

# The Preparation of Molecularly Imprinted Poly(*o*-phenylenediamine) Membranes for the Specific *O,O*-Dimethyl- $\alpha$ -hydroxyphenyl Phosphonate Sensor and Its Characterization by AC Impedance and Cyclic Voltammetry

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**ABSTRACT:** A molecularly imprinted polymer (MIP) membrane for sensing *O,O*-dimethyl- $\alpha$ -hydroxyphenyl phosphonate (DHP) has been prepared by electropolymerizing *o*-phenylenediamine on the glassy carbon electrodes in the presence of DHP. Optimization studies with the aim to enhance insulating properties and response kinetics of the polymer membrane were carried out with respect to template molecular concentration, the monomers concentration, the polymer membrane thick and scan rate. Cyclic voltammetry and electrochemical impedance have been used to

characterize the behavior of MIP polymer membrane. The capacitive measurements were also certified the imprinting effect of the polymer layers. The experimental results showed that DHP imprinted polymer has better recognition property for the template than that of a blank polymer. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 101: 2222–2227, 2006

**Key words:** molecularly imprinted polymer membrane; cyclic voltammetry; electrochemical impedance; electropolymerization

## INTRODUCTION

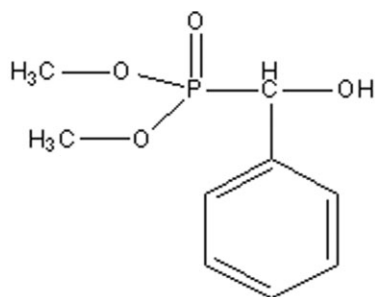
In recent decades, a novel technology, namely molecularly imprinting technique, has become a powerful method for the preparation of polymeric membranes that have the ability to bind a specific chemical species.<sup>1</sup> The imprinting process involves polymerization of functional monomers in the presence of the template molecule, which is typically the target molecule or a molecule of similar size and chemical functionality. Once the template is removed, the molecular recognition site of appropriate size and chemical functionality is produced to bind the target. Imprinted polymeric materials have been successfully used as enzyme-mimic catalysts,<sup>2,3</sup> binding assays,<sup>4,5</sup> and solid phase extraction materials.<sup>6</sup> But they are finding increasing use in imprinted membranes as sensitive components for the development of chemical sensors.<sup>7–9</sup>

For the construction of the molecularly imprinted polymers (MIPs)-based sensor, different signal transducers, such as chemically modified field effect transistor,<sup>10</sup> fluorescence quenching,<sup>11</sup> surface plasma resonance,<sup>12</sup> electrochemical measurements,<sup>13–16</sup> and piezoelectric quartz crystal technique<sup>17,18</sup> have been applied. In contrast to the numerous MIPs-based sensors employing other signal transducing mechanism reported in literatures, capacitive sensors were relatively less available. For the construction of a capacitive sensor, an ultrathin membrane of perfect insulating properties is very important. Thus, to acquire satisfactory insulating films, the electropolymerization of *o*-phenylenediamine was carried out by cyclic voltammetry (CV) in a potential range of 0–0.8 V with 4 cycles. These membranes show satisfactory performance in the selective recognition of *O,O*-dimethyl- $\alpha$ -hydroxyphenyl phosphonate (DHP).

Organophosphate esters are commonly used as persistent pesticides and nerve agents. Thus, it is highly desirable to develop methods for the determination of these compounds. The most common methods involve their preconcentration on a solid phase and subsequent detection by HPLC or gas chromatography (GC), often coupled to MS.<sup>19,20</sup> Several studies used electrochemical methods to detect electroactive or-

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**Figure 1** Chemical structure for *O,O*-dimethyl- $\alpha$ -hydroxyphenyl phosphonate.

ganophosphate compounds<sup>21–23</sup> or applied electrochemical and gravimetric techniques in conjunction with enzymes, predominantly choline esterase and organophosphate hydrolase, to detect organophosphate compounds have been reported.<sup>24–27</sup> In the present work, a MIP membrane for the detection of DHP has been developed by electropolymerizing *o*-phenylenediamine on the GC electrode in the presence of the template. Electrochemical impedance measurements were employed to verify the imprinting effect of MIP membranes.

