

A novel electrochemical sensor based on molecularly imprinted polymers for caffeine recognition and detection

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Abstract A sensitive and selective electrochemical sensor based on molecularly imprinted polymers (MIPs) was developed for caffeine (CAF) recognition and detection. The sensor was constructed through the following steps: multi-walled carbon nanotubes and gold nanoparticles were first modified onto the glassy carbon electrode surface by potentiostatic deposition method successively. Subsequently, *o*-aminothiophenol (ATP) was assembled on the surface of the above electrode through Au–S bond before electropolymerization. During the assembled and electropolymerization processes, CAF was embedded into the poly(*o*-aminothiophenol) film through hydrogen bonding interaction between CAF and ATP, forming an MIP electrochemical sensor. The morphologies and properties of the sensor were characterized by scanning electron microscopy, cyclic voltammetry, and differential pulse voltammetry. The recognition and determination of the sensor were observed by measuring the changes of amperometric response of the oxidation-reduction probe, $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$, on modified electrode. The results demonstrated that the prepared sensor had excellent selectivity and high sensitivity for CAF, and the linear range was $5.0 \times 10^{-10} \sim 1.6 \times 10^{-7} \text{ mol L}^{-1}$ with a detection limit of $9.0 \times 10^{-11} \text{ mol L}^{-1}$ (S/N=3). The sensor was also successfully employed to detect CAF in tea samples.

Keywords Electrochemical sensor · Molecular imprinted polymers · Multiwalled carbon nanotubes · Gold nanoparticles · Caffeine

Introduction

Caffeine (CAF) (1, 3, 7-trimethylxanthine) is an active alkaloid widely distributed in natural products, including tea, coffee bean, cocoa, and cola nuts [1, 2]. CAF has many important pharmacological effects, such as the stimulation of the central nervous system in diuresis, positive effect on cardiovascular system, promotion of secretion of gastric acid, and alleviation of migraine. Therefore, the investigation and determination of CAF not only have clinical significances but can also give beneficial guidance to people's health and life.

The most common analytical methods used for the determination of CAF are high-performance liquid chromatography [2, 3], capillary electrophoresis [4, 5], tandem mass spectrometry [6], infrared linear dichroic and raman spectroscopy [7], and solid-phase molecular fluorescence [8]. These approaches are perfectly adequate in terms of their accuracy, but expensive instrumentation, highly skilled technicians, complicated, and time-consuming procedures are required. Electrochemical methods provide useful alternatives due to their simplicity, high sensitivity, good stability, low-cost instrumentation, and on-site monitoring. Several articles reported the electrochemical detection of CAF on different electrodes. Orlando et al. [9] prepared a boron-doped diamond electrode for the determination of paracetamol and CAF with a linear range of 5.0×10^{-7} to $8.3 \times 10^{-7} \text{ mol/L}$. Graphene was employed by Huang et al. [10] to fabricate a modified electrode for the sensitive determination of CAF with a detection limit of $1.2 \times 10^{-7} \text{ mol/L}$. Ly et al. [11] developed a new kind of CAF sensor by immobilizing DNA onto a carbon nanotube paste electrode. Compared with the ordinary carbon nanotube paste electrode, the former exhibited a broader linear range and a lower detection limit. However, electrochemical method is still not a very common way for CAF determination since its oxidation occurs at high

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potential, which overlaps with that of the discharge of the electrolytic solution [12].

As it is well-known, theophylline (ThPh) and other purin alkaloids coexist with CAF in real samples, which would influence the detection of CAF [3]. Many techniques have been employed to improve the selectivity of CAF determination. Molecular imprinting technique, the design and construction of a biomimetic receptor system with predetermined recognition for target molecule, has been proposed and developed rapidly in recent years [13–15]. Owing to the complementarity in shape and binding sites, the created nanocavities in synthesized molecularly imprinted polymers (MIPs) can act as artificial antibodies and exhibit high selectivity towards the imprinted molecules. The significant advantages in easy preparation, low cost, predictable specific recognition and high stability, enable MIPs to be applied in wide fields, such as solid-phase extraction, chromatography separation, drug releases, catalysts, and sensors [16–20]. Therefore, a sensor, combining the electrochemical method and molecularly imprinted technique, could be supposed to achieve the determination and recognition of CAF simultaneously. Yoshimi et al. [21] polymerized a thin layer of MIPs on electrically conductive indium-tin oxide electrode surface in the presence of theophylline. The modified electrode could recognize theophylline when CAF was used as an analogue. Nevertheless, no quantitative detection for a template molecule was provided. Alizadeh et al. [22] synthesized a CAF-selective MIP particle, which was further used for a carbon paste electrode preparation. The MIPs functioned as selective recognition elements and preconcentrator agents for CAF determination with good results, while it needed a long time for the synthesis and pretreatment of MIP particles.

