Highly selective and sensitive determination of dopamine by the novel molecularly imprinted poly(nicotinamide)/CuO nanoparticles modified electrode

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A B S T R A C T

A novel electrochemical sensor was proposed for the determination of dopamine (DA) based on the molecularly imprinted electropolymer (MIPs)/copper oxide (CuO) nanoparticles modified electrode. MIPs were firstly prepared by using nicotinamide as an environment-friendly monomer to selectively recognize the template molecules. CuO nanoparticles were used to enhance the number of imprinted sites per unit surface area of the electrode and then improve the selectivity and sensitivity of the electrochemical sensor. Thus, the obtained electrochemical sensor could effectively minimize the interferences caused by ascorbic acid (AA), uric acid (UA) and sample matrix. The linear range for the detection of DA was changed from 0.02 μmol L \(^{-1}\) to 25 μmol L \(^{-1}\) with the detection limit of 8 nmol L \(^{-1}\) (S/N = 3), which was lower than those of the reported MIPs-based sensor. Finally, the proposed method was applied to measure dopamine in serum samples. The spiked recoveries were changed from 96.9% to 105.9% and the RSD was not higher than 8.8%. It was shown that the proposed sensor exhibited significant promise as a reliable technique for the detection of DA in human serum samples.

1. Introduction

The development of chemical sensors for specific molecule analysis at low concentration has attracted increased attention. Different recognition elements have been introduced to enhance the selectivity of sensors and meet various application requirements. Owing to the complementarity in functional group orientation and spatial structure, molecularly imprinted polymers (MIPs) have selective recognition toward the template molecule, even in the presence of similarly structured interfering analogues. With significant advantages in the physical and chemical stability, ease of preparation, low cost and applicability in harsh environmental condition, MIPs have been utilized in various fields, such as clinical analysis, medical diagnostics, environmental monitoring and drug delivery (Li et al., 2014; Qian et al., 2014; Zhao et al., 2014). Especially for the electrochemical detection of dopamine (DA), MIPs-based sensor is necessary, because DA, ascorbic acid (AA) and uric acid (UA) coexist in biological fluids, and DA has an overlapping oxidation peak with AA and UA at traditional electrodes (Hong et al., 2013; Qian et al., 2014; Wu et al., 2013).

Initially, MIPs are mostly developed by the free-radical polymerization, resulting in nonconducting polymers, which are tested as recognition units of chemical sensors utilizing different transduction schemes. Subsequently, electropolymerization is utilized to directly prepare rigid, uniform, and compact MIP films on transducer surfaces (Sharma et al., 2012). This technique has some advantages over other preparation procedures of MIPs-based sensors (drop-coating, composite-making, and spin-coating), such as simplicity and speed of preparation, thickness and morphology controlling, high reproducibility and the possibility of preparation and operation in aqueous solutions (Rezaei et al., 2014; Roy et al., 2013). More importantly, MIPs film prepared by electropolymerization has high stability, electrocatalytical activity and conductivity, which could improve the sensitivity and selectivity of sensors and then its practical applications.

Unfortunately, there are two major problems with the molecularly imprinted electropolymerization. One of the problems is the choice of electroactive functional monomers. Nowadays, o-aminophenol (Li et al., 2009), o-phenylenediamine (Wu et al., 2013), pyrrole (Kan et al., 2012; Qian et al., 2014), 3-acrylamidophenylboronic acid (Hong et al., 2013), and p-aminobenzenethiol...
(Xue et al., 2013) are always used to synthesize molecularly imprinted electropolymers in the presence of template (DA). However, these monomers have acute toxicity and mutagenicity, which may cause long-term adverse effects in the aquatic environment. Sensors constitute important technological advances in many application fields. But, they are often developed without regard for their environmental impact, and there has become a growing interest in the development of sensors using “green” chemistry (Grinenval et al., 2014). Green chemistry involves the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances (Zhang et al., 2010). Nicotinamide (3-pyridine carboxylic acid amide, NA) is the main constituent of vitamin B complex and the reactive moiety of nicotinamide adenine dinucleotide. Electrochemical behaviors of NA have been widely investigated in the past decades due to their biological reactivity (Wang et al., 2006). However, the usage of NA as an environment-friendly monomer for fabrication of MIPs-based sensors has never been reported.

