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Au–TiO₂/Chit modified sensor for electrochemical detection of trace organophosphates insecticides

Yunhe Qu, Hong Min, Yinyin Wei, Fei Xiao, Guoyue Shi*, Xiaohua Li, Litong Jin*

Department of Chemistry, East China Normal University, Zhongshan Road (N), Shanghai 200062, PR China

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ABSTRACT

In this paper, Au–TiO₂/Chit modified electrode was prepared with Au–TiO₂ nanocomposite (Au–TiO₂) and Chitosan (Chit) as a conjunct. The Au–TiO₂ nanocomposite and the films were characterized by electrochemical and spectroscopy methods. A set of experimental conditions was also optimized for the film's fabrication. The electrochemical and electrocatalytic behaviors of Au–TiO₂/Chit modified electrode to trace organophosphates (OPs) insecticides such as parathion were discussed in this work. By differential pulse voltammetry (DPV) measurement, the current responses of Au–TiO₂/Chit modified electrode were linear with parathion concentration ranging from 1.0 ng/ml to 7.0×10^3 ng/ml with the detection limit of 0.5 ng/ml. In order to evaluate the performance of the detection system, we also examined the real samples successfully in this work. It exhibited a sensitive, rapid and easy-to-use method for the fast determination of trace OPs insecticides.

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1. Introduction

OPs are known to be highly neurotoxic and they disrupt the cholinesterase that regulates acetylcholine, a neurotransmitter needed for proper nervous system function [1]. As toxic compounds, the OPs may cause negative effect on the visual system, sensory function, cognitive function, and nervous system which result in severe health problems in both animals and humans. For example, parathion is one kind of OPs that is very toxic with LD_{50} of 3 mg/kg in rats, and may be responsible for more death among agricultural field workers than any other pesticides [2]. Thus, OPs insecticides pose major security and environmental risks in present time. The detection of trace OPs is of tremendous importance and there is a necessity for accurate, sensitive, rapid, easy-to-use and portable method to facilitate the detection, quantification and remediation of these OPs present in the natural aquatic system or remain in food.

Currently, several detection techniques such as HPLC, GC/MS, capillary electrophoresis and colorimetry have been developed to measure the OPs insecticides in environment and in food [3–11]. A majority of these methods are time-consuming, expensive, and require trained personnel, which may not be suitable for on-site

use [12]. Electrochemical methods are very attractive for on-site monitoring of OPs, like parathion [13–17]. The electrochemical system possesses high sensitivity, good stability, minimal space and power requirement and low-cost instrumentation [18]. To enhance the sensitivity and stability of the measurements, the electrodes modified with designable molecules have been used in electrochemical determination. The target compounds such as OPs would be adsorbed onto the chemically modified surface which makes the trace detection possible.

In recent years, nanometer-scale materials have been widely used in the fabrication of electrochemical sensors for their large surface area, large pore size, uniform pore structure, and high loading capacity [1,19–25]. These favourable characteristics of the modified materials make the detection of different substrate possible. In the previous articles, the nanocomposite Au–TiO₂ has been applied to the detection of nitric compounds and it has greatly enhanced the sensitivity of the detection system [26–28]. To the best knowledge of the authors, there are little papers which take advantage of Au–TiO₂ nanocomposite modified electrodes to detect the OPs.

In this paper, an Au–TiO₂/Chit/glass carbon electrode (GCE) was prepared for the fast detection of trace OPs. The film was composed of Au–TiO₂ nanocomposite and Chit as a conjunct. In the nanocomposite, TiO₂ nanoparticles have a strong affinity to the OPs which possess a phosphate group and Au nanoparticles have an excellent conductivity. This modified electrode relies on the two important characteristics to detect ultrace parathion and a lower detection limit that than other methods is achieved [29–31]. The





^{*} Corresponding authors. Tel.: +86 21 62232627; fax: +86 21 62232627.

E-mail addresses: gyshi@chem.ecnu.edu.cn, iamshgy@hotmail.com (G. Shi), ltjin@chem.ecnu.edu.cn (L. Jin).

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nanoparticles were characterized with UV–vis and scan electron microscope (SEM). DPV were employed to optimize the experimental parameters for the detection of pesticides in solution and vegetable samples. The interferences for the determination were also studied in this work. The results illustrate that this method is fast, simple and sensitive, which will have wide application in the fields of foodstuff security and environmental protection.

