A novel electrochemical sensor for paracetamol based on molecularly imprinted polymeric micelles

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\textbf{A B S T R A C T}

A novel molecular imprinted polymeric (MIP) micelle was prepared via macromolecule self-assembly of an amphiphilic photo-crosslinkable copolymer in combination with a molecular imprinting technique using paracetamol as the template molecule, and applied as a molecular recognition element to construct paracetamol (PCM) electrochemical sensor. The template molecules (PCM) were imbedded in the copolymer micelle during the self-assembly micellization of amphiphilic copolymers through the interactions between PCM and copolymer chain. A robust MIP film was formed in situ on the electrode surface by electrodeposition of the MIP micelles and subsequent photo-crosslinking, leading to successful construction of a MIP sensor. Using differential pulse stripping voltammetry (DPSV), selective detection of PCM in a linear concentration range of 1 μM–4 mM was obtained, revealing wider linear response and higher upper detection limit of detection compared to previously reported PCM electrochemical sensors, which was attributed to numerous effective recognition sites among the polymer matrix due to the large specific surface area of MIP micelles. In addition, this MIP sensor showed excellent selectivity to PCM, and the interferences from structure similar analogs were effectively avoided. Excellent stability and repeatability has also been exhibited. Finally, it was successfully applied to detect PCM in real samples with good recoveries. Together, these results indicate that our MIP sensor is a promising platform for accurate and reproducible detection of PCM.

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1. Introduction

Paracetamol (N-acetyl-p-aminophenol or acetaminophen, PCM) is the most widely used antipyretic and analgesic drug in the world [1]. It is not only an effective and safe analgesic agent used for the relief of mild to moderate pain associated with headache, arthritis, backache, toothaches and postoperative pain, but also used for reduction of fevers of viral and bacterial origin [2,3]. Generally, paracetamol does not exhibit any harmful side effects. However, abnormal level of paracetamol is believed to be associated with the formation of some liver and nephrotoxic metabolites [4,5]. In addition, the use of acetaminophen in children younger than one year may cause an increase in rhinoconjunctivitis, asthma, and eczema [6]. Therefore, determination of PCM in biological samples and quality control in pharmaceuticals is very important considering the enormous interest of PCM for therapeutic purposes. Many analytical methodologies based on different principles, such as titimetry [7], spectrophotometry [8], chromatography [9], capillary electrophoresis [10], chemiluminescence [11] and flow-injection analysis [12] have been developed for the analysis of PCM. However, these methods require expensive instruments, long analysis time, highly skilled technician and laborious sample pretreatment, which make them unsuitable for routine analysis. Taking the above mentioned above and the high electroactivity of PCM into consideration, electrochemical analytical techniques for PCM determination has been widely explored method due to simplicity, high sensitivity, low cost, easy operation and possibility to miniaturization [13–16]. However, during the electrochemical determination of PCM, the interferents such as dopamine, aminophenol, ascorbic acid, uric acid all have the similar oxidation potential to PCM [17–19]. Especially, dopamine and aminophenol even have similar structure with PCM. Thus, the development of highly selective, sensitive and rapid analytical methods for determination of PCM in the presence of excess interferents is of great importance.

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In recent years, molecular imprinting has become a powerful tool for the preparation of polymeric materials with special recognition ability [20,21]. Molecular imprinting technique involves forming a complex of template molecules and functional monomers and subsequent copolymerization with crosslinking monomers. Then removal of template molecules by extraction process generates the recognition cavities complementary to the shape, size and functional groups of template. In this way, an end product MIPs with high specificity can be achieved. In addition, the resultant MIPs have the advantages of good mechanical/chemical stability, easy and cheap preparation, the flexibility in choosing copolymerizing monomers and low cost, which render them promising alternatives to enzymes, antibodies, and natural receptors for use in areas including biosensors, bioseparation, medical diagnostics, catalysis and drug delivery [22–24]. Although the bulk MIPs prepared by the conventional method exhibit high selectivity, some disadvantages such as time-consuming and complicated preparation process, low-affinity binding, high diffusion barrier, low-rate mass transfer and poor site accessibility [25,26].

