Electrochemical Preparation of a MIP-Glassy Carbon Electrode for the Determination of Dimethoate

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Abstract. In this work a dimethoate-polypyrrole (dim-PPy) MIP films were electropolymerized by cyclic voltammetry (CV) on the surface of glassy carbon electrode (GCE), using pyrrole (Py) as the monomer and dimethoate (dim) as the template. Dimethoate is electro-inactive, therefore an electroactive $K_3[Fe(CN)_6]$ solution was used as probe in the CV and square wave voltammetry (SWV) for the evaluation of the performance of the imprinted (MIP) and non-imprinted (NIP) films. To investigate the analytical performance of the MIP system in the dimethoate detection, the dim-free MIP films electrode, obtained after the removal of the dimethoate, was placed in solutions containing dimethoate at different concentrations for the analyte rebinding. After the rebinding step, for the MIP films there was a decrease of the response and the current was lower than that for the dim-free MIP films. The decrease of the response could thus be used to indirectly detect the analyte quantitatively. For the NIP films, the response of $K_3[Fe(CN)_6]$ was very small and showed no obvious difference with different dimethoate concentrations in the rebinding step. These results illustrated that the dim-PPy MIP film system is simple to construct and easy to operate and could be used to recognize dimethoate.

Keywords: Dimethoate · Molecularly imprinted polymer · Polypyrrole · Electrochemical sensor

1 Introduction

Dimethoate is an organophosphate pesticide (OP) that has an inhibitory effect on the function of the enzyme acetylcholinesterase (AChE); the latter hydrolyses the neurotransmitter acetylcholine and this effect leads to a pathologic excess of acetylcholine in the body. Toxicity of OPs affect many organs [1], particularly the nervous system [2]. Detection of organophosphate pesticides in food samples has been extensively studied using biosensors based on AChE [3, 4]; this system is quite sensitive but the selectivity

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is poor. Because of wide use and acute toxicity of OPs, it is important to develop rapid, sensitive and selective detection methods.

Molecularly imprinted polymers (MIPs) have received considerable attention in analytical chemistry, primarily because specific recognition sites are formed in the MIP matrix, and excellent selectivity toward the analyte is achieved [5]. Molecularly imprinting is a process by which selected functional monomers are polymerized around a target analyte (template). After polymerization, the template molecule is extracted and a polymer matrix, which is complementary in shape and functionality to the template, is obtained. Thus, the polymer has the ability to selectively link to the target analyte. The chemical and mechanical stability, the facility of preparation and the relatively low cost of the polymers make them attractive for several analytical applications and in some cases they are used as replacers of natural receptors and enzymes [6]. The electropolymerization process is frequently used in the development of molecularly imprinted electrochemical sensors. The advantages are the control of the polymer thickness, which can be regulated by electrochemical conditions, the simple preparation procedure and the formation of very thin films that are beneficial to rapid response [7]. The electropolymerization of polypyrrole (PPy) has been widely used for the preparation of molecularly imprinted electrochemical sensors, due to its excellent biocompatibility and the facility of the immobilization of different compounds [8].

In the present study, MIPs were prepared by electropolymerization of pyrrole (Py) in the presence of dimethoate (template) on the glassy carbon electrode surface, for the electrochemical detection of pesticide. Dimethoate (dim) is electro-inactive, therefore an electroactive $K_3[Fe(CN)_6]$ solution was used as the probe in the cyclic voltammetry (CV) and square wave voltammetry (SWV). To investigate the analytical performance of the MIP system in the dimethoate detection, the dim-free MIP films electrode, obtained after the removal of the dimethoate, was placed in solutions containing dimethoate at different concentrations for the analyte rebinding. After the rebinding step, for the MIP films there was a decrease of the response and the current was lower than that for the dim-free MIP films. The decrease of the response is indirectly related to the amount of the analyte.

2 Materials and Methods

2.1 Chemicals and Apparatus

All the chemicals were obtained from Sigma-Aldrich. Pyrrole was distilled under vacuum until a colorless liquid was obtained, purged with argon and kept in darkness at −30 °C.

Electrochemical studies were carried out using a BASi potentiostat-galvanostat controlled by a Bas100 Software. A three-electrode system was used for all measurements: a MIP-glassy carbon electrode (3 mm diameter) as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl/NaCl (3 M) as the reference electrode.

