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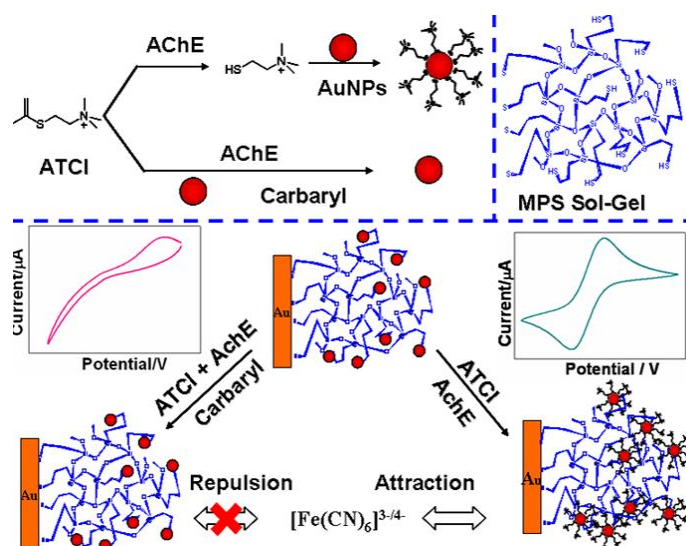
A Simple Electrochemical Biosensor based on AuNPs/MPS/Au Electrode Sensing Layer for Monitoring Carbamate Pesticides in Real Samples

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Graphical abstract



Highlights

- A novel and simple biosensor was developed for quantitative determination of carbamate Pesticides.
- The biosensor was developed based on AuNPs/MPS/Au electrode as a sensing layer.
- The principle was based on the different electrochemical response toward $\text{Fe}(\text{CN})_6^{3-}$.
- The biosensor has also been used to determine carbamate Pesticides in real samples.

Abstract

A simple electrochemical biosensor for quantitative determination of carbamate pesticide was developed based on a sensing interface of citrate-capped gold nanoparticles (AuNPs)/(3-mercaptopropyl)-trimethoxysilane (MPS)/gold electrode (Au). The biosensor was fabricated by firstly assembling three-dimensional (3D) MPS networks on Au electrode and subsequently assembling citrate-capped AuNPs on 3D MPS network via Au-S bond. The interface of AuNPs/MPS/Au was negatively charged originating from the citrate coated on AuNPs that would repulse the negatively charged ferricyanide ($[\text{Fe}(\text{CN})_6]^{3-/4-}$) to produce a negative response. In the presence of acetylcholinesterase (AChE) and acetylthiocholine (ATCl), the AChE catalyzes the hydrolysis of ATCl into positively charged thiocholine which would replace the citrate on AuNPs through the strong Au-S bond and convert the negative charged surface to be positively charged. The resulted positively charged AuNPs/MPS/Au then attracted the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to produce a positive response. Based on the inhibition of carbamate pesticides on the activity of AChE, the pesticide could be quantitatively determined at a very low potential. The linear range was from 0.003 to 2.00 μM . The sensing platform was also proved to be suitable for carbamate pesticides detection in practical sample.

Keywords: Electrochemical biosensor; Carbamate pesticide; Acetylcholinesterase; (3-mercaptopropyl)-trimethoxysilane network; Ferricyanide

1. Introduction

Carbamate pesticides are extensively used in agricultural fields owing to their high insecticidal activity [1-4]. However, the trace amounts of carbamate pesticides in the environment will result in a potential hazard for ecosystem and human healthiness due to their inhibitory effect on acetylcholinesterase (AChE), a key enzyme regulates acetylcholine (a neurotransmitter needed for proper nervous system function) [5-9]. Therefore, it is of great importance to find ways for detection of carbamate pesticide residues. Enzyme-based electrochemical biosensors have emerged in recent years as the most promising alternative to traditional methods for carbamate pesticide detection due to their high sensitivity, rapid response and easy operation [10-13]. Among them, AChE biosensors based on the inhibition of AChE showed satisfactory results for pesticide analysis [14-17]. AChE could catalyze the hydrolysis of acetylthiocholine (ATCl) to produce an electroactive thiocholine as a marker for carbamate pesticides detection. The inhibition of carbamate pesticides on AChE resulted in the decline of produced thiocholine and the oxidation current decreased accordingly. Then carbamate pesticides could be detected by measuring the decline of oxidation current of thiocholine [18-20]. However, the oxidation potential of thiocholine is very high and the oxidation peak is very weak, which results in a poor sensitivity and serious interference.

To overcome these serious problems, nanocatalysts were employed to catalyze the oxidation of thiocholine to reduce the work potential. For example, AChE/Prussian blue (PB)-chitosan (CHIT)/glassy carbon electrode (GCE) exhibited a good electrocatalytic activity toward the oxidation of thiocholine [1]. The oxidation potential of thiocholine was decreased from 0.68 V to 0.32 V (v.s. saturated calomel electrode (SCE)) and the sensitivity and selectivity of the biosensor were improved accordingly. The AChE/polyaniline/multi-walled carbon nanotubes/GCE catalyzed the oxidation of thiocholine at +0.25 V (v.s. Ag/AgCl (saturated KCl)) and used for carbamate pesticides detection in fruit and vegetables accordingly [14]. Another method was based on AChE and cholineoxidase co-immobilized electrode by using parabenzoquinone as a mediator [21]. Although good performances have been achieved, the strategies of immobilizing enzymes on solid supports still suffer from inherent drawbacks as followed [22]. First, sequential immobilization procedures are tedious and complicated [10,23,24]. Second, sensitivity and stability are fragile during transportation and storage because enzymes tend to lose their activity in contact with a supporting surface owing to unfavorable orientation and unfolding [25]. Third,

the reproducibility and regenerative ability are typically low [20,25-28].

