

Determination of Parathion in Vegetables by Electrochemical Sensor Based on Molecularly Imprinted Polyethyleneimine/Silica Gel Films

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A novel sensor for the determination of parathion based on coupled grafting of the functional macromolecule polyethyleneimine on surfaces of silica gel particles via a surface imprinting method using molecular imprinting technology was fabricated. The electrochemical behavior of parathion at the imprinted sensor was characterized by cyclic voltammetry and linear sweeping voltammetry. The imprinted films showed high selectivity toward parathion in comparison to similar organophosphates. A linear response over parathion concentration in the range of 0.015–15 mg kg⁻¹ was exhibited with a detection limit of 0.003 mg kg⁻¹ (S/N = 3). The imprinted film sensor has been applied to the determination of parathion in spiked vegetable samples and shows promise for fast and selective determination of trace levels of parathion in real samples.

KEYWORDS: Parathion; vegetables; molecular imprinting; polyethyleneimine; silica gel; electrochemical sensor

INTRODUCTION

Organophosphate pesticides (OPs) have played an important role in increasing agricultural productivity. They are widely used in pre- and postharvest treatments to control diseases of fruits and vegetables (1–3). However, concerns are being raised because of their persistence, bioaccumulation, and potential for toxicity, both in animals and in humans (4, 5). Legislation imposes several limits on the presence of these pesticides as residues. The European Union has set maximum admissible concentrations of 0.1 μg L⁻¹ for individual pesticides and their related compounds in drinking water and of 0.05 mg kg⁻¹ for food of plant origin (6). Parathion is one kind of OP that is very toxic, with an LD₅₀ of 3 mg kg⁻¹ in rats, and may be responsible for more deaths among agricultural field workers than any other pesticide (7, 8). For the sake of human health protection and environmental control, the detection of trace OPs is of tremendous importance.

The methods used to determine organophosphate pesticides in fruits and vegetables are mainly based on gas chromatography or high-performance liquid chromatography coupled with various detections (9–11): mass spectrometry (12), thermospray mass spectrometry (13), and flame photometry (14). However, when the content of OPs is trace in food or drinking water, it is difficult to detect, or very expensive instruments (LC-GC)/MS/MS (12) are needed. The majority of those techniques may not be suitable for field or online monitoring. Consequently, the development of a simple, rapid, inexpensive, and sensitive analytical method for routine OP screening in fruits and vegetables is of particular significance and necessity. The electrochemical system possesses

high sensitivity and good stability, requiring only minimal space and low-cost instrumentation. Several electrochemical methods are also studied to detect electroactive OP compounds (15, 16) or applied electrochemical and gravimetric techniques in conjunction with enzymes, predominantly acetylcholine esterase and organophosphate hydrolase (17–19).

Molecular imprinting technology is a synthetic approach to imitate natural molecular recognition, and it has been proved to be an efficient method to produce functionalized materials that have the ability to recognize the specific template from a mixture of closely related compounds. The application of molecularly imprinted polymers (MIPs) has been utilized as materials of molecular recognition in many scientific and technical fields, such as solid-phase extraction, chromatographic separation, membrane separations, sensors, drug releases, and catalysts (20–24). Recently, the use of MIPs as specific preconcentration elements or detection platforms for OPs has been developed. A number of studies have recognized the importance of the selective binding of organophosphates by molecular imprinting for application in sensing (25–27). Surface molecular imprinting is one of the important types of molecular imprinting. The imprinted polymer not only possesses high affinity and selectivity but also can avoid the lower mass transfer to some extent, compared to the acrylic-based MIP prepared according to the conventional method.

