Amperometric detection of dopamine in human serum by electrochemical sensor based on gold nanoparticles doped molecularly imprinted polymers

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Abstract

In this work, a highly sensitive and selective biomimetic electrochemical sensor for the amperometric detection of trace dopamine (DA) in human serums was achieved by gold nanoparticles (AuNPs) doped molecularly imprinted polymers (MIPs). Functionalized AuNPs (F-AuNPs), a novel functional monomer bearing aniline moieties on the surface of the AuNPs, were prepared via a direct synthesis method and then used to fabricate the conductive MIPs film on the modified electrode by electropolymerization method in the presence of DA and p-aminobenzenethiol (p-ATP). The obtained electrochemical sensor based on the conductive film of AuNPs doped MIPs (AuNPs@MIPs) could effectively minimize the interferences caused by ascorbic acid (AA) and uric acid (UA). The linear range for amperometric detection of DA was from 0.02 μmol L⁻¹ to 0.54 μmol L⁻¹ with the detection limit of 7.8 nmol L⁻¹ (S/N=3). Furthermore, the AuNPs@MIPs modified electrode (AuNPs@MIES) was successfully employed to detect trace DA in different human serums.

Keywords:
Dopamine
Functionalized AuNPs
Doping
Molecularly imprinted polymers
Electrochemical sensor

1. Introduction

Dopamine (DA), a naturally occurring catecholamine, is extensively distributed in the mammalian central nerve system (Araujo et al., 2012), which has a significant role in human metabolism as well as in the central nervous and renal systems (Liu et al., 2005). It is involved in drug addictions (Gorwood et al., 2012), schizophrenia (Grace, 2012) and Parkinson’s disease (Darbin, 2012; Shiner et al., 2012). As is well-known, ascorbic acid (AA) and uric acid (UA) usually coexist with DA in biological samples (Noroozifar et al., 2012). Therefore, it is highly desirable for analytical research and clinical application to establish a sensitive, selective and quick method to detect trace DA in biological samples.

Although there have been a lot of analytical methodologies developed to detect DA (Kim et al., 2012; Wang et al., 2007), the electrochemical sensor has many advantages over other methods, such as high sensitivity, good controllability, rapid response and real-time detection (Junior et al., 2000; Thiagarajan and Chen, 2007), and can detect DA conveniently because of its electroactive nature (Xue et al., 2004). However, the selectivity becomes the bottleneck in its development due to the existing of AA and UA in biological samples, which concentrations are several orders of magnitude higher than that of DA, and have similar oxidation potential to DA (Tang et al., 2008; Kaya and Volkan, 2012). Recently, the molecularly imprinted technique (MIT) has been demonstrated as one of the most promising techniques in the sensor field for its high selectivity, low cost, chemical stability and easy preparation (Hu et al., 2008; Liang et al., 2009; Xie et al., 2010; Ma et al., 2011; Ouyang et al., 2007). Owing to the complementarity in functional group orientation and spatial structure, molecularly imprinted polymers (MIPs), the recognition elements, can act as artificial antibodies toward the target molecules (Wang et al., 2011; Yuan et al., 2011; Ouyang et al., 2008; Xing et al., 2012). MIPs have considerable promise for applications in clinical analysis, medical diagnostics, environmental monitoring and drug delivery (Bassi et al., 2007; Wang et al., 2010). However, the MIPs modified sensor (MIES) typically suffered from low sensitivity because MIPs are lack of conductivity and electrocatalytic activity (Li et al., 2012).

