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Detection of Dopamine Based on Tyrosinase-Fe_3O_4 Nanoparticles-chitosan Nanocomposite Biosensor

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Abstract

A tyrosinase biosensor based on Fe_3O_4-chitosan nanocomposite has been developed for the amperometric detection of dopamine by the biocatalytically liberated dopaquinone at −0.25V vs. saturated calomel electrode. The obtained bio-nanoparticles, which were attached to the surface of a glassy carbon electrode (GCE), showed excellent electrochemical characteristics and at the same time acted as mediator to transfer electrons between the enzyme and the electrode. Under optimal conditions, the biosensor showed broad linear response of 2.0×10^{-8} to 7.5×10^{-5} mol L^{-1}, with the low detection limit of 6.0×10^{-9} mol L^{-1} and the high sensitivity of 46 μA/mM for the determination of dopamine in the presence of ascorbic acid. Such tyrosinase biosensor exhibits great promise for rapid, simple and cost-effective analysis of dopamine in the samples. This immobilization approach effectively improved the stability of the electron transfer mediator and is promising for construction of biosensor and bioelectronic devices.

Keywords: Fe_3O_4 nanoparticles; Dopamine; Tyrosinase; Chitosan; Biosensor.

1. Introduction

Dopamine (DA), which is the most important neurotransmitter among the catecholamines, plays an important role in the function of central nervous, renal, hormonal and cardiovascular systems. The change of DA has been proved to be a very effective route toward brain tissues, and the loss of DA-containing neurons may result in serious diseases, such as Parkinson's disease [1]. In recent years, extensive methods have been used for the detection of DA, such as high performance liquid chromatography [2], chemical luminescence [3], and gas chromatography–mass spectrometry [4]. Although these strategies are effective for the detection of DA, there are still some drawbacks, such as time-consuming, low sensitivity and expensive equipment. Electrochemical techniques are an attractive method for the determination of DA because of their high sensitivity as well as

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their applicability to real-time detection of DA in brain tissues. Tyrosinase (Tyr) based electrochemical biosensor is a promising and effective tool for the determination of DA [5, 6].

Tyrosinase, also known as polyphenol oxidase, is a divalent copper ion with the enzyme protein binding of metal enzymes. Tyr widely exists in animals, plants, micro-organisms in vivo. The key issue in the development of Tyr biosensor is the effective immobilization of Tyr on the electrode surface. Different approaches, such as polymer entrapment [7], electropolymerization [8], sol-gels [9], self-assembled monolayer [10], and covalent linking [6] have been widely used to construct Tyr biosensors. However, some of these methods are relatively complex. The solvents are disadvantageous to the environment. Therefore, searching for a simple and reliable scheme to immobilize Tyr is of considerable interest. In recent years, magnetic nanoparticles as special biomolecular immobilizing carriers have become the focus of research. Due to their special properties, magnetic nanoparticles have been used in immunology [11] and cell separation process [12]. The successful applications of magnetic nanoparticles in the immobilization of biomolecular have been reported [13, 14]. Because of their good biocompatibility, strong superparamagnetic, low toxicity and easy preparation process, magnetic nanoparticles have been employed to immobilize enzyme in different matrices [15]. Reetz et al. [13] reported mechanically stable lipases-Fe_3O_4 nanoparticle-sol-gel biocatalysts by simultaneous entrapment of lipase and nanostructured Fe_3O_4 in hydrophobic sol-gel materials. Cao et al. [14] reported an electrochemical biosensors utilizing electron transfer in heme proteins immobilized in Fe_3O_4 nanoparticles. Chitosan (CHIT) has been extensively used for the immobilization of enzymes and the construction of biosensors. Owing to its excellent characteristics, such as biocompatibility, film forming ability, nontoxicity, physiological inertness and high mechanical strength, it has a very important purpose [17-18].

In this work, we prepared a novel Tyr biosensor based on Fe_3O_4 magnetic nanoparticles-CHIT nanocomposite modified glassy carbon electrode (GCE), and it was further applied to detect DA. The proposed biosensor exhibits fast and sensitive amperometric responses to DA in the presence of ascorbic acid (AA). In addition, the analytical performances of the biosensor with respect to repeatability and stability were also evaluated. The biosensor shows great promise for rapid, simple, and cost-effective analysis of DA in the samples. The proposed strategy can be extended for the development of other enzyme-based biosensors.