In this study, a novel sensor for the determination of parathion based on polyethyleneimine (PEI) as functional macromolecule and ethylene glycol dimethacrylate (EGDMA) as cross-linker was fabricated by surface imprinting method using molecular imprinting technology. The electrochemical behavior of parathion at the imprinted film sensor was characterized by cyclic voltammetry and linear sweep voltammetry. The factors affecting

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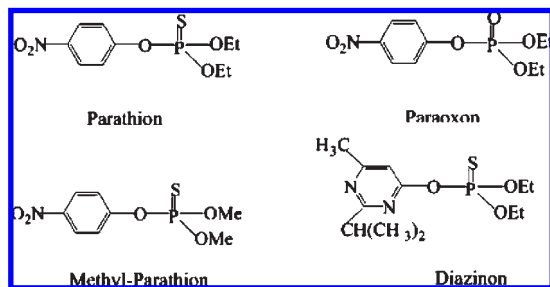


Figure 1. Chemical structures of the studied compounds.

adsorption and separation of the analytes are optimized to establish a simple and sensitive method as a potential analytical strategy to monitor the parathion in real samples.

MATERIALS AND METHODS

Materials and Reagents. Silica gel (100–140 mesh, Shanghai Chemical Reagent Co., Shanghai, China) was used as the support material to prepare the imprinted functionalized polymer. PEI, EGDMA, and γ -chloropropyltrimethoxysilane (CP) (Sigma, St. Louis, MO) were used in this study. Chitosan was obtained from Sinopharm Chemical Reagent. Figure 1 shows the chemical structures of the organophosphates used in this paper. Parathion, paraoxon, methyl-parathion, and diazinon were purchased from Dr. Ehrenstorfer GmbH Co. Stock solutions of individual pesticide standards were prepared in ethanol and kept at 0 °C until used. Then, they were diluted to the required concentration using 0.1 mol L⁻¹ phosphate buffer solution (PBS). PBS, pH 6.5, was prepared from NaH₂PO₄ and Na₂HPO₄ with distilled water. Cucumber and cabbage samples were purchased in a supermarket in Shanghai. Other chemicals used were of analytical grade, and all compounds were used without further purification.

Apparatus and Equipment. Electrochemical data were obtained with a three-electrode system using a CHI 660C electrochemical workstation (Shanghai Chenhua Instruments Co., Shanghai, China). The parathion sensor was used as a working electrode, a platinum wire as a counter electrode, and a saturated calomel electrode (SCE) (Jiangsu Electroanalytical Instruments Factory, China) as the reference electrode. The FT-IR spectrum was obtained at ambient temperature using a NEXUS470 optical bench (Nicolet) to confirm the chemical structure change before and after molecular imprinting. A thermogravimetric analyzer (TGA/SDTA851, Mettler Toledo) was used to calculate PEI amount (g/100 g) of grafting.

Preparation of Imprinted Polymer. The composite material PEI/SiO₂ was prepared according to the steps described in refs 28–30; PEI was grafted onto the surfaces of silica gel particles via the coupling grafting method (“grafting to” method). The typical preparation process was as follows. First, 10 g of silica gel particles was mixed with 60 mL of 33% methanesulfonic acid in a three-neck flask and refluxed with stirring for 8 h. Following this, the activated silica gel was reacted with CP at 80 °C for 6 h by using xylene as a solvent into which 1 mL of water was added, and chloropropylation silica (CP-SiO₂) was prepared. Finally, CP-SiO₂ was added into a 10% PEI aqueous solution, reaction was carried out at 90 °C for 6 h, PEI was grafted onto the silica gel surface in a coupling manner, and the composite adsorption particles of PEI/SiO₂ were prepared. The grafting degree of PEI/SiO₂ was determined with the TGA method. The PEI/SiO₂ particles used in this study have a grafting degree of 15.41 g/100 g. To prepare the MIP-PEI/SiO₂, PEI/SiO₂ (0.6 g) with a grafting degree of 15.41 g/100 g was added into 50 mL of parathion solution of 4 mmol/L, and the pH value was adjusted to 8 with dilute NaOH solution, and a certain amount of cross-linking agent EGDMA (0.1 g, 0.6 mmol) was added. The cross-linking reaction was performed at room temperature for 8 h with continuous stirring. The polymer was fully washed with hydrochloric acid solution of 0.1 mol L⁻¹ to remove the template. Then, the parathion-imprinted polymer MIP-PEI/SiO₂ was isolated by filtration and washed with pure water. Finally, the product particles were dried under vacuum at 80 °C for 12 h. The infrared spectra of PEI/SiO₂ and MIP-PEI/SiO₂ were determined to confirm the chemical structure change of PEI/SiO₂ after molecular imprinting. Complete extraction was

