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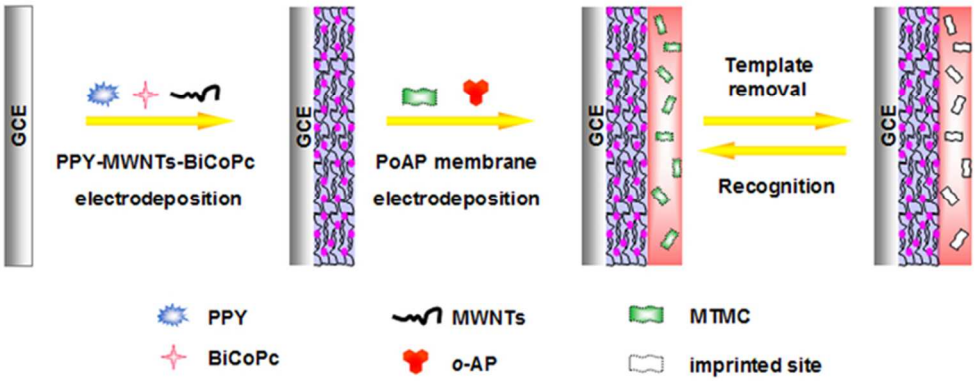

Graphical Abstract

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Electrochemical sensor based on a bilayer of PPY/MWCNTs/BiCoPc composite and molecularly imprinted P*o*AP for sensitive recognition and determination of metolcarb

Ling-Jie Kong, Ming-Fei Pan, Guo-Zhen Fang, Xin-lei He, Yin-qiang Xia, Shuo Wang*

Key Laboratory of Food Nutrition and Safety, Ministry of Education of China, Tianjin University of Science and Technology, Tianjin 300457, China

*Corresponding author: Shuo Wang

Tel: (+86 22) 60601456

Fax: (+86 22) 60601332

E-mail: s.wang@tust.edu.cn

Abstract

A molecularly imprinted electrochemical sensor for metolcarb (MTMC) detection was designed and constructed by electropolymerizing a poly-*o*-aminophenol (P*o*AP) membrane in the presence of MTMC after the modification of a composite that consisted of polypyrrole (PPY), functionalized multiwalled carbon nanotubes (MWNTs) and binuclear phthalocyanine cobalt (II) sulfonate (BiCoPc) on a glassy carbon electrode (GCE) surface. The modified electrodes were characterized by scanning electron microscopy (SEM) and cyclic voltammetry (CV). The molecularly imprinted based sensor had a good binding ability toward MTMC upon measuring the variation of the amperometric response of the oxidation-reduction probe, $K_3Fe(CN)_6$. The relative peak current response was found to be proportional to the concentration of MTMC in the range of 1.0×10^{-8} to 0.6×10^{-6} mol L^{-1} with a detection limit of 7.88 $\times 10^{-9}$ mol L^{-1} (S/N = 3). This desirable sensitivity may be attributed to the presence of the PPY-MWNTs-BiCoPc composite layer, which enhanced the electrode surface area and amplified the current signal. The sensor showed good selective affinity toward MTMC, compared with similar molecules, with good reproducibility and long-term stability. The prepared sensor was successfully applied to the determination of MTMC residue in spiked vegetable samples with satisfactory recoveries ranging from 88.8% to 93.3%.

Keywords: molecularly imprinted P*o*AP membrane, metolcarb, polypyrrole, functionalized multiwalled carbon nanotubes, binuclear phthalocyanine cobalt (II) sulfonate.

1. Introduction

The development of biological and chemical sensors for specific molecule analysis has received considerable recent attention with research being conducted in various fields including food safety [1], bioprocess control [2, 3], environmental monitoring [4] and clinical field [5-7] . Their high selectivity toward target molecules depends on the recognition element, which is usually derived from living organisms like enzymes [8, 9] and antibodies [10, 11]. However, most of these materials are expensive natural biomacromolecules and they generally have poor stability against high temperatures, harsh chemical environments, or extreme pH conditions, and this makes their wide application difficult.

Molecularly imprinted polymers (MIPs), also referred to as biomimetic receptors, are artificial polymeric materials that have the ability to specifically bind molecules with significant advantages in terms of ease of use and low preparation cost as well as good mechanical and chemical stability under extreme conditions [12-14]. Therefore, MIPs may be used to overcome the limitations of biomacromolecules and can be used as sensitive components in the development of biological and chemical sensors [15-17]. For the construction of MIP-based sensors, in situ electropolymerization is a promising method by which an ultrathin polymeric membrane can be easily synthesized and adhered to a transducer surface of any shape, size and thickness by controlling the amount of charge transferred [18-22]. However, because of the high density and poor conductivity of electropolymerized molecularly imprinted membranes, it will have fewer imprinted sites and a slow electron transfer

rate on modified electrode surfaces, which restricts the analytical efficiency of the proposed MIP-based sensors.

Multiwalled carbon nanotubes (MWNTs), since their discovery in 1991, have been the subject of comprehensive studies in various fields [23]. Owing to their unique three-dimensional structures, high mechanical and chemical stability, large surface-to-volume ratios, low resistivities and good biocompatibility, MWNTs can be used as promising nanomaterials in the construction of electrochemical biosensors because they increase the electrode's surface area and they facilitate electron transfer [24-26]. Polypyrrole (PPY) is one of the most extensively studied conducting polymers and has been widely used in the development of sensor devices because it can be polymerized in a facile manner at neutral pH to form a stable membrane under ambient conditions [27, 28]. To improve the efficiency and broaden the applications of PPY, a dopant is usually part of the PPY polymer matrix [29]. Among the various dopants, phthalocyanines, particularly binuclear phthalocyanines (such as binuclear phthalocyanine cobalt (II) sulfonate, BiCoPc) are desirable because of their large π electron conjugated system, excellent electron storage and transfer ability, and their strong electrocatalytic behavior [30]. Producing a composite of PPY, MWNTs and binuclear phthalocyanine should combine the advantages of these materials. Composite materials may possess complementary properties because of synergistic effects and thus increase the total performance of the sensing component.