The other problem is fewer imprinted sites formed on the surface of electrode, because of the relatively high density of electropolymers (Xue et al., 2013), which will decrease the selectivity and sensitivity of MIPs-based sensor. With the development of nanotechnology, functional nanomaterial is often used to improve the conductivity and catalytic ability of the sensors and then dramatically enhance the intensity of the electrochemical signal (Wen et al., 2014). To the best of our knowledge, there was no report about nanomaterial as an enhancer of template immobilization in the MIPs-based sensor to increase the number of imprinted cavities. Copper oxide (CuO) is a p-type metal oxide semiconductor with a narrow band-gap that has been effectively used in the fabrication of electrical, optical and photovoltaic devices, heterogeneous catalysis, magnetic storage media and lithiumion electrode materials (Reddy et al., 2012). In this study, CuO nanoparticles were used to enhance the number of imprinted sites per unit surface area and then improve the selectivity and sensitivity of sensors.

Dopamine (DA) levels are found to be correlated with the severity and progression of neurological disorders such as Parkinson’s and Alzheimer’s diseases. Sensitive and selective detection of dopamine is of great importance for the understanding and diagnostics of neurological diseases. In this article, a novel electrochemical sensor was developed based on the MIPs@CuO modified electrode for sensitive and selective determination of DA. CuO nanoparticles with high capacity for DA were firstly introduced on the surface of glassy carbon electrode (GCE), and then the MIPs film was constructed by the electropolymerization using NA as an environment-friendly monomer. Then, the obtained sensor was used to determine trace DA in serum samples.

2. Materials and methods

2.1. Reagents and materials

All chemicals were of analytical grade and used without further purification. DA, AA, UA and NA were purchased from Sigma Company (St. Louis, MO, USA). Copper acetate, acetic acid, sodium hydroxide, ethylene glycol, and ethanol were procured from Sinopharm Chemical Reagent Co., Ltd., China. The phosphate buffer solution (PBS) was prepared by mixing the stock solution of NaH₂PO₄ and Na₂HPO₄. Deionized water was applied for all polymerization and reaction processes. Human serum samples were kindly provided by a team of volunteers and then stored at −20 °C until analysis.

2.2. Apparatus

The identification of crystalline phase of CuO nanoparticles was performed using an X-ray diffractometer (PANalytical BV Company X’Pert PRO) of monochromatized with Cu Kα radiation over the 2θ range 10–80° at a rate of 4° min⁻¹. The surface morphologies of MIPs@CuO film were observed by a field-emission scanning electron microscope (FESEM, Sirion 200, FEI, Holland). The particle size distributions of CuO nanoparticles were performed on Delsa™ Nano particle analysis instrument (Beckman Coulter, CA, USA).

The electrochemical determination was performed using an electrochemical workstation (CH Instruments 660C, Shanghai Chenhua Co. Ltd., China) and a standard three-electrode system with modified GCE as the working electrode, a platinum wire as the counter-electrode and a saturated calomel electrode (SCE) as the reference electrode.
2.3. Fabrications of the MIPs@CuO@GCE and NIPs@CuO@GCE

The synthesis of CuO nanoparticles was performed as described in the literature (Sahooli et al., 2012). Briefly, we mixed 2.7 g copper acetate and 2 mL acetic acid with 600 mL ethylene glycol in a round-bottomed flask. Then, 40 mL NaOH (1 mol L$^{-1}$) was gradually dropped into the solution under magnetic stirring at 78 °C. Being cooled at room temperature, CuO nanoparticles were purified several times with absolute ethanol and deionizer water, respectively.