With the development of nanoscience and nanotechnology, many nanomaterials have been extensively used to improve the sensitivity of electrochemical sensors. Multiwalled carbon nanotubes (MWNTs) and gold nanoparticles (GNPs), with high surface areas, outstanding electrical conductivity, good chemical and thermal stability, have often been adopted in fabricating electrochemical sensors [23–27]. Moreover, the interesting synergistic property of the composite of MWNTs and GNPs enable this kind of nanocomposite to become an attractive material in the preparation and application of electrochemical sensors [28].

Herein, MIPs and the nanocomposite of MWNTs and GNPs were combined to construct a novel electrochemical sensor for CAF recognition and detection. A functional monomer, *o*-aminothiophenol (ATP) assembled on the surface of the nanocomposite through Au-S bonds between ATP and GNPs [29]. The template molecule, CAF, was embedded simultaneously through the hydrogen bonding interaction between ATP and CAF. The prepared electrochemical sensor was characterized by scanning electron microscopy (SEM) and differential pulse voltammetry (DPV). The prepared electrochemical sensor

exhibited a high recognition capability toward CAF as well as a broad linear range and a low detection limit.

Experimental

Chemicals and reagents

Multiwalled carbon nanotubes (MWNTs) (diameter, 40–60 nm; length, 1–2 μm) were purchased from Shenzhen Carbon Nanotechnologies Co., Ltd. Caffeine (CAF), theophylline (ThPh), and theophylline-7-acetic acid (ThPh-7) were purchased from Alfa Aesar. Guanine (GUN) was obtained from Sanland-chem International, Inc. *o*-Aminothiophenol (ATP), tetrachloroaurate (III) acid (HAuCl_4), and tetrabutylammonium perchlorate were obtained from Aladdin Reagent Co., Ltd. Other reagents were commercially available as analytical reagent grade and were used without further purification. Phosphate-buffered solutions (PBS) (pH 6.5) were prepared by using $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{HPO}_4$ and KH_2PO_4 . Double distilled water was used throughout the experimental process.

Apparatus

All electrochemical measurements were performed on a CHI 660B electrochemical workstation (Chenhua Instruments Co., Shanghai, China) with a standard three-electrode configuration. A modified glassy carbon electrode (GCE) was served as a working electrode (3.0 mm in diameter), a saturated calomel electrode was used as a reference electrode, and a platinum wire electrode was employed as an auxiliary electrode. The morphology of a modified electrode surface was investigated by SEM (Hitachi S-4800, operated at 10 kV). The actual pH values were determined with a pH/ion analyzer model pHs-3CT (Da Pu Instrument Co., Ltd., Shanghai, China).

Pretreatment of MWNTs

Crude MWNTs of 0.5 g was added into the mixed solution of 50 mL of HNO_3 and 150 mL of H_2SO_4 under ultrasonication for 4 h at room temperature. The resulted suspension was filtered to recover the MWNTs. Then the solid was washed repeatedly with water for several times until the pH value of the filtrate reached 7.0. The collected solid was dried overnight, obtaining carboxylic groups functionalized MWNTs.

Preparation of GNP- and MWNT-modified GCE (GNPs/MWNTs/GCE)

Prior to the surface modification, the GCE was polished with 0.3 and 0.05 μm of alumina slurries, washed with water, and finally sonicated for 10 s. Then 2.5 mg of functionalized MWNTs was added to 5.0 mL of water and sonicated for

30 min to obtain a stable diluted suspension of MWNTs. After the three electrodes were dipped into the suspension, a potentiostatic deposition method was used to prepare the MWNT modified GCE (MWNTs/GCE) at a potential of 1.7 V for 1 h [30]. Then, the same method was employed to fabricate GNPs modified MWNTs/GCE (GNPs/MWNTs/GCE) by immersing of MWNTs/GCE into the mixed solution of $4.0 \times 10^{-3} \text{ mol L}^{-1}$ HAuCl₄ and 0.1 mol L^{-1} KNO₃ at a potential of -0.2 V for 180 s [31, 32].