2. Experiments

2.1 Reagents

Tyr (EC 1.14.18.1, 5000U $mg^{-1}$from mushroom) and CHIT were bought from Sigma-Aldrich (St.Louis, Missouri). Fe_3O_4 magnetic nanoparticles with size of about 30nm were purchased from Anhui Maanshan Powder Engineering Co. (Anhui, China). The injection of DA Hydrochloride was obtained from Yabang Johnson Pharmaceutical Co. (Jiangsu, China). All other chemicals were of analytical grade and were used as received without further purification. Water used in all the experiments was purified through an ion exchange system with a resistance about 18.0 MΩ cm^{-1} (Nanopure, Barnstead, USA). The supporting electrolyte was 0.1mol L^{-1} phosphate buffer solution (PBS) prepared with Na_2HPO_4 and KH_2PO_4 and the pH was adjusted with NaOH or H_3PO_4.

2.2 Apparatus

All electrochemical measurements were performed with a model CHI660A electrochemical workstation (CH Instruments, Chenhua Co., Shanghai, China) controlled by a personal computer. A conventional three-electrode system equipped with a bare GCE or the prepared Tyr-Fe_3O_4-CHIT-GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode, was used for all electrochemical measurements. The pH of solution was determined by using a pHS25 acidity meter (LIDA Instrument, Shanghai, China). Branson 2000 ultrasonic cleaner (Branson Ultrasonics Co.,USA) was used for cleaning the electrodes.

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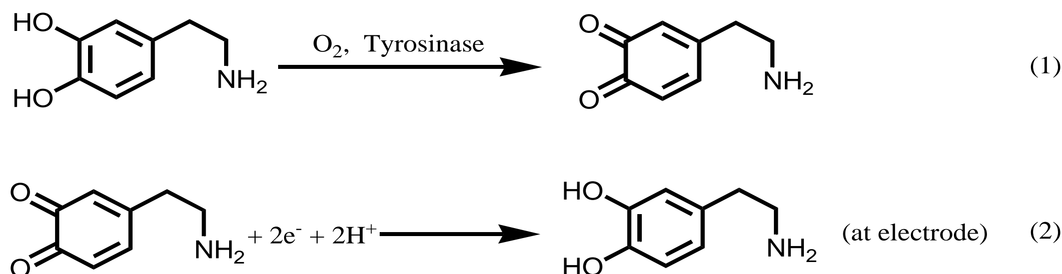
2.3 Fabrication of Tyr-Fe_3O_4 nanoparticle-CHIT biosensor

The preparation of Tyr-Fe_3O_4-CHIT nanocomposite biosensor and electrochemical measurements were the same with our previous work [19]. Tyr solution (6.4 mg mL^{-1}) was prepared by dissolving Tyr in 0.1 mol L^{-1} PBS (pH 6.5). CHIT solution (5 mg mL^{-1}) was prepared with acetic acid. 1.7 mg Fe_3O_4 was dispersed in 1.0 mL doubly distilled water and agitated in an ultrasonic bath for about 2h. The Fe_3O_4 magnetic nanoparticles, CHIT, and Tyr were mixed thoroughly with a volume ratio of 1: 1: 2, then 10 μL of the mixture were dropped on the surface of GCE. Finally, the electrode was allowed to dry at room temperature. Atomic force microscopy (AFM) and alternating current impedance (AC impedance) were used to characterize the Tyr-Fe_3O_4 nanoparticles-CHIT nanocomposite, as described in our previous work [19].

3. Results and discussion

3.1 Electrochemical sensing characteristics of Tyr-Fe_3O_4-CHIT nanobiocomposite biosensor

In the current study, DA was used to investigate the electrochemical characteristics of the Tyr-Fe_3O_4-CHIT nanobiocomposite biosensor. Fig. 1 shows the typical cyclic voltammograms of DA at the Fe_3O_4-CHIT-GCE (curve a), Tyr-GCE (curve b) and Tyr-Fe_3O_4-CHIT-GCE (curve c). As expected, there is no redox peak observed at CHIT-Fe_3O_4-GCE in the absence of Tyr even at a concentration of DA up to 1.0×10^{-6} mol L^{-1}. It indicates that there is no redox activity for DA in the selected potential range. A well defined reduction peak at the potential of −0.25V was observed at the Tyr-GCE (curve b) and Tyr-Fe_3O_4-CHIT-GCE (curve c). Obviously, the observed reduction peak was attributed to the direct reduction of dopaquinone liberated from the enzyme-catalyzed reaction on the electrode surface. The electrochemical behavior of DA may be very close to two consecutive single-electron processes. The steps of the enzymatic reaction were shown as follows [20] :