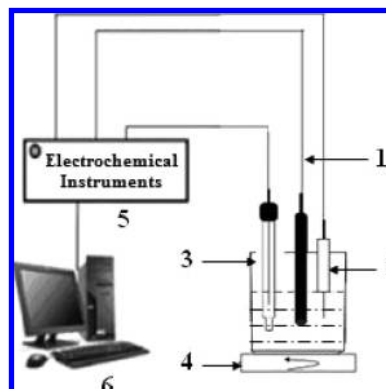


Figure 2. Layout of the three-electrode system: (1) working electrode; (2) counter electrode; (3) reference electrode; (4) magnetic stirrer; (5) CHI workstation; (6) computer.

confirmed by cyclic voltammetry (CV). For comparison, the nonimprinted polymer was also prepared following the same procedure, but without the addition of parathion.

Preparation of Parathion Sensor. The glassy carbon electrode was polished with a 0.05 μ m alumina slurry followed by thorough flushing with twice-distilled water, and then ultrasonically in 1:1 nitric acid, 1 mol L⁻¹ NaOH, acetone, and twice-distilled water. A certain amount of MIP-PEI/SiO₂ (1 mg) and a 1 mg mL⁻¹ chitosan solution were mixed together thoroughly, and then 4 μ L mixtures were dropped onto the surface of the pretreated glassy carbon electrode. After drying for 20 min, the modified electrode was rinsed with doubly distilled water. When the electrode is not in use, it should be stored in PBS (pH 6.5) at 4 °C.

Electrochemical Measurements. Figure 2 shows the layout of the three-electrode system. CV of a glassy carbon electrode coated with imprinted film was recorded in a solution of 0.1 mol L⁻¹ PBS (pH 6.5) between +0.4 and -1.4 V with a scan rate of 100 mV s⁻¹. Linear sweeping voltammetry (LSV) was performed in the potential range from -0.2 to -1.0 V. The scan rate, sample interval, and quiet time were 0.1 V s⁻¹, 0.001 V, and 2 s, respectively. The bound parathion was extracted by immersion of the electrode in ethanol for 15 min. Rebinding of parathion was achieved by incubation of the electrode in different concentrations of parathion solution in 0.1 mol L⁻¹ PBS. The electrode was then removed, rinsed with distilled water, and transferred into an electrochemical cell containing a 5 mL of PBS for electrochemical measurements as mentioned above. The supporting electrolyte used in the experiments was thoroughly deoxygenated by bubbling nitrogen (N₂ 99.99%) before each experiment.

Uptake kinetics of parathion by the imprinted PEI/SiO₂ polymer was also examined. The parathion-imprinted sensor was immersed in a 3 mg kg⁻¹ parathion solution with magnetic shaking for 5, 10, 15, 20, 30, 40, 50, and 60 min at room temperature. Following each incubation step, the electrodes were rinsed for 30 s with water, to avoid carry-over of the analyte. The electrodes were then transferred into fresh 0.1 M PBS, and the linear sweeping voltammograms were recorded.

Preparation of Samples. Extraction of 10 g of the homogenized cucumber or cabbage sample was performed with ether (30 mL). The extract was filtered with 0.45 μ m membrane filters and then evaporated to dryness. Ethanol (2 mL) was added to the dry residue, and after mixing, the solution was diluted to 100 mL with pH 6.5 phosphate buffer solution. Spiking of the sample was achieved by injecting a parathion stock solution into the sample and then homogenizing.

