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RESEARCH PAPER

Broad-spectrum electrochemical immunosensor based on one-step electrodeposition of AuNP–Abs and Prussian blue nanocomposite for organophosphorus pesticide detection

Haowei Dong[1,2,3] · Qingxue Zhao[1,2,3] · Jiansen Li[1,2,3] · Yaodong Xiang[1,2,3] · Huimin Liu[1,2,3] · Yemin Guo[1,2,3] · Qingqing Yang[1,2,3] · Xia Sun[1,2,3]

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Abstract

Broad-spectrum antibodies can effectively recognize substances with similar structures and have broad application prospects in field rapid detection. In this study, broad-spectrum antibodies (Abs) against organophosphorus pesticides (OPs) were used as sensitive recognition elements, which could effectively recognize most OPs. Gold nanoparticles (AuNPs) have good biocompatibility. It combined with Abs to form a gold-labeled probe (AuNPs–Abs), which enhances the effective binding of antibodies to nanomaterials. Prussian blue (PB) was added to electrodeposition solution to enhance the conductivity, resulting in superior electrochemical performance. The AuNP–Abs–PB composite film was prepared by electrodeposition on the electrode surface to improve the anti-interference ability and stability of the immunosensor. Under the optimal experimental conditions, the immunosensor had a wide detection range (IC_{20}–IC_{80}: 1.82×10^{-3}–3.29×10^{4} ng/mL) and high sensitivity. Most importantly, it was simple to be prepared and could be used to detect multiple OPs.

Keywords Broad-spectrum antibodies · Gold-labeled antibody probes · One-step electrodeposition · Electrochemical immunosensor · Organophosphorus pesticides

Introduction

Pesticides play an important role in improving the quality of agricultural products, and organophosphorus pesticides (OPs) are widely used for their high efficiency and wide spectrum [1, 2]. However, people's abuse of pesticides has led to a series of agricultural safety problems, public health problems and environment sustainable development problems [3, 4]. Therefore, the detection method of OPs residues is developing constantly. Traditional pesticide detection methods include mass spectrometry, gas chromatography, high performance liquid chromatography and their combination methods, which have high sensitivity and high accuracy [5]. However, complicated sample pretreatment, time-consuming and laborious detection process, and the need for professional operation have hindered the use and popularization of these methods [6].

At the same time, some portable and accurate new detection technology has been concerned by researchers [7]. Among them, an antibody is a kind of high efficiency and durable sensitive recognition element [8–10], which has the characteristics of high sensitivity, strong specificity, and good anti-interference ability [2, 11]. Although there have been many reports on antibody-based electrochemical biosensors, most of them only target one kind of target [12]. It was reported that more than 200 OPs have been commercialized and dozens of them are commonly used [6, 13]. And it is significant and necessary to construct a simultaneous detection method of OPs. Multiple antibody recognition is a good way to solve this problem. Multiple recognition antibodies, including bifunctional antibodies and broad-spectrum antibodies, can recognize multiple targets simultaneously. Lan et al. [14] recently reported a multi-linked immunoassay

✉ Yemin Guo
gym@sdut.edu.cn

1 School of Agricultural Engineering and Food Science, Shandong University of Technology, No. 266 Xincun Xilu, Zibo 255049, China

2 Shandong Provincial Engineering Research Center of Vegetable Safety and Quality Traceability, No. 266 Xincun Xilu, Zibo 255049, China

3 Zibo City Key Laboratory of Agricultural Product Safety Traceability, Zibo, China

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method for the simultaneous detection of seven pesticides, which could achieve qualitative and quantitative detection of the targets. They immobilized seven antigens on a microarray chip and used gold-labeled probes as tracers to construct and optimize a 7-linked immune array analysis method based on an indirect model. Shu et al. [15] used a bifunctional antibody (BfAb) that could simultaneously recognize methyl parathion and imidacloprid, and labeled them with horseradish peroxidase (HRP) and alkaline phosphatase (ALP) as a chemiluminescent probe marking two haptens respectively. An indirect competitive multiple immunochromatographic test strip based on a time-resolved chemiluminescence method was constructed, with a linear range of 0.1–250 ng/mL and a detection limit of 0.058 ng/mL. The entire detection process could be performed within 22 min carry out. Up to now, immunosensors for the detection of multiple pesticide residues were mostly based on the superposition of a single detection [16, 17]. For a large variety of OPs, it is far from meeting the actual detection needs. There had been many reports about broad-spectrum antibodies against OPs [18], which could effectively identify most OPs. Based on broad-spectrum antibodies, it is very promising to carry out research of immunosensors that can detect the entire OPs.