Comparing the reduction peak currents of DA at the Tyr-GCE and Tyr-Fe_3O_4-CHIT-GCE, It can be seen that the Fe_3O_4 in the nanobiocomposite dramatically enhances electrochemical response of DA. The results indicated that Fe_3O_4 played an important role in immobilizing Tyr and enhancing the enzyme catalytic sites accessible to substrate molecules. The phenomenon also indicated that CHIT-Fe_3O_4 composite membrane played an important role in maintaining the biological activity of the Tyr and the promotion of enzyme catalytic activity close to the substrate molecule. It greatly improved the detection sensitivity. Additionally, using the proposed Tyr-Fe_3O_4-CHIT-GCE, there was no need to use any electron mediators. As reported in the literature [21], when the Tyr was immobilized on the GCE with CHIT, extra electron mediators such as $Fe(CN)_6^{4-}$ had to be employed.

3.2 Optimization of experimental parameters

The principle of the typical electrochemical Tyr biosensor is based on the amperometric detection of the enzymatic product dopaquinone, which is generated during the course of the Tyr-catalyzed oxidation of DA in the presence of dissolved oxygen. In this work, amperometric measurements were carried out in air-saturated PBS.

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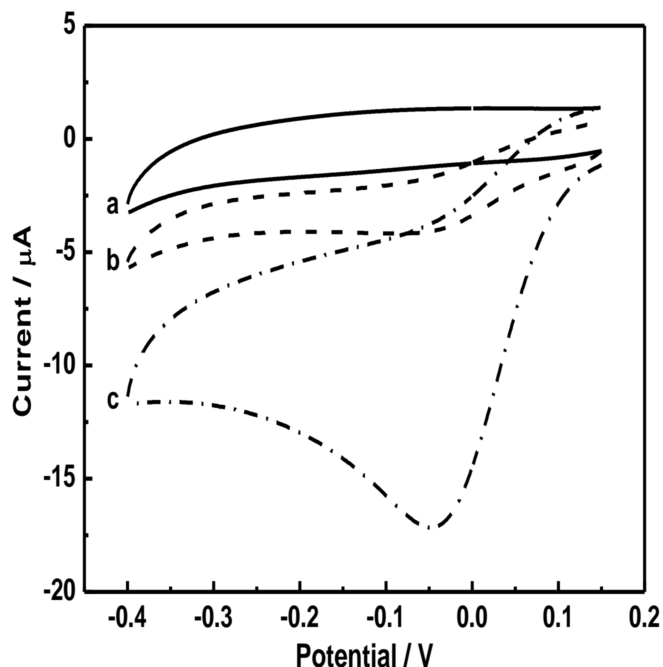
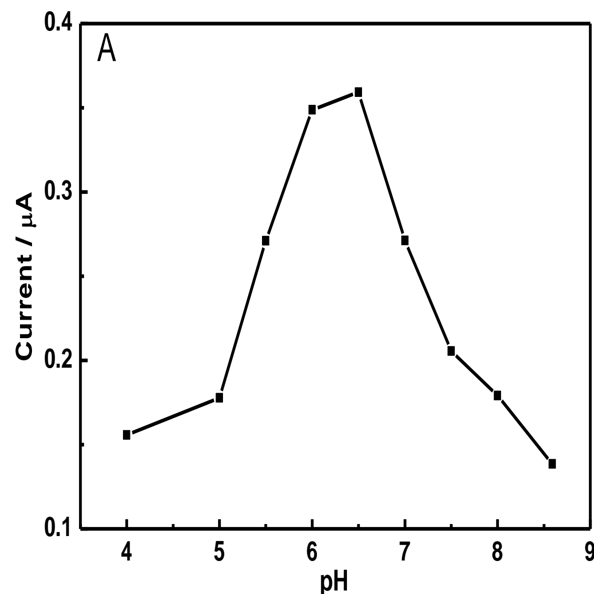


Figure 1. Cyclic voltammograms of 1.0×10^{-6} mol L^{-1} DA at (a) Fe_3O_4-CHIT-GCE; (b) Tyr-GCE; (c) Tyr-Fe_3O_4-CHIT-GCE. Scan rate: 50 mV s^{-1}; supporting electrolyte: 0.1 mol L^{-1} PBS (pH 6.5).