RESULTS AND DISCUSSION

Characteristics of Imprinted and Nonimprinted Polymers by FT-IR Spectra. To ascertain the presence of parathion in the PEI/SiO₂ composite materials, FT-IR spectra of imprinted and nonimprinted PEI/SiO₂ are compared in Figure 3. Imprinted and nonimprinted polymers showed similar locations and appearances of the major bands. The observed features around 1093 and 950 cm⁻¹ indicated Si-O-Si and Si-O-H stretching vibrations, respectively. O-H vibration reflected at 1630 cm⁻¹

indicated that it had adsorbed water (20). The bands around 801 and 467 cm^{-1} resulted from Si–O vibrations. The adsorptions of flex vibration and bend vibration for N–H bond appear at 3430 and 1630 cm^{-1} , respectively, and the adsorption of bend vibration for the C–N bond appears at 1488 and 1560 cm^{-1} . The appearances of these absorption bands show that PEI macromolecules have been grafted onto silica gel surface, and PEI/SiO₂ composite particles in a coupling manner have been formed. Characteristic features of imprinted PEI/SiO₂ when compared with nonimprinted PEI/SiO₂ were obvious infrared absorbance peaks at 1665 and 1384 cm^{-1} , which were attributed to the stretching vibration of N=O. These results suggested that PEI had been combined with the surface of the functionalized silica gel particle and that it had reacted with parathion in the imprinted materials through very strong hydrogen bond interaction between PEI and parathion. When hydrochloric acid is used as eluent, the hydrogen bond interaction between parathion

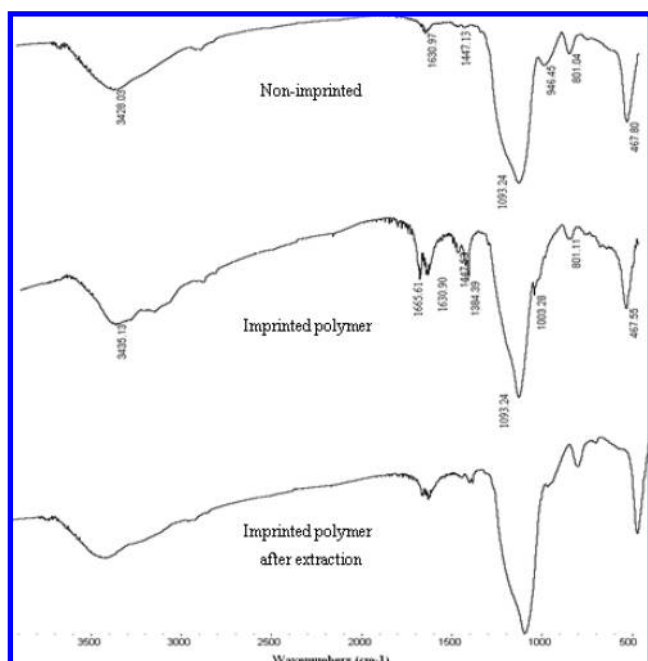


Figure 3. FT-IR spectra of the imprinted, nonimprinted polymer, and imprinted polymer after extraction in 0.1 mol L⁻¹ hydrochloric acid for 6 h.