Metolcarb (MTMC) is an important N-methylcarbamate pesticide and is widely used in agricultural production because of its broad spectrum of activity and low

bioaccumulation potential [31]. However, its pesticidal action results from the inhibition of acetylcholinesterase transmission at nerve endings is potentially hazardous to human health [32, 33]. Until recently, analytical methods for MTMC residue determination in agricultural products were generally based on chromatography [34, 35] and immunoassays [36, 37]. Although these methods are sensitive and specific, a number of shortcomings still restrict their practical use and these include expensive instrumentation, time-consuming procedures, and the poor chemical or physical stability of antibodies and enzymes. The development of an inexpensive, rapid and stable but sensitive method for MTMC residue analysis is still a challenge.

We thus report a rapid electrochemical strategy for MTMC determination by electrodepositing a molecularly imprinted poly-*o*-aminophenol (P*o*AP) membrane on a GC electrode surface modified with a PPY-MWNTs-BiCoPc composite. As far as we know, this is the first attempt to fix the PPY-MWNTs-BiCoPc composite material onto the surface of a GC eletrode to construct a MIP-based sensor. The resultant sensor demonstrated that the modified composite layer can effectively improve the performance of the imprinted electrodeposited membrane to provide a more sensitive analysis of MTMC.

2.1 Reagents and chemicals

Pyrrole, template MTMC, and the other analytes tested including carbaryl, isoprocarb and propoxur (Fig. 1) were purchased from Sigma–Aldrich (Madrid, Spain). *o*AP, MWNTs and BiCoPc were purchased from Alfa Aesar (Tianjin, China),

Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China) and Shanghai Dibo Chemical Technology Co., Ltd., (Shanghai, China), respectively. Potassium ferricyanide ($K_3[Fe(CN)_6]$) and the other chemicals used in our experiments were purchased from Tianjin No. 1 Chemical Reagent Factory (Tianjin, China) and were of analytical grade. Doubly deionized water (DDW, 18.2 MΩ cm) was used throughout and was obtained from a Water Pro water purification system (Labconco, Kansas City, USA).

Stock solutions of the individual analytes (MTMC, carbaryl, isoprocarb and propoxur) were prepared in methanol with a concentration of 1.0 mmol L^{-1} and stored at 4 °C. The corresponding working solutions were obtained by diluting the individual stock solutions with DDW.

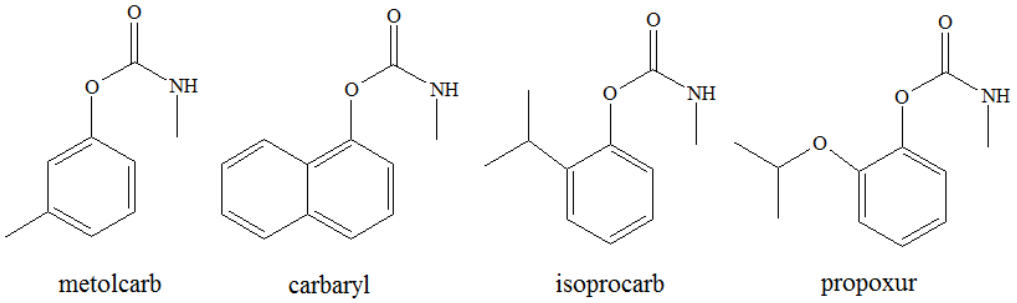


Fig.1 Chemical structures of MTMC, carbaryl, isoprocarb and propoxur

2.2 Apparatus

All the electrochemical experiments were carried out at room temperature using a LK 2006 electrochemical workstation (Tianjin Lanlike Chemical and Electronic High Technology Co., Ltd., China) connected to a personal computer. A typical three-electrode configuration was employed consisting of a bare or modified GC electrode as the working electrode, a saturated calomel electrode (SCE) and platinum foil as the reference electrode and counter electrode, respectively. Scanning electron

microscopy (SEM, SU1510, Hitachi, Japan) was used to observe the morphology of the surface of various modified electrodes.

2.3 Preparation of the Imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC Electrode

500.0 mg of crude MWNTs were added to 60.0 mL of concentrated HNO_3 under sonication for 10 min. The mixture was then refluxed under stirring at 85 °C for 12 h. After cooling to room temperature, the mixture was separated by filtering through a 0.22 μm polycarbonate membrane and it was washed thoroughly with DDW until the pH was neutral. The obtained solid product was denoted MWNTs-COOH, and it was found to enhance the dispersion and stability of the crude MWNTs [34].

The preparation of the PPY-MWNTs-BiCoPc-GC electrode was conducted as follows: the bare GC electrode (d = 4 mm) was polished carefully to a mirror-like surface with 0.3-0.05 μm alumina aqueous slurry and it was then successively cleaned with nitric acid (1:1, v/v), ethanol and DDW. MWNTs-COOH (6.0 mg) was dispersed into the pyrrole aqueous solution (50.0 mmol L^{-1}, 6.0 mL) containing BiCoPc (6.0 mg). After sonication for 1 h, chronoamperometry was employed to electrodeposit the PPY-MWNTs-BiCoPc composite onto the bare GC electrode surface by immersing the electrode in the above-mentioned solution using a constant potential of 0.7 V for 180 s. Additionally, we prepared a PPY-GC electrode, a PPY-MWNTs-GC electrode and a PPY-BiCoPc-GC electrode using the same conditions as above in a relevant solution.