Subsequently, 0.5 μL CuO nanoparticles (1 mg mL$^{-1}$) were dropped on the surface of glassy carbon electrode and then the resulting electrode was incubated in a 0.1 mol L$^{-1}$ PBS buffer (5 mL, pH=7.5), containing 10 μmol L$^{-1}$ NA, 6.5 μmol L$^{-1}$ DA

![Fig. 2. Optimization of different conditions affecting the determination of DA. Variation of electrode response for DA (25 μmol L$^{-1}$) with changing of (A) the volume of aqueous solution containing CuO nanoparticles; (B) the mole ratio of template and monomer; (C) pH value of solution; (D) scan rate and (E) the number of cycles.](image-url)
and 0.1 mol L⁻¹ KCl, for 20 min at room temperature to complete the adsorption of DA and pre-assembly between template and monomer. Cyclic voltammetry was performed to obtain the MIPs film from −1.0 V to +2.0 V for 20 cycles at the scan rate of 0.1 V s⁻¹. A brief scheme of the sensor fabrication is shown in Fig. 1. The embedded DA was removed by scanning between −0.2 V and +0.8 V in a 0.1 mol L⁻¹ PBS buffer for several cycles until no obvious peak could be observed. Preparation processes of non-imprinted polymers (NIPs) film were as same as the method mentioned above but without the addition of DA.

2.4. Experimental detection

In order to understand the feasibility of electrochemical sensor in practical application, the MIPs@CuO@GCE was used to determine DA in real human serum samples. Interfering proteins from 0.5 mL serum were removed by precipitation and then centrifugation (10,000 rpm, 5 min). The serum samples were diluted 1:10 with 0.1 mol L⁻¹ PBS buffer (pH=7.5) and then detected by cyclic voltammetry method. It was performed in the potential range from −0.2 V to 0.8 V at a scan rate of 0.1 V s⁻¹. All the experiments were carried out at room temperature.

3. Results and discussion

3.1. Preparation and characterization of MIPs@CuO@GCE

In this study, CuO nanoparticles were p-type metal oxide semiconductors with a narrow band-gap. Fig. S1 (Supporting information) shows the XRD pattern of CuO nanoparticles. All the peaks can be assigned to monoclinic symmetry of CuO (space group C2/c; α₀=4.688 Å, β₀=3.423 Å, γ₀=5.132 Å; JCPDS Card no. 48-1548). No other peak was observed belonging to any impurity semiconductors with a narrow band-gap. Fig. S1 (Supporting information). Çakır et al. described that NA can bind to metal ions with pyridine N to form the mixed ligand complexes and catalyze the reduction of metal ions in the complex (Çakır et al., 2001). Thus, the reduction peak (a1) was found at −0.67 V and then disappeared in the subsequent CVs, which indicated that the electropolymers were completely coated on the surface of CuO@GCE in the first scan. Then, we can find that three oxidation peaks were generated at −0.02 V (b1), 0.18 V (b2) and 1.7 V (b3) and two reduction peaks were generated at about 0.05 V (a3) and −0.55 V (a2) in the electropolymORIZATION process (Fig. 2). The possible reactions are shown as the following:

Based on the literatures (Kotkar and Srivastava, 2008; Wang et al., 2006; Zhu and Lin, 2009), it was observed that NA as the monomer could obtain single electron to form a transition state (reduction procedure), which dimerized at the sixth position. At a potential considerably more positive than that of the first reduction wave, the dimer can be oxidized back to NA (oxidation peaks b3). In fact, with the increasing of circle number, the reduction peak current was gradually increased, the current of oxidation peak b1 was rapidly decreased (transition state→NA) and the current of oxidation peak b3 was very weak. All of these results demonstrated the chain propagation for polymerization and then the formation of imprinted cavities. It was interesting to note that the current of oxidation peaks (b2), absenting in the NIPs@CuO@GCE, was not changed in the electropolymORIZATION process, implying the formation of imprinted cavities without the self-polymerization and electrochemical catalysis of DA (template). In fact, the detailed mechanism needs to be further investigated. In this study, we focus our attention mainly on the possibility of NA
as an environment-friendly monomer for fabrication of MIPs-based sensors.