The parameter affecting the amperometric response of the biosensor is the pH of supporting electrolyte. Fig.2A presents the pH of the amperometric response of 5×10^{-6} mol L^{-1} DA in the pH range 4.0 − 8.0. It can be seen that the response current increases with increasing pH from 4.0 to 6.5, and then decreases as pH increases further. At low pH, the increase in amperometric response with an increasing pH was attributed to the increase of the enzyme activity. When the pH>6.5, the decrease of the amperometric response was due to the involvement of protons in the reduction reaction of dopaquinone. The optimum response was achieved at pH 6.5, which is consistent with the optimum pH range of 5.0 − 8.0 reported for the Tyr [8]. It indicated that the immobilization procedure had not altered the activity of Tyr significantly. Moreover, the Tyr would lose activity irreversibly at lower or higher pH. Therefore, a 0.1 mol L^{-1} PBS with pH 6.5 was selected as the supporting electrolyte in the current study.



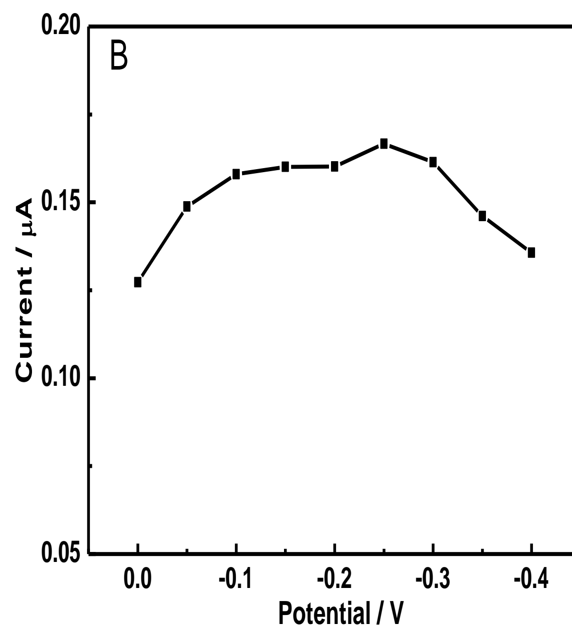


Figure 2. **(A)** Effect of pH on the biosensor response to 5×10^{-6} mol L^{-1} DA in 0.1 mol L^{-1} PBS. Operating potential: −0.25 V vs. SCE.; **(B)** Effect of working potential on the biosensor response to 5×10^{-6} mol L^{-1} DA in 0.1 mol L^{-1} PBS (pH 6.5).

Although Fig.1 provides voltammetric information of dopaquinone on the Tyr-Fe_3O_4-CHIT nanobiocomposite biosensor, it is not accurate to obtain optimal working potential for the sensitive detection of enzymatic produced dopaquinone. To determine optimum potential for

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the biosensor operation, hydrodynamic voltammetric studies were carried out with 5×10^{-6} mol L^{-1} DA. The working electrode was operated at a different potential (between −0.4 and 0 V, 50 mV potential step), and the transient currents were allowed to decay to a steady-state value. The relationship between the difference of response current and background current and the potential was shown in Fig.2B. With the work of the negative potential shift, signal current and background current increased the margin. At −0.25 V the ratio of the signal and background current was the maximum. When the operating potential was lower than −0.25 V, the response current increased, but at the same time the background current also increased. Moreover, lower potential can bring interferences from electroactive species. Therefore, a working potential of −0.25V was selected for the future amperometric measurements.