To electrochemically synthesize a P*o*AP membrane onto the surface of the

PPY-MWNTs-BiCoPc composite the resulting electrode was immersed in a N_2-deoxygenated perchloric acid solution (0.1 mol L^{-1}, pH 5.5) containing *o*AP (20.0 mmol L^{-1}), MTMC (10.0 mmol L^{-1}) with a small amount of methanol as cosolvent because of the poor solubility of MTMC in water. The electropolymerization was performed by eight consecutive cyclic voltammetry scans over the potential range of -0.1 - 0.8 V (versus the SCE) at a scan rate of 50 mV s^{-1}. After the electropolymerization, the P*o*AP membrane modified PPY-MWNTs-BiCoPc-GC electrode was rinsed with a water:methanol solution (4:6, v/v) three times for 30 min each to remove the template molecules trapped within the polymer matrix. Control studies were performed by fabricating a non-imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode without an additional template during electropolymerization. A schematic diagram of the whole imprinted sensor preparation procedure is shown in Fig. 2.

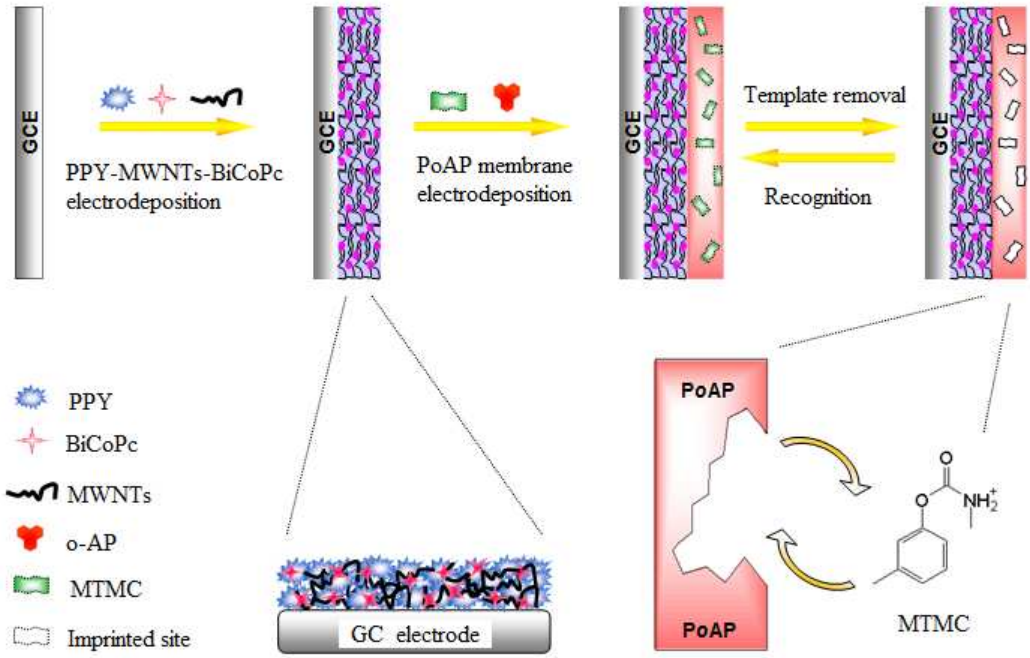


Fig. 2 Preparation procedure for the imprinted PPY-MWNTs-BiCoPc-GC electrode

2.4 Measurement experiments

Cyclic voltammetry (CV) was used to characterize the different modified electrodes and to evaluate the rebinding of MTMC onto the imprinted and non-imprinted PPY-MWNTs-BiCoPc-GC electrodes. The CV experiments were performed from -0.2 to +0.6 V at a scan rate of 100 mV s^{-1} in a standard solution of $K_3[Fe(CN)_6]$ (1.0 mmol L^{-1}) containing KNO_3 (0.2 mol L^{-1}). In the template rebinding experiments, when the initial current response was stable, a small aliquot of analyte solution was injected by microsyringe and the resulting current responses were recorded and used for data analysis. All measurements were taken at room temperature.

2.5. Sample preparation

Cucumber and cabbage samples obtained from a local market were determined to be free of MTMC by HPLC and were used to perform spiking and recovery studies. The samples were cut and then 2.0 g of a sample was packed into a 50 mL polypropylene centrifuge tube and spiked with MTMC at 40.0 μg kg^{-1}, 200.0 μg kg^{-1}, and 400.0 μg kg^{-1}. 10.0 mL methanol was added to the tube and it was thoroughly shaken (5 min). The obtained mixture was centrifuged at 4000 rpm for 10 min. The extraction procedure was repeated twice and the supernatant was combined and evaporated to dryness at 60 °C under reduced pressure. Finally, the dried extract was redissolved in 10.0 mL of 5% methanol-standard $K_3[Fe(CN)_6]$ solution and filtered using a 0.22 μm filter before electrochemical analysis.