Preparation of MIP-modified GNPs/MWNTs/GCE

MIP films on the GNPs/MWNTs/GCE were synthesized in the following steps. First, GNPs/MWNTs/GCE was immersed into $5.0 \times 10^{-3} \text{ mol L}^{-1}$ tetrabutylammonium perchlorate solution, containing $1.5 \times 10^{-2} \text{ mol L}^{-1}$ ATP and $5.0 \times 10^{-3} \text{ mol L}^{-1}$ CAF, for self-assembling about 12 h. Second, the above electrode was electropolymerized via cyclic voltammetry scan at a potential range of -0.4 to $+1.4 \text{ V}$ in the same solution at 50 mV s^{-1} for 30 cycles. Finally, the electrode was immersed into 0.2 mol L^{-1} HCl under stirring for 1 h to remove template molecules, obtaining MIP-modified GNPs/MWNTs/GCE (MIPs/GNPs/MWNTs/GCE). The preparation procedure of the imprinted sensor is shown in Scheme 1.

As a control, non-imprinted polymer (NIP)-modified electrodes (NIPs/GNPs/MWNTs/GCE) were prepared in the same way except for the addition of template molecules during the assembled and electropolymerized process.

Electrochemical measurements

$[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ was chosen as an electrochemical probe to investigate the properties of the prepared sensors due to the nonelectroactivity of CAF in chosen potential window. Electrochemical experiments were carried out in PBS containing 10 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$. Differential pulse voltammetry (DPV) were used to investigate the changes of peak current intensity of electrochemical probe. In DPV measurements, the scan was performed from -0.2 to 0.8 V at a rate of 50 mV s^{-1} , pulse width, pulse period, and quiet time were 0.05, 0.2, and 2 s, respectively. ThPh, GUN, and ThPh-7 were

chosen as the structural similar molecules to evaluate the recognition capacity of the prepared sensor. The oxidized peak currents of 10 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in PBS solution containing a tenfold excess of ThPh, GUN, or ThPh-7 over CAF were recorded.

Results and discussion

SEM characterization of the modified electrodes

Surface morphology and microstructure of a sensor greatly affect its performance. The SEM images of MWNTs/GCE, GNPs/MWNTs/GCE, and MIPs/GNPs/MWNTs/GCE are shown in Fig. 1a, b, c, respectively. As shown in Fig. 1a, MWNTs are distributed on the surface of GCE and form a three-dimensional network structure, which enable the MWNTs/GCE to have a much higher specific area. This modified MWNT layer is much more stable than that which is modified by dropping method, which also facilitates the modification of the subsequent steps. It was obvious that the GNPs were coated uniformly onto the MWNT surface to form the composite of GNPs/MWNTs on the GCE surface, as shown in Fig. 1b. This kind of composite would promote the electron-transfer rate and improve the sensitivity of sensor. Moreover, GNPs could form Au-S bonds with polymerized monomer ATP when GNPs/MWNTs/GCE was immersed into ATP solution, as illustrated in Scheme 1. The coexisted CAF could be further assembled onto the GNPs/MWNTs/GCE surface through hydrogen bonding interaction between residual amino groups of ATP and the nitrogen/oxygen atom of CAF. After the assembled and electropolymerized procedures, the morphology of the sensor surface could be seen in Fig. 1c. Compared to the GNPs/MWNTs/GCE, the size of the network structure increased remarkably, indicating the formation of the MIPs on the surface of GNPs/MWNTs/GCE.

Optimization of imprinted sensor working conditions

The pH value of PBS would affect the electrochemical performance of the sensor. As shown in Fig. 2, the imprinted sensors