To solve these problems, an effective approach has been developed by the preparation of nano-sized MIPs. This is done because nanostructured imprinted materials have a small dimension with extremely high surface-to-volume ratio, most of template molecules are situated at or approximate to the materials surface, which are expected to possess several remarkable advantages over normal imprinted materials (e.g., more complete removal of templates, higher binding capacity and faster binding kinetics) [27]. Up to now, molecular imprinting has been reported at nanostructures such as nanospheres, nanoshells and nanowires/nanotubes [28–31]. Despite the great achievements, the exploration of new imprinted materials ideally suitable for molecular recognition elements in chemo-sensors remains a great challenge. Recently, Li et al. reported molecular imprinting at polymer micelles by self-assembling an amphiphilic block copolymer in an organic-selective media [32]. The self-assembly micellization of amphiphilic copolymers is a kind of micro-phase separation behavior that generally happens in a selective solvent: a mixture of two immiscible solvents, one of which is a non-solvent for at least one component in the amphiphilic copolymer [33,34]. Thanks to certain interactions between the template molecule and the polymer, numerous template molecules are trapped into the self-assembled aggregates and thus fulfill the molecular imprinting. Due to the small size of molecular imprinting micelle, it showed high rebinding capacities toward the template molecules, which provided a new platform as molecular imprinting matrix. However, in Ding’s work, the molecular imprinting was carried out in organic solvent which significantly limit their applications in the field of biotechnology.

Here, a novel kind of molecularly imprinted polymer micelles was synthesized by self-assembly micellization of amphiphilic copolymers in aqueous solution containing PCM and applied as molecular recognition element to construct a novel electrochemical MIP-based sensor for voltammetric selective and sensitive detection of PCM. PCM templates were facilely imprinted into copolymer micelles during the self-assembly micellization of amphiphilic copolymers through the interactions between PCM and copolymer chain as well as hydrophobic effect. Then, the MIP micelles were deposited onto the electrode to form a MIP film via direct electrodeposition, and the subsequent photocrosslinking locked the recognition cavities in the polymer matrix and enhanced the film’s robustness. Finally, the removal of the PCM template from the film results in spatially organized rebinding sites (cavities) to generate the MIP electrode which have affinity and selectivity toward the analyte. The whole strategy is depicted in Fig. 1. The obtained MIP sensor showed a sensitive and selective detection of PCM in aqueous solution.

2. Experimental

2.1. Chemicals and reagents

PCM, dopamine and 4-aminophenol (analytical grade) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the other reagents and chemicals were of analytical grade and used as received. All solutions were prepared with ultra-pure water (18.2 MΩ cm) from a NW Ultra-pure Water System (Henan, China).

2.2. Instruments

MIP micelles morphology was examined using a Hitachi H-7500 transmission electron microscope (TEM) operating at 80 kV for TEM observations. UV-vis spectra were recorded on a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). Dynamic light scattering (DLS) was carried out using an ALV-5000 laser light scattering spectrometer. The zeta potential and Z-average diameter results were obtained from a Zetasizer Nano ZS90 (Malvern, UK) instrument. The irradiation light for photo-crosslinking was obtained from a UV–vis spot curing system (PowerARC UV 100, Blue Sky Special Lamps Co., Ltd., Hebei, China) combined with a 365 nm filter, and was applied vertically from above the electrode. The morphology of the MIP film on the electrode surface was observed using a Hitachi S-4800 field emission scanning electron microscope (FESEM) operating at 5 kV. Controlled potential electrolysis (CPE), differential pulse stripping voltammetry (DPSV) and cyclic voltammetry (CV) were performed in a three-electrode system with an Epsilon electrochemical work-station (BAS, USA). The bare gold electrode (4 mm diameter; Aida, Tianjin, China), the MIP electrode or the MIP sensor was used as the working electrode. A Pt plate electrode and saturated calomel electrode (SCE, Aida) were used as counter and reference electrodes.

Fig. 1. Schematic illustration of the MIP sensor fabrication using the self-assembly MIP micelles and electrodeposition technique.
respectively. All potentials applied to the working electrode were referred to SCE.