Herein, a novel strategy for quantitative determination of carbamate pesticides was developed based on the citrate-capped gold nanoparticles (AuNPs)/(3-mercaptopropyl)-trimethoxysilane (MPS)/Au electrode (Au) as a sensing interface. The negatively charged AuNPs/MPS/Au interface repulsed the same charged ferricyanide ($[\text{Fe}(\text{CN})_6]^{3-/4-}$) to produce a negative response. While, in the presence of AChE and ATCl, AChE would catalyze the hydrolysis of ATCl into positively charged thiocholine which could replace the citrate on the citrate-capped AuNPs through Au-S bond to convert the negatively charged surface into positively charged one and attract the negatively charged $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to give a positive response accordingly. Based on the inhibition of carbamate pesticides on the activity of AChE, the carbamate pesticides could be quantitatively determined. The resulted biosensor could be performed at low potential and used to determine trace amounts of carbamate pesticides in practical samples accordingly. The experimental conditions related to the preparation of AuNPs/MPS/Au and the performance of the resulted sensor was investigated in detail.

2. Experimental section

2.1 Chemicals and solutions

The AChE (type C3389, 500 U mg^{-1} from electricel), ATCl and MPS were purchased from Sigma-Aldrich (St. Louis, USA). Chloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) and trisodium citrate were purchased from EM Sciences. Other reagents of analytical reagent grade were purchased from Beijing Chemical Reagent Factory (Beijing, China). 0.2 M phosphate buffer solution (PBS pH 7.0) was prepared from 0.2 M sodium dihydrogen phosphate (NaH_2PO_4) and 0.2 M disodium hydrogen phosphate (Na_2HPO_4). All solutions were prepared with ultra-pure water purified by a Millipore-Q System ($\rho \geq 18.2 \text{ M}\Omega \text{ cm}$).

2.2 Preparation of citrate-capped AuNPs

The citrate-capped AuNPs were prepared according to previous procedure [29]. Briefly, HAuCl_4 solution (10 mL, 24.0 mM) was heated to boiling, and then trisodium citrate (1.0 mL, 38.8 mM) was added. The mixture was kept boiling under continuous stirring. The solution was kept boiling for another 15 min under vigorous stirring after its color was changed from light yellow to dark red. Then the solution was cooled down to room temperature and stored at 4 °C. The

formation of citrate-capped AuNPs was confirmed by transmission electron microscopy (TEM) and UV-vis absorbance spectroscopy (Figure S1, Supporting Information).

2.3 Preparation of MPS sol-gel networks

According to the previous literatures [30,31], aqueous MPS sol-gel was prepared by mixing MPS with water at a 1:4 ratio, 10% (v/v) of ethanol, and 3.3% (v/v) of 0.1 M hydrochloric acid. The mixture was sonicated for 30 min until a clear and homogeneous solution was formed and subsequently stored at room temperature for 3 h. Then the homogeneous and pellucid solution was used to prepare the MPS/Au electrode

2.4 Preparation of AuNPs/MPS/Au

The cleaned Au electrode was thoroughly rinsed with water and immersed in the MPS sol-gel for 10 min at room temperature to prepare MPS/Au electrode. The resulted self-assembled electrode was thoroughly rinsed with water to remove physically adsorbed MPS. Then it was immersed in the citrate-capped AuNPs solution for 10 h to prepare AuNPs/MPS/Au which was then stored at 4 °C before use (Scheme 1A).

2.5 Instruments

All electrochemical experiments were performed using a CHI 750D electrochemical workstation (Shanghai, China). A three-electrode configuration was used and contained a platinum wire as the auxiliary electrode, a SCE as the reference electrode, and a bare or modified Au electrode as the working electrode. UV-vis absorption spectra were recorded by a Lameda 35 UV-vis Spectrophotometer using a transparent quartz glass substrate. Atomic force microscopy (AFM) measurements were carried out with an AJ-III (Shanghai Aijian Nanotechnology) in tapping mode. Standard silicon (Si) cantilevers (spring constant, 0.6-6 N/m) were used under its resonance frequency (typically, 60-150 kHz). To examine the size of citrate-capped AuNPs, the sample was characterized by a JEM-2100HR TEM at 200 kV. Fourier transform infrared (FTIR) spectroscopy was obtained by a Perkin-Elmer Spectrom 100 spectrometer (Perkin-Elmer Company, USA) with KBr power.

The pretreated AuNPs/MPS/Au was firstly immersed into 0.2 M PBS (pH 7.0) containing ATCl and AChE in the absence and presence of different concentrations of carbamate pesticides for 10 min. Then the AuNPs/MPS/Au electrode was removed out from the above solution, rinsed with ultra-pure water and put into 0.2 M PBS (pH 7.0) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to record cyclic voltammograms (CVs) response subsequently. The inhibition of carbamate pesticides was

calculated as followed:

$$\text{inhibition (\%)} = \frac{i_{p, \text{control}} - i_{p, \text{exp}}}{i_{p, \text{control}}} \times 100 \quad (1)$$

where $i_{p, \text{control}}$ and $i_{p, \text{exp}}$ are the peak currents on AuNPs/MPS/Au electrode which was immersed in 0.2 M PBS (pH 7.0) containing ATCl and AChE without and with pesticide inhibition, respectively.