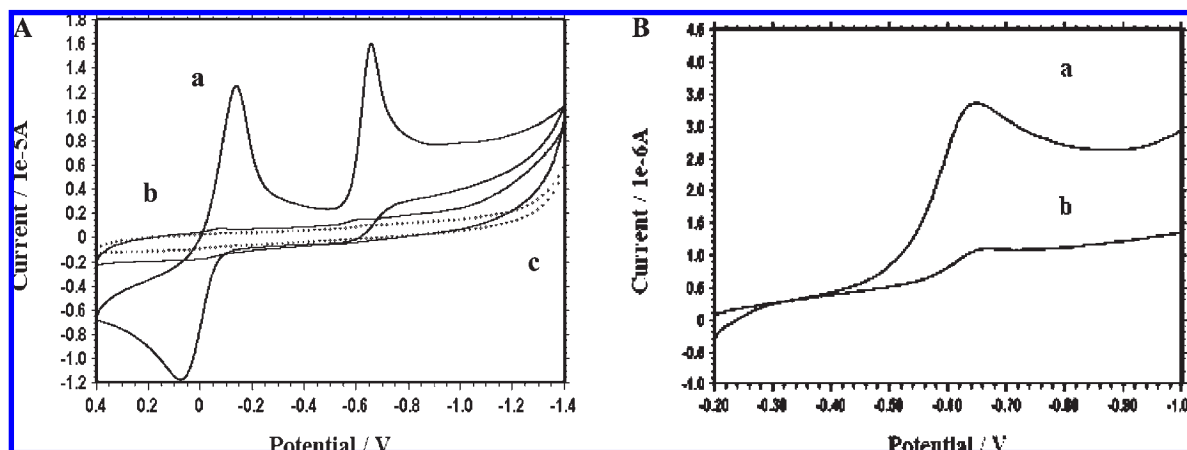


Figure 4. (A) Cyclic voltammetry of GCE coated with parathion-imprinted PEI/SiO₂ film (a) and nonimprinted PEI/SiO₂ film (c, dashed line). The voltammogram was recorded after the imprinted PEI/SiO₂ composite material was ultrasonically extracted in 0.1 mol L⁻¹ hydrochloric acid for 6 h (b). (B) Linear sweeping voltammetry of a GC electrode coated with a parathion-imprinted film (a) and nonimprinted film (b) after incubation in a 3 mg kg⁻¹ solution of parathion in 0.1 mol L⁻¹ PBS (pH 6.5) for 50 min.

and PEI is disrupted and, subsequently, parathion is released. Therefore, the corresponding characteristic adsorptions vanish.

Electrochemical Detection. Parathion exhibits well-defined CV in aqueous solution (25) resulting from the reduction of the nitrophenyl group to hydroxylamine via a four-electron irreversible reduction process at -0.6579 V versus SCE. Subsequently, with the potential turn more positive, hydroxylamine could be oxidized to nitroso through a two-electron transfer reaction. The oxidation peak is observed at 0.077 V, and the peak current increases gradually in the following scan. Another reductive peak appearing at -0.1364 V should be ascribed to a two-electron transfer reversible reaction corresponding to the transformation between nitroso and hydroxylamine.

Figure 4A(a) shows the CV of a parathion imprinted PEI/SiO₂ at the sensor in a 0.1 mol L⁻¹ PBS (pH 6.5) in the potential ranging from 0.4 to -1.4 V. Clear reduction-oxidation waves can be seen. Because the electrochemical measurement was carried out in a parathion-free solution, this implies that the oxidation-reduction waves are entirely due to the parathion embedded inside the PEI/SiO₂ composite materials. After the ultrasonic extraction of parathion in 0.1 mol L⁻¹ hydrochloric acid for 6 h, as shown in **Figure 4A(b)**, the CV of the imprinted film shows significant current decrease at both peaks. This indicates that parathion was efficiently removed in hydrochloric acid. Nevertheless, the small redox peaks of CV after parathion removal indicate that some unextractable templates were still left in the PEI/SiO₂ matrix. In the highly cross-linked rigid matrix of silica, those original templates situated at the center of PEI/SiO₂ particles cannot be completely removed, and no peaks could be observed in the NIP film (**Figure 4A(c)**).