Scheme 1 Scheme of fabrication of MIPs/GNPs/MWNTs/GCE

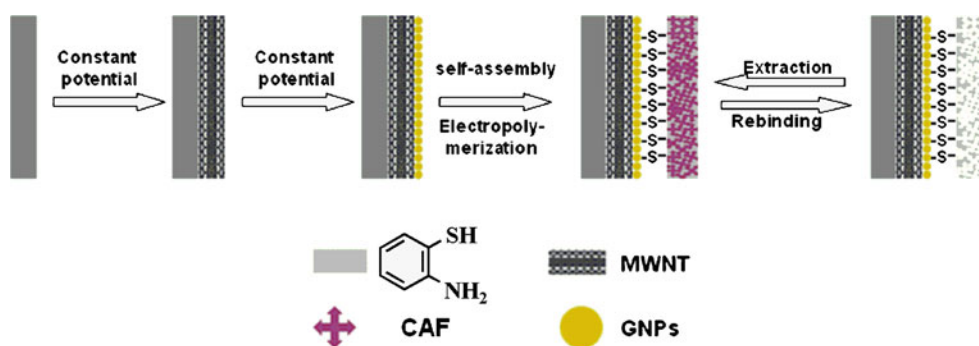
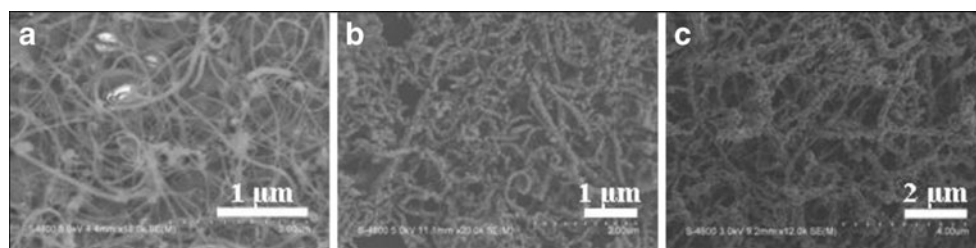


Fig. 1 SEM images. **a** MWNTs/GCE, **b** GNPs/MWNTs/GCE, **c** MIPs/GNPs/MWNTs/GCE



were tested by DPV at a constant concentration of CAF (5 mmol L^{-1}) in PBS with the pH value range from 5.0 to 9.0, containing 10 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$. The current intensity difference of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ on a sensor was observed before and after immersing the sensor into CAF solution. It was obvious that the current intensity difference increased with the increase of pH at the beginning and then decreased as the pH increased further, and the highest current intensity difference was obtained at pH 6.5. Therefore, pH 6.5 PBS was used in the further study.

The incubation time was also an important factor for estimating the performance of the imprinted sensor. The incubation time experiments of the imprinted sensor were carried out in 0.1 mol L^{-1} PBS (pH 6.5) containing 10 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ and 5 mmol L^{-1} CAF, and the results are shown in Fig. 3. The current intensity of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ almost reached an equilibrium after 8 min, revealing a rapid dynamic adsorption of CAF molecules to the prepared sensor. Compared to the incubation time of other CAF MIPs reported, the present sensor only needed a remarkably shorter time, which could be contributed to the network structure of the imprinted film [33]. Most imprinted cavities situated in the surface and approximately to the surface of the MIP films, which made the recognition sites much more accessible for the template molecules and took a shorter time to gain adsorption equilibrium. Thus, the optimum incubation time should be 8 min for the determination of CAF.

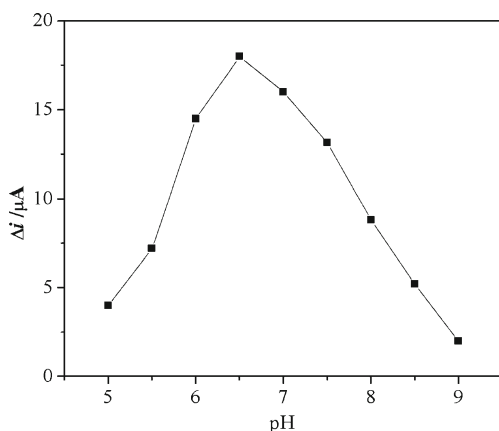


Fig. 2 pH influence on the imprinted sensor in PBS containing 10 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ and 5 mmol L^{-1} CAF

The ratio of ATP to CAF during electropolymerization also influences the performance of imprinted sensors. The sensor was investigated by varying the ATP to CAF molar ratio of 5:1, 4:1, 3:1, 2:1, and 1:1. Five imprinted sensors were fabricated according the proposed ratio with a constant concentration of CAF (5 mmol L^{-1}), respectively. The results showed that the sensor with the ratio of 3:1 exhibited the largest current intensity of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$. Therefore, the ratio 3:1 of ATP to CAF was chosen for the imprinted sensor construction.