3. Results and discussion

3.1 Characterization of AuNPs/MPS/Au electrode

AFM is an effective tool to observe surface topography [32]. To monitor the formation of MPS networks on the gold substrate and examine the distribution of the citrate-capped AuNPs, AFM was used to follow each self-assembled step. Figure S2 (Supporting Information) show the AFM images of differently modified gold substrates. The AFM image of bare gold substrate surface, shows a small root mean square (RMS) roughness of 10 nm, indicating a smooth surface (Figure S2A, Supporting Information). After the three-dimensional (3D) MPS sol-gel networks were self-assembled on the gold substrate surface, the RMS roughness increased to 28 nm (Figure S2B, Supporting Information). The rough surface enlarged the specific surface area of the MPS/Au electrode significantly. The 3D MPS networks contained a large number of -SH groups and the citrate-capped AuNPs could be strongly bound to the networks via Au-S bond [33,34]. Figure S2C (Supporting Information) reveals the citrate-capped AuNPs in the 3D MPS networks and accordingly the RMS roughness increases to 39 nm. Such a distribution could provide a necessary conduction pathway, a significant increase of effective electrode surface and a nice response toward $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

FTIR spectroscopy was also performed to confirm the MPS/AuNPs assembly (Figure S2D, Supporting Information). The FTIR spectrum of MPS film show peaks at 2929 and 2877 cm^{-1} for asymmetric and symmetric $-\text{CH}_2$ stretching vibration, and 2550 cm^{-1} for S-H stretching vibration, respectively. The -SH stretching vibration at 2554 cm^{-1} in FTIR spectrum of MPS/AuNPs film obviously decreased, indicating that the -SH has been combined with AuNPs to form the Au-S [35-37]. The results clearly proved the formation of MPS/AuNPs.

CVs of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is a valuable and convenient tool to monitor the assembly of the modified

electrode [38], because the electron transfer between the solution species and the electrode must occur either through the barrier or the defects in the modified electrode. Therefore, it was chosen to investigate each assembly step. Figure 1A shows the CVs of various modified electrodes in 0.2 M PBS (pH 7.0) containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Well-defined CVs, characteristic of a diffusion-limited redox process, were observed at the bare Au (curve a). After the bare Au was dipped in the MPS sol-gel solution, an obvious decrease in the anodic peak and disappearance of the cathodic peak were observed (curve b), which was agreeable with previous results that the electrochemical reversibility of $\text{Fe}(\text{CN})_6^{3-/4-}$ strongly decreased at a MPS modified electrode as a consequence of the MPS barrier [31]. The MPS networks could act as the inert electron and mass transfer blocking layer to hinder the diffusion of $\text{Fe}(\text{CN})_6^{3-/4-}$ toward the electrode surface. The citrate-capped AuNPs was chemisorbed on the MPS/Au electrode and formed negatively charged films on the electrode. The remarkable decrease of current was observed in curve c (Figure 1A) owing to the excess negative charge of carboxyl group from the citrate to repulse the same charged $\text{Fe}(\text{CN})_6^{3-/4-}$ [31].

3.2 The principle of the novel biosensor

Figure 1B described the CVs of AuNPs/MPS/Au in 0.2 M PBS (pH 7.0) containing 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ before (curve a) and after (curve b, c) immersing in 0.2 M PBS (pH 7.0) containing AChE and ATCl without (curve b) and with (curve c) carbamate pesticides (carbaryl), respectively. As discussed above, no obvious peak was observed at the AuNPs/MPS/Au before it was immersed in 0.2 M PBS (pH 7.0) containing AChE and ATCl. After the AuNPs/MPS/Au was immersed into 0.2 M PBS (pH 7.0) containing AChE and ATCl and incubated at 37.5 °C for 10 min, a quasi-reversible oxidation peak and reduction peak appeared at 0.27 V and 0.17 V, respectively (curve b). As shown in Scheme 1B, ATCl could be catalyzed by AChE into positively charged thiocholine which could substitute the negatively charged citrate on the surfaces of AuNPs due to the strong Au-S bond to form a positively charged interface [39]. The positively charged interface attracted negatively charged $\text{Fe}(\text{CN})_6^{3-/4-}$ to result in the large peak current. However, after the AuNPs/MPS/Au electrode was immersed in 0.2 M PBS (pH 7.0) containing AChE, ATCl and carbaryl, the oxidation and reduction peak currents were both smaller than that in the absence of carbamate pesticides due to the decrease of thiocholine resulted from the inhibition of AChE activity by carbamate pesticides. Since the oxidation and reduction peak currents were dependent on the concentration of thiocholine which was related to the

concentration of carbamate pesticides, the peak current could be used as a marker for carbamate pesticide detection.