The extraction of parathion results in the formation of sites in the matrix that can selectively rebind the template molecules. The affinity and selectivity of the parathion-imprinted film were characterized using LSV. A glassy carbon electrode coated with parathion-imprinted or nonimprinted films was placed in a 3 mg kg⁻¹ parathion solution for 50 min. **Figure 4B** shows LSV of the parathion-imprinted and nonimprinted film electrodes. Clear signals assigned to the reductive peak of the bound parathion are observed with parathion-imprinted film. At the same time, nonimprinted polymer modified electrodes have bound very small amounts of parathion. These results clearly indicate the significant affinity of imprinted versus nonimprinted films toward parathion, which demonstrated that the binding affinity of the

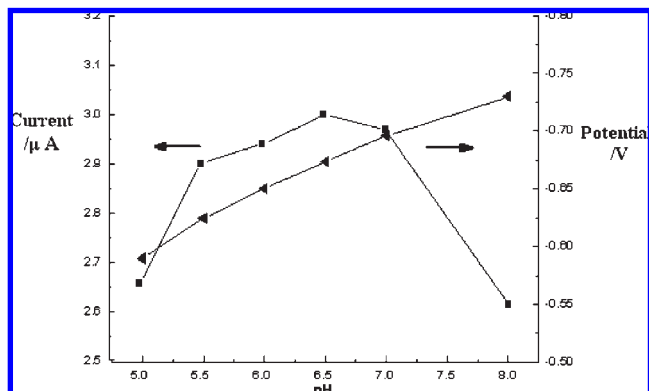


Figure 5. Effect of pH on the electrochemical response of the imprinted sensor: linear dependence of the reduction current on the pH (■); linear dependence of the reduction peak potential on the pH (▲). The concentration of parathion is 3 mg kg^{-1} .

imprinted film was from the specific sites formed by the imprinting effect.

Effect of pH on the Reduction of Parathion at the Imprinted Sensor. Both the sensing system and the electrochemical reaction may be affected by the pH value of the electrolyte solution. The effect of pH value in the test medium on the current response of parathion was examined in the range of pH 5–8. **Figure 5** presents the effect of pH on the responses of the parathion. We can see that the irreversible reduction peak potential changes to a more positive potential with the decrease of the pH of the solution, which shows a linear dependence of the reduction peak potential on the pH in the range of 5–8. A slope of 45.8 mV pH^{-1} suggested that the numbers of protons and electrons involved in the reaction are equal. This was consistent with the redox mechanism of parathion. We can also observe that the response current increases with an increase of pH up to 6.5, and then it decreases at higher pH. This indicates that the lower pH value is advantageous to electronation reaction much as the mechanism shown above, but the excessively lower pH may also lead to the hydrogen side reaction, which may affect the PEI/SiO₂ composite materials to the parathion adsorption and cause the decrease of electrochemical signal. Thus, according to the current enhancement and shape, pH 6.5 was selected for further experiments.

Uptake Kinetics of Parathion by the Imprinted PEI/SiO₂ Sensor. The uptake kinetics of parathion by the imprinted PEI/SiO₂ sensor (3 mg kg^{-1} parathion onto the imprinted sensor) was also examined and is shown in **Figure 6**. The results indicated that the imprinted sensor had fast uptake kinetics: 63.5% of binding was obtained within a short shaking period of 5 min, and adsorption equilibrium was almost reached within 50 min. If the concentration of parathion was lower, the time to saturation would be correspondingly shorter. The rapid adsorption kinetics of the imprinted film is an obvious advantage for its application in the online detection. This means that surface imprinting greatly facilitates diffusion of the template to the binding sites.

Selectivity of the Imprinted Sensor for Parathion. A series of closely related compounds were used to further examine the selectivity of the designed molecularly imprinted sensor. Selective recognition property studies of imprinted polymer and nonimprinted polymer were performed with four OPs at 3 mg kg^{-1} . Results (**Figure 7**) demonstrate that the reductive current value of imprinted polymer toward parathion was higher than that of the three other OPs, whereas the adsorption capacities of nonimprinted polymer were almost same. The competitive selective property of parathion on the imprinted PEI/SiO₂ sensor was investigated using PBS containing 0.06 mg kg^{-1} parathion and