Metallic nanoparticles (MNPs) have emerged as a kind of inspiring material and have played important roles in a wide range of fields, such as sensing, electronics, catalysis, biolabeling and optoelectronics (Haes and Van Duyne, 2002; Lewis, 1993; Haruta and Date, 2001; Chan and Nie, 1998; Nicewarner-Pena et al., 2001; Kumat, 2002), due to their special characteristics.
Among all kinds of MNPs, gold nanoparticles (AuNPs) have been extensively used in fabrication of different kinds of sensors because of their good catalytic activity, excellent biocompatibility, ease of further functionalization and other related properties (Luo et al., 2005; Rashid et al., 2006; Zhou et al., 2006; Sohn et al., 2009). The integration of AuNPs into MIPs could solve the shortcomings of MIPs by enhancing the conductivity and catalytic activity of MIPs, which in turn can dramatically improve the sensitivity of imprinted sensor (Shankar et al., 2011; Sheffer and Mandler, 2009; Akamatsu et al., 2012). Functionalized monolayer-protected AuNPs have found increased use in many recent applications (Grainger and Castner, 2008; Kalimuthu and John, 2010; Park et al., 2011). They can give access to establishing a new method to fabricate the nanocomposites of AuNPs doped MIPs (AuNPs@MIPs), which have significant application value in the sensor field (Riskin et al., 2009; Riskin et al., 2008).

In this article, a novel AuNPs@MIES was proposed and employed for the sensitive and selective detection of DA in the coexistence of AA and UA. AuNPs were firstly introduced to modify the gold electrode (AuE), and then the conducting film of AuNPs@MIPs was constructed by the electropolymerization at a constant potential using DA as template molecules, functionalized AuNPs (F-AuNPs) as functional monomers and p-aminobenzenethiol (p-ATP) as cross-linkers. The AuNPs@MIPs were evaluated to confirm its electrochemical properties, such as selectivity, conductivity, electrocatalytic activity and stability. Furthermore, the obtained sensor was employed to detect the trace DA in human serums.

2. Experimental section

2.1. Reagents and materials.

All chemicals were of analytical grade and used without further purification. DA was purchased from Changzhou Yabang Pharmacy Co. Ltd., China. Sodium borohydride (NaBH₄), sodium citrate, AA and UA were purchased from Sinopharm Group Chemical Reagent Co., Ltd., China; p-ATP and chloroaic acid (HAuCl₄) were purchased from Xiya reagent Co., Ltd.; the acetate buffer solution (ABS) was prepared by mixing the stock solution of NaAc and HAc, and the phosphate buffer solution (PBS) was prepared by mixing the stock solution of Na₂HPO₄ and NaH₂PO₄. Human serum samples were obtained from Jiangsu Province Hospital. Dopamine enzyme-linked immunosorbent assay (ELISA) kit was obtained from IBL Co., Ltd., Germany.

2.2. Apparatus.

Electrochemical data were obtained with a three-electrode system using an electrochemical analyzer (CHI 660D, China). UV–vis spectra were obtained on a Shimadzu UV-2450 spectrophotometer. Scanning electron microscope (SEM) images and Energy Dispersive Spectrometer (EDS) were recorded with JSM-5900 (JEOL, Japan). The silicon slice sprayed with gold was introduced to imitate the surface structure of AuE. Enzymatic activity was measured using SpectraMax M2e microplate reader (Molecular Device Co., USA).

2.3. Synthesis of AuNPs.

AuNPs were synthesized via reduction of HAuCl₄ by NaBH₄ according to a reported method (Brown et al., 1996). Briefly, 0.5 mL of 1% trisodium citrate and 5.0 mL of 1% HAuCl₄ solution were added rapidly to 20 mL H₂O with continuous stirring for 10 min. Then, 0.5 mL of 0.075% NaBH₄ was added dropwise while stirring for another 30 min. After filtration, the obtained wine-red colloidal solution of AuNPs was stored at 4°C.

2.4. Synthesis of F-AuNPs.

The F-AuNPs were prepared by mixing a 10 mL solution containing 32 mg of HAuCl₄ in ethanol and a 5 mL solution containing 6 mg p-ATP in methanol. The two solutions were stirred in the presence of 0.4 mL of glacial acetic acid in an ice bath for 1 h. Subsequently, 2.5 mL of aqueous solution of 0.5 mol L⁻¹ NaBH₄ was added dropwise, resulting in a brown solution. The solution was stirred for an additional hour in an ice bath and then for 10 h at room temperature. The product was successively washed and centrifuged with methanol and ethanol, respectively (Riskin et al., 2008).

Fig. 1. Schematic representation for the preparation of the AuNPs@MIES.
2.5. Fabrication of AuNPs@MIES.