3. Results and discussion

3.1. Molecular imprinting electropolymerization

Figure 3A shows representative cyclic voltammograms during the P*o*AP membrane electropolymerization process at the PPY-MWNTs-BiCoPc-GC electrode surface in the presence of MTMC. A large *o*AP anodic peak was observed at a potential of 0.43 V in the first positive-direction potential scan and it completely disappeared in the second anodic scan. No cathodic peak was obtained during the negative-direction potential scan. These results suggest that the process was an entirely irreversible oxidation process and that the active groups ($–NH^{3+}$) of *o*AP can be utilized to immobilize the template molecules during the positive potential scan [39]. The current intensity decreased sharply under continuous cyclic scanning and stabilized after five cycles and this seems to be related to the progressive formation of an insulating P*o*AP membrane that covers the surface of the PPY-MWNTs-BiCoPc-GC electrode leading to a suppression of the voltammetric response. No significant differences were observed for the cyclic voltammograms in the presence or absence (Fig. 3B, curve c) of MTMC, which indicates that MTMC did not show electrochemical activity in the chosen potential window. Therefore, its structure was not electrochemically altered during electropolymerization. Additionally, the cyclic voltammograms for the electropolymerization of the P*o*AP membrane on the PPY-MWNTs-BiCoPc-GC electrode exhibited the highest anodic peak current (≈ 180 μA) compared with the membrane electrodeposited onto the bare GC electrode (Fig. 3B, curve a, ≈ 70 μA) and the PPY-BiCoPc-GC electrode (Fig. 3B, curve b, ≈

120 μA). This high anodic peak current can be explained by the excellent electrical conductivity and large surface area of the modifed PPY-MWNTs-BiCoPc composite layer.

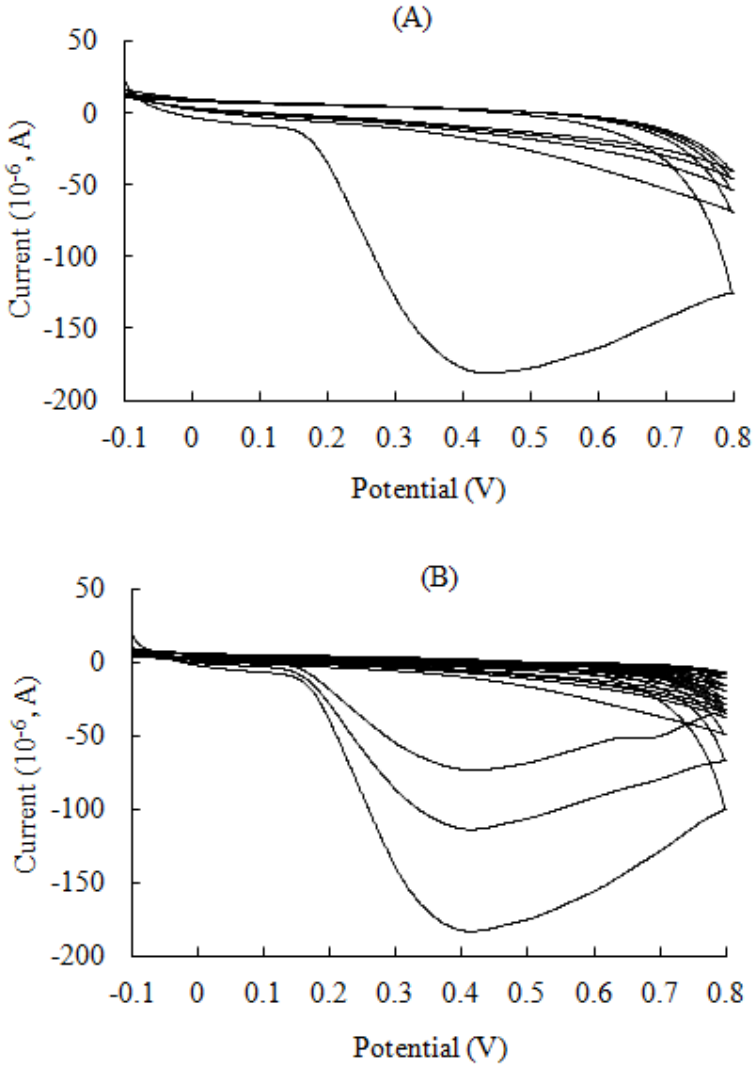


Fig. 3 (A) Cyclic voltammograms for the electropolymerization of the PoAP membrane at the surface of the PPY-MWNTs-BiCoPc-GC electrode in the presence of MTMC. Scan rate: 50 mV s^{-1}, cycle number: 5. (B) Cyclic voltammograms for the electropolymerization of the PoAP membrane at the surface of (a) the bare GC electrode, (b) the PPY-BiCoPc-GC electrode, and (c) the PPY-MWNTs-BiCoPc-GC electrode in the MTMC absence. Scan rate: 50 mV s^{-1}, cycle number: 8.

3.2. Characteristics of the modified electrode

3.2.1. Morphological characterization

The surface morphology of the various modified electrodes were compared by SEM. Fig. 4A shows a SEM image of the PPY-MWNTs-GC electrode and it is clear that the MWNTs were distributed over the GC electrode surface with a typically three-dimensional tubular structure and numerous clusters were formed, indicating that the MWNTs tend to aggregate in the PPY layer. When BiCoPc was doped into the PPY layer, a vastly different morphology results (Fig. 4B) as the homogeneous PPY layer contains a larger amount and more uniform MWNTs. This rough composite layer structure increased the electrode surface area and influenced the amount of imprinted sites formed for the following electropolymerization. As shown in Fig. 4C, after the electrodeposition of the P*o*AP membrane at the surface of the PPY-MWNTs-GC electrode the size of the network structures composed of MWNTs and BiCoPc increased remarkably. This can be explained by the sufficient encapsulation of the composite layer by the polymer membrane. These results demonstrated that the P*o*AP membrane was easily electrochemically deposited onto the PPY-MWNTs-BiCoPc composite layer surface with the formation of many rough network structures. The resulting modified electrode therefore had a large specific surface area for binding to target molecules.