During the application of immunosensors, antibodies are often attached to the electrode surface modified with specific nanomaterials [19, 20]. Through the specific recognition of the target by antibodies, the target was captured and connected to the electrode surface. Unsatisfactorily, antibodies being immobilized onto the electrode surface, some researchers had used the method of directly dropping the antibody solution [5, 21, 22], or inserting the electrode into the antibody solution [23–25], then using for detection after a simple incubation [25]. For the competitive immunoassay model, the detected signal decreases with the increase of the concentration of the target, and shows the trend of signal closing [20, 26]. In this case, the amplification of electrochemical signal is particularly important. Researchers usually used high affinity antibodies and appropriate markers to amplify the electrochemical signal [27–29]. As a kind of nanomaterial with excellent performance, gold nanoparticles are often used as biomarkers [30, 31]. Therefore, it was of great significance to properly and effectively immobilize antibodies onto the electrode surface [32]. Lah et al. [33] recently reported a method for detecting HER2 cancer biomarker. They used the Pb-based quantum dots as a label in a sandwiched immunosensor, used square wave voltammetry to study the relationship between changes in electrochemical signals and target concentrations. Gao et al. [34]designed a novel reverse colorimetric immunoassay (RCIA) strategy and utilized for sensitive detection of low-abundance proteinin biological fluids. They used functional gold nanoparticles as enzymatic bioreactors and sandwich immunoassay to detect the target. The catalytic efficiency of magnetic bead-based peroxidase simulation for TMB was reduced. Similarly, Jampasa et al. [35] also used the sandwiched method. But it was different that they labeled the secondary antibody with Au. They used the gold signal response as an indicator, and performed the electrochemical detection by differential pulse voltammetry (DPV) method. AuNPs had excellent properties such as high electrical conductivity and biocompatibility [36], which could provide ideal active sites for antibody binding in the construction of electrochemical immunosensors [37–39]. Based on the above, we tried to couple broad-spectrum antibodies with gold nanoparticles (AuNPs) to form a gold-labeled antibody probe (AuNP–Abs). This method could not only reduce the waste of antibodies, but also enhance the binding of antibodies to nanomaterial [34, 40].

Prussian blue (PB) has good redox properties and is one of the most widely used compounds in the manufacture of unlabeled electrochemical immunosensors [41]. Haji-Hashemi et al. [42] continuously electrodeposited PB and AuNPs on the surface of glassy carbon electrodes, which effectively improved the stability and electrical activity of PB as an electron mediator. Due to the electrodeposition method was simple and effective, some researchers [43] used it to make nanocomposite films on electrode surfaces. It could make nanomaterials uniformly dispersed and firmly immobilized on the electrode surface [44], and could be well used to construct an electrochemical sensing platform [45]. Shaikh et al. [46] prepared antibody probes labeled with nanomaterials, and then electrodeposited the nanoprobes on the electrode surface by dielectrophoresis. The prepared immunosensor was successfully applied to actual detection. This method was often used in the detection of tumor markers in clinical medicine, but there were few reports on the detection of pesticide residues.

Here, we attempted to construct an immunosensor with a simple preparation process that could recognize multiple OPs by using broad-spectrum antibodies and electrodeposition method. By coupling the broad-spectrum antibody with AuNPs, the gold-labeled probe (AuNP–Abs) was prepared, which enhanced the effective binding of the antibody to the nanomaterial. The electrodeposition solution was prepared using AuNP–Abs and PB, and an AuNP–Abs–PB composite film was formed on the electrode surface by electrodeposition. OPs concentration was quantitatively determined by monitoring the electrochemical behavior changes on the electrode surface.

Experiment

Reagent

Screen-printed carbon electrode (SPCE, TE100) was bought from Zensor R&D (Taiwan). Phosphate buffer solution

(PBS, 0.01 M) was prepared using $Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4{\cdot}12H_2O$, which were purchased from Sinopharm Chemical Reagent Co., Ltd. Bovine serum albumin (BSA) and chitosan (CS) were purchased from Sigma-Aldrich (USA). The reagents such as $HAuCl_4{\cdot}3H_2O$, K_2CO_3, $K_3[Fe(CN)_6]$ and other chemicals were all analytically pure. Parathion, coumaphos, quinalphos, parathion-methyl, chlorpyrifos, triazophos, deltamethrin and carbofuran were purchased from Beijing Yihuatongbiao Technology Co., Ltd. The broad-spectrum monoclonal antibodies against OPs were purchased from Beijing Biodragon Immunotechnologies Co., Ltd.