Prior to modification, the bare AuE was polished to a mirrorlike surface and electrochemically cleaned by cycling the potential between −0.2 V and 1.5 V in 0.5 mol \( L^{-1} \) \( H_2SO_4 \) at a scan rate of 100 mV s\(^{-1}\) until a stable voltammogram was obtained. Then the electrode was incubated in a solution of p-ATP (10 mmol L\(^{-1}\) in ethanol) for 24 h at room temperature. After being rinsed with ethanol and water, the p-ATP modified AuE was further immersed into the colloidal solution of AuNPs (pH 5.0) for 10 h to generate the AuNPs modified electrode.

An electrolyte solution containing 1 mmol L\(^{-1}\) DA, 10 mmol L\(^{-1}\) F-AuNPs, 7 mmol L\(^{-1}\) p-ATP and 0.1 mol L\(^{-1}\) ABS (pH 5.0), was kept in dark under a nitrogen atmosphere at room temperature for 6 h to complete the pre-assembly between DA and F-AuNPs through the hydrogen-bond interaction. The AuNPs modified electrode was immersed into the electrolyte solution and the AuNPs@MIES was prepared by the electropolymerization at a constant potential of 1.0 V for 400 s. After that, the electrode was immersed in 0.5 mol L\(^{-1}\) \( H_2SO_4 \) and treated with a constant potential of −0.5 V for 400 s to remove the templates and dried under nitrogen flow. The fabrication process of AuNPs@MIES was indicated in Fig. 1. For the control experiments, the AuNPs doped nonimprinted electrochemical sensor (AuNPs@NIES) and MIES (undoped with AuNPs) were prepared but without DA or F-AuNPs, respectively.

2.6. Experimental detection.

Electrochemical measurements were carried out in a 10.0 mL aqueous solution containing 10 mmol L\(^{-1}\) of \([Fe(CN)_6]^{3-/4-}\) and 0.1 mol L\(^{-1}\) of KCl. Cyclic voltammetry (CV) method was performed in the potential range from −0.2 V to 0.6 V at a scan rate of 100 mV s\(^{-1}\). Differential pulse voltammetry (DPV) measurement was performed in the scan range from −0.2 V to 0.6 V at a scan rate of 100 mV s\(^{-1}\), with the pulse width, pulse period and quiet time were 0.02 s, 0.1 s and 2 s, respectively. Electrochemical impedance spectroscopy (EIS) experiment was carried out at a potential of 0.2 V over the frequency range from 100 mHz to 10 kHz, using an amplitude of 10 mV.

3. Results and discussion

3.1. Characterization of the modified AuE.

The morphology of AuNPs self-assembled film and AuNPs@MIPs was characterized by SEM. In Fig. S2 (A), AuNPs (demonstrated by EDS curve in the inset) were quite uniformly distributed due to the the method of self-assembly (Freeman et al., 1995; Huang et al., 2007; Sardar et al., 2008), and the diameter of the AuNPs was about 12 nm. The image in Fig. 2 (A) showed relatively rough and flower-like structure doped with AuNPs (demonstrated by EDS curve in the inset), suggesting that the AuNPs@MIPs film was fabricated by electropolymerization of F-AuNPs in the presence of DA and p-ATP. In the EDS curve, the signals of O, S and N were derived from the the MIPs. Fig. S2 (B) showed the cross section of AuNPs@MIES. The thickness of the polymer film was about 1.3 \( \mu \)m.

3.2. Optimization of experimental parameters.

The current response that reached to the top was choosen as the optimization criterion. The amount of the imprinted sites of MIPs was affected by the ratio of templates, monomers and cross-linkers. The results in Fig. S3 (A) and (B) revealed that the largest current response was obtained when the ratio among DA, F-AuNPs and p-ATP was 1:10:7. The amount of F-AuNPs directly impacted on the number and structure of the imprinted cavities and the amount of doped AuNPs. Low amount of F-AuNPs resulted in lacking of enough imprinted cavities and doped AuNPs, while excessive F-AuNPs brought about wasting of monomers. p-ATP with appropriate amount could provide sufficient cross-linking and fill the defect originated from the pre-assembly between DA and F-AuNPs to fabricate more effective recognition sites in imprinted cavities.