Fig. S4 (Supporting information) shows the CVs of MIPs@CuO@GCE and NIPs@CuO@GCE in the template-removing process. Disappearance of DA characteristic peaks implied the complete elution of template and successful fabrication of imprinted cavities. Furthermore, the peak currents of DA on MIPs@CuO@GCE were higher than those on MIPs@GCE, implying the enhancement of CuO nanoparticles for imprinting effect (Fig. S3B). It could also be developed for the determination of other target molecules based on a MIPs-based sensor prepared by NA as a monomer.

3.2. Electrochemical behavior of the proposed sensors

Cyclic voltammograms was an effective tool for probing the electrochemical properties of the modified electrodes. Fig. 3 shows the CV responses of four different assembled electrodes involving CuO@GCE, MIPs@GCE, MIPs@CuO@GCE and NIPs@CuO@GCE for the template molecules (DA). Because of the weak catalysis, a poor redox peak could be observed on the CuO@GCE, even though CuO nanoparticles had a high adsorption capacity for DA (84.3 mg g\(^{-1}\)). However, there were well-defined redox peaks on the MIPs@GCE, implying that NA could be used to prepare electropolymers and

![Fig. 3. CVs of CuO@GCE, MIPs@GCE, MIPs@CuO@GCE and NIPs@CuO@GCE in 0.1 mol L\(^{-1}\) PBS solution containing 25 \(\mu\)mol L\(^{-1}\) DA. Scan rate: 0.1 V s\(^{-1}\).](image)

![Fig. 4. Current responses of DA, NE, EP, AA and UA at MIPs@CuO@GCE and the structural formulas of five compounds. Concentration of each compound is 25 \(\mu\)mol L\(^{-1}\). The \(\Delta I\) value of UA was obtained at 0.24 V. Inset: the CVs of 2 \(\mu\)mol L\(^{-1}\) DA in the absence and presence of 50 \(\mu\)mol L\(^{-1}\) UA and 50 \(\mu\)mol L\(^{-1}\) AA. The concentrations were equal to the upper limit of normal reference in serum.](image)
this film had highly catalytic ability towards the oxidation of DA. Moreover, current response of DA on the MIPs@CuO@GCE was higher than that on the NIPs@CuO@GCE, indicating the successful fabrication of imprinted cavities and then highly selective recognition effect. As shown in Fig. 3, it was observed the anodic peak current of DA on the MIPs@CuO@GCE (6.8 μA) was higher than the summation (2.9 + 0.4 μA) of that on the MIPs@GCE and CuO@GCE, respectively. Experimental results indicated that CuO nanoparticles as an enhancer played an important role in the preparation of MIPs film, due to their high adsorption capacity for the template, increased surface area and good conductivity.

For investigation of the reaction mechanism, scan rate dependent experiments were carried out for the reaction system at the MIPs@CuO@GCE. It was shown that both anodic and cathodic peak currents increased clearly with increasing potential scan rates. In the range of 0.02–0.2 V s⁻¹, both the anodic and cathodic peak currents were linearly dependent on the scan rate, suggesting that this system was an adsorption controlled process. Actually, to obtain sensitive and reproducible detection of DA, the electrode should be immersed in the solution for a period of time to reach adsorption equilibrium. However, the results demonstrated that the anodic and cathodic peak currents of DA were not changed between 30 s and 5 min, indicating the rapid adsorption at the initial contact time. The possible reason was high adsorption of DA on the surface of CuO@GCE and then growing of MIPs film in an ordered arrangement.

3.3. Molecular recognition by the proposed sensors

As imprinted materials, one of the main functions of the proposed MIPs@CuO film was to improve the selectivity of electrochemical sensors. Thus, with respect to the possible interference in the determination of DA, epinephrine (NE) and norepinephrine (EP) were determined as structural analogues and AA and UA were investigated as coexisting interferents. As shown in Fig. 4, due to the extremely similar structure, the current responses of NE and EP were higher than those of AA and UA, indicating the high selectivity of the proposed sensor. Furthermore, the current responses of NE and EP were only 24–33% as high as that of DA, because of different functional groups and molecule size. It was shown that MIPs film using NA as a monomer played an important role in the confining responses of NE and EP. For the AA and UA, because of the obviously different structures, the cavities formed in the imprinting process could not bind them tightly, which resulted in much lower response.