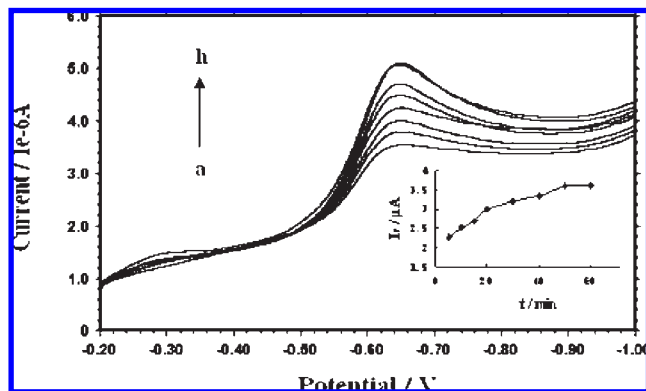


Figure 6. LSV responses at increasing incubation times: 5, 10, 15, 20, 30, 40, 50, 60 min (a–h). (Inset) Kinetic uptake plot of the imprinted polymer.

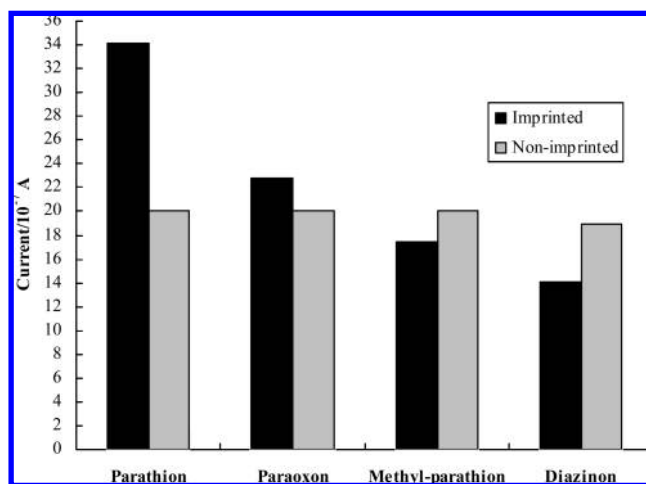


Figure 7. Selective adsorption of four OPs by the imprinted and non-imprinted sensors.

Table 1. Competitive Selectivity of the Imprinted Sensor toward the OPs

interfering substance	changes of current response in different ratios of interfering substance to parathion (0.06 mg kg^{-1})		
	10:1	50:1	100:1
paraoxon	+2.5%	+8.1%	+16.5%
methyl-parathion	+2.0%	+3.0%	+6.7%
diazinon	+0.6%	+2.9%	+5.5%

different amounts of potential interfering substances as incubation media (**Table 1**). Methyl-parathion and diazinon were added to parathion solution up to 50 times the concentration, respectively; no perceivable change in the peak current was observed, whereas the addition of paraoxon, which differs from parathion in only one atom, at 100:1 ratio can increase the current response about 16.5% and brought on positive interference for electrochemical detection of parathion. The greatly similar structure contributes to its higher current response. The selectivity of the parathion-imprinted cavity toward parathion is rather remarkable in its magnitude, especially when the close structural resemblance of the investigated organophosphates to parathion is taken into account. The high selectivity originates from the rigid structure of the cavity and the nature of the prepolymerization complex between parathion and the functional monomers. Another contributing factor can be the different solubilities of the organophosphates in the aqueous medium. We can see that the selectivity of the imprinted film sensor to parathion is good, and an excellent parathion sensor has been fabricated successfully.

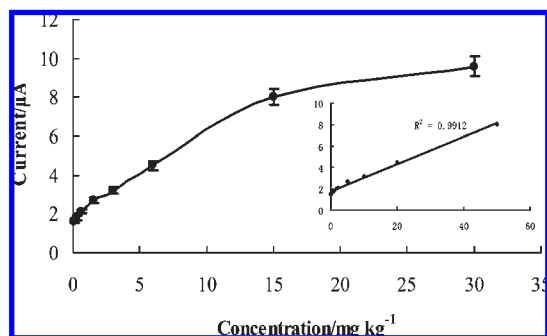


Figure 8. Calibration curve of imprinted PEI/SiO₂ sensor response for parathion.