The performance of the AuNPs@MIPs film was related to pH value of the electrolyte for electropolymerization. The electropolymrization effect was investigated at a range of pH 4–8. As observed in Fig. S3 (C), the AuNPs@MIES obtained at pH 5 had maximum current response.

In the process of the electropolymrization, the constant potential of 1.0 V was used to form the AuNPs@MIPs film. The thickness of the film could easily be adjusted by controlling the scan time during the procedure. The obtained film was yellow green, and the color was deepened with the extension of the scan time. As shown in Fig. S3 (D), the current response reached maximum with 400 s, and then decreased with extension of scan time.

3.3. Electrochemical behavior of the sensors.

CV and EIS were effective tools for probing the electrochemical properties of the surface-modified electrodes. Fig. 2 (B) showed the cyclic voltammograms of the bare AuE and modified electrodes. The CV curve of the bare AuE showed a well-defined quasi-reversible peaks (curve a). The redox peak currents and area increased apparently after the bare AuE was modified with the AuNPs (curve b), which might be attributed to excellent electrical conductivity and high specific surface area of the AuNPs. When the AuNPs@MIPs or MIPs film was modified on the AuNPs modified electrode (curve c, d), the peak currents both decreased. However, the peak current of the AuNPs@MIES was higher than that of the MIES, and the redox peak separation of the AuNPs@MIES was less than that of MIES. This indicated that the conductivity and electrocatalytical activity of AuNPs@MIPs film was better than that of the MIPs film doped with AuNPs. The redox peak of the AuNPs@NIES (curve e) was almost not appeared, this is likely because the electron transfer of \([Fe(CN)_6]^{3-/4-}\) was blocked by the AuNPs@NIPs film. All these demonstrated that the imprinted cavities were formed in the AuNPs@MIPs and MIPs films, and the molecules of \([Fe(CN)_6]^{3-/4-}\) could reach the electrode surface, but the AuNPs@MIPs possessed better electron transfer property and electrocatalytical activity to the probing molecules.

Fig. S4 illustrated the results of electrochemical impedance spectra of the bare AuE and modified AuE. It is obvious that the AuNPs modified AuE (curve b) exhibits a small radius circular arc, which reveals that the heterogeneous charge-transfer resistance decreased, implying that the AuNPs can promote electron transfer of \([Fe(CN)_6]^{3-/4-}\). And the apparent increase in the semicircle diameter was observed after the AuNPs@MIPs (curve c) or MIPs (curve d) film was constructed on the modified electrode. This indicated that the imprinted film can hinder the electron transfer of \([Fe(CN)_6]^{3-/4-}\). Moreover, the semicircle diameter of AuNPs@MIES was smaller than that of MIES. The semicircle diameter of the AuNPs@NIES (curve e) was the largest among the five sensors, this is likely because the electron transfer of \([Fe(CN)_6]^{3-/4-}\) was blocked by the AuNPs@NIPs film. The experimental results recorded by CV and EIS method implied that the AuNPs@MIPs film owned better conductivity and electrocatalytical activity than MIPs film did. By appropriately doping AuNPs into MIPs, the drawbacks of MIPs, such as low conductivity and electrocatalytical activity, can be overcome.
3.4. Optimization of the analytical condition.

The optimization criterion was the current response that reached the maximum value. The effect of pH value of the supporting electrolyte solution on the current response of 1 μmol L\(^{-1}\) DA was tested by DPV method, and the results can be revealed in Fig. S5. The current response of DA on the AuNPs@MIES gradually increased as the pH value increased from 4 to 7 and then decreased with higher pH value. Therefore, PBS (pH 7) solution was used for the detection of DA.

3.5. Molecular recognition by AuNPs@MIES.

Generally, coexisting electroactive components such as AA and UA show serious interference in the electrochemical detection of DA. Therefore, the amperometric responses of AA and UA were tested. The normal physiological level of AA and UA is generally much higher than that of DA. Thus, the interfering effects of successive additions of 3 μmol L\(^{-1}\) AA and 6 μmol L\(^{-1}\) UA were examined with reference to 0.03 μmol L\(^{-1}\) DA and presented in Fig. 2 (C). If the amperometric response of DA was set as 100%, the responses of AA and UA were only 2.67% and 0.74%.