2.2 Preparation of MIP Film Electrodes and Electrochemical Measurements

A bare glassy carbon electrode (GCE) was polished using 1, 0.3 and 0.05 μm alumina paste on microcloth pads and rinsed thoroughly with distilled water until a mirror-like surface was obtained. Then it was sonicated in 1:1 (v/v) ethanol and distilled water for 10 min. Finally the electrode was washed with distilled water and allowed to dry at room temperature before use. Then the GCE was immersed in PBS solution containing 30 mM pyrrole and 10 mM dimethoate for the electropolymerization step by using cyclic voltammetry in the potential range between −0.4 and +1.5 V during 10 cycles at a scan rate of 50 mV/s. After the electropolymerization, in order to extract dimethoate from the imprinted polymer, the dim-PPy MIP films were immersed in a pH 2 HCl solution and stirred for 20 min (dim-free MIP films). The dimethoate molecules were extracted from the polymer matrix to give a surface complimentary in shape and functionality to the template. For rebinding step, the MIP films after the dimethoate removal were incubated into dimethoate solutions at different concentrations (0.01–10 nM) for 15 min (dim-rebinding MIP films).

Electrochemical measurements for the characterization of the electrodes were performed by using CV and SWV in a pH 7 PBS containing 1 mM $K_3[Fe(CN)_6]$ and 0.1 M KCl solution in the potential range from −0.3 to +0.8 V (CV-SWV) and from +0.8 to −0.3 V (SWV). The $K_3[Fe(CN)_6]$ was used as the probe because the dimethoate is electro-inactive. All measurements were performed at room temperature. A control electrode (non imprinted polymer electrode, NIP) was prepared under the same conditions but without adding dimethoate during the electropolymerization.

3 Results and Discussion

The dim-PPy MIP films were obtained by electropolymerization on the surface of GCE using CV in potential range between −0.4 and +1.5 V during 10 cycles (scan rate 50 mV/s) in PBS solution containing 30 mM pyrrole and 10 mM dimethoate (Fig. 1). An oxidation peak of pyrrole was observed on the first scan at about 1 V, then the peak decreased under continuous cyclic scans. The decrease is related to the formation of PPy films that hinder pyrrole monomer further access to the surface of the GCE. Figure 2a shows cyclic voltammogram obtained with bare GCE and a well-defined redox peaks were observed. After the electropolymerization, for the dim-PPy MIP films no response was observed (Fig. 2b) because of the polymeric matrix and the dimethoate molecules that cover the surface of the electrode. After washing with HCl solution for the dimethoate removal a redox peak appeared again (Fig. 2c) because the electrochemical probe had easier access to the electrode surface. After the template removal, the electrode was incubated in dimethoate solution with different concentration for 15 min for the rebinding step. After incubation in a dimethoate solution, a decrease in the redox peaks currents was observed (Fig. 2d) and the current was lower than that for the dim-free MIP films, because of the ability of the electrode to incorporate again the dimethoate molecules, blocking the diffusion of the probe. ΔIp was the difference between the catodic (ΔIpc) or anodic peak (ΔIpa) current of the probe at a

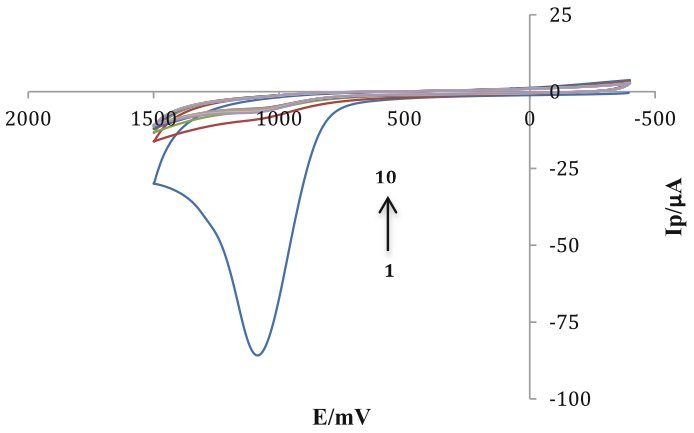

Fig. 1. Cyclic voltammogram for the electropolymerization of 30 mM Py at GCE surface in PBS containing 10 mM dimethoate; scan rate 50 mV/s; 10 cycles