2.3. Preparation

2.3.1. Preparation of the amphiphilic copolymer

The amphiphilic copolymer was prepared as follows (see SFig. 1 in Supporting Information): an acrylic copolymer was synthesized via free radical polymerization using dimethylamino ethylmethacrylate (DMA, 20 mmol), 2-hydroxy ethylacrylate (HEA, 20 mmol), 2-ethylhexyl acrylate (EHA, 32 mmol) and styrene (St, 28 mmol). Further postfunctionalization introduced a urethane group and cross-linkable acrylate side groups into the polymer to form the novel photo-crosslinkable amphiphilic copolymer using isophorone diisocyanate (IPDI) as bridges. The polymers formed were then dissolved in acetone and recovered by precipitation into excess of 1:4 (v/v) methanol/water and dried in vacuum at room temperature. The NMR and FT-IR characterization have been supplemented in the supporting information (SFigs. 2 and 3), which gave convincing evidence to the structure of the copolymer.

2.3.2. Preparation of MIP micelles

The photo-crosslinkable amphiphilic copolymer, the template molecules PCM and the diphenylketone as photoinitiator were dissolved in tetrahydrofuran (THF) to give a 20 wt% copolymer solution, containing 5 wt% PCM and 0.2 wt% diphenylketone. Meanwhile, a certain amount of lactic acid was added and the resulted solution was stirred overnight to induce PCM complexation with the copolymer. An equal amount of water as non-solvent, was added drop wise into the acidic polymer solution with stirring to induce the self-assembly and micellization of the copolymer. During this process, the solution had blue opalescence. After the addition of water, the obtained solution was stirred for 2 h and then added to a large amount of distilled water to stabilize the micelles. The final prepared MIP micelles solution contained approximately 20 mg/mL of the photo-crosslinkable copolymer. Non-imprinted polymeric (NIP) micelles were similarly prepared but in the absence of the template molecules.

2.3.3. Preparation of the MIP sensor

A bare gold electrode (4 mm in diameter) was cleaned by polishing with alumina (Aida) under sonication in ethanol or water after each step. The electrode was then cleaned with a hot mixture of “piranha” solution (a 1:3 mixture of 30% H2O2 and conc. sulfuric acid), rinsed with ultra-pure water and dried. The MIP micelles solution prepared in Section 2.3.2 was used as a bath solution for electrodoposition. After electrodoposition was carried out via CPE at −2 V for 120 s, the electrode was covered with a MIP film which was then subjected to UV light irradiation for 3 min (UV light generated by a spot-curing system with a wavelength of 365 nm and a power of 16 mW/cm²). Finally the template molecules were removed from the film by extraction with a mixture of acetone and methanol at a ratio of 1:9 (v/v) overnight. Extraction of the templates was monitored by differential pulse stripping voltammetry (DPSV), until the oxidation peaks of PCM disappeared in the DPSV voltammogram. The obtained electrode, the so-called “MIP sensor”, was stored in ultra-pure water before use to avoid any contamination from dust. For comparison, a non-imprinted control electrode (NIP sensor) was fabricated following the same procedure for the MIP sensor, but using non-imprinted micelles instead of MIP micelles.

2.4. Electrochemical measurements

The MIP electrode was immersed into a sample solution containing a certain concentration of PCM, which is the accumulation process, for different time. After rinsing with double distilled deionized water to remove the PCM non-specifically adsorbed on the film, the electrode was transferred into PBS solution. DPV measurements were then applied to detect the rebinding. After the measurements, the template-entrapped electrode was submitted to washing by a mixture of acetic acid and methanol with the purpose of removing PCM molecules in the MIP film and regeneration of the MIP sensor.

3. Results and discussion

3.1. Preparation of the molecularly imprinted polymer micelles

The synthetic route of the amphiphilic copolymer was shown in SFig. 1. DMA, HEA, EHA and St were chosen as the polymerizing units to prepare the copolymer. After the addition of lactic acid, the DMA unit is ionized bearing and becomes highly hydrophilic whereas EHA and St are hydrophobic, so the obtained copolymer (molecular weight $M_n = 5.6 \times 10^4$ g/mol) was an amphiphilic macromolecule whose micellization could be induced via the self-assembly in aqueous solution. In addition, the cationic property of DMA makes it possible for the MIP micelle to be electrodeposited onto the gold electrode. HEA provides a site through which a urethane group and cross-linkable acrylate side groups into the polymer using isophorone diisocyanate (IPDI) as bridges. The introduction of the double bond into the copolymer endows it cross-linking capability under UV light exposure, which would contribute to the subsequent formation of compact and robust MIP film and stabilization of the binding sites.