All the aqueous solutions used in the experiment were prepared by LS MK2 Pall ultrapure water system (18.2 MΩ·cm, USA). The electrochemical measurement process was performed on a CHI660D electrochemical workstation (Shanghai Chenhua Co., Ltd., China). The prepared AuNP–Abs were centrifuged by a centrifugal machine (TGL-20B, China). The structural characterizations of AuNP–Abs–PB were completed by an electron scanning microscope (S-3000N, Japan).

Preparation of immunosensor

Preparation of gold-labeled antibody probes (AuNP–Abs)

The AuNPs was prepared by the reduction of chloroauric acid by trisodium citrate. Prepare it according to the method of Yao et al. [47]. And then we coupled AuNPs with Abs. We added 18 μL K_2CO_3 solution to 1 mL AuNPs to create an alkaline environment. Added 60 μL of broad-spectrum antibody (0.1 mg/mL), and shook at room temperature for 30 min, so that the surface negative charge of gold nanoparticles and the positively charged groups of proteins formed a firm bond due to electrostatic adsorption. Added 119 μL BSA (10%, m/v), and continued shaking reaction for 30 min, and sealed up nonspecific binding site. Incubated it at 4 ℃ for 2 h. After taking it out, centrifuged (12,000 rpm, 30 min) and discarded the supernatant. Added 100 μL borate buffer solution (2.0 mM), oscillated to make it even. Repeated the above operation once and stored at 4 ℃.

Preparation of electrodeposition solution

First, we prepared the Prussian-blue (PB): 0.0625 g $FeCl_3$, 0.0822 g $K_3[Fe(CN)_6]$, 0.7455 g KCl and 1 mL hydrochloric acid were added to 100 mL chitosan-acetic acid solution (0.05%, m/v), and dispersed by ultrasound at room temperature until a stable dark green dispersion was obtained. And then, 1 mL diluted AuNP–Abs was added to PB and then dispersed by ultrasound at room temperature for 0.5 h until completely dissolved.

Preparation of the AuNP–Abs/PB/SPCE immunosensor

The preparation of the immunosensor was an electro-reduction method, and after an electrodeposition treatment, an AuNP–Abs–PB composite film was formed on the surface of SPCE. First, immersed the pre-treated electrode in the electrodeposition solution (mixed AuNP–Abs and PB), and used cyclic voltammetry to process for a certain time in the potential range of −0.3 to +0.3 V (scan speed: 100 mV/s) to obtain AuNP–Abs–PB/SPCE. Then, rinsed with ultrapure water and blew dry with nitrogen gas, and stored at 4 ℃. Figure 1 was a schematic diagram of the electrodeposition process and the detection principle of the immunosensor.

Electrochemical detection methods

When the target was detected, some changes in electrochemical behavior occurred on the electrode surface of the immunosensor. To monitor this change, a differential pulse voltammetry (DPV) scan was performed in the $[Fe(CN)_6]^{3-/4-}$ solution (pH 7.4, 5.0 mM). The scanning potential range was from −0.6 to +0.6 V, the potential increment was 4 mV/s, and the amplitude was 100 mV. The DPV response of each bare electrode was measured in advance, denoted as I_1. Under the optimal experimental conditions, several immunosensors were prepared, and their initial DPV

