the *RSD* of current signals for the same concentration of catechol at six independently prepared biosensors was 3.6 %, which proved good reproducibility of the biosensor preparation. When stored in 0.1 M pH 6.0 PBS at 4 °C, the CuO/SBA-15/Au/Tyr modified electrode was measured once every 2 days, no obvious decrease in the response to catechol was observed after one week of storage. After 20 days the response still retained 86 % value of the initial response, suggesting acceptable lifetime of the immobilized tyrosinase. This implied that the modified GCE was highly efficient for retaining the bioactivity of enzyme and preventing its leakage from the membrane.

3.6. Analysis of Dopamine Hydrochloride Injection

To study the real feasibility of the proposed biosensor, the proposed biosensor was employed to determine DA content in dopamine hydrochloride injection. Without special sample pretreatment, the injection was divided into three samples: the original solution (I), 5 times dilution (II), 25 times dilution (III). The detection results of the three samples were presented in Table 3. The errors were less than 4.9 %, indicating that the proposed biosensor could give acceptable accuracy.

4. Conclusions

In this work, a promising material, CuO doped mesoporous silica, was firstly used as enzyme immobilization matrix for the biosensor construction. Due to the special properties such as high surface activity and good biocompatibility, the CuO/SBA-15 hybrid could immobilize a large amount of Tyr and retain their bioactivity efficiently. Based on the advantages of co-entrapped CuO and gold nanoparticles, the resulting biosensor accelerated the enzymatic oxidation of phenolic compounds by oxygen for quick formation of *o*-quinone, leading to a rapid and sensitive amperometric response to catechol. The biosensor showed a wide linear range and good analytical performance with short response time, good repeatability and acceptable stability. The metal oxide doped mesoporous silica provided an insight avenue for construction of enzyme based biosensors in various applications.

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