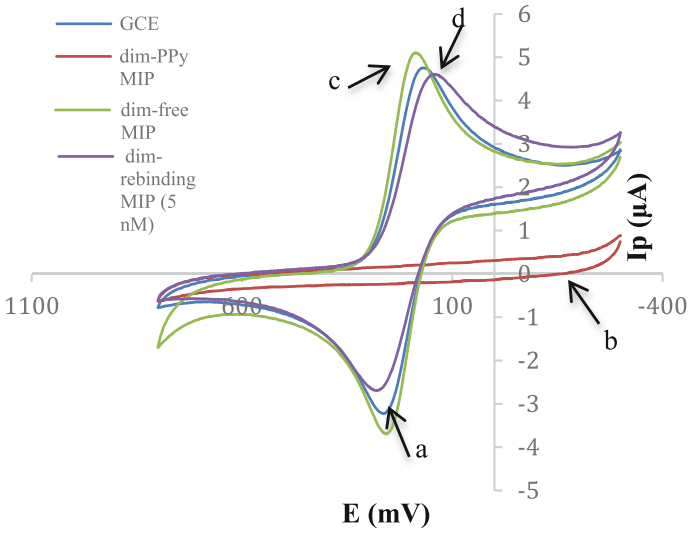

Fig. 2. CVs of 1 mM $K_3[Fe(CN)_6]$ at 10 mV/s in pH 7.0 PBS at **a** bare GCE, **b** dim-PPy MIP film electrode, **c** dim-free MIP film electrode and **d** dim-rebinding MIP film electrode after 5 nM dimethoate rebinding

dim-free MIP film electrodes and that at the dim-rebinding MIP film electrodes. A relationship between the ΔIp and the logarithm of the concentration was evaluated in the range 0.01–10 nM and a linear response was observed. These results indicate that the decrease of the response could thus be used to indirectly detect the analyte quantitatively. For the NIP films, the response of $K_3[Fe(CN)_6]$ was very small and showed no obvious difference with different dimethoate concentrations in the rebinding step

because there is no recognition site for dimethoate (Fig. 3). An initial evaluation of the selectivity of the MIP sensor was carried out using other organophophate pesticides, such as malathion and parathion during the rebinding step. There was no significant decrease of the peak currents for the interferents after the rebinding step (Fig. 4), indicating a very promising selectivity of the sensor. The reproducibility of the MIP-GCE was estimated by determination of 5 nM dimethoate using three different electrodes that were prepared under the same conditions. The relative standard deviation was found to be 3.2% for the ΔIpa and 1.8% for the ΔIpc, demonstrating that the preparation of sensor has a good reproducibility.

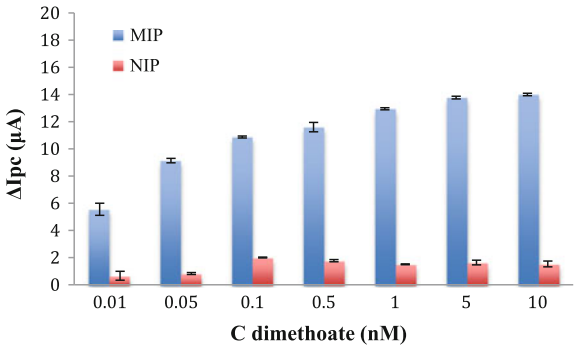

Fig. 3. ΔIpc (SWV) of the MIP and NIP films where ΔIpc represents the difference between the reduction peak current of the probe for the dim-free MIP or NIP films and that after the dimethoate rebinding for 15 min

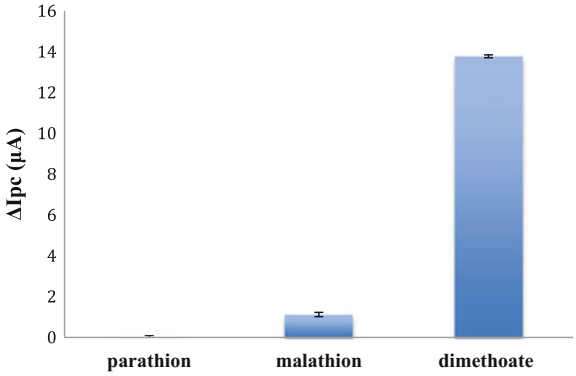

Fig. 4. ΔIpc (SWV) of the MIP films where ΔIpc represents the difference between the reduction peak current of the probe for the dim-free MIP films and that after the dim-free MIP films were placed in 5 nM dimethoate, parathion and malathion solutions for 15 min

4 Conclusion

In this work, a MIP-GCE for the determination of dimethoate was developed, by cyclic voltammetric electropolymerization of a polypyrrole film on the surface of GCE. The difference of the catodic or anodic peak current of probe (ΔIpc or ΔIpa) at electrodes between dim-free MIP and dim-rebinding MIP films could be used to measure dimethoate quantitatively. The developed sensor showed a good reproducibility, repeatability and selectivity. These results illustrated that the dim-PPy MIP film system is simple to construct and easy to operate and could be used to selectively detect dimethoate in real samples.

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