Furthermore, matrix interference of serum samples and coexisting interferences of AA and UA with high concentrations (the concentration was equal to the upper limit of normal reference range) were the major problem for the practical application of proposed sensor in clinical diagnosis. Compared with background buffer solution, there was almost no change in the oxidation peak current for blank serum samples (RSD = 6.2%), which demonstrated that sample matrix could not affect the determination of DA and enhance background noise. Furthermore, it was shown that 25-fold of AA and UA over DA (simulation of real sample) hardly caused interference, implying the MIPs@CuO@GCE could be used to directly determine DA in complex serum sample.

3.4. Validation of the analytical principle

In order to test the feasibility of the proposed method for the determination of DA in real serum samples, the relationship between the change of oxidation peak current and the concentration of DA was studied. Some experimental parameters were optimized, and the results are shown in Fig. 5. The change of oxidation peak current exhibited a good linearity with the concentration of DA in the range of 0.02–25 μmol L⁻¹, and the detection limit was obtained as 8 ± 1.8 nmol L⁻¹ (S/N = 3). Compared with NIPs@CuO@GCE, the lower limit of linear range of the MIPs@CuO@GCE was 10 times lower than that of NIPs@CuO@GCE, indicating the electrochemical enhancement effect of MIPs film prepared by NA as the monomer. The comparison of different MIPs modified electrodes for DA determination was displayed in Table 1 (Gao et al., 2007; Kan et al., 2008; Lakshmi et al., 2009; Mao et al., 2011; Wu et al., 2013). It was shown that the low detection limit of DA was achieved at the MIPs (AABA, 3-acrylamidophenylboronic acid)-Au electrode (Hong et al., 2013), AuNPs@MIPs(p-ATP, p-amino-phenzenethiol)-Au electrode (Xue et al., 2013) and MIPs(NA)@CuO@GCE (the proposed method). However, the proposed sensor has the advantages of low cost (without AuNPs) and being environmentally friendly, and wide linear range, indicating the

![Fig. 5. The corresponding current responses of MIPs@CuO@GCE and NIPs@CuO@GCE for different concentrations of DA. The relative standard deviations (RSD) were changed from 3.4% to 9.8.](image-url)
excellent electrochemical performance and widely practical application.

The reproducibility of the proposed method was evaluated by determining DA solution (0.1 μmol L\(^{-1}\)) using a series of electrodes modified with different batches of MIPs@CuO and relative standard deviation (RSD) was lower than 5.3% (n=5). After consecutive analyzing the same solution using one modified electrode, RSD of current changes was lower than 4.8% (n=5), which exhibited a good reproducibility of the proposed method. The long-term stability of MIPs@CuO@GCE was also determined by weekly measure. The results showed that the peak current only decreased by 2.5% after 7 days, indicating that the proposed MIPs@CuO@GCE was considerably stable.

In addition, in order to investigate the practical perspective of the proposed method, the amounts of DA in 12 human serum samples were determined by the proposed method (X) and the classical LC–MS/MS method (Y) (Huang et al., 2014), respectively. Those results were statistically compared by the paired t-test. It was shown that the two methods had a good consistency, with a regression equation of Y=1.01 X+0.014, and the correlation coefficient was 0.9977 (Fig. S5, Supporting information). However, the proposed MIPs-based sensor has many advantages over the classical LC–MS/MS methods, such as simple operation, inexpensive and portable instrument, short analysis time and low running cost. These results illustrated that the proposed sensor exhibited significant promise as a reliable technique for the detection of DA in human serum samples.

3.5. Determination of DA in serum samples

To evaluate the practicability of the developed sensor for routine analysis, the proposed sensor based on MIPs@CuO@GCE was used to determine DA content in real serum samples. The results indicated that spiked recoveries were changed from 96.9% to 105.9% and RSD was lower than 8.8% (Table S1, Supporting information). It was also noted that the DA level could be detected in the original serum sample and the concentrations were found from 22 nmol L\(^{-1}\) to 36 nmol L\(^{-1}\). Therefore, it is necessary to develop a selective and sensitive analytical method for determination of trace DA in real serum samples.