3.3 Optimizing parameters for the performance of biosensor

Since the peak current mainly resulted from the electrostatic interactions between $\text{Fe}(\text{CN})_6^{3-/4-}$ and AuNPs/MPS/Au electrode, some factors involved in the performance of biosensor were investigated (Figure S3, Supporting Information). The effect of AChE concentration on the performance of biosensor in the absence of carbamate pesticides was firstly investigated. Figure 2A showed the plot of amperometric response of biosensor versus the AChE concentration. The experiment was performed by incubating the AuNPs/MPS/Au in 0.2 M PBS (pH 7.0) containing 2.0 mM ATCl and different concentrations of AChE for 10 min. Then the CVs of the AuNPs/MPS/Au was measured in 0.2 M PBS (pH 7.0) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. With the increasing of AChE concentration, the peak current increased gradually and reached the maximal value at about 4.0 mM. Then the peak current slightly decreased as the AChE concentration continued increasing. Since the hydrolysis of ATCl into thiocholine was catalyzed by AChE, it was expected that when more AChE was added into the solution, the hydrolysis reaction would become faster and more thiocholine would be generated. Therefore, more AChE would catalyze the hydrolysis of more ATCl into thiocholine to produce a large number of positive charges on AuNPs/MPS/Au, which could attract more $\text{Fe}(\text{CN})_6^{3-/4-}$ on AuNPs/MPS/Au to enhance peak current. However, excess AChE might result in aggregation on the AuNPs/MPS/Au surface to hinder the electron transfer of $\text{Fe}(\text{CN})_6^{3-/4-}$ and accordingly lead to the slight decrease of peak current.

The amount of ATCl was another important factor related to the performance of the biosensor. A control experiment was performed under similar conditions with varied ATCl concentration where the concentration of AChE was maintained at 4.0 mM. Figure 2B displays the plot of the amperometric response of biosensor versus the ATCl concentrations. With the increasing of ATCl concentration, the peak current increased gradually and reached the maximal value at about 2.0 mM. Thus, 4.0 mM AChE and 2.0 mM ATCl were used in the following work for the determination of carbamate pesticides.

The assembling time of Au electrode in MPS solution was another important factor related to the performance of biosensor. Figure 2C displays the plot of the amperometric response of biosensor versus assembling time of Au electrode in MPS solution. The experiment was

performed under the above optimal conditions. As could be seen in Figure 2C, with the increasing of the assembling time, the peak current decreased gradually, which indicated more and more MPS was immobilized on the electrode surface. Here, 10 min was used to construct the MPS/Au electrode in the following experiments.

The amperometric response of biosensor depended greatly on the reaction time of AuNPs/MPS/Au in 0.2 M PBS (pH 7.0) containing AChE and ATCl. Figure 2D shows the plot of amperometric response of the biosensor versus different immersing time. With the increasing of immersing time, the peak current increased gradually and reached the maximal value at 10 min. Thus, 10 min was chosen as the optimum immersing time of the AuNPs/MPS/Au in 0.2 M PBS (pH 7.0) containing AChE and ATCl and used in the following experiments for the determination of carbamate pesticides.

The optimum pH and temperature were also explored as shown in Figure S4 (Supporting Information). The bioactivity of AChE depended greatly on the pH of electrolyte solution. Figure S4A (Supporting Information) shows the plot of amperometric response of the biosensor versus different pH in 0.2 M PBS (5.0 – 9.0) in the presence of 4 mM AChE and 2 mM ATCl. The biosensor shows highest response in a buffer solution of pH 7.0. Thus, the optimum pH of 7.0 was selected in this work for the determination of carbamate pesticides. Another important aspect effecting enzyme activity was the temperature. As shown in Figure S4B (Supporting Information), with the increasing of the temperature from 0 to 37.5 °C, the amperometric response of the biosensor increased gradually. When the temperature was higher than 37.5 °C, the amperometric response decreased rapidly because the enzyme activity was lost under the higher temperature. Therefore, the 37.5 °C was chosen as the optimum temperature. The optimizing parameters for the performance of biosensor were listed in Table S1 (Supporting Information).

3.4 Detection of carbamate pesticides in a standard solution

In order to quantify the concentration of carbamate pesticides, the CVs of the AuNPs/MPS/Au immersed in 0.2 M PBS (pH 7.0) containing ATCl and AChE with optimal concentration in the absence and presence of different concentrations of carbamate pesticides for 10 min were measured. Carbaryl (1-naphthyl methylcarbamate), as one of the carbamate pesticides, is used in the following experiments. As shown in Figure 3A, with the increasing of the carbaryl concentration, a noticeable decrease of CVs' signal at AuNPs/MPS/Au was observed. The decrease of peak current mainly resulted from the decrease of AChE's activity inhibited by

carbaryl and accordingly resulted in the decrease of produced thiocholine. As one of the carbamate pesticide, carbamate pesticides exhibited high toxicity to inhibit the activity of AChE irreversibly. Thus, the thiocholine from the hydrolysis of ATCl catalyzed by AChE also decreased. At high concentration, the inhibition of carbaryl on the activity of AChE tended to a maximum value, indicating its binding interaction with active target group in AChE could reach saturation, leading to the maximum inhibition. Figure 3B shows the inhibition of AuNPs/MPS/Au immersed in 0.2 M PBS (pH 7.0) containing 2 mM ATCl and 4 mM AChE with optimal concentration in the absence and presence of different concentrations of carbaryl for 10 min. Under the optimal experimental condition, the carbaryl inhibition to AuNPs/MPS/Au was proportional to its concentration in the range from 0.003 to 2.00 μM (the inset in Figure 3B) with the correlation coefficients of 0.997. The detection limit was estimated to be 1.0 nM and the sensitivity was $32.04 \mu\text{A cm}^{-2}/\text{mM}$ based on the criterion of a signal-to-noise ratio of 3.

