Short communication

Novel electrochemical sensing platform based on magnetic field-induced self-assembly of Fe₃O₄@Polyaniline nanoparticles for clinical detection of creatinine

Tingting Wen, Wanying Zhu, Cheng Xue, Jinhua Wu, Qing Han, Xi Wang, Xuemin Zhou, Huijun Jiang

* School of Pharmacy, Nanjing Medical University, Nanjing 210029, PR China
b The First People’s Hospital of Kunshan, Kunshan 215300, PR China

ARTICLE INFO

Article history:
Received 15 November 2013
Received in revised form
21 December 2013
Accepted 3 January 2014
Available online 10 January 2014

Keywords:
Electrochemical sensor
Self-assembly
Magnetic field-induced
Molecularly imprinted polymers
Creatinine

ABSTRACT

A novel electrochemical sensing platform based on magnetic field-induced self-assembly of Fe₃O₄@Polyaniline nanoparticles (Fe₃O₄@PANI NPs) has been for the first time fabricated for the sensitive detection of creatinine in biological fluids. The template molecule, creatinine, was self-assembled on the surface of Fe₃O₄@PANI NPs together with the functional monomer aniline by the formation of N–H hydrogen bonds. After pre-assembled, through the magnetic-induction of the magnetic glassy carbon electrode (MGCE), the ordered structure of molecularly imprinted polymers (MIPs) were established by the electropolymerization and assembled on the surface of MGCE with the help of magnetic fields by a simple one-step approach. The structural controllability of the MIPs film established by magnetic field-induced self-assembly was further studied. The stable and hydrophilic Fe₃O₄@PANI can not only provide available functionalized sites with which the template molecule creatinine can form hydrogen bond by the abundant amino groups in PANI matrix, but also afford a promoting pathway for electron transfer. The as-prepared molecularly imprinted electrochemical sensor (MIES) shows good stability and reproducibility for the determination of creatinine with the detection limit reached 0.35 nmol L⁻¹. In addition, the highly sensitive and selective MIES has been successfully used for the clinical determination of creatinine in human plasma and urine samples. The average recoveries were 90.8–104.9% with RSD lower than 2.7%.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Creatinine, the dehydrogenated form of creatine, is an important clinical index of renal glomerular filtration rate, diabetic nephropathy, renal failure and muscular dystrophy (Hallan et al., 2004; Jacobi et al., 2008). The normal range of serum creatinine is 0.6–1.5 mg dL⁻¹ for a healthy adult. When one’s serum creatinine rises above 8–10 mg dL⁻¹, hemodialysis or peritoneal dialysis may be required to maintain the normal functions of kidney. It is essential to monitor serum creatinine for a patient with chronic kidney disease (Coresh et al., 2003).

The most commonly used methods for the clinical determination of creatinine are based on colorimetry, a nonenzymatic method, in which the chromogenic agents react with creatinine to form a colored complex (Husdan and Rapoport, 1968). However, the applicability of these methods is usually limited to monitoring the level of creatinine rapidly and reliably for two reasons (Shin et al., 2001). Firstly, the processes are complicated, costly and time-consuming. Secondly, most methods are of poor selectivity due to severe interferences from the metabolites in biological samples (Weber and van Zanten, 1991). There are also several promising techniques developed for monitoring creatinine in blood, including enzymatic catalysis of creatinine (direct and indirect) and antibody-based affinity detection (Chou et al., 2009; Khan and Wernet, 1997; Lad et al., 2008). The existing detections mostly are based on enzymatic electrocatalysis of creatinine. However, the enzymes had several disadvantages such as high cost for practical applications and relatively poor stability. Therefore, searching for new biomimetic sensing method for the accurate and rapid determination of creatinine in biological fluids is of great significance.