2. Experimental

2.1. Chemicals and apparatus

TiO₂ (P-25, Φ = 30 nm) was purchased from Degussa Company. Parathion, Dichlorvos, Methamidophos and Trichlorfon were purchased from Dr. Ehrenstorfer GmbH Company. Chitosan was purchased from Sinopharm Chemical Regent. All the reagents were used without further purification and the double-distilled water was used in this work.

Au–TiO₂ nanoparticles were characterized by SEM (HITACHI S-4800) and UV–vis spectrophotometer (Cary–50).

All the electrochemical experiments were performed with a CHI 1030 analyzer (CH Instruments). A three-electrode configuration was employed, consisting of a bare glassy carbon electrode (d=3 mm, Bioanalytical system, Tokyo, Japan) or a Au–TiO₂/Chit modified electrode serving as working electrode, while a saturated calomel electrode (SCE) and platinum wire served as the reference and counter electrode, respectively. Electrochemical experiments were carried out in a 10.0 ml electrochemical cell at room temperature ($25 \circ C \pm 1$). All potentials were referred to the SCE reference electrode.

2.2. Synthetic of Au–TiO₂ nanocomposite

Nano-TiO₂ solution was prepared by dispersing nano-TiO₂ particles in the double-distilled water and sonicated for 5 min with a concentration about 0.1 mg/ml. The previous solution was stirred with 1.88 ml 0.1 mol/l sodium citrate for 10 min to exchange OH⁻ with citrate anions. Then, 0.125 ml of 1% HAuCl₄ were incrementally added (with at least 10 min between additions) along with an excess of 0.07 ml 0.1 mol/l NH₂OH·HCl. Stirring for about 12 h, the clear solution became purple and 1.02×10^{-3} mol/l Au–TiO₂ nanoparticles were synthesized. The amount of Au was about 0.1 mol of Au per mol of TiO₂. Nano-Au solution was synthesized by repeating previous procedure without adding TiO₂ particles.

2.3. Fabrication of (Au-TiO₂/Au/TiO₂)/Chit/GCE

The prepared GCE was immerged into $0.5 \text{ mol/l } H_2SO_4$ and performed CV until stabilization before used. $10.0 \,\mu$ l Au–TiO₂ solution and $2.0 \,\mu$ l 1 mg/ml Chit solution were mixed together, and then $8.0 \,\mu$ l mixtures were added to the surface of GCE. Finally, the electrode was dried in the air. For contrast, the TiO₂/Chit electrode and Au/Chit electrode were also prepared in this work.

2.4. Electrochemical experiments

DPV measurements were performed to optimize the experimental conditions and determine the OPs compounds at room temperature in the potential range from -0.3 to -0.8 V with the scan rate of 100 mV/s. The supporting electrolyte used in the experiments was thoroughly deoxygenated by bubbling nitrogen (N₂ 99.99%) before each experiment.



Fig. 1. SEM image of Au–TiO₂ particles and the inset shows UV–vis spectra of nanoparticles. (A) Au–TiO₂ nanoparticles and (B) TiO₂ nanoparticles.

2.5. Preparation and determination of real samples

The onion, lettuce and cabbage were purchased from the market and cleaned three times using the double-distilled water. Different concentrations of parathion solution were sprinkled on the surface of different vegetables [32,33]. After about 24 h, all the samples weighing 10–20 g were chopped and meshed before 1 ml of acetone and 9 ml of 0.1 M phosphate buffer (pH 8.0) were added to each sample. All the experiments above maintained on the nitrogen atmosphere and the mixtures were treated in ultrasonic for 15 min. The suspensions were centrifuged (10 min, 2000 rpm) and the supernatants were directly detected by DPV without any extraction or preconcentration step. The content of parathion in the samples can be achieved from the calibration curve.

3. Result and discussion

3.1. Characterization of Au–TiO₂ by SEM and UV–vis

In pure Au nanoparticles, the collective oscillations of free electrons, known as the surface plasmon, cause an absorption peak (\sim 520 nm) to appear in the visible region [34]. Fig. 1 inset is the UV–vis spectra of nanoparticles of Au–TiO₂ (A) and TiO₂ (B). We find that there is a big absorption peak at 525 nm in curve A and no obvious absorption peak in curve B. Thus, the UV–vis spectra of the nanocomposites confirms that the Au nanopaticles were embedded in the TiO₂ particles.