## EXPERIMENTAL

### Instruments and reagents

A GC electrode ( $d = 3$  mm) was used as the working electrode, a saturated calomel electrode and a platinum wire as the reference and counter electrode, respectively. A CHI 660A electrochemical workstation (CH Instruments, Texas) in conjunction with a three-electrode cell was used for the electropolymerization and the electrochemical measurements.

DHP was supplied by Chemical Department of Central China Normal University. Figure 1 shows the molecular structure of the template. The stock solutions were prepared by dissolving in ethanol and kept at 0°C. *o*-Phenylenediamine was purchased from Chemical Reagent (Shanghai, People's Republic of China). All chemicals were of analytical grade, and all compounds were used without further purification. Double-distilled water was used throughout all the experiment.

### Preparation of MIP

GC electrode was polished to a mirror finished with polish paper and 0.3–0.05  $\mu\text{m}$  alumina slurry, and cleaned thoroughly in an ultrasonic cleaner with 1:1 nitric acid, alcohol, and double-distilled water. The reaction solution comprises 0.01M template, 0.01M *o*-phenylenediamine and 1/15M phosphate buffer (PB, pH 6.98). After deoxygenating the solution by

bubbling nitrogen gas for about 15 min, the electropolymerization was performed by CV in the potential range of 0–0.8 V, with a scan rate of 50 mV/s and 4 cycles. After the electropolymerization, the modified electrode was washed with alcohol and double-distilled water alternately to remove the imprinting molecules. With these steps, the electrodes modified with DHP imprinted polymer were prepared. Reference controlled electrode was prepared under the same procedure except the absence of template.

### Extraction and binding measurements

In stirring condition, the template was removed from the imprinted polymer by repeated extraction with ethanol. Complete extraction was confirmed by HPLC and CV. Reference controlled electrodes were rinsed in ethanol to remove traces of unreacted materials. To confirm the complete extraction, the extracted polymer was repetitively incubated with 2 mL ethanol. No trace of the template was found in the repeated extraction.

After extraction of the template, the extracted membrane was soaked in 10 mL 0.01 mM DHP in PB (pH 6.98) for 2 h at 25°C. Then, the extracted membrane was washed by the buffer solution to remove physically adsorbed material and was stored in clean test tubes.

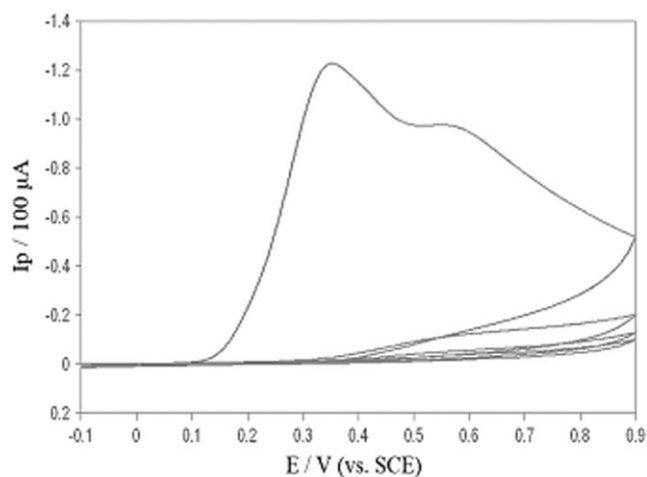
### Electrochemical measurements

Cyclic voltammetric measurements were performed in the presence of 10 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6](1:1)$  (0.2M NaCl used as the supporting electrolyte). CVs of the imprinted membranes were recorded in the potential range of 0–0.8 V with a scan rate of 50 mV/s. Impedance measurements were carried out at the open potential with a frequency range of 0.002–10,000Hz and an amplitude of 5 mV. After electropolymerization, the imprinted polymer was rinsed and then dried in a nitrogen flow for impedance measurements.