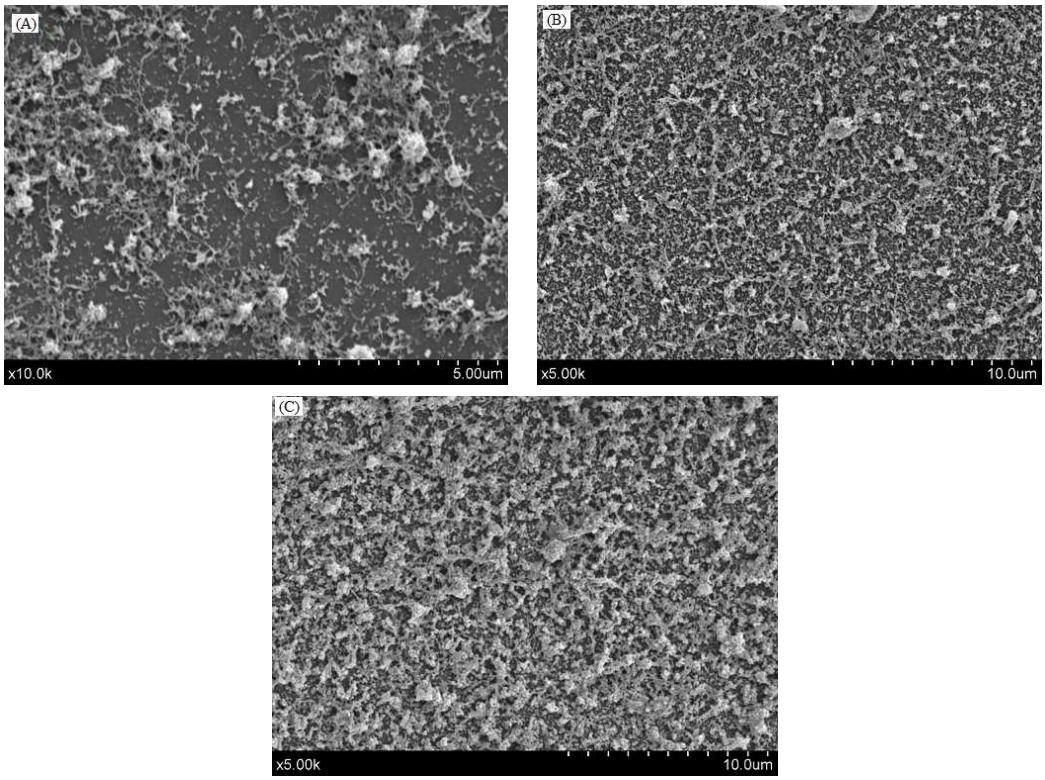
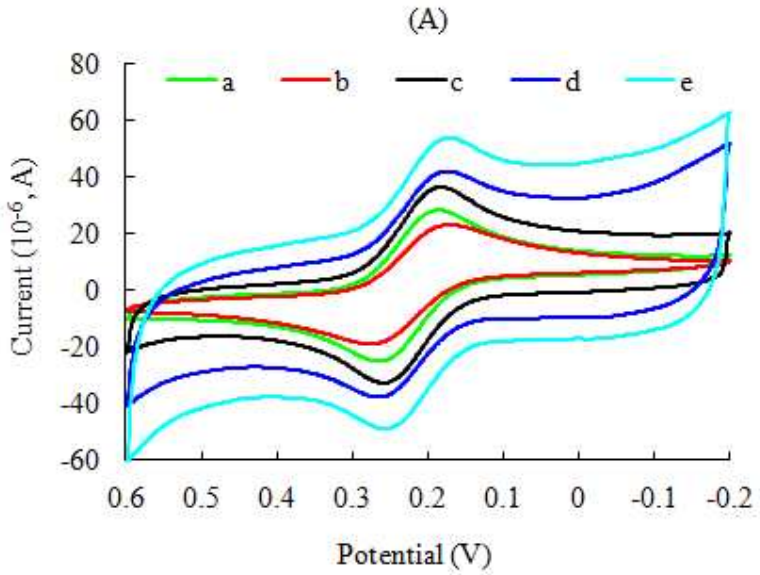


Fig. 4 SEM images of the surfaces of (A) the PPY-MWNTs-GC electrode, (B) the PPY-MWNTs-BiCoPc-GC electrode and (C) the PPY-MWNTs-BiCoPc-GC electrode after the electrodeposition of the PoAP membrane

3.2.2. Electrochemical characteristics of the PPY-MWNTs-BiCoPc composite layer

Cyclic voltammetry is a convenient and effective method to investigate electron transfer in surface modified electrodes [40]. In this work, the electrochemical behavior of the electrodes prepared using different modification processes was studied in a 1.0 mmol L^{-1} $K_3[Fe(CN)_6]$ solution containing 0.2 mmol L^{-1} KNO_3. A comparison of the cyclic voltammograms of the bare GC electrode, the PPY-GC electrode, the PPY-MWNTs-GC electrode, the PPY-BiCoPc-GC electrode and the PPY-MWNTs-BiCoPc-GC electrode is shown in Fig. 5A. The cyclic voltammogram

of the bare GC electrode showed a typical quasi-reversible electrochemical reaction with a couple of redox peaks (curve a). After PPY was polymerized onto the GC electrode's surface (curve b) its reversibility deteriorated indicating that a compact PPY layer was formed and this passivated the electrode surface. When the MWNTs (curve c) and BiCoPc (curve d) were doped into PPY by electropolymerization the redox peak currents of the obtained electrodes increased significantly. However, a maximum peak current was observed only when the MWNTs and BiCoPc were doped simultaneously (curve e). This result can be attributed to a synergistic effect in the modified PPY-MWNTs-BiCoPc composite layer wherein the electron transfer rate accelerated and the effective area of the electrode surface increased.