According to the literatures, the particles size could be calculated in virtue of the following expression:

$$D = (-6.6521 \times 10^{-8})\lambda + (1.9557 \times 10^{-4})\lambda - (9.2352 \times 10^{-2})\lambda + 13.29.$$

 λ is the wavelength of the UV peak (525 nm). The results show that the particle diameter of the as-prepared Au nanoparticles is about 8.00 nm. Fig. 1 is the SEM image of the Au–TiO₂ nanoparticles which confirms the successfully formation of the nanoparticles. Clearly, Au nanopaticles can be found on the surface of TiO₂ particles and they are dispersed evenly throughout the TiO₂ particles. The Au nanoparticles formed in our work possess an average size of 8–9 nm which is consistent with the diameter that was calculated from the UV–vis spectra. So we confirm that Au–TiO₂ nanocomposite was successfully synthesized in our work.



Fig. 2. Cyclic voltammograms of Au–TiO₂/Chit/GCE in a phosphate buffer solution (pH 5.0) with 0.5 μ g/ml parathion solution (A) and in the absence of parathion (B).

3.2. Electrochemical behavior of parathion

Fig. 2 shows the voltammograms of parathion/Au–TiO₂/Chit /GCE (A) electrode and Au–TiO₂/Chit/GCE (B) in a phosphate buffer solution (pH 5.0). A pair of rather well-defined redox peaks (E_{pa1} , 0.179 V and E_{pc1} , 0.026 V) and an irreversible reduction peak (E_{pc2} , -0.573 V) were observed in the potential range from 0.4 to -1.0 V (Fig. 2, curve A). The irreversible reduction peak corresponds to the reduction of the nitro group to the hydroxylamine group (Fig. 3, reaction 1), and the reversible redox peaks are attributed to a twoelectrode-transfer process (Fig. 3, reaction 2). The mechanism for this process was widely reported [3]. A control experiment was performed under the same conditions in the absence of parathion; no redox peak was observed (Fig. 2, curve B). So we can conclude that the redox peaks correspond to the reaction of the parathion.

3.3. Comparison of different modified electrodes

Fig. 4 shows the current responses to parathion with the electrodes of Au–TiO₂ (a), Au (b), bare (c) and TiO₂ (d). Compared with curves b–d, the curve a of Au–TiO₂ electrode exhibits the biggest current response to the same concentration of parathion and the best linearity. It is because that the nanocomposite has bigger surface area and more active-center. Furthermore, the TiO₂ nanoparticles have a strong affinity to the OPs which possess a phosphate group but the conductivity of the particles is not very well. The better conductivity is due to the Au nanoparticles which play an important role similar to a conducting wire, which was easier for the electron transfer to take place. Curves b and c are the current responses of Au modified electrode and bare electrode, respectively. As can be seen the responses of the two electrodes are similar and much smaller than the nanocomposite modified elect-



Fig. 4. Current responses of electrodes modified with different nanoparticles. (a) Au–TiO₂, (b) Au, (c) bare and (d) TiO₂. The concentration of parathion is from 0.2 to $1.0 \,\mu$ g/ml, in the PBS (pH 5.0) DPV scanning potential range, -0.3 to -0.8 V, the scanning rate, $100 \,\text{mV/s}$.

trode because either the Au or bare electrode surface do not have a good affinity to the parathion. Curve d is the current response of the TiO_2 modified electrode which has the smallest response to parathion even though TiO_2 has a good affinity to OPs because the TiO_2 nanoparticles baffles the electron transfer from the TiO_2 to the electrode surface. Thus, the Au– TiO_2 nanocomposite that possesses the strong affinity and better conductivity play a really important role in the detection system.

3.4. Optimum experimental parameters

We compared the DPV current responses by the electrodes modified with different loadings of nanoparticles in 0.2 µg/ml parathion solution. Theoretically, the nanoparticle films will have more active-centers and larger surface area with the loadings increment. In this work, we found that the electrode modified with 8.0 μ l Au–TiO₂ solution (1.15 × 10⁻⁷ mol/cm²) resulted in the biggest responses. Deficient loadings modified on the electrode may result in less active-centers and smaller surface area. But we also found the superfluous loadings modified on the electrode were brushed off from the surface and reduced the rate of electron transfer (figure not shown). Furthermore, the excess loadings of Au-TiO₂ may affect the reproducibility and sensitivity of the OPs determination. Therefore, we choose 1.15×10^{-7} mol/cm² ($C_{Au-TiO_2} \times V/S_{GCE}$) Au-TiO₂ nanoparticles as the optimum amount in our experiment. We also examined the stability of the optimum sensor (Fig. not shown). The sensor keeps 85% current responses to the same concentration parathion after used 10 days.