Electrochemical sensors have many advantages, such as high sensitivity, good controllability, rapid response and real-time detection (Wu et al., 2012), and can detect creatinine conveniently because of its electroactive nature (Lad et al., 2008). Nonetheless, selectivity becomes its bottleneck in the development due to the complex matrix in biological samples. Recently, molecularly imprinted electrochemical sensor (MIES), a newly analytic device which combined molecularly imprinted technique (MIT) with electrochemical sensor, has received...
much attention of all the feasible approaches to solve the problem (Huynh et al., 2013; Wen et al., 2012; Xue et al., 2013; Xie et al., 2010; Yuan et al., 2011). Owing to the three-dimensional specific molecular recognition of molecular imprinted polymers (MIPs) films, the MIES has high selectivity towards the target analyte. The as-prepared MIPs could be regarded as the artificial receptor to recognize the target molecule by the stereo-shape ability as well as the covalent or non-covalent interactions (Ma et al., 2011). However, the MIPs modified sensor (MIES) typically suffered from low sensitivity because MIPs are lack of conductivity and electrocatalytic activity.

In recent years, magnetic nanoparticles (MNPs) have been attracting much interest in the fields of separation science, biological and medical applications (Majetich and Jin, 1999; McHenry et al., 1994; Sung et al., 2013; Sun et al., 2000; Zeng et al., 2002). Especially, functionalized Fe₃O₄ NPs have new features such as higher hydrophilicity and stability and which may prompt the applications in many fields such as electrochemical devices, electromagnetic interference, non-linear optics, microwave-absorbing materials, electromagnetic shielding, molecular electronics, catalysis, etc. (Gangopadhyay and De, 2000; Hong et al., 2005; Malinauskas, 2001; Yang et al., 2009).

Self-assembly of functional nanoparticles (NPs) is one of the most promising methods for the preparation of novel materials and devices with exceptional properties (Chien et al., 2002; Li et al., 2011; Puntes et al., 2001; Tanase et al., 2001), which has been identified as an important process where the building blocks spontaneously organize into ordered structures by thermodynamic and other constraints (Jia and Gao, 2008; Nandiyanotty et al., 2013; Sheparovych et al., 2006; Sun and Murray, 1999). NPs were organized into ordered structures either through direct interactions (e.g. by interparticle forces) or indirectly using a template or an external field. Although the layer-by-layer (LBL) self-assembly technique has been so far extensively used in electrochemical sensors (Araki et al., 2013; Li et al., 2013), it is a usually time-consuming scheme with the complicated preparing process (Pedro et al., 2013; Zhu et al., 2010). More recently, external fields (e.g. magnetic field, electric field, electron beam, light and laser, etc.) have been emerged as key methods to direct the assembly of NPs. Specifically, through magnetic field, MNPs can be oriented and assembled by dipolar interactions (Chen et al., 2010; Hoffmann et al., 2013; Lattuada et al., 2011; Paterno et al., 2012), which gives rise to a specific directional self-assembly, enabling MNPs to form orderly geometric structure.

Herein, by magnetic field-induced self-assembly of Fe₃O₄@PANI NPs, a novel MIES for clinical detection of creatinine was prepared through a one-step approach. The conducting MIPs film was constructed by the electropolymerization at a constant potential in the pre-assemble solution containing creatinine, aniline and hydrophilic Fe₃O₄@PANI NPs by one-step approach. Moreover, the ordered structure and thickness of the film can be easily controlled by changing the concentration of Fe₃O₄@PANI NPs. The experimental condition has been optimized by both quantum chemical computation and the experimental results. Fe₃O₄@PANI NPs and the structure of the MIP film have been characterized by Raman spectra, Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscope (SEM). The prepared MIES was also characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Furthermore, the MIES has been successfully applied to detect trace creatinine in human plasma and urine.

2. Experimental

2.1. Reagents and materials

Creatinine was purchased from Shenzhen Maxchentech Co., Ltd. (Shenzhen, China). Sarcosine, bilirubin, creatine were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Ascorbic acid (AA) and uric acid (UA) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ammonium persulfate and aniline were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade. The phosphate buffer (PB) solution was prepared by mixing the stock solution of Na₂HPO₄ and Na₂HPO₄. The electrolyte solution was prepared by double distilled water and purged with nitrogen for 20 min before using.

2.2. Apparatus

MGCE (ϕ=5 mm, magnetic flux density=0.066 T) was purchased from Tianjin Incole Union Technology Co., Ltd. (Tianjin, China). Electrochemical data were obtained with a three-electrode system using an electrochemical analyzer (CHI660D, China). The scanning electron microscope (SEM) characterization was carried out by S-3400NRT (Hitachi company, Japan). Raman spectra were performed by using DXR Smart Raman Spectrometer excited with 633 nm laser radiation (Thermo Scientific, USA).

2.3. Samples

Human plasma and urine were obtained from healthy subjects. Human plasma was placed under −20 °C before analysis. The urine was refrigerated after adding methylbenzene (1%) as antiseptic.

2.4. Synthesis of Fe₃O₄@PANI

Fe₃O₄ NPs were synthesized according to the literature reported (Wang et al., 2013). 1.72 g FeCl₂·4H₂O and 4.72 g FeCl₃·6H₂O were dissolved in 80 mL of water. The mixture was magnetically stirred and purged with nitrogen gas, and then 10 mL aqueous ammonia was added. The reaction was stirred for 1 h at 80 °C. After that, the Fe₃O₄ NPs were washed by deionized water until neutral. Then the obtained Fe₃O₄ NPs were dispersed in 100 mL of 1 mol L⁻¹ HCl by stirring for 15 min, 4 mL aniline was added with stirring for another 30 min. Subsequently, 8 mL of 0.1 mol L⁻¹ ammonium persulfate was added dropwise, and incubated for 3 h at 0–5 °C. The dark green Fe₃O₄@PANI NPs were collected by an external magnetic field, washed by 1 mol L⁻¹ HCl, ethanol, deionized water in sequence, and dried in vacuum.

2.5. Preparation of MIES

A mixed 5 mL PB solution (pH=6.5) containing 45 mmol L⁻¹ aniline, 2.5 mmol L⁻¹ creatine and 0.25 mg mL⁻¹ Fe₃O₄@PANI NPs, was placed in dark at room temperature. After incubating for 4 h under a nitrogen atmosphere, the functional monomers and template molecules were pre-assembled through the hydrogen-bond interaction, and the nanocomposite of Fe₃O₄@PANI–creatinine–aniline was formed. The freshly polished MGCE was immersed in the pre-assembled solution and then physically attracted the nanocomposite through magnetic force for 10 min. Subsequently, the MIES was prepared by constant potential electrochemical polymerization at 0.8 V for 500 s. Finally, the electrode was immersed in 0.5 mol L⁻¹ H₂SO₄ and treated with a constant potential of −0.4 V for 3 min to remove the template molecules, and dried under nitrogen. The route for the fabrication of MIES was demonstrated in Fig. 1. Non-molecular imprinted electrochemical sensor (NMIES) was identically prepared except for the addition of template molecules.
2.6. Experimental measurements

Electrochemical measurements were carried out either in 5.0 mL aqueous solution containing 1 mmol L\(^{-1}\) of K\(_3[Fe(CN)]_6\) and 0.1 mol L\(^{-1}\) of KCl or in PB solution (pH = 6.5). The CV method was employed with potential range from 0.2 V to 0.4 V, scan rate 100 mV s\(^{-1}\). In differential pulse voltammetry (DPV) measurements, the scan was performed from 0.4 V to 0.6 V, pulse width, pulse period and quiet time were 0.025 s, 0.3 s and 2 s, respectively.

3. Results and discussion

3.1. Characterization of Fe\(_3O_4@PANI\) NPs

In the FT-IR spectrum of Fe\(_3O_4@PANI\) NPs (Fig. S2), the peaks at 1563 and 1135 cm\(^{-1}\) are attributed to C=Ns\(ftenging\)–N\(=
\(tftenging\)–C=C\(=\) stretching vibration and C−C stretching mode for the benzenoid rings, respectively. The band at 812 cm\(^{-1}\) assigns to aromatic C−H bending out of the plane of the 1,4-disubstituted aromatic ring, which suggests that PANI has been synthesized. The peak appeared at 594 cm\(^{-1}\) in the composite is due to Fe−Os\(ftenging\) stretching band of Fe\(_3O_4\) (Reddy et al., 2008). The results of Raman spectrum of Fe\(_3O_4@PANI\) NPs were consistent with the FT-IR (Fig. S3).

3.2. Optimization of experimental parameters

The suitable amount of functional monomer and template is a significant factor for the study as it will affect the affinity and imprinting effect. When creatinine is adsorbed, the specific imprinted sites will be occupied. A higher \(\Delta I_p\) indicates more creatinine adsorption, which suggests that more specific imprinted cavities have been formed. When the mass concentration ratio of creatinine to aniline was 1:14.8 with creatinine mass concentration 0.283 g L\(^{-1}\), the highest response would be obtained (Figs. S4A and S4B).

The amount of Fe\(_3O_4@PANI\) NPs affected the structure and thickness of the MIPs film. Excessive Fe\(_3O_4@PANI\) NPs formed a too thick MIPs film and the templates embedded too deep to remove, which would directly impact on the number of the imprinted cavities and the conductivity of the electrode. The mass concentration ratio of creatinine to Fe\(_3O_4@PANI\) NPs was optimized (Fig. S4C) and the best ratio was 1:14.8:0.9 when the MIES had maximum sensitivity.

The performance of the MIPs film is related to electropolymerization time. The obtained film was green, and the color was deepened with the extension of the scan time. The current response increased with the prolongation of the electropolymerization time (Fig. S4D). However, when the time is longer than 500 s, the shape of CV redox peaks in the [Fe(CN)\(_6\)]\(^{3−/4−}\) probe solution was unobvious for the interference by the redox peaks of PANI. For this reason, 500 s has been chosen.

The pH value relates to the existence form of the template molecule and affects the MIPs film adsorption. The pH of the analysis solution was investigated in the range of 6.0–8.0. Under acidic conditions, creatinine molecules are mainly keto form in which the amount of negative oxygen ions is low while PANI is with positive charge. In that case, the adsorption was decreased due to charge exclusion. When pH increased from 6.0 to 6.5, the amount of negative oxygen ions enhanced, and the protonation of the N atom of creatinine’s guanidine group is weakened. These two factors may promote the MIPs film adsorption. Therefore, \(\Delta I_p\) increased with the pH lower than 6.5. When the pH value is further increased, the PANI could not form quaternary ammonium cations effectively, which may result in the decrease adsorption of creatinine. The MIES obtained at pH 6.5 had maximum current response (Fig. S4E).

3.3. SEM characterization

Fig. 2A, B and C showed the cross section of the MIPs film prepared in the optimal concentration ratio obtained from the above experiment with the Fe\(_3O_4@PANI\) NPs concentration of 0.1 mg mL\(^{-1}\), 0.25 mg mL\(^{-1}\), 0.4 mg mL\(^{-1}\), respectively. The thickness of MIPs film increases with the increasing concentration of Fe\(_3O_4@PANI\) NPs, which indicates that the thickness of the
polymer at the microscopic level could be controlled by varying the Fe3O4@PANI NPs concentration. Fig. 2D and E showed the MIPs film prepared with and without magnetic field (the magnet in the MGCE can be removed). It is obvious that the MIPs film synthesized with magnetic field is uniform distribution. Compared with Fig. 2D, the SEM image in Fig. 2E showed relatively sparse and scattered structure. This suggested the Fe3O4@PANI NPs can be uniformly arranged on the surface of MGCE by external magnetic field, which can effectively and conveniently control the formation of sensing film.

3.4. Electrochemical behavior of MIES

CV has been used to characterize the preparation process of the imprinted sensor. Fig. 3A showed the cyclic voltammograms of bare electrode and modified electrode in the [Fe(CN)6]3−/4− probe solution. A pair of well-defined quasi-reversible peaks is shown at the bare MGCE (curve a). After electropolymerization, the redox peak currents and area of the cyclic voltammogram increased (curve b). It might be contributed to the excellent electrical conductivity of PANI and Fe3O4@PANI NPs which could greatly enhance the electron transfer rate of [Fe(CN)6]3−/4−. After template elution, the redox peak currents and area of the cyclic voltammogram increased once more, indicating that imprinted cavity caused by the removal of creatinine molecule formed the channels for the probe [Fe(CN)6]3−/4− to arrive at the electrode surface (curve c). MIES’s adsorption to creatinine caused the decrease of redox peak current value, which may be because the imprinted cavities have been occupied by creatinine and the electron transfer of [Fe(CN)6]3−/4− was blocked (curve d).

Fig. 3B illustrated the results of the CV method of MIES prepared with and without Fe3O4@PANI NPs. It is obvious that the peak current and area of MIES prepared with Fe3O4@PANI NPs (curve c) was higher and larger than the one prepared without Fe3O4@PANI NPs (curve b), indicating that the MIES prepared with Fe3O4@PANI NPs owned better conductivity and electrocatalytic activity than the one without Fe3O4@PANI NPs. By appropriately doping Fe3O4@PANI NPs into MIPs, the drawbacks of MIPs, such as low conductivity and electrocatalytic activity, can be overcome. Meanwhile, the MIES can be constructed more conveniently by a one-step approach based on magnetic field-induced self-assembly of Fe3O4@PANI NPs.

3.5. Evaluation of the adsorption characteristic of MIES and NMIES

The adsorption kinetics of MIES was investigated with three concentration levels of creatinine solution at different time intervals. We could deduce that the adsorption reached the equilibrium within approximately 20 min (Fig. S5). Moreover, the adsorption isotherms of MIES and NMIES have been plotted in Fig. S6.

The selectivity property of MIES was investigated with 0.5 μmol L−1 creatinine and 100-fold mass ratio of interferents containing AA, sarcosine, bilirubin, creatine and UA (structure shown in Fig. S7). Fig. 3C showed that the DPV response in PB solution (pH=6.5) of creatinine was almost consistent. This indicates that the MIES had a high selectivity for recognizing creatinine in the presence of interferents.

3.6. Reproducibility and stability

Five batches of MIES were investigated to compare their $I_p$ after adsorption in 0.5 μmol L−1 creatinine PB solution and the RSD was
3.8%, confirming that the preparation method was highly reproducible. The reproducibility of MIES was investigated by comparing \( I_p \) at room temperature for 10 times detection with the same electrode. The RSD calculated was 1.9%, which demonstrated that the MIES had good reproducibility. The MIES retained a response of 95% after 15 days storage at 4°C and decreased within 10% after 30 days, which indicated a good long-term stability.

3.7. Method validation and sample analysis

The DPV response in PB solution (pH=6.5) of MIES shows a good linear relation with serious concentration of creatinine solution. As shown in Fig. 3D, the linear range was \( 2.0 \times 10^{-10} \)–\( 1.0 \times 10^{-8} \) mol L\(^{-1}\). The linear regression equation was expressed as \( I (\mu A) = 0.173 (\mu mol L^{-1}) + 0.047 \) with the correlation of 0.996. The limit of detection (LOD) was 0.35 nmol L\(^{-1}\) (S/N = 3), which was compared with the other methods in Table S1. The as-proposed MIES was also used for the determination of creatinine in human plasma and urine. The analytical results were 0.18 \( \mu \)mol L\(^{-1}\) for human plasma sample and 0.25 \( \mu \)mol L\(^{-1}\) for urine sample, and the recoveries were 90.8–104.9% with RSD < 2.3% for human plasma sample and 93.3–101.3% with RSD < 2.7% for urine sample (Table S2).

4. Conclusions

A biomimetic MIES based on magnetic field-induced self-assembly was fabricated for the sensitive detection of creatinine for the first time. In our system, the \( \text{Fe}_3\text{O}_4@\text{PANI} \) NPs can be oriented and assembled orderly on the surface of MGCE by magnetic field-induced self-assembly, leading to the formation of uniformly MIPs film with high sensitivity. The thickness of the MIPs film could be easily controlled and the assembly can be completed by a one-step approach under the directional induction of the magnetic electrodes. Furthermore, the predetermined property of MIPs prompts their applications for detection of different targets in complex matrix by changing template molecules. The simple, controllable, inexpensive MIES with high sensitivity, selectivity and preferable dynamics characteristic was established and realized the rapid determination of creatinine in biological fluid. While the influences on the structure and properties of MNPs–MIPs nanocomposites by magnetic field-induced have been received considerable attention, the role of magnetic field in self-assembly and fabrication of MIES will be further studied.

Acknowledgment

This work was supported by National Natural Science Foundation of China (Nos. 81273480 and 21175070).

Appendix A. Supplementary materials

Supplementary materials associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.01.013.