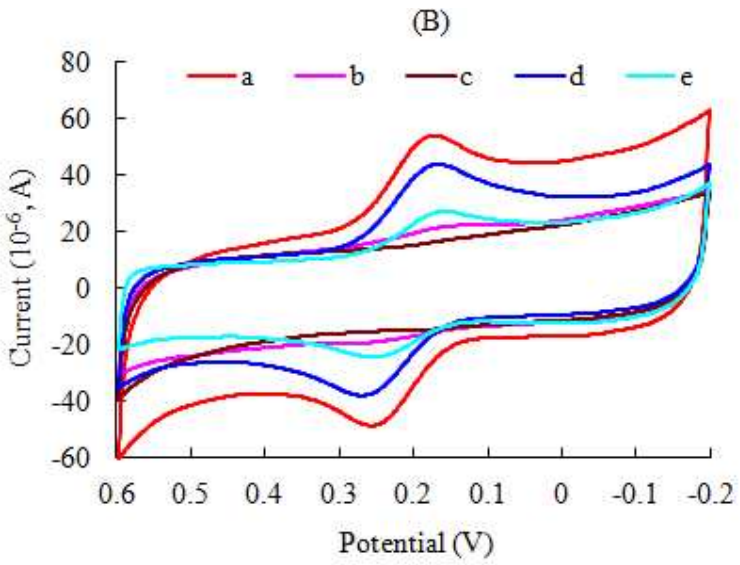


Fig. 5 (A) Cyclic voltammograms of (a) the bare GC electrode, (b) the PPY-GC electrode, (c) the PPY-MWNTs-GC electrode, (d) the PPY-BiCoPc-GC electrode and (e) the PPY-MWNTs-BiCoPc-GC electrode in an aqueous solution consisting of 1.0 mmol L^{-1} $K_3[Fe(CN)_6]$ and 0.2 mol L^{-1} KNO_3. Scan rate: 100 mV s^{-1}. (B) Cyclic voltammograms of (a) the PPY-MWNTs-BiCoPc-GC electrode, (b) the non-imprinted PoAP-PPY-MWNTs-BiCoPc-GC electrode, the imprinted PoAP-PPY-MWNTs-BiCoPc-GC electrode (c) before and (d) after removing the template MTMC and (e) the imprinted PoAP-PPY-MWNTs-BiCoPc-GC electrode after incubating with 6 × 10^{-7} mol L^{-1} MTMC and obtained from a 1.0 mmol L^{-1} $K_3[Fe(CN)_6]$ solution containing 0.2 mmol L^{-1} KNO_3. Scan rate: 100 mV s^{-1}.

3.2.3. Electrochemical characteristics of the imprinted and the non-imprinted electrodes

The cyclic voltammograms of the PPY-MWNTs-BiCoPc-GC electrode and the various P*o*AP membrane modified PPY-MWNTs-BiCoPc-GC electrodes were compared and are shown in Fig. 5B. As shown in curve a, the PPY-MWNTs-BiCoPc-GC electrode showed a remarkable current response with a

couple of redox peaks. After modification with the non-imprinted P*o*AP membrane only a small peak current was observed (curve b) because of the formation of a non-conducting P*o*AP membrane that covered the surface of the PPY-MWNTs-BiCoPc-GC electrode. This hindered the electron transfer pathways. However, for the imprinted PPY-MWNTs-BiCoPc-GC electrode, the redox peaks were restored after the template elution process (curve c and curve d), which suggests that cavities were produced in the imprinted P*o*AP membrane after MTMC removal. $[Fe(CN)_6]^{3-}$ could diffuse through these cavities toward the surface of the electrode resulting in a redox reaction. Furthermore, after immersing the imprinted PPY-MWNTs-BiCoPc-GC electrode in 6.0×10^{-7} mol L^{-1} MTMC, the redox peak currents decreased obviously (curve e), which can be explained by the rebinding of MTMC onto the produced imprinted cavities, which blocks the arrival of $[Fe(CN)_6]^{3-}$ on the electrode surface.

3.3. Optimization of the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode's preparation conditions

The constitution of the PPY-MWNTs-BiCoPc composite layer will affect the electrochemical performance of the modified electrode and, therefore, we optimized the amount of MWNTs and BiCoPc doped into the PPY layer at different ratios (BiCoPc/MWNTs ratio, 1:4, 1:2, 1:1, 3:2, 2:1 and the concentration of MWNTs was fixed at 1 mg mL^{-1}). We found that the redox peak currents of $Fe(CN)_6^{3-/4-}$ increased significant at BiCoPc/MWNTs ratios up to 1:1 after which they tended to be flat. The growth interface conductivity may be attributed to a composite action between

BiCoPc and MWNTs through π-π interactions which reduces the aggregation of MWNTs and improves the properties of the PPY lay. However, excessive BiCoPc doping and excessive deposition time will generate a polarization phenomenon in the PPY-BiCoPc leading to the poor stability of the current response. As a result, a BiCoPc/MWNTs ratio of 1:1 and a deposition time of 180 s were selected to prepare the composite layer for electrode modification.

For the construction of a MTMC imprinted P*o*AP membrane, the amount of imprinted sites available for the selective rebinding template molecules is directly influenced by the ratio of template to monomer in the electropolymerization solution. To detemine the most suitable ratio, the P*o*AP membranes were synthesized and compared using template:monomer molar ratios of 1:1, 1:2 and 1:3. The results show that the membrane synthesized at a ratio of 1:2 exhibited the largest current response change and the shortest adsorption equilibrium time during CV analysis. Therefore, it was chosen for the following experiments.