Investigation of Reproducibility and Stability of the Imprinted Sensor. To repetitively employ the modified electrode, a simple procedure for its regeneration had to be developed. Interestingly, almost no changes in the electrochemical signal of the bound parathion were observed upon continuous cycling of the electrode. This indicates that the template is strongly bound to the cavity and that CV is not sufficient to remove the electroactive species from the matrix. On the other hand, immersing the electrode in ethanol made it possible to reuse it for numerous analytical cycles. For example, when the concentration of parathion was controlled at 0.06 mg kg⁻¹, good reproducibility was observed with a relative standard deviation (RSD) of 5.4% for eight parallel detections. The sensor retained a response of 96% of the initial current after 10 days of storage in PBS at 4 °C, and it showed no obvious current decline after 30 uses. The results demonstrate that our sensor is sufficiently perfect for the determination of trace parathion.

Linearity and Loading Capacity of the Imprinted Sensor. The linearity of parathion was checked over a concentration range of 0.015–15 mg kg⁻¹ (Figure 8), for reductive peak current (~-0.65 V), which is wider than that at ZrO₂ nanoparticle-based electrochemical sensor (5) and that by multiresidue method using solid-phase extraction and gas chromatography–microelectron capture detection (31). A good linear relationship was observed with a good correlation coefficient ($R^2 = 0.9912$). The detection limit of the sensor was 0.003 mg kg⁻¹ (S/N = 3), which is much lower than that obtained at a potentiometric OPH-based enzyme electrode (18) and is comparable to those obtained at the amperometric bienzymatic sensor based on a FePC-modified carbon paste electrode (2) and the disposable screen-printed thick-film electrodes (17). In addition, the saturation level of the imprinted sensor was 30 mg kg⁻¹.

Determination of Parathion Levels in Vegetable Samples. High sensitivity of the sensor enabled us to determine parathion in vegetable samples according to the recommended method. For the validation studies the vegetable samples were divided into two fractions for electrochemical methods and HPLC analyses. The results obtained with both methods were same. None of cucumber and cabbage samples from a local supermarket contained any parathion, so they were spiked with three levels of parathion (0.12, 0.3, 0.6 mg kg⁻¹) and analyzed. At each concentration, three measurements were performed. The analytical data are shown in Table 2. We achieved good recoveries of cucumber and cabbage samples ranging from 97.7 to 102.5% and from 94.7 to 107.5%, respectively.

In summary, a simple molecular imprinting procedure was developed to synthesize a highly selective parathion-imprinted PEI/SiO₂ sensor by a surface molecular imprinting technique. The prepared sensor shows high affinity and selectivity and good site accessibility for parathion. The analytical characterization of

Table 2. Recoveries of Parathion in 10.0 g Cucumber and Cabbage Samples (Mean ± RSD, $n = 3$)

sample	spiked (mg kg ⁻¹)	recovery (%)
cucumber		
1	0.12	102.5 ± 2.0
2	0.30	97.7 ± 4.5
3	0.60	102.3 ± 5.5
cabbage		
1	0.12	107.5 ± 1.0
2	0.30	94.7 ± 1.5
3	0.60	100.8 ± 0.6

parathion at this sensor indicated that the sensor was practical for the determination of parathion in vegetable samples.

ABBREVIATIONS USED

OPs, organophosphate pesticides; MIPs, molecularly imprinted polymers; PEI, polyethyleneimine; EGDMA, ethylene glycol dimethacrylate; CP, γ -chloropropyltrimethoxysilane; PBS, phosphate buffer solution; CV, cyclic voltammetry; LSV, linear sweeping voltammetry.

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