## RESULTS AND DISCUSSIONS

### Preparation of MIP membranes

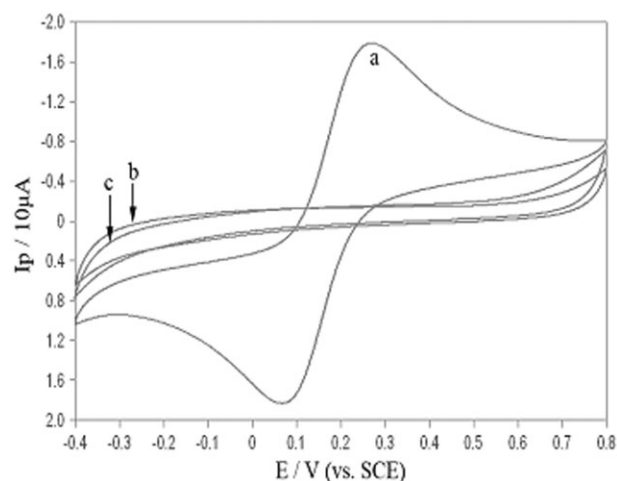
Figure 2 shows the typical cyclic voltammograms recorded in the presence of 0.01M DHP during the electropolymerization of *o*-phenylenediamine. Voltammetric behavior of *o*-phenylenediamine showed that it is entirely irreversible oxidation process. The peak current decreased significantly under continuous cyclic scan, which means that the insulating polymer was formed and bound to the electrode surface. It is thought that for slow diffusion of analytes into a polymer film, a layer which is too thick would not be



**Figure 2** Typical cyclic voltammograms for the electropolymerization of *o*-phenylenediamine. 0.01M *o*-phenylenediamine in 1/15M phosphonate buffer containing 0.01M *O,O*-dimethyl- $\alpha$ -hydroxy benzenyl phosphonate. Number of scans: 4; scan rate: 50 mV/s.

beneficial for fast response kinetics. Thus, formation of ultrathin polymer films on the electrode surface is preferred to improve the sensitivity of the devices. The thickness of polymer can easily be adjusted by controlling the scan rate and the number of cycles during electropolymerization. It was demonstrated that four scans were suitable to enable efficient template incorporation during the electropolymerization and enhance the current response of the MIP membrane simultaneously. Similar electrochemical behavior has been observed in the absence of template, as DHP has no electroactivity on GCE under the experimental conditions. It also indicates that the structure of DHP is not electrochemically altered during the imprinting process.<sup>28</sup> The driving force of recognition process would be originated from the rigid structure of the imprinted cave and the nature of the prepolymerization complex between DHP and the functional monomers. In the imprinted membrane, DHP is encapsulated in the imprinted cave and is strongly adsorbed through " $\pi$ - $\pi$ " noncovalent binding and hydrophobic interactions. The formation of the imprinting membrane might be attributed to the hydroxyl bonding between the P=O bond of template molecules and the N-H bond of functional monomers.

CV experiments were run to verify the formation of the DHP-imprinted membrane on the electrode surface. Curve a in Figure 3 shows the cyclic voltammogram using the bare GCE in the presence of 10 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  (1:1)-mixture. A pair of typical redox peaks can be found. Curve b and c show the cyclic voltammograms of the GCE modified with nonimprinted membrane and DHP-imprinted membrane under the same condition. It can be found that the peak current in curve b is lower than that in curve a,

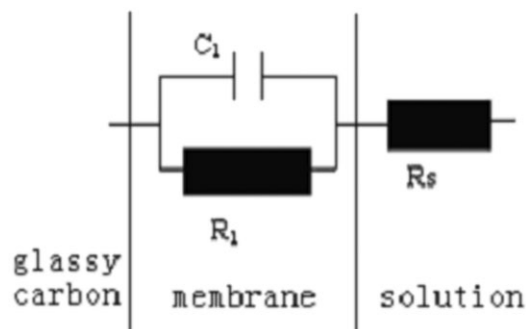


**Figure 3** Voltammograms of glassy carbon electrode in the presence of 10 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  (1:1)-mixture: (a) bare electrode; (b) GCE modified with nonimprinted membrane, scan rate is 50 mV/s; (c) GCE modified with DHP-imprinted membrane, scan rate is 50 mV/s.

and the peak current in curve c is much lower than that in curve a. The result means that the nonconducting DHP-imprinted membrane assembled on the electrode gradually and retarded the electron-transfer between the electrolyte and the electrode.