To prepare the MIP micelles, water as a non-solvent, was added carefully to the THF solution of the amphiphilic copolymer and PCM. As shown in Fig. 1, when water was gradually added to the THF solution of the copolymer, the hydrophobic moieties of the copolymer started to associate because of the enhanced hydrophobic interaction between the hydrophobic moieties and the selected solvent mixture of THF and H2O. Meanwhile, the hydrophilic segments tended to be exposed to the aqueous phase to maintain and stabilize the formed hydrophobic microphase. The self-assembly behavior of the copolymer was studied by the turbidity method [35]. The absorbance of a THF solution of the amphiphilic copolymer at 621 nm was measured for different water contents (by adding water dropwise), and the turbidity was calculated according to the following equation: $\tau = 1 - A^{-1}$, where $A$ is the absorbance of the solution. As shown in SFig. 4, the turbidity of the solution increased with the water content, and when the water content reached 11.6 vol.%, a turbidity jump occurred. This water content is defined as the critical water content (CWC) at which the hydrophobic segments start to aggregate. The inset in SFig. 4 shows a simple Tyndall scattering experiment for the obtained solutions. A strong light scattering signal was observed in the solution of the copolymer, confirming the formation of micelles [36].

During the assembly of the copolymer, PCM as the template molecule was entrapped into the resultant micelles through hydrogen bond (formed between ester and carbamate groups in the copolymer and hydroxyl and amide groups in PCM), constructing three-dimensional structures around the PCM molecule and producing numerous binding sites. Besides the hydrogen bonding, the hydrophobic effect also plays role in driving PCM into the copolymer micelle. The formation of micelles during the self-assembly process was further confirmed by the DLS and TEM study. As shown in Fig. 2A, the diameter of MIP micelle depended dramatically on the pH of solution. As the pH increased, the hydrodynamic radius $R_h$ of the MIP micelle decreased. The decrease of $R_h$ with the increasing pH is attributed to the weakening of the copolymer hydrophilicity with the deprotonation of DMA unit, leading to micelle
contraction and thus the decrease of the micelle size. A minimum $R_h$ of around 68 nm was achieved at about pH = 4.5 (Fig. 2B). When the pH value was higher than 4.5, the hydrodynamic radius increased significantly, which is possibly due to the aggregation of micelles. Considering that the smaller the micelles, the more efficient molecular imprinting, the pH of the MIP micelle solution was kept around 4.5. The inset of Fig. 2B presents the TEM images of the MIP micelle at pH 4.5. It can be observed that spherical-like MIP micelles were formed and dispersed with average hydrodynamic diameters of about 60 nm, which is in agreement with the result of DLS. These nano-dispersed MIP micelles are beneficial for molecular recognition due to their high specific surface area, which can provide a large number of recognition cavities. It should be also noted that at pH 4.5, the zeta potential of the MIP micelles was about +24 mV (SFig. 5) owing to the protonation of DMA, which ensures the following electrodeposition step.