The thickness of the imprinted polymer membrane was a critical parameter that affected the permeation rate and stability of the recognition element. Although more P*o*AP deposition can embed more of the template molecule and give better membrane stability, the incomplete removal of the template from an excessively thick membrane will reduce the number of accessible imprinted sites and finally result in low binding capacity and slow binding kinetics [41, 42]. Because of the use of the electrodeposition method, the thickness of the P*o*AP membrane is easily controlled by adjusting the number of CV scanning cycles. We found that the current response

change for the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode toward the same concentration of MTMC increased with CV cycle number up to eight cycles and it then decreased gradually with additional cycles. Thus, eight cycles were chosen for the electrodeposition of the P*o*AP membrane to give a suitable thickness for MTMC detection.

3.4 Evaluation of the binding performance of the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode

The binding affinity of the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode toward MTMC was evaluated by measuring the CV response upon the addition of the MTMC working solution.

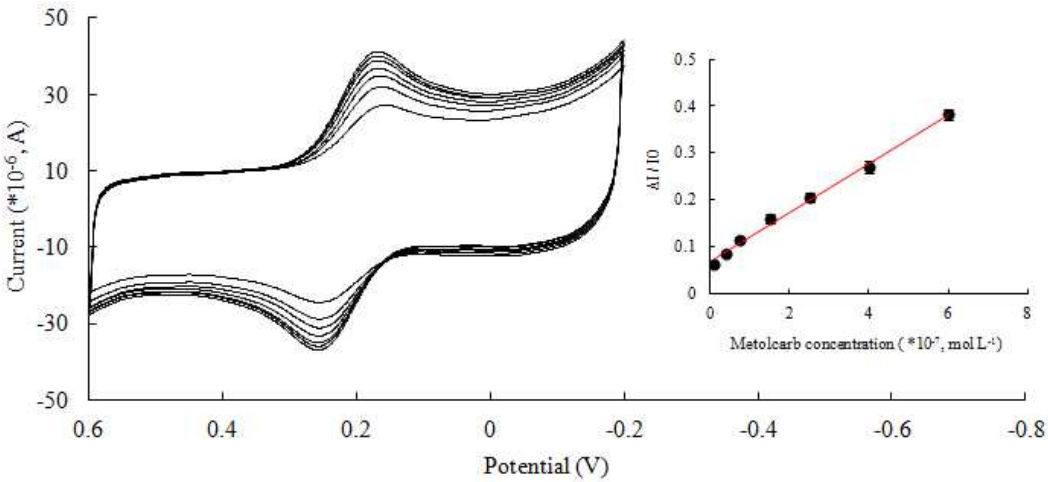


Fig. 6 Cyclic voltammograms of the imprinted PoAP-PPY-MWNTs-BiCoPc-GC electrode in response to different concentrations of MTMC. Scan rate: 100 mV s^{-1}. Inset: linear relationships of the relative current change vs. MTMC concentrations.

As shown in Fig. 6, an increase in the concentration of MTMC leads to a significant decrease in current response, indicating that the increased adsorption of

MTMC molecules onto the imprinted electrode surface hinders the diffusion of the probe $[Fe(CN)_6]^{3-}$. When the concentration is high the change in current tends to stabilize, and this can be attributed to the sufficient occupation of imprinted cavities by MTMC molecules. The relative current change is defined as $\Delta I/I_0$, where $\Delta I = I_0 - I_c$ is the change in anodic peak current, and I_0, I_c refer to the anodic peak current values at analyte concentrations of 0 and c mol L^{-1}, respectively. The results show that the relative current change is proportional to the concentration of MTMC in the range of 1.0×10^{-8} to 0.6×10^{-6} mol L^{-1} with a correlation coefficient of 0.9938 (Fig. 6, inset). The limit of detection (LOD, S/N = 3) was calculated to be 7.88×10^{-9} mol L^{-1} (about 1.3 μg L^{-1}), which could be comparable with the results of chromatographic method with more than 6.455 μg kg^{-1} [35] and immunoassay with 1.2 μg L^{-1} [36] and biomimetic sensing with 2.21 μg L^{-1} [43]. On the contrary, the decrease in the response current was much lower and independent by comparison with the imprinted electrode when the non-imprinted electrode was used as the working electrode (Fig. 7). This may be because the non-imprinted electrode lacked suitable imprinted sites for MTMC binding. Moreover, the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode also had a shorter equilibrium time (≈ 8 min) for analyte measurements. The rapid adsorption equilibrium characteristic is attributed to the electrodeposition of P*o*AP onto a very rough PPY-MWNTs-BiCoPc composite layer, which enhances the penetration of the analyte into the imprinted P*o*AP membrane (Fig. 4C).

3.5. Selectivity experiment

The selectivity of the proposed sensor, based on the specific binding interactions between the analyte and the recognition sites is an important characteristic that should be investigated. In this study, compounds with structures similar to MTMC such as isoprocarb, propoxur and carbaryl were used to evaluate the current response of the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode at a series of analyte concentrations (1.5×10^{-7}, 4.0×10^{-7}, 6.0×10^{-7} mol L^{-1}). For simplicity, we define the selectivity coefficient (SC) as SC = $\alpha_{MTMC} / \alpha_{analyte}$, where α is the gradient of the respective linear curves for the different analytes.