#### Characterization of MIP membranes by alternating current (AC) impedance

AC impedance spectroscopy, which is an effective method to probe the features of surface-modified electrode,<sup>29</sup> was employed to characterize the electrodes modified with imprinted polymer. In the present study, the capacitance behavior was investigated using impedance measurements. A simple equivalent circuit is used to simulate the imprinted polymer electrode (Fig. 4). There is a certain transformation rela-



**Figure 4** Simple equivalent circuit of the imprinted polymer electrode.  $C_1$  is the capacitance of the polymer membrane,  $R_1$  is an ohmic resistance of the polymer membrane, and  $R_s$  is the resistance of the electrolyte solution and reference electrode.

tionship between the equivalent circuits for similar plots on an impedance complex plane and on a capacitance complex plane.<sup>30</sup>

The transfer role for an equivalent circuit being composed of the resistance and the capacitance is given by following derivation. The value of AC impedance measurements can be expressed as

$$Z = Z' - jZ'' \tag{1}$$

where  $Z'$  is the real part of impedance,  $Z''$  is the imaginary part of impedance.

Equation (1) can be substituted for eq.(2a):

$$Y = Y' + jY'' \tag{2a}$$

where  $Y'$  is the real part of mobility,  $Y''$  is the imaginary part of mobility.

The angle frequency  $\omega$  ( $\omega = 2\pi f$ ,  $f$  is the frequency) divided mobility, then eq. (2a) can be derived as

$$Y/\omega = Y'/\omega + Y''/\omega \tag{2b}$$

the complex mobility of capacitance is described as

$$Y = j\omega c = \omega c'' + j\omega c' \tag{3a}$$

Equation (3a) divided angle frequency ( $\omega$ ), then can be derived as

$$Y/\omega = c'' + jc' \tag{3b}$$

In contrast eqs. (2b) and (3b), we can see  $Y'/\omega = c''$  is the imaginary part of capacitance;  $Y''/\omega = c'$  is the real part of capacitance. Thus, the data of AC impedance experiments can be exhibited by using the plot on the  $Z''-Z'$  or the plot on the  $c''-c'$  plane. A typical spectrum of complex capacitance is observed a semicircle. The semicircle diameter corresponds to the capacitance of membrane.

The capacitive detection is based on the principle of plate-capacitor. When an electrode is immersed in the electrolyte, an electrical double-layer will come into being and the electrode/electrolyte interface behavior can be considered as a plate-capacitor. The capacitance is expressed as the following equation.

$$C_{dl} = \epsilon_0 \epsilon_r A/d \tag{4}$$

where  $\epsilon_0$  is the permittivity of the free space,  $\epsilon_r$  is the dielectric constant of electrical double-layer that separates the electrode from the electrolyte,  $A$  is the surface area,  $d$  is the closet distance from the electrode to the electrolyte. As can be seen from eq. 1 any insulating membrane adsorbed onto the electrode surface will change the thickness and the dielectric properties of the dielectric layer. The structure is considered as a

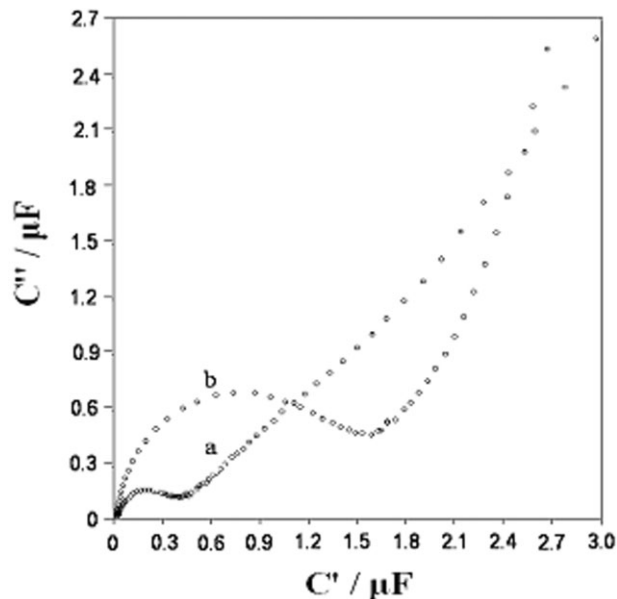


Figure 5 Typical complex capacitance plane of the electrode modified with DHP imprinted polymer before (a) and after being (b) washed with ethanol. Number of scans: 4 cyclic; scan rate: 50 mV/s.