A comparison of the analysis performance of this biosensor with previous amperometric AChE biosensor for carbamate pesticides detection was listed in Table 1. It clearly indicated that the detection limit of AuNPs/MPS/Au was lower than most of the reported literatures. Therefore, the AuNPs/MPS/Au has an advantage in the sensing performance of trace amounts of carbamate pesticides.

The interferences from some chemicals such as nitrophenol, ascorbic acid, uric acid, Cu^{2+} , Cd^{2+} , Pb^{2+} , Hg^{2+} , SO_4^{2-} and NO_3^- were investigated. No obvious inhibition behavior could be observed. As shown in Figure 4A, the prepared electrode was reusable after it was potentially scanned in NaOH solution from -0.1 V to 1.0 V at 100 mV s^{-1} and subsequently immersed in trisodium citrate for 80 min. The small peak current of $\text{Fe}(\text{CN})_6^{3-/4-}$ (curve b) due to electrostatic repulsion between $\text{Fe}(\text{CN})_6^{3-/4-}$ and the AuNPs/MPS/Au became larger (curve a) after the AuNPs/MPS/Au was immersed in the ATCl+AChE solution. However, the large peak current of $\text{Fe}(\text{CN})_6^{3-/4-}$ was depressed (curve c) after the AuNPs/MPS/Au was potential scanned from -0.1 V to 1.0 V at 100 mV s^{-1} and subsequently immersed in trisodium citrate for 80 min, suggesting the thiocholine molecules were removed off from the AuNPs/MPS/Au and the citrate adsorbed on the electrode surface again. After the AuNPs/MPS/Au was immersed in the ATCl+AChE solution for 10 min again, the large peak current was recovered. The reusability was tested by repeating the above experiments for five times, the 5.32% relative standard deviation (RSD) was obtained. The consequence demonstrated the good reproducibility of the electrode. As shown in Figure 4B, with

the increasing of immersing time in trisodium citrate, the current gradually decreased, which might result from the adsorption of more citrates onto electrode surface to repulse the negatively charged $\text{Fe}(\text{CN})_6^{3-/4-}$. After 80 min, the peak current became the smallest, suggesting 80 min was essential for the reproducibility of the electrode. The proposed biosensor also has the advantage of long-term storage stability for the practical application. The prepared AuNPs/MPS/Au biosensor was stored at 4 °C when it was not in use. No obvious decrease in the CVs response was observed in 3, 7 and 10 day storage. After a 28 day storage period, the biosensor retained 88% of its initial current response, indicating good storage stability.

3.5 Detection of carbamate pesticides in a practical sample

The detection of carbamate pesticides in fruit samples was performed on the AuNPs/MPS/Au with standard addition method. To verify the accuracy of our methods, the original concentration of carbamate pesticides in fruit samples was firstly determined by HPLC method. Then the standard AChE and ATCl solution with different concentration of carbamate pesticides was injected into the fruit samples and the measurements were carried out by our method. The standard addition method was used to eliminate matrix effect in the detection of carbamate pesticides in fruit samples. Correlation of the quantification results for carbamate pesticides in fruit samples with HPLC/biosensor analysis (x-axis) and the new designed biosensor (y-axis) suggested that the biosensor might be applied in practical sample (Table S2 and Figure S5, Supporting Information).

4. Conclusions

A new approach for the detection of carbamate pesticides in aqueous solution was developed based on the citrate-capped AuNPs/MPS/Au sensing layer. The unique feature of this assay method was to detect the carbamate pesticides detection was basis of the response of the AuNPs/MPS/Au to $[\text{Fe}(\text{CN})_6]^{3-/4-}$ under relative low work potential. The citrate-capped AuNPs was well diffused into MPS sol-gel network. Such a distribution could provide a necessary conductive pathway, a significant increase of effective electrode surface and a increased loading of citrate-capped AuNPs, which all contributed to a good response toward $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Furthermore, in this strategy, the enzymes exist in a solution and accordingly their bioactivity could reach the maximum. The simply modified electrode was used in this strategy, which would

improve the reproducibility, regeneration and stability of biosensors. Simultaneously, the strategy is simple and low-cost owing to the simple preparation of the electrode and simple technique. The amperometric current changed proportionally toward carbamate pesticides concentration from 0.003 to 2.00 μM . The sensing platform was also proved to be suitable for carbamate pesticides in a practical sample. This work not only gives a way to detect carbamate pesticides but also provides a potential strategy for the detection of non-electroactive species.

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Figure captions

Figure 1. (A) CVs of various modified electrodes in 0.2 M PBS (pH 7.0) containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at 100 mV s^{-1} : bare Au (a), MPS/Au (b) and AuNPs/MPS/Au (c). (B) CVs of AuNPs/MPS/Au (a: before; b: after) immersed in 4 mM AChE + 2 mM ATCl solution under 37.5 °C and (c) 4 mM AChE + 2 mM ATCl + 0.8 μM carbaryl in 0.2 M PBS (pH 7.0) containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at 100 mV s^{-1} under 37.5 °C.