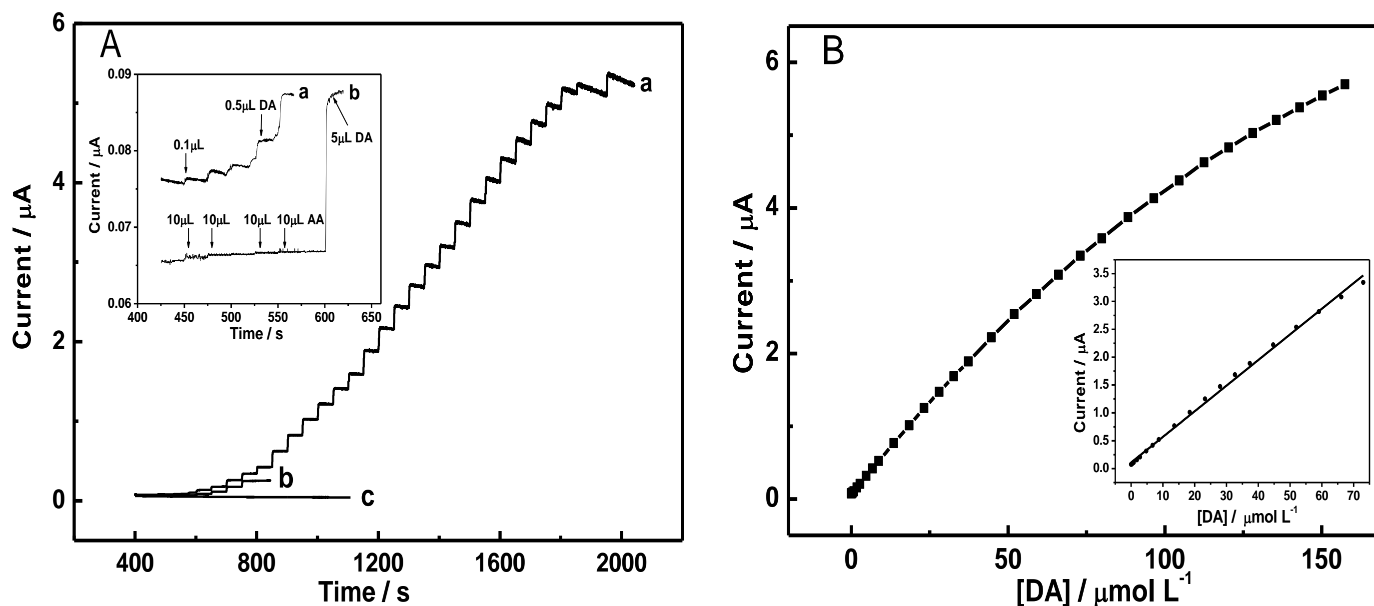

Figure 3. (A) Typical current-time response curves for the successive addition of adding 1.0×10^{-3} mol L^{-1} DA in presence of 1.0×10^{-4} mol L^{-1} AA (curve a), addition of 1.0×10^{-3} mol L^{-1} AA followed by successive additions of 1.0×10^{-3} mol L^{-1} DA (curve b) in 0.1 mol L^{-1} pH 6.5 PBS and the blank solution (curve c) at the Tyr-Fe_3O_4-CHIT-GCE. Operating potential: −0.25 V (vs. SCE). Inset: the magnification of the first several steps. **(B)** Plots of catalytic peak current vs. the concentration of DA. Inset: linear plots of catalytic peak current vs. the concentration of DA.

3.3 Analytical performance of the biosensor

The electrochemical biosensing of DA was performed under the optimized experimental conditions. Fig.3A (curve a) displays a typical current-time plot for the Tyr-Fe_3O_4-CHIT-GCE on successive additions of 1.0×10^{-3} mol L^{-1} DA to 0.1 mol L^{-1} PBS (pH 6.5) at an applied potential of −0.25 V. When an aliquot of DA was added to the supporting solution containing 1.0×10^{-4} mol L^{-1} AA, an almost instantaneous DA response is observed with steady-state currents attained less than 2−3 s. Such fast response was attributed to the rapid diffusion of DA from bulk solution to the immobilized Tyr. To further confirm that the modified electrode was able to determine DA selectively, current-time response was obtained by successive addition of 10 μL AA followed by successive additions of 5 μL DA. Experiments show that the sensor had almost no response current toward AA and only generated a very small background current (Fig. 3A, curve b) prior to the additions of DA. After the addition of DA, the sensor had a fast response, which indicated that Tyr-Fe_3O_4-chitsan-GCE also had a good selectivity. The sensor response of the DA calibration curve was shown in Fig.3B, the reduction peak currents increase linearly with the concentration of DA in the linear range of

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$2.0\times10^{-8}\sim7.5\times10^{-5}$ mol L^{-1} (r = 0.999). The linear regression equation of DA can be expressed as i_p (µA) $=1.089\times10^{-7}+0.046c$ (µmol L^{-1}). The detection limit is 6.0×10^{-9} mol L^{-1} (S/N=3) and the sensitivity is 46 µA/(mmol L^{-1}). Compared to the method of determination of DA in the latest reports [22, 23], the scope of the linear range is wider and the limit of detection is lower an order of magnitude. It is clear that the sensor provides a good way for the selective determination of DA.