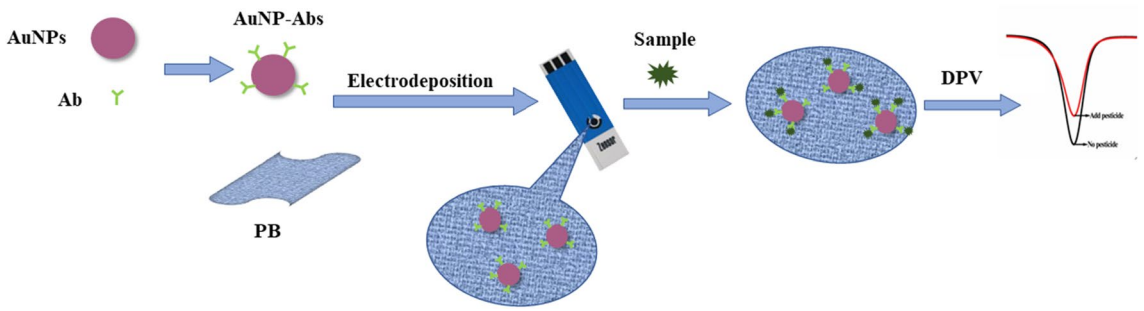

Fig.1 Electrodeposition process and the detection principle of the immunosensor

response values were measured, denoted as I_2. The immunosensor was placed in an organophosphorus pesticide solution with a certain concentration, incubated at room temperature for a certain time. After washing and blow-dried, the electrochemical response was measured again, denoted as I_3. Calculate current change $\Delta I'$ value ($\Delta I' = I_2 - I_1$), through which to evaluate effect of electrodeposition. And calculated the current change ΔI value ($\Delta I = I_3 - I_2$), analyzed the relationship between ΔI and pesticide concentration.

Pretreatment of samples

The samples of baby cabbages and spinach were purchased from the market, and then fully crushed. We weighed 25.0 g of the crushed samples, then added 80.0 mL of methanol-PBS (70%, v/v). After mixing, the samples were homogenized for 2 min, and filtered with a filter paper (0.45 μm) to produce the sample solution. The sample solution was mixed with PBS at a volume ratio of 1:6 to prepare a sample matrix solution for subsequent experiments.

Results and discussion

Characterization of AuNP–Abs–PB/SPCE

Scanning electron microscopy (SEM) and cyclic voltammetry (CV) were used to characterize the surface morphology and electrochemical characteristics of electrodeposited electrodes. As shown in Fig. 2A, there were obvious spherical protrusions on the electrode surface, indicating that the antibody and AuNPs were successfully coupled. In addition, the element analysis on the electrode surface was carried out by SEM. It could be seen from Fig. 2B, C, Fe and N elements were evenly distributed on the surface of the electrode surface, indicating that PB and AuNPs had successfully electrodeposited on the surface of the electrode.

Moreover, the electrochemical behavior of different composites electrodeposited on the electrode surface was characterized by CV (Fig. 2D). Compared with a bare SPCE (Fig. 2D-a), the electrode modified by PB (Fig. 2D-b) showed a higher peak current. When PB and AuNPs were