3.2. The fabrication of MIP sensor

The MIP micelles were subsequently electrodeposited on the gold electrode to form MIP film using the controlled potential electrolysis (CPE). SFig. 6 depicted the mechanism of MIP micelles electrodeposition. It shows that during the electrodeposition process high localized pH is generated electrochemically at the cathode surface due to the hydrogen evolution reaction, and the MIP micelles switched from soluble to insoluble due to the deprotonation of the tertiary amino groups. As a result, they were then deposited on the electrode when they arrived at the electrode surface. The generation of a pH gradient at the cathode surface is well-established in electrochemical systems and has been used to explain the electrodeposition of chitosan [37,38]. Profiting from electrodeposition, the formation of MIP film was controllable and could be adjusted by deposition conditions, such as deposition time. Fig. 3A shows the current–time curve of MIP micelles electrodeposition process. It can be observed that during the electrodeposition the current decreased significantly within the first 20 s and then tended to be stable, suggesting the formation of MIP film on the electrode surface. SEM investigation further confirmed the formation of MIP film. As shown in Fig. 3B(b), after electrodeposition for 10 s, a large amount of nanoparticles were formed on the electrode, indicating the deposition of MIP micelles on the electrode. With elongating deposition time, more and more micelles were deposited onto the surface of electrode and gradually converted into a MIP film. Just as we expected, a continuous MIP film with a rough surface was observed after depositing 120 s, as shown in Fig. 3B(c). Therefore, the electrodeposition method for preparing the MIP sensor was conducted at $-2$ V for 120 s. Due to the existence of a large amount of double bond in the copolymer, UV irradiation could induce the crosslinking of the obtained MIP film. After photocrosslinking, the structure of the MIP film was locked and the stability was improved, leading to the generation of a robust MIP film which was able to resist the solvent extraction step.

Fig. 2. (A) The $Z$-average diameter (●) of the MIP micelles as a function of the solution pH. The sample solution was 0.1 wt% MIP micelles solution with 0.01 M NaCl and the pH was adjusted by lactic acid and NaOH aq. (B) DLS plot and TEM image (inset) of the MIP micelles formed by the amphiphilic copolymer self-assembly. The MIP micelles solution was 0.01 wt%, pH = 4.5.

Fig. 3. (A) The current–time curve of MIP micelles electrodeposition performed with CPE. Bath solution: 20 mg/mL MIP micelles solution, applied potential: $-2$ V, deposition time limit: 120 s. (B) SEM images of the surface of the bare gold electrode (a), the electrode covered with the MIP film ($-2$ V, 10 s) (b) and ($-2$ V, 120 s) (c).
As the blocking efficiency of the MIP film has great effect on the performance (e.g., selectivity and repeatability) of the resulted MIP sensor, cyclic voltammetry technique was used to investigate the quality of the MIP film on the gold electrode. Fig. 4 presents the voltammograms on the bare gold electrode and the electrode covered with the MIP film (deposited for 120 s) using K$_3$[Fe(CN)$_6$] as an electroactive probe. A pair of redox peaks characteristic of K$_3$[Fe(CN)$_6$] was observed on the bare gold electrode (curve a). After deposition for 120 s and crosslinking by UV irradiation, no redox peaks could be observed (curve b), indicating a compact MIP film was formed with good blocking efficiency on the electrode surface. Once the template PCM was extracted from the crosslinked MIP film by soaking, the K$_3$[Fe(CN)$_6$] redox peaks reappeared (curve c) because the formation of vacant recognition sites or binding cavities made mass and electron transfer possible, and K$_3$[Fe(CN)$_6$] could therefore pass through the cavities of the MIP film to reach the electrode surface. In the meanwhile, the peak current of K$_3$[Fe(CN)$_6$] reflects the effective surface area of the electrode which can be determined by the Randles–Sevcik method [39]. It can be observed that deposition of the MIP film on gold electrode reduced electrode surface area by approximately 98%, indicating the total loss of the conductive area, which further confirm that the gold electrode has been completely covered by a compact MIP film with good blocking efficiency. After the template was extracted, the electrode surface area was increased to some degree compared to that before template removal, supporting the presence of vacant cavities in the MIP film for K$_3$[Fe(CN)$_6$] transfer and signal transduction.

3.3. Performance of the MIP sensor

3.3.1. Electrochemical behavior of paracetamol on the MIP electrode

The electrochemical behavior of paracetamol was investigated on MIP and NPC electrode using differential pulse voltammetry (DPV). It is well known that PCM in aqueous solution gives a well-defined oxidation peak originating from the oxidation of paracetamol to N-acetyl-p-quinoneimine on solid electrodes [40]. As shown in Fig. 7, a distinct characteristic oxidation peak of PCM at around 485 mV could be observed on the MIP electrode after incubation of the MIP electrode in 2 mM PCM solution. As a comparison, no such oxidation peak could be observed on the NPC electrode since the non-imprinted polymer micelles have no cavities for binding PCM. As a consequence, the above results demonstrated that the MIP sensor has recognition capacity toward PCM molecules, and the recognition capacity was originated from the imprinting effect.