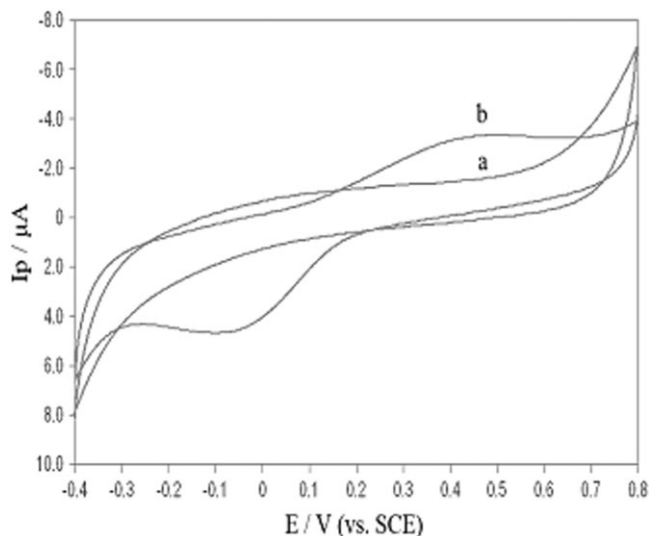
serial capacitor and the capacitance is therefore calculated as

$$1/C_t = 1/C_i + 1/C_m \tag{5}$$

where the  $C_t$  is the total capacitance,  $C_i$  is the initial capacitance;  $C_m$  is the capacitance of the insulating membrane.

Figure 5 shows the change of the plot on the  $c''-c'$  plane of the electrode modified with DHP imprinted polymer after it was treated differently. The initial capacitance of membrane is 0.4  $\mu\text{F}$ . After immersing the electrode in ethanol for 40 min, the capacitance substantially increased to 1.6  $\mu\text{F}$ . Equation (4) shows that the increase in capacitance is attributed to the  $A/d$  arise. This means that the effective area of imprinted film increases or the distance of electrolyte to electrode decreases. Nevertheless, no obvious change was observed in the nonimprinted polymer under the same experimental conditions.

Curve a in Figure 6 shows the cyclic voltammogram of the imprinted film in the presence of 10 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$  (1:1)-mixture. No redox peak is observed on CV. However, it can be seen from curve b that  $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$  (1:1)-mixture has obvious redox signals after immersing the imprinted membrane in ethanol for 40 min. It suggested that the template was removed from the imprinted membranes and reveals that pores have been produced in the polymer. The porosity enhances the diffusion of  $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$  through the ply-

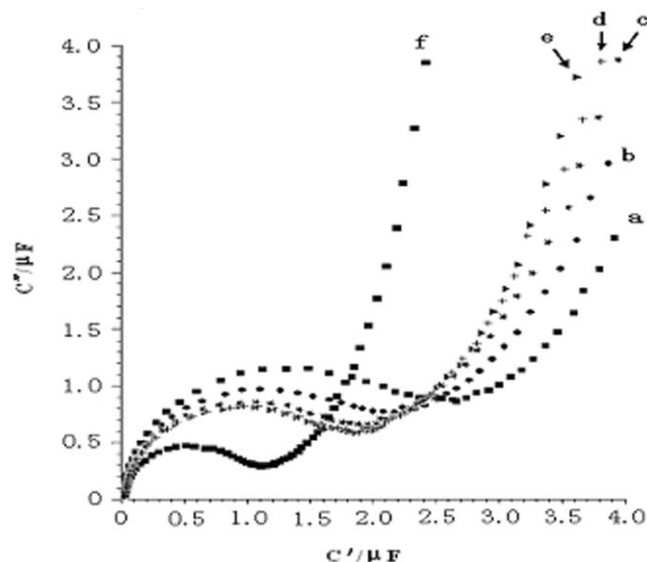


**Figure 6** Voltammograms of glassy carbon electrode in the presence of 10 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  (1:1)-mixture: (a) after 4 cycles electropolymerization; (b) the same electrode after being washed with ethanol for 40 min.

mer and can promote redox reaction on the electrode surface.