Figure 2. (A) The plot of peak current versus AChE concentration. (B) The plot of peak current versus ATCl concentration. (C) The plot of peak current versus the immersing time in MPS solution. (D) The plot of peak current versus the time to immerse AuNPs/MPS/Au in 4 mM AChE + 2 mM ATCl solution.

Figure 3. (A) CVs of the AuNPs/MPS/Au electrode immersed in 4 mM AChE + 2 mM ATCl + different concentration carbaryl (a-e: 0, 0.5, 1.0, 1.5 and 2.0 μM) for 20 min in 0.2 M PBS (pH 7.0) containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at the scan rate of 100 mV s^{-1} under 37.5 °C. (B) Plot of the inhibition ration versus carbaryl concentration. Inset: calibration curves of the biosensor to carbaryl concentration.

Figure 4. (A) CVs of AuNPs/MPS/Au before (b) and after (a) immersed in 4 mM AChE + 2 mM ATCl for 10 min, and then was potential scanned from -0.1 V to 1.0 V at 100 mV s^{-1} in (c) 1.0 M NaOH and subsequently immersed in 30 mM citrate for 80 min and (d) in 4 mM AChE + 2 mM ATCl for 10 min in 0.2 M PBS (pH 7.0) containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at the scan rate of 100 mV s^{-1} under 37.5 °C. (B) CVs of AuNPs/MPS/Au immersed in 1.0 M NaOH + 30 mM citrate for different time in 0.2 M PBS (pH 7.0) containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at the scan rate of 100 mV s^{-1} under 37.5 °C.

Scheme 1. Schematic illustration of the stepwise fabrication process and principle of the novel biosensor based on AuNPs/MPS/Au sensing layer.