3.3.2. Effect of incubation time

The incubation is a fairly important step since its direct influence on the degree of adsorption on the surface of electrode and the sensitivity of the electrochemical sensor. The dependence of the peak current of PCM at the preconcentration time on the sensitivity properties was studied under 2 mM PCM (pH = 7.0). Fig. 5 shows the change of the peak current with the incubation time (0–500 s). It was found that the peak current recorded with the MIP sensor increased significantly at the initial stage and then reached a steady-state current after 350 s, suggesting that the adsorption of PCM has reached a saturation state at the surface of the sensor. Obviously, the adsorption kinetics of PCM on the MIP film are very fast and completes within 350 s. Therefore, the incubation time was set as 350 s for the determination of PCM on the MIP electrode.

3.3.3. Analytical performance

In order to obtain an analytical curve of the sensor for the target PCM, the sensitive DPV measurements at various concentrations of PCM were performed on the MIP electrode. As shown in Fig. 6A, the peak current intensity of PCM was found to increase with increasing PCM concentration. Under the same conditions, the response of the non-imprinted control electrode was independent of the PCM concentration, and stayed at very low values for all the concentrations within the test range. This result confirms the formation of recognition sites in the MIP film, which benefited PCM to bind with the MIP film. The calibration curve for the peak current versus the concentration of PCM exhibited a linear response ranged from 1 μM to 4 mM for the MIP sensor as shown in Fig. 6B. The linear regression equation was $i_{pa} \text{(Ma)} = 1.5009 + 5.0158C_{PCM} \text{(mM)}$ with a correlation coefficient of 0.994. The detection limit was estimated to be $3.3 \times 10^{-7}$ M based on the signal corresponding to three times the noise of the response. And the performance of the PCM detection on the basic of MIP electrode was compared with published PCM electrochemical sensors as shown in Table 1. It was exciting to discover that our proposed MIP sensor had much wider detection range with high upper limit for PCM sensing than other electrochemical sensors in the previous reports. The wider detection range with high upper limit was attributed to the large specific surface area of the MIP micelles. The nano-sized micelles (68 nm) has a small dimension with high surface-to-volume ratio and thus more recognition sites could be accommodated, which resulted in a larger number of effective binding caves in the fabricated MIP film.
Table 1
Comparison of the performance of our proposed MIP sensor with other published electrochemical sensors for PCM sensing.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Detection method</th>
<th>Linear range (µM)</th>
<th>Detection limit (µM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C60/GCE</td>
<td>DPV</td>
<td>50–1500</td>
<td>5</td>
<td>Goyal et al. [41]</td>
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<tr>
<td>PIP/PGE</td>
<td>DPV</td>
<td>5–500</td>
<td>0.79</td>
<td>Ozcan and Sahin [42]</td>
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<tr>
<td>PANI/MWCNTs/GCE</td>
<td>SWV</td>
<td>1–100</td>
<td>0.25</td>
<td>Li and Jorg [43]</td>
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<td>CILE</td>
<td>DPV</td>
<td>1–2000</td>
<td>0.3</td>
<td>Guan et al. [44]</td>
</tr>
<tr>
<td>Nafion/TiO2–graphene/GCE</td>
<td>DPV</td>
<td>1–100</td>
<td>0.21</td>
<td>Fan et al. [45]</td>
</tr>
<tr>
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<td>1.18</td>
<td>Seong et al. [15]</td>
</tr>
<tr>
<td>Boron-doped diamond electrode</td>
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<td>0.4–100</td>
<td>0.21</td>
<td>Svrca et al. [46]</td>
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<td>Wang et al. [47]</td>
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<td>12–120</td>
<td>2.0</td>
<td>Kumar et al. [48]</td>
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<tr>
<td>MIP</td>
<td>DPV</td>
<td>1–4000</td>
<td>0.33</td>
<td>Present work</td>
</tr>
</tbody>
</table>

Abbreviations: GCE: glassy carbon electrode; DPV: differential pulse voltammetry; PIP: paracetamol imprinted polypyrrole; PGE: pencil graphite electrode; SWV: square-wave voltammetry; CILE: carbon ionic liquid electrode; PGA: poly(glutamic acid); PAY: poly(acid yellow 9).