3.4 Reproducibility and stability of the biosensor

The repeatability of the same Tyr-Fe_3O_4-CHIT-GCE biosensor was examined by the detection of 5.0×10^{-6} mol L^{-1} DA. A relative standard deviation (RSD) of 2.1% was obtained for ten successive determinations, which indicated a good repeatability of the measurements with no need to apply a complicated pretreatment procedure to the electrode. The long-term stability of the biosensor was evaluated by measuring its response to 5.0×10^{-6} mol L^{-1} DA for one week. When not in use, the electrode was stored at 4℃ under dry condition. The biosensor retained about 95% of its original response after one week. The relatively good stability of the biosensor may be due to that the film could provide a biocompatible microenvironment and the specific ability of Tyr could be protected effectively.

3.5 Determination of actual samples

The concentration of DA hydrochloride injection is 5.27×10^{-2} mol L^{-1}. In 5.00 ml PBS (pH 6.5), adding DA to the sample solution, the average concentration was measured to be 5.47×10^{-2} mol L^{-1}. A relative standard deviation (RSD) value is 5.18 % (n=5). The recovery of the 6 samples was calculated by the use of the calibration curve in Figure 3 B that was shown in Table 1. The recovery was acceptable, showing that the proposed methods could be efficiently used for the determination of DA in human blood serum.

Table 1. Recovery test of DA

No.	Samples (10^{-6} mol L^{-1})	Added (10^{-6} mol L^{-1})	Found (10^{-6} mol L^{-1})	Recoveries (%)
1	2.68	1.00	3.73	105.0
2	2.96	1.00	4.00	104.0
3	3.00	1.00	4.02	102.0
4	2.72	2.00	4.78	102.9
5	2.96	2.00	5.14	109.0
6	2.72	4.00	7.01	107.3

4. Conclusion

In this paper, we have developed an enzyme biosensor based on the immobilization of Tyr in Fe_3O_4 magnetic nanoparticles-CHIT nanobiocomposite. The nanobiocomposite film provided a suitable microenvironment, which could effectively present large loading amount of enzyme and prevent from leaching the immobilized enzyme. The proposed biosensor exhibited a good analytical performance for the amperometric detection of DA, and showed high sensitivity, low detection limit and good reproducibility in the presence of AA. The results clearly suggest that Fe_3O_4 magnetic nanoparticles provide an attractive matrix for effective immobilization of biomolecules.

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References

[1] Liu, A.H.; Honma, I.; Zhou, H.S. Electrochemical biosensor based on protein–polysaccharide hybrid for selective detection of nanomolar dopamine metabolite of 3,4-dihydroxyphenylacetic acid (DOPAC), *Electrochem. Commu.*, 2005, 7, 233–236.

[2] Gil-Loyzaga, P.; Parés-Herbute, N. HPLC detection of dopamine and noradrenaline in the cochlea of adult and developing rats, *Developmental Brain Research*, 1989, 48,