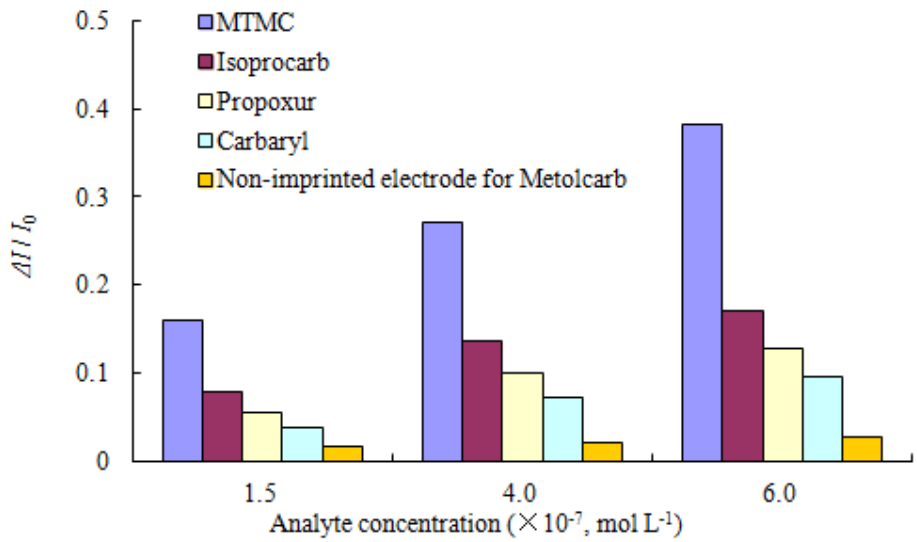


Fig. 7 Relative current change corresponding to the imprinted PoAP-PPY-MWNTs-BiCoPc-GC electrode for different concentrations of MTMC, carbaryl, isoprocarb and propoxur and the non-imprinted PoAP-PPY-MWNTs-BiCoPc-GC electrode for the determination of MTMC.

As shown in Fig. 7, the analytes isoprocarb, propoxur and carbaryl exhibited less relative current changes than MTMC with SC values of 2.45, 3.06 and 3.95, respectively. This indicates good selectivity of the imprinted

P*o*AP-PPY-MWNTs-BiCoPc-GC electrode toward the template molecule over its structural analogs. Moreover, the relatively high current changes for isoprocarb compared with the other two analogs in the selectivity experiment is attributed to its structure being more similar to MTMC and its smaller steric hindrance, which enabled it to easily enter the imprinted cavities in the P*o*AP membrane.

3.6. Reproducibility and stability

The reproducibility of the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode was estimated by detection the current response at a controlled MTMC concentration of 1.5×10^{-7} mol L^{-1}. The measurements were carried out with seven, freshly prepared, imprinted electrodes under the same conditions and an acceptable relative standard deviation (RSD) of 4.1% was obtained. After each detection, the electrode was rinsed with a water/methanol solution (4:6, v/v) to extract the analyte molecules. The RSD for five repeated analyses using a single imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode was calculated to be 5.3%, indicating that the sensor had good reproducibility.

The stability of the imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode over time was estimated over a month by weekly measurements of its current response toward a 1.5×10^{-7} mol L^{-1} MTMC test solution. A slight decrease in the relative current change to about 88% of its initial value was found after the first two weeks of storage in DDW at 4 °C, and it retained 83% of its initial value after one month of storage. This good durability of the developed sensor is attributed to the rigid structure

of the imprinted P*o*AP membrane synthesized by electropolymerization and the excellent stability of the PPY-MWNTs-BiCoPc composite layer.

3.7 Analysis of vegetable samples

To demonstrate a feasible application of the fabricated imprinted P*o*AP-PPY-MWNTs-BiCoPc-GC electrode, cucumber and cabbage samples spiked with MTMC at three concentrations (40.0 μg kg^{-1}, 200.0 μg kg^{-1}, 400.0 μg kg^{-1}) were evaluated using the sensor. The concentration of MTMC was detected using three replicate measurements for each sample under the same conditions and the results are listed in Table 1. Good recoveries were obtained and ranged from 88.8 % to 93.3% with RSDs from 3.3 % to 5.1%, suggesting that the proposed sensor could accurately and reliably be used to analyze the MTMC residue in the vegetable sample.

Table 1. Recoveries of MTMC from spiked cucumber and cabbage samples, as determined by the developed electrochemical sensor

Vegetable samples	Spiked *conc.* (μg kg^{-1})	Theoretical *conc.* (μg kg^{-1})	Detected *conc.* (μg kg^{-1})	Recovery %	RSD % (n = 3)
	40.0	8.0	7.42	92.8	3.3
Cucumber	200.0	40.0	36.40	91.0	4.2
	400.0	80.0	73.60	92.0	3.8
	40.0	8.00	7.29	91.1	3.6
Cabbage	200.0	40.0	35.52	88.8	4.0
	400.0	80.0	74.67	93.3	5.1

4. Conclusions

In this study, a novel PPY-MWNTs-BiCoPc composite layer and a molecularly imprinted P*o*AP electrodeposited membrane were modified in a stepwise manner on a GC electrode surface for the development of a MIP-based electrochemical sensor for MTMC determination. We found that the P*o*AP-PPY-MWNTs-BiCoPc-GC electrode performed well in the sensitive and selective determination of the target molecule. This can be ascribed to a synergistic effect in the PPY-MWNTs-BiCoPc functional layer and a number of selective binding sites in the imprinted P*o*AP membrane. The sensor was found to have good reproducibility, long-term stability and could be used to analyze for trace amounts of MTMC in spiked vegetable samples. This work provides a convenient and inexpensive method for the preparation of sensing elements and broadens the application of MIP-based sensors in the field of drug residue determination.

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