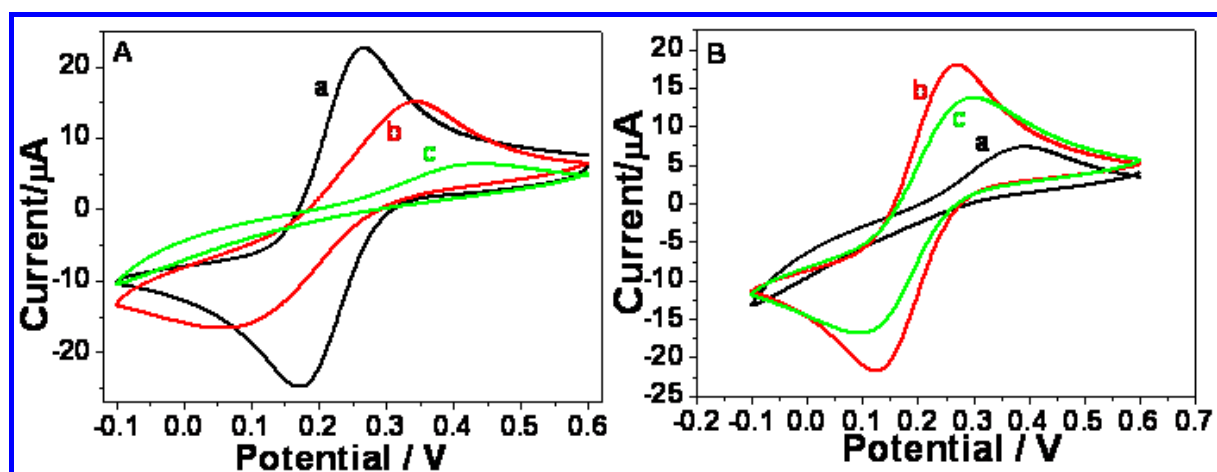


Figure 1

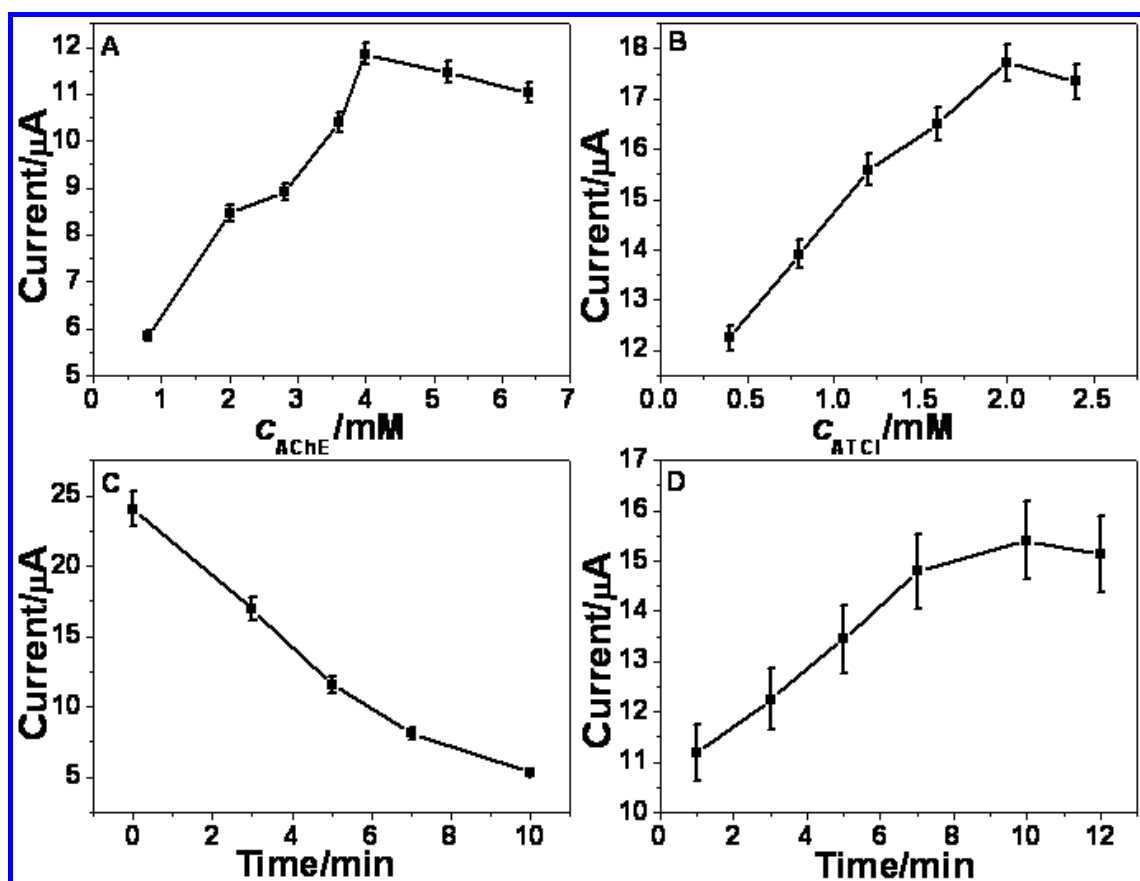


Figure 2

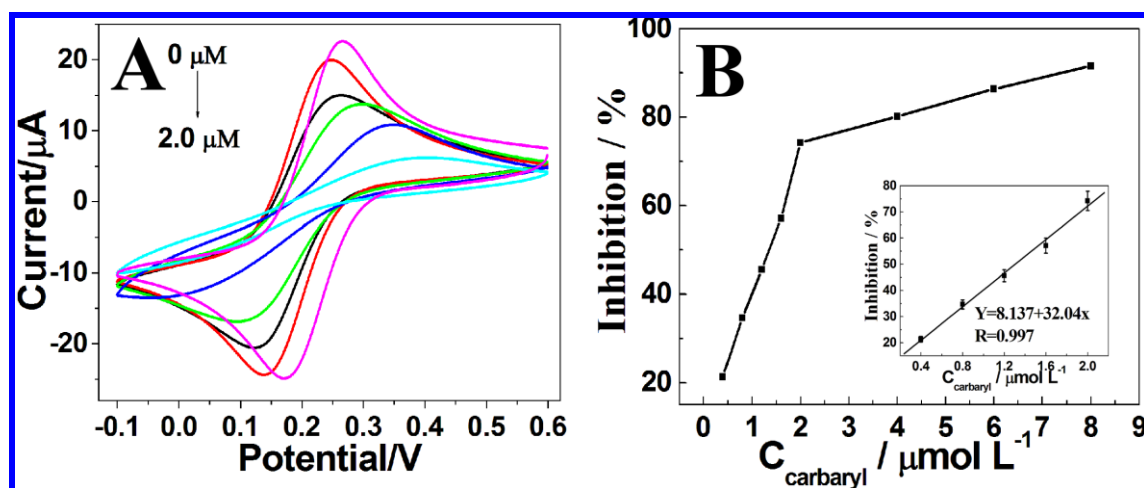


Figure 3

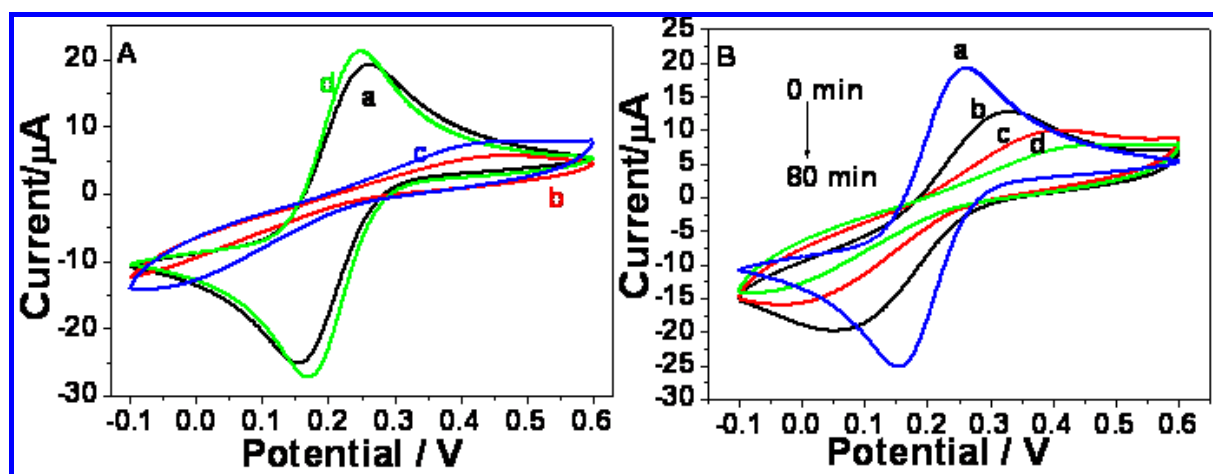
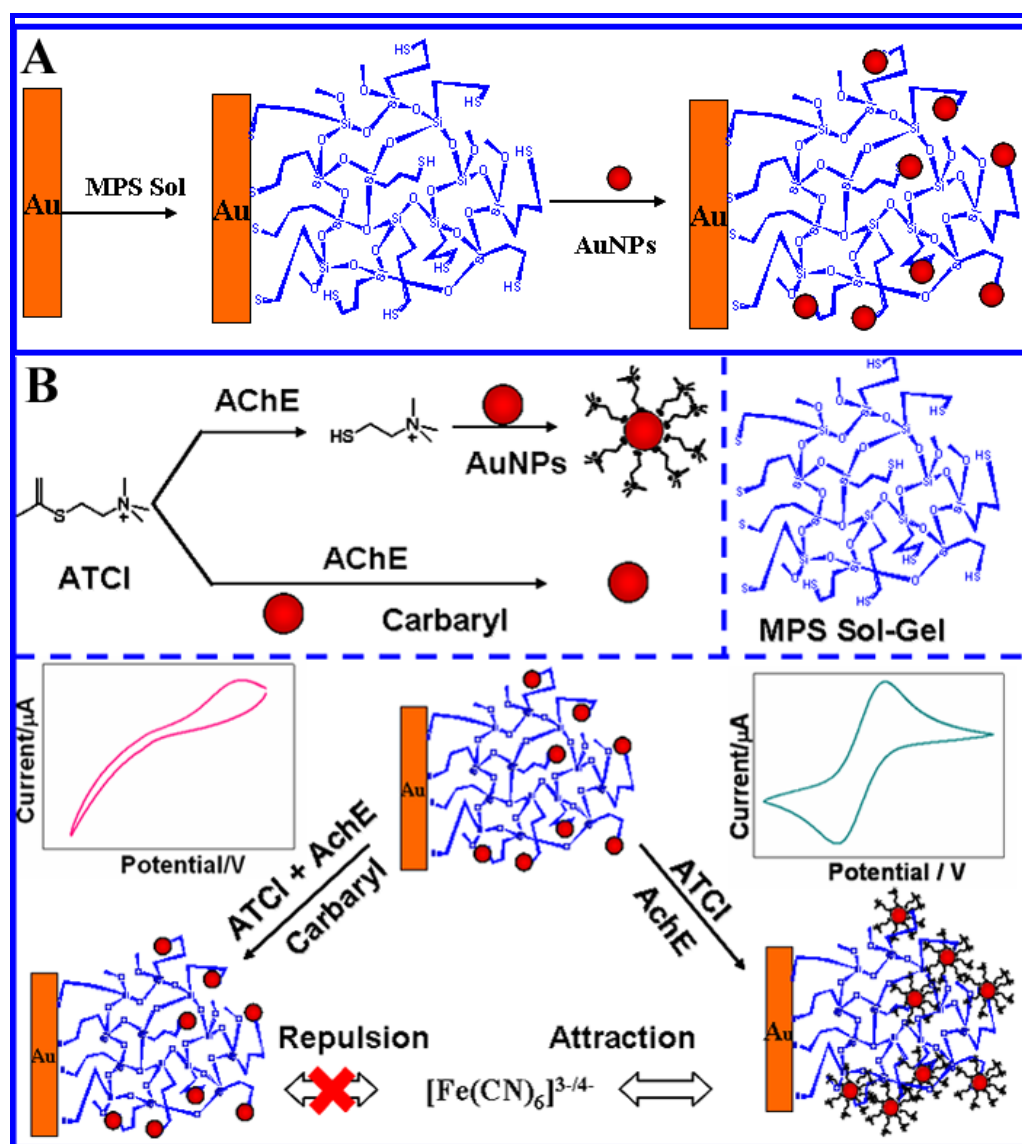


Figure 4



Scheme 1

Table 1 Comparison of analytical performance of biosensors

Electrode	Detection limit (nM)	Linear range (μM)	References
CoO/rGO ^a /GCE	37.5	0.5-200	18
MIP ^b /GR ^c -IL ^d -Au/CHIT ^e -AuPtNPs/GCE	8.0	0.03-6.0	13
AChE-CdS-GR-CHIT/GCE	3.5	0.010-10.0	11
Au-MPA ^f -AChE/ChO ^g SAMs ^h /gold electrode	5.96	0.01-1.0	20
AChE/PB ⁱ -CHIT ^j /GCE	3.0	0.01-0.4 1.0-5.0	1
AChE/PANI ^j /MWCNT ^k /GCE	1400	9.9-49.6	14
AChE/pRGO ^l -CHIT/GCE	2.5	0.005-0.25	10
AChE-TiO ₂ -GR/GCE	1.5	0.005-0.075 0.075-10.0	15
Co-phthalocyanine-MWCNT/GCE	5.46	0.33-6.61	[40]
AuNPs/MPS/Au	1.0	0.003–2.0	This work

^aReduced oxide graphene; ^bMolecularly imprinted polymer; ^cGraphene; ^dIonic liquid; ^eChitosan; ^fMercaptopropionic acid; ^gCholineoxidase; ^hSelf-assembled monolayers;

ⁱPrussian blue; ^jPolyaniline; ^kMulti-walled carbon nanotubes; ^lPorous reduced graphene oxide;