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157–160.
[3] Zhang, H.M.; Li, N.Q.; Zhu, Z.W. Electrocatalytic response of dopamineat a DL-homocysteine self-assembled gold electrode, *Microchem. J.*, 2003, 64, 77–82.
[4] Dzierzega-Lecznar, A.; Kurkiewicz, S.; Stepien, K.; et al., GC-MS analysis of thermally degraded neuromelanin from the human substantia nigra, *J. American Society for Mass Spectrometry*, 2004, 12, 920–926.
[5] Tembe, S.; Kubal, B.S. ; Karve, M. ; et al. Glutaraldehyde activated eggshell membrane for immobilization of tyrosinase from Amorphophallus companulatus: Application in construction of electrochemical biosensor for dopamine, *Anal. Chim. Acta*, 2008, 612, 212–217.
[6] Zhou, Y.L.; Tian, R.H.; Zhi, J.F. Amperometric biosensor based on tyrosinase immobilized on a boron-doped diamond electrode, *Biosens. Bioelectron.*, 2007, 22, 822–828.
[7] Dempsey, E.; Diamond, D.; Collier, A. Development of a biosensor for endocrine disrupting compounds based on tyrosinase entrapped within a poly(thionine) film, *Biosens. Bioelectron.*, 2004, 20, 367–377.
[8] Xue, H.G.; Shen, Z.Q. A highly stable biosensor for phenols prepared by immobilizing polyphenol oxidase into polyaniline–polyacrylonitrile composite matrix, *Talanta*, 2002, 57, 289–295.
[9] Zhang, T.; Tian, B.Z.; Kong, J.L.; et al. A sensitive mediator-free tyrosinase biosensor based on an inorganic–organic hybrid titania sol–gel matrix, *Anal. Chim. Acta*, 2003, 489, 199–206.
[10] Tatsuma, T.; Sato, T. Self-wiring from tyrosinase to an electrode with redox polymers, *J. Electroanal. Chem.*, 2004, 572, 15–19.
[11] Ao, L.M.; Gao, F.; Pan, B.F.; et al. Fluoroimmunoassay for Antigen Based on Fluorescence Quenching Signal of Gold Nanoparticles, *Anal. Chem.*, 2006, 78, 1104–1106.
[12] Sonti, S.V.; Bose, A. Cell Separation Using Protein-A-Coated Magnetic Nanoclusters, *J. Colloid Interface Sci.*, 170 (1995) 575–585.
[13] Reetz, M.T.; Zonta, A.; Vijayakrishnan, V.; et al. Entrapment of lipases in hydrophobic magnetite-containing sol-gel materials: magnetic separation of heterogeneous biocatalysts, *J. Mol. Catal. A: Chem.*, 1998, 134, 251–258.
[14] Cao, D.F.; He, P.L.; Hu, N.F. Electrochemical biosensors utilising electron transfer in heme proteins immobilised on Fe_3O_4 nanoparticles, *Analyst*, 2003, 128, 1268–1274.
[15] Zhao, G.; Xu, J.J.; Chen, H.Y. Fabrication,characterization of Fe_3O_4 multilayer film and its application in promoting direct electron transfer of hemoglobin, *Electrochem. Commum.*, 2006, 8, 148–154.
[16] Chen, L., Gorski, W. Bioinorganic Composites for Enzyme Electrodes, *Anal. Chem.*, 2001, 73, 2862–2868.
[17] Feng, J.J.; Zhao, G.; Xu, J.J.; et al. Direct electrochemistry and electrocatalysis of heme proteins immobilized on gold nanoparticles stabilized by chitosan, *Anal. Biochem.*, 2005, 342, 280–286.
[18] Rosane, W.Inês; O.Zwirtes de; V.Iolanda, C. Immobilization procedures for the development of a biosensor for determination of hydroquinone using chitosan and gilo (Solanum gilo), *Enzyme and Microbial Technology*, 2006, 38, 449–456.
[19] Wang, S.F.; Tan, Y.M.; Zhao, D.M.; et al. Amperometric tyrosinase biosensor based on Fe_3O_4 nanoparticles-chitosan nanocoposite, *Biosens. Bioelectron*., 2008, 23, 1781–1787.
[20] Yang, H.S.; Peng, T.Z.; Shen, B.E.; et al. Thin-layer spectroelectrochemical investigation of dopamine oxidation on carbon electrodes, *Journal of Zhejiang University (Science Edition)*, 2001, 28, 294–298.
[21] Wang, G.; Xu, J.J.; Ye, L.H.; et al. Highly sensitive sensors based on the immobilization of tyrosinase in chitosan, *Bioelectrochemistry*, 2002, 57, 33–38.
[22] Wang, G.Y.; Wang, Z.H.; Xiao, S.F.; et al. Electrocatalytic Separation for Dopamine and Epinephrine at Multi-Wall Carbon Nanotube Modified Electrode and Simultaneous Determination, *Chinese Journal of Analytical*

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Chemistry, 2003, 31, 1281–1285.
[23] Liang, R.P.; Qiu, J.D.; Huang, T.T.; et al. Simultaneous Determination of Dopamine and Epinephrine with Electrode Modified by Nickelous Macrocyclic Complex Film, *Journal of Instrumental. Analysis*, 2002, 21, 31–34.

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