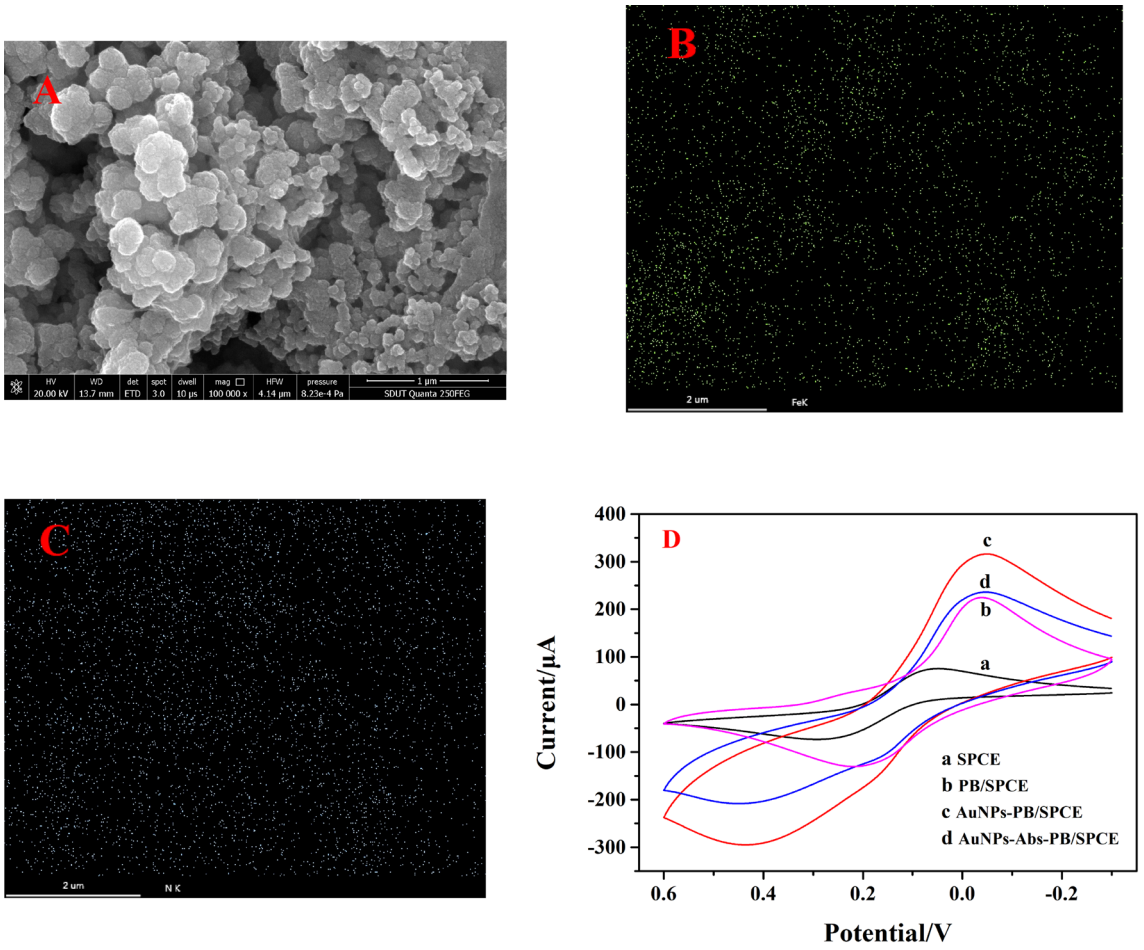

Fig. 2 SEM image of **A** AuNP–Abs–PB/SPCE; the element analysis of **B** Fe and **C** N; **D** CV image of AuNP–Abs–PB/SPCE

deposited on the electrode at the same time (Fig. 2D-c), the resulting PB-AuNPs/SPCE had a larger current peak, indicating that the conductive AuNPs was successfully modified on the electrode surface. When Abs were added to the electrodeposition solution (Fig. 2D-d), the peak value of REDOX was decreased significantly because Abs negatively charged increased electron transfer resistance. The characterization results indicated that electrodeposition was a feasible method for the preparation of electrochemical immunosensor.

Condition optimization of the immunosensor

The coupling ratio of antibody to AuNPs was an important factor which affected the detection efficiency of immunosensors. It was well known that more antibodies could improve the sensitivity of detection, but with them comes the cost of detection. At the same time, the coupling of excessive antibodies would lead to the AuNP–Abs conductivity decreased. In this study, the effects of different doses of antibodies coupled with AuNPs on the conductivity of electrodeposited composite films were investigated. Different doses of antibodies (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μg) were added to the same amount (1 mL) of AuNPs (0.4 mg/mL). The ability to transfer $[Fe(CN)_6]^{3-/4-}$ onto the electrode surface after deposition was studied by DPV. As can been seen from Fig. 3a, when the dose of antibody was 3 μg, the $\Delta I'$ value was relatively large. Most importantly, the mean deviation of the results was minimal, indicating that the immunosensor prepared with this electrodeposited solution has the best stability. Therefore, 3 μg was chosen as the best antibody additive.

Figure 3b showed the effect of AuNP–Abs concentration on immunosensor performance. Experiments found that along with the increase of AuNP–Abs concentration, the ΔI value increased gradually. When the concentration of AuNP–Abs was 1:4000, the ΔI value reached the maximum. But with the further increase of AuNP–Abs concentration, the ΔI value dropped. It indicated that excessive AuNP–Abs might lead to hybridization between molecules, causing less AuNP–Abs being immobilized on the electrode. This made the electrode surface can capture the target to reduce, the ΔI value was smaller. Therefore, during the preparation of the immunosensor, the concentration of AuNP–Abs was set at 1:4000.

The time of electrodeposition was an important factor to control the thickness of the deposited film, which directly affected the signal amplification ability of the immunosensor. In this part, different deposition times (i.e., different CV cycles) were set to prepare the immunosensor, and the $\Delta I'$ value was measured. As shown in Fig. 3c, the $\Delta I'$ value of the prepared immunosensor gradually increased with the increase of the electrodeposition CV cycle times. In the 15th cycle, the $\Delta I'$ value was the largest. And as the deposition time continued to increase, the $\Delta I'$ value remains almost unchanged, that was, the deposition amount on the electrode surface had reached the maximum. Therefore,