The thickness of the MIP film was controlled by changing the number of scan cycles, which was found to affect the sensitivity and stability of the sensor. The scan cycles varied from 10 to 50 in this research in order to optimize film thickness. Polymer films formed less than 20 scan cycles were found to be unstable. However, higher scan cycles formed a thicker film with less accessible imprinted sites, leading to the gradual decrease of current intensity with further increase in the number of scan cycles. The current intensity of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ at MIPs/GNPs/MWNTs/GCE indicated that the number of optimum electropolymerization cycles was 30.

It is difficult to achieve a satisfied sensitivity and reproducibility if template molecules cannot be extracted from the imprinted film completely. Ethanol, HCl (0.1 mol L^{-1} , 0.2 mol L^{-1}), H_2SO_4 (0.1 mol L^{-1} , 0.2 mol L^{-1}), and NaOH (0.1 mol L^{-1} , 0.2 mol L^{-1}) were used as extraction solutions to remove template molecules, respectively. The largest current response of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ was obtained after an electrode was incubated into 0.2 mol L^{-1} of HCl for 1 h under

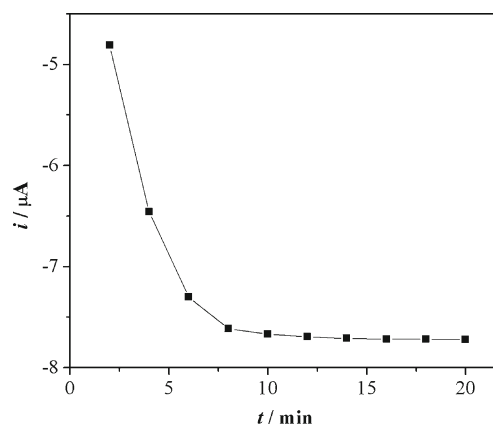


Fig. 3 Effect of incubation time on the current response on MIPs/GNPs/MWNTs/GCE

continuous stirring. In addition, the MIPs/GNPs/MWNTs/GCE could be regenerated for several times by using this kind of elution. Therefore, soaking in 0.2 mol L⁻¹ HCl for 1 h was chosen as the best condition for template molecule extraction.

Electrochemical performance of the sensor

Determination of CAF

The dependence of the reduction peak current of electrochemical probe at MIPs/GNPs/MWNTs/GCE under different CAF concentration was recorded by DPV. As shown in Fig. 4, the absolute current intensity decreased obviously with the increase of CAF concentration, which indicated that an increased adsorption of CAF on the sensor surface occurred. This portion of CAF blocked the electrochemical probe to pass through the MIP film and reach the surface of the electrode, leading to the decrease of absolute current intensity. Detailed experimental results revealed that the peak currents were proportional to the concentrations of CAF in the range of 5.0×10⁻¹⁰ to 1.6×10⁻⁷ mol L⁻¹ with a detected limit of 9.0×10⁻¹¹ mol L⁻¹ (S/N=3). Compared with MIPs/MWNTs/GCE, MIPs/GNPs/MWNTs/GCE exhibited a broader linear range and a lower detected limit. The reason may be the fact that the GNPs promoted the electron transfer rate of [Fe(CN)₆]³⁻/[Fe(CN)₆]⁴⁻ [34].

Selectivity of MIPs/GNPs/MWNTs/GCE

Different potential interferent or structure similar substance, such as ThPh, GUN, or ThPh-7 was added into the solution to evaluate the selectivity of MIPs/GNPs/MWNTs/GCE. As shown in Fig. 5, a tenfold excess of ThPh, GUN, or ThPh-7 over CAF hardly caused the significant change of current intensity of [Fe(CN)₆]³⁻/[Fe(CN)₆]⁴⁻. In addition, the selectivity of NIPs/GNPs/MWNTs/GCE was also evaluated. No perceivable change on the current intensity of [Fe(CN)₆]³⁻/[Fe

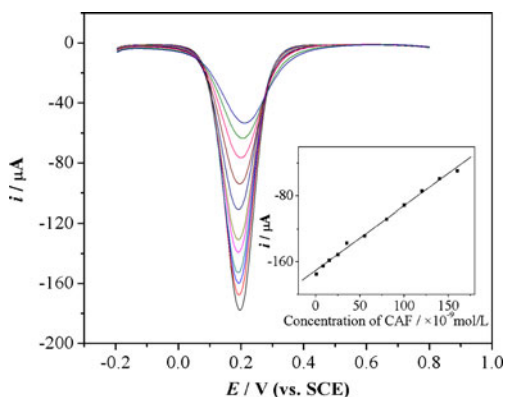


Fig. 4 DPV net current responses of MIPs/GNPs/MWNTs/GCE after 8 min of incubation with different concentrations of CAF in PBS. Inset: standard curve of CAF concentration vs. current