4. Conclusions

Nicotinamide as an environment-friendly monomer was firstly used to construct a MIPs-based sensor for DA sensing. Owing to the high adsorption capacity, CuO nanoparticles could effectively enhance the immobilized amounts of template per unit surface area and then improve the selectivity and sensitivity of sensor. The proposed sensor based on MIPs@CuO@GCE had good selectivity and sensitivity for the determination of DA without the matrix interference of serum sample. Under the optimized condition, the linear range was changed from 0.02 μmol L\(^{-1}\) to 25 μmol L\(^{-1}\), and the detection limit was 8 nmol L\(^{-1}\), which was lower than those of the reported MIPs-based sensor. In a word, the proposed sensor provided a simple and reliable technique for DA detection in biological samples and a notion to perfect the MIPs-based sensor for trace detection.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.07.053.

References


Table 1

Comparison of the proposed method with other electrochemistry methods based on MIPs in the determination of DA.

<table>
<thead>
<tr>
<th>Layer description</th>
<th>Linear range (mol L(^{-1}))</th>
<th>Detection limit (mol L(^{-1}))</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP-Si:ITO electrode</td>
<td>(2.0 \times 10^{-6} – 8.0 \times 10^{-4})</td>
<td>(2.0 \times 10^{-6})</td>
<td>Gao et al. (2007)</td>
</tr>
<tr>
<td>MWNTs-MIPs:GCE</td>
<td>(5.0 \times 10^{-7} – 2.0 \times 10^{-4})</td>
<td>(5.0 \times 10^{-7})</td>
<td>Kan et al. (2008)</td>
</tr>
<tr>
<td>MIPs-hybrid-Au electrode</td>
<td>(2.3 \times 10^{-7} – 1.4 \times 10^{-4})</td>
<td>(2.3 \times 10^{-7})</td>
<td>Lakshmi et al. (2009)</td>
</tr>
<tr>
<td>GSCR–MIPs:GCE</td>
<td>(1.0 \times 10^{-7} – 8.3 \times 10^{-4})</td>
<td>(1.0 \times 10^{-7})</td>
<td>Mao et al. (2011)</td>
</tr>
<tr>
<td>MIPs(MWNTs)/GCE</td>
<td>(6.3 \times 10^{-7} – 1.0 \times 10^{-4})</td>
<td>(6.0 \times 10^{-8})</td>
<td>Kan et al. (2012)</td>
</tr>
<tr>
<td>MIPs (OPD)-Au electrode</td>
<td>(3.2 \times 10^{-9} – 4.6 \times 10^{-7})</td>
<td>(7.0 \times 10^{-7})</td>
<td>Wu et al. (2013)</td>
</tr>
<tr>
<td>MIPs (AABA)-Au electrode</td>
<td>(5.0 \times 10^{-9} – 2.0 \times 10^{-6})</td>
<td>(2.0 \times 10^{-8})</td>
<td>Hong et al. (2013)</td>
</tr>
<tr>
<td>AuNP/MIPs: (p-ATPG)-Au electrode</td>
<td>(2.0 \times 10^{-3} – 5.6 \times 10^{-7})</td>
<td>(7.8 \times 10^{-9})</td>
<td>Xue et al. (2013)</td>
</tr>
<tr>
<td>MIPs(NA)/CuO@GCE</td>
<td>(2.0 \times 10^{-9} – 2.5 \times 10^{-5})</td>
<td>(8.0 \times 10^{-9})</td>
<td>This work</td>
</tr>
</tbody>
</table>

* Si:ITO- silanized indium tin oxide electrode.
* MWNTs: multiwalled carbon nanotubes.
* GSCR: graphene sheets/congo red.
* OPD: o-phenylenediamine.
* AABA: 3-acrylamidophenylboronic acid.
* AuNP: gold (Au) nanoparticles.
* p-ATP: p-aminobenzenethiol.
Yang, S., Li, G., Yin, Y., Yang, R., Li, J., Qu, L., 2013. J. Electroanal. Chem. 703, 45–51.
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