Fig. 5 presents the effect of pH on the responses of the parathion. We can see that the irreversible reduction peak potential changes to



Fig. 3. Scheme of the electron-transfer process.



Fig. 5. Effects of pH on the electrochemical response of the sensor. The concentration of parathion is $0.2 \ \mu$ g/ml. Electrochemical detection conditions, same as Fig. 4.

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 3.0–7.0. A slope of 50.0 mV/pH suggests that the numbers of proton and electron involved in the reaction are equal which is consistent well with the redox mechanism of parathion as shown in Fig. 3 (reaction 1). We can also see that the response current increases with an increase of pH up to 5.0, and then it decreases at higher pH. It indicates that the lower pH value is advantageous to electronation reaction march as the mechanism showed above, but the excessively lower pH such as 3.0 may lead to the analyzing hydrogen side reactions, which may affect the Au–TiO₂ nanoparticles to the parathion adsorption and cause the decrease of electrochemical signal.

3.5. The detection of parathion and the analyze of real samples

Under the above optimal parameters, Au–TiO₂/Chit/GCE was used to detect parathion with DPV in this work. Fig. 6 shows the DPV responses recorded in PBS (pH 5.0) containing different concentration of parathion by the modified electrode. As Fig. 6 shown, there is



Fig. 6. DPV responses of increasing parathion concentration, from bottom to top, 0, 1.0×10^{-3} , 5.0×10^{-2} , 0.1, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 µg/ml, respectively. The inset shows the calibration curve. Electrochemical detection conditions, same as Fig. 4.

Table 1	
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The detection results of real samples

Sample	The amount of parathion sprinkled on the vegetables (ng)	The amount of parathion detected by the modified electrode (ng)
Onion Lettuce Cabbage	1000 1000 1000	890 910 880

a well reduction peak at -0.575 V even in 1.0 ng/ml parathion solution and the response currents enhance gradually with the increase of the parathion concentration. It indicates that the parathion can be reduced at the potential of -0.575 V, corresponding to the formation of hydroxylamine with the addition of parathion the reduction current was increased equally with the increase of parathion's concentration [3]. The inset is the calibration curve of the detection system. The current responses are linear with parathion ranging from 1.0 ng/ml to 7.0×10^3 ng/ml with the detection limit of 0.5 ng/ml which indicates that Au-TiO₂/Chit modified electrode displays a better sensitivity to the fast determination of parathion.

To evaluate the performance of the developed modified sensor, the contents of parathion in real samples of onion, lettuce and cabbage were detected by Au–TiO₂/Chit/GCE. Additionally, a blank sample was prepared from the noncontaminated vegetables and analyzed with the above method. From the data in Table 1, it can be seen that the parathion amount in the vegetable samples were about 10% lower when analyzed by a modified sensor compared to the amount of parathion sprinkled on the vegetables. The lower result can be attributed to the insufficient oxidation of the parathion.

3.6. Interferences experiments

The effect of interferences of electroactive nitrophenyl derivatives and oxygen-containing inorganic ions were evaluated during the detection of parathion The control experiments were performed with $0.4 \,\mu$ g/ml parathion in PBS (pH 5.0) in the absence or presence of $0.4 \,\mu$ g/ml dichlorvos, $0.4 \,\mu$ g/ml methamidophos, $0.4 \,\mu$ g/ml trichlorfon, $0.1 \,\text{mol/l} \,\text{NO}_3^-$ or $0.1 \,\text{mol/l} \,\text{SO}_4^{2-}$, respectively. 95–108% current responses at $-0.575 \,\text{V}$ were remained in the above solutions.

4. Conclusion

We have demonstrated an electrochemical approach for the fast determination of OPs with the use of Au–TiO₂/Chit modified electrode incorporating a three-electrode configuration. The material of nanocomposite Au–TiO₂ exhibits large surface area and good affinity of OPs which help the electrode adsorption and determination of parathion. The sensitivity of the modified electrodes has been investigated by electrochemical methods. The results show that the Au–TiO₂/Chit modified electrode exhibits high sensitivity for the trace determination of parathion in solution and real samples.

We believe that the nanocomposite $Au-TiO_2/Chit$ modified sensor on the basis of electrochemical method may have promising application for OPs detection in environmental field.

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