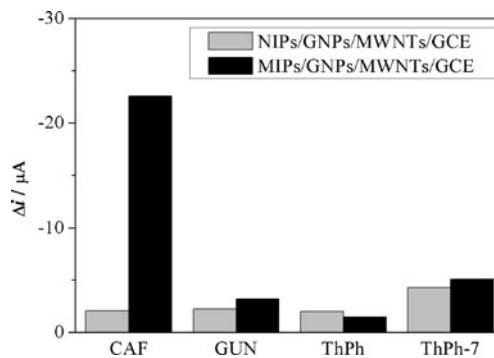


Fig. 5 Selectivity of MIPs/GNPs/MWNTs/GCE

(CN)₆]⁴⁻ could be observed with the increase of CAF, ThPh, ThPh-7, or GUN concentration on the surface of NIPs/GNPs/MWNTs/GCE. These results indicated that MIPs/GNPs/MWNTs/GCE had a good recognition capability to CAF.

Regeneration and stability of MIPs/GNPs/MWNTs/GCE

The reproducibility of the sensor was investigated by comparing the current of DPV response to 10 mmol L⁻¹ [Fe(CN)₆]³⁻/[Fe(CN)₆]⁴⁻ in the presence of 1×10⁻³ mol L⁻¹ CAF at five MIPs/GNPs/MWNTs/GCE prepared independently. Relative standard deviation is 3.3 %, which indicated that the sensor displayed an acceptable reproducibility. After the first electrochemical determination of CAF, MIPs/GNPs/MWNTs/GCE was immersed in 0.2 mol L⁻¹ HCl to remove the bound CAF from the MIPs film. MIPs/GNPs/MWNTs/GCE was then incubated in [Fe(CN)₆]³⁻/[Fe(CN)₆]⁴⁻ solution containing the same concentration of CAF for the next electrochemical measurement. The current intensity of [Fe(CN)₆]³⁻/[Fe(CN)₆]⁴⁻ decreased to about 91.2 % of the initial value after being used more than ten binding/detection/extraction cycles.

The stability of the imprinted sensor was tested in 0.1 mol L⁻¹ PBS containing 10 mmol L⁻¹ [Fe(CN)₆]³⁻/[Fe(CN)₆]⁴⁻ and 1×10⁻³ mol L⁻¹ CAF. The current response of the imprinted sensor decreased to 96.9 % after storing for 2 weeks at 4 °C. The results demonstrated that the prepared electrochemical sensor had excellent regeneration property and stability, which provided a new class of polymer-modified electrodes for sensor applications.

Table 1 The content of caffeine in different tea samples

Sample	CAF (mg/100 g)	Recovery (%)
1	15.2	103.5
2	10.6	97.7
3	8.7	105.3
4	6.4	99.2

Analysis of real samples

Four kinds of commercial tea leave samples were treated according to the literature with light modification [35]. The tea samples were dried at 80–90 °C overnight and weighed. Each of the dry tea leaves (5 g) was incubated in 50 mL of boiling water three times and then centrifuged. Three milliliters of supernatant was diluted ten times by $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ for further detection. The samples were spiked with different amounts of standard CAF solution, and then the total amount of CAF was measured under the optimum conditions and calculated by calibration curve (analytical equation: $y = -174.80558 + 0.82446x$). The recovery rates of the spiked samples were estimated to be between 97.7 % and 105.3 % as shown in Table 1. The results were in acceptable agreement and good reproducibility, which suggested that the proposed approach would be a promising method for CAF determination.

Conclusions

In this work, we have developed a novel electrochemical sensor for CAF recognition and detection by combining MIPs and the nanocomposite of MWNTs and GNPs. Compared to the MIPs/MWNTs/GCE, the MIPs/GNPs/MWNTs/GCE showed a broader linear range and a lower detection limit. Moreover, the MIPs/GNPs/MWNTs/GCE possessed a specific recognition capability for CAF over other structures similar compounds as well as excellent stability and regeneration. Therefore, the novel, simple strategy reported here can be further used to recognize and detect other template molecules.

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