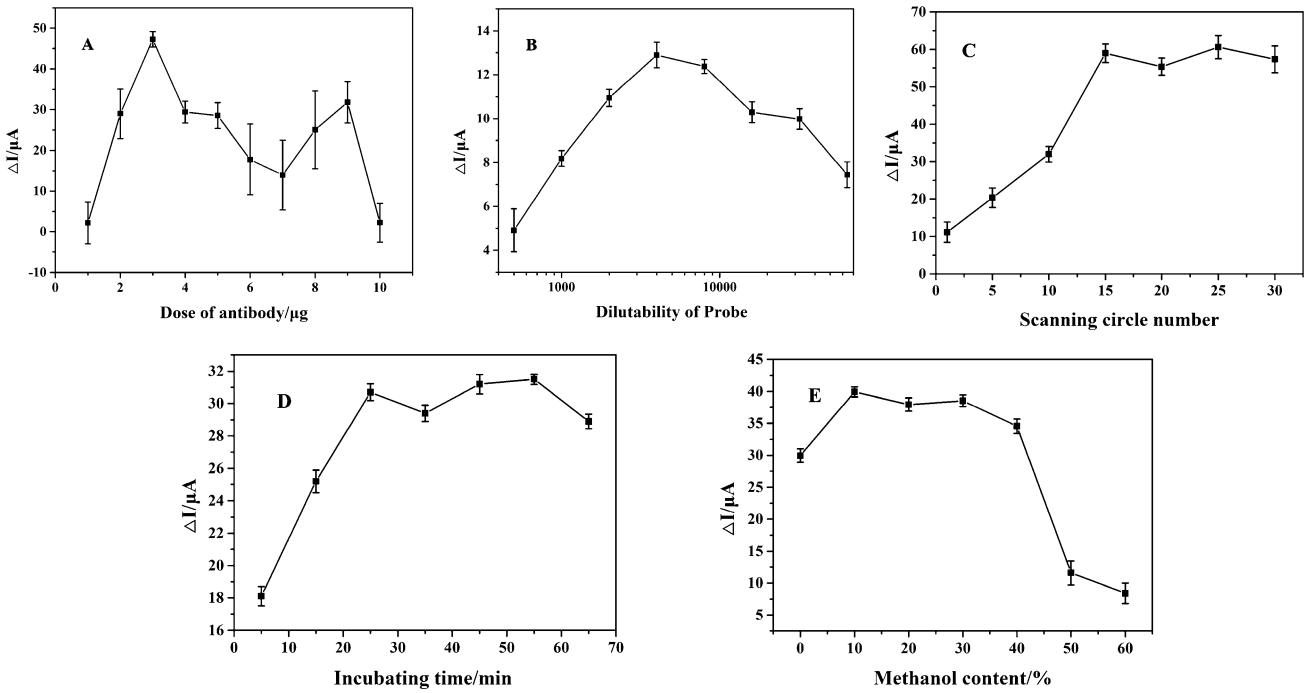

Fig. 3 Optimizing the experimental conditions: **a** dose of antibody; **b** dilutability of probe; **c** scanning circle number; **d** incubation time; **e** methanol content

15 cycles were used as the optimal electrodeposition time of the immunosensor.

In the presence of the target pesticide (i.e., organophosphorus pesticide), an OPs-Ab complex formed on the electrode surface hindered electron transfer, causing the peak current to decrease. The amount of this complex was closely related to the specific binding time between the target and antibody. Therefore, incubation time between the immunosensor and the target solution was another important factor affecting the performance of the immunosensor. As shown in Fig. 3d, with the passage of time, the electrode surface captured more and more pesticide molecules, leading to electron transfer, enabling ΔI gradually increased. Reached a tipping point (25 min), the sensor surface compounds reached saturation. And further increased the incubation time, the ΔI value would not change significantly. Therefore, 25 min was selected as the optimal incubation time of the immunosensor with the pesticide target.

Because pesticides were soluble in organic solvents. In the sample processing, methanol was often used as the extraction solvent. The methanol content in the matrix solution was too low to be effectively extracted into the pesticide in the sample. But at the same time, antibody was a kind of sensitive recognition element with biological activity, and too high methanol content would affect its activity and reduced its recognition ability. In this experiment, the suitable range of methanol content in the sample solution was studied (Fig. 3e). It was found that when the methanol content (v/v) was within the range of 10–40%, the sample could be effectively extracted and the antibody could keep the optimal activity. Therefore, the methanol concentration (v/v) range suitable for this immunosensor was 10–40%.

Electrochemical detection of organophosphorus pesticides

Under the best experimental conditions, the relationship between the pesticide concentration (C) and the corresponding electrochemical signal change value (ΔI) was studied. In this experiment, the standard solutions of parathion, phoronidin, quetifosion and methyl parathion were mixed