### Optimization of experimental conditions

In the experiment, the best electropolymerization conditions for the preparation of the imprinted polymer were selected by testing the template concentration, the monomers concentration, scan rate, and cyclic numbers. The mainly examining items were capacitance change of the imprinted membrane before and after being extracted with ethanol for 40 min, and the results were summarized in Table I. We can see from Table I that the template molecular concentration has no notable effect on the film capacitance. However, the membrane capacitances decrease with increase in the



**Figure 7** Complex capacitance plane of the electrode modified with DHP imprinted film (a) DHP-imprinted film, (b) after interaction with nitrophenol ( $10 \mu M$ ) for 40 min, (c) after interaction with ascorbic acid ( $10 \mu M$ ) for 40 min, (d) after interaction with dopamine ( $10 \mu M$ ) for 40 min, (e) after interaction parathion ( $10 \mu M$ ) for 40 min, and (f) after interaction with DHP ( $10 \mu M$ ) for 40 min.

monomer concentration. The change of the imprinted polymer capacitance decreases obviously with increase in scan rate (No. 3 in Table I). That is to say, an ultrathin film can be obtained at a fast scan rate. The data clearly indicate that selective binding sites have been formed in the imprinted films. According to the earlier discussion, we can draw a conclusion that the best conditions for the construction of a DHP imprinted membrane are No. 8 in Table I.

The selectivity of the imprinted sensor was investigated by testing its response towards some potential interferences. In the experiments, dopamine, ascorbic acid, parathion, and nitrophenol were selected to test

**TABLE I**  
Optimal Parameters and Results for the Imprinted Polymer Membranes Obtained at Different Experimental Conditions

No.	Scan rate (v/s)	Cyclic numbers	Monomer concentration (mol/L)	Template concentration (mol/L)	Capacitance before extraction ( $\mu F$ )	Capacitance after extraction ( $\mu F$ )
1	0.015	2	0.0025	0.01	0.23	1.21
2	0.05	2	0.0025	0.01	0.40	1.60
3	0.1	2	0.0025	0.01	0.45	0.96
4	0.05	4	0.0025	0.01	0.38	1.42
5	0.05	8	0.0025	0.01	0.36	1.39
6	0.05	12	0.0025	0.01	0.18	1.22
7	0.05	4	0.005	0.01	0.24	1.59
8	0.05	4	0.01	0.01	0.20	1.50
9	0.05	4	0.02	0.01	0.15	0.90
10	0.05	4	0.01	0.0025	0.21	1.47
11	0.05	4	0.01	0.005	0.24	1.68

its selectivity. Figure 7 shows the capacitance change of the imprinted membranes upon interaction with  $1.0 \times 10^{-5}M$  DHP, dopamine, ascorbic acid, parathion, nitrophenol for 40 min, respectively. As can be seen from Figure 7, more DHP has been accumulated at the imprinted polymer than these interferences under the same experimental conditions. That is to say, the selectivity of the imprinted membrane is satisfactory to DHP.

The repeatability of the imprinted polymer was investigated in  $1.0 \times 10^{-5}M$  DHP solution. The RSD is about 4.1% ( $n = 7$ ). After the imprinted sensor was washed with ethanol and then immersed in the PB solution (pH 6.98, 1/15M) for 7 days, the capacitance change upon interaction with  $1.0 \times 10^{-5}M$  DHP solution decreased only 1%. The response of DHP at the imprinted sensor was studied after it being used for 10, 30, and 50 times, no obvious change was observed after 50 times. Reproducibility for the fabrication of seven sensors, independently made, gave an acceptable reproducibility with a relative standard deviation of 4.7% for the detection of  $1.0 \times 10^{-5}M$  DHP.

The capacitance change of the imprinted membrane is linearly related to the *O,O*-dimethyl- $\alpha$ -hydroxy benzoyl phosphonate concentration in the range of  $5.0 \times 10^{-8}$  to  $5.0 \times 10^{-5}M$  ( $r = 99.91\%$ ) with a detection limit of  $1.0 \times 10^{-8}M$ .

## CONCLUSIONS

In the presented work, an MIP membrane specific for an electroinactive DHP has been developed based on electropolymerization of *o*-phenylenediamine. The imprinted polymer exhibits satisfactory selectivity and specificity for the template molecule. This method can be easily adapted to the development of chemical sensor for organophosphates or other small molecules, in conjunction with a proper transduction mechanism. AC impedance measurements may be a convenient and sensitive method for studying these imprinted membranes even if the embedded species are nonelectroactive.

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