Fig. 2 (D) showed DPVs of the AuNPs@MIES for DA in a concentration range of 1.0–2.4 μmol L\(^{-1}\), in the presence of 0.1 mmol L\(^{-1}\) AA and 0.2 mmol L\(^{-1}\) UA. The AuNPs@MIES resolved the overlapping voltammetric wave into the anodic peak of DA at 0.13 V and AA at −0.04 V, but no obvious peak for UA existed. The linearity curve (the inset) with a correlation coefficient of \(R=0.9932\) illustrated that there was significant linear correlation between current response and the concentration of DA in the presence of AA and UA.

3.6. Reproducibility, repeatability and stability of AuNPs@MIES.

To investigate the reproducibility and repeatability of the AuNPs@MIES, the experiments were performed in the PBS (pH 7.0) solution containing 1 μmol L\(^{-1}\) DA. The AuNPs@MIES was expected to be regenerated with RSD of peak currents was 4.4% using five different imprinted sensors. The good repeatability was observed with RSD of 3.7% after continuous using the same sensor for 30 times. It revealed that the imprinted sensor had good reversibility. The stability of the AuNPs@MIES has been examined and monitored the current response for 1 μmol L\(^{-1}\)DA PBS solution. The sensor was dipped in PBS (pH 7.0) solution at 4 °C when not in use. The apparent decrease was not observed in 10 days. The current response retained 96% after 20 days, and retained 92% after a month. These demonstrated that the imprinted sensor possessed excellent stability.

3.7. Calibration curve and sample analysis.

The amperometric response of the AuNPs@MIES to successive additions of DA was further evaluated under optimized experimental condition. Fig. S7 showed the amperometric current–time response of DA at 0.16 V. As illustrated, upon addition of DA to the stirring PBS (pH 7.0), the oxidation current increased steeply and reached a steady-state current within average response time of 4 s. The amperometric signal showed a good linear correlation to DA concentration in the range from 0.02 μmol L\(^{-1}\) to 0.56 μmol L\(^{-1}\). The linear regression equation was expressed as \(I_{pa}(\mu A)=0.24427C_{DA}(\mu mol L^{-1})−2.02×10^{-3}\) with a correlation coefficient of \(R=0.9987\). The detection limit is 7.8 nmol L\(^{-1}\) (S/N=3).
Human serum samples were used for analysis of DA. Interfering proteins from 0.5 ml serum were removed by precipitation and centrifugation. The serum samples were diluted 1:10 with PBS (pH 7.0) and then were detected. The analytical results were shown in Table 1 and the recoveries were in a range between 96.7% and 104.9% with RSD < 4.1%. For evaluating the analytical reliability, the average content of DA in the same serum sample (0.033 μmol L⁻¹, RSD = 4.6%, n = 3) was detected by ELISA (Kim et al., 2008) and the detection result was in accordance with the electrochemical method established in this work (0.029 μmol L⁻¹, RSD = 3.8%, n = 3).

### Table 1

<table>
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<tr>
<th>Samples</th>
<th>Determined level (μmol L⁻¹)</th>
<th>Spiked level (μmol L⁻¹)</th>
<th>Total found (μmol L⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
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<td>0.271</td>
<td>97.1</td>
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</table>

### 4. Conclusions

In summary, we have constructed an AuNPs@MIES for sensitive and selective detection of DA in human sera. F-AuNPs, the novel functional monomers, were fabricated and employed to prepare the AuNPs@MIES film possessed better conductivity and electrocatalytic activity. And the imprinted sensor could effectively avoid the interferences caused by AA and UA which coexist with DA in biological samples. Moreover, the AuNPs@MIES was applied for detecting trace DA in human serum with satisfactory results. Therefore, this new AuNPs@MIES composites can offer a strategy to enhance the electrochemical performance of MIES and be used as the material to construct sensitive and selective sensors for determination of trace target molecules in complex matrix.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.04.022.