3.3.4. Selectivity of the MIP sensor

One of the important analytical factors for an amperometric chemsensor is its ability to discriminate the interfering species having electroactivities and structures similar to the target analyte. The selectivity experiments were carried out by using dopamine (DA) and 4-aminophenol as the similarities toward PCM detection and characterized by DPV. Among various analogs, DA and 4-aminophenol were tested due to their structural similarity and electrochemical activity (an overlapping oxidation potential on the bare electrode with PCM). Fig. 7 showed that the current responses of MIPs toward PCM were much higher than the other two analogs, and the ratio of peak current of DA and 4-aminophenol to PCM was 0.14 and 0.13, respectively. The non-specific adsorption was intended as a contribution to the current response of DA and 4-aminophenol on the MIP electrodes. Obviously, the MIP sensor gave good selectivity for the target analyte PCM. The high specificity was probably due to the shape of the cavities in the imprinted polymer matrix just fitting for the unique molecular structure of PCM, so they cannot bind other analogs tightly despite of the similar structure of DA and 4-aminophenol to PCM [49,50]. Thus, the above results suggested that the MIP electrode had molecular recognition and discrimination capabilities.

3.3.5. Stability and reproducibility of the MIP sensor

The long-term stability of our MIP sensor was investigated by examining the current response of the same MIP electrode in PCM solution after an interval of one week during storage in a refrigerator at 4°C. Fig. 8 shows that the MIP electrode exhibited only a small decrease in the signal current with a relative standard deviation (RSD) of 2.4%, demonstrating that the sensor response was quite stable and reversible. These results revealed that PCM could reversibly interact with the binding sites, and our MIP sensor was therefore expected to be able to be regenerated and used repeatedly. We considered that the excellent stability and repeatability was ascribed to the photo-crosslinking protocol during the sensor

Fig. 6. (A) DPV voltammograms of PCM of different concentration in PBS (pH = 7). From (a) to (e): 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.4, 0.8, 1, 2, 3, 4, 6, 7, 8 mM. (B) The plot of peak currents as a function of the concentration of PCM on MIP electrode (a) and NIP electrode (b). The red line indicates the calibration curve. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 7. The plot of peak currents of the different concentration of (a) PCM, (b) DA and (c) 4-aminophenol on MIP electrode.
preparation, which not only improved solvent resistance of the MIP film, but also locked the size and shape of the recognition caves.

3.3.6. Detection of acetaminophen in real samples

In order to evaluate the practical application of the proposed MIP sensor, the MIP electrode was applied to the analysis of a kind of paracetamol (150 mg/tablet) commercial tablet. The tablets were ground to powder and dissolved in 0.1 M PBS (pH 7.0). All the samples were determined in triplicate under the same conditions. The results were listed in Table 2. As shown in Table 2, the content of PCM in the tablet was calculated to be 143.1 mg/tablet on average, which is in good agreement with the label amount. In addition, the recovery of three independent experiments varied from 95.3% to 98.9%. These results indicate that this MIP electrode was reliable, effective and sensitive enough for the determination of acetaminophen in real pharmaceutical samples.

4. Conclusions

In this work, preparation of a novel molecularly imprinted polymer (MIP) micelle and its recognition property for paracetamol are investigated. The MIP micelle was prepared via macromolecule self-assembly of an amphiphilic copolymer in the presence of PCM and electrodeposited on the gold electrode to fabricate an electrochemical MIP sensor for detection of PCM. The resulting MIP sensor can specifically recognize PCM and determine PCM with a wide linear range from 1 μM to 4 mM. Compared to previously reported PCM electrochemical sensors, the wide linear response and high upper detection limit of our MIP sensor was attributed to numerous effective recognition sites among the polymer micelle matrix due to the nano-size and thus large specific surface area of the MIP micelle, confirming the promising prospect of micelles as imprinting matrix. We also showed that the fabricated MIP sensor could successfully and accurately detect the amount of PCM present in real pharmaceutical samples, indicating that the MIP electrode is a promising analytical platform for detecting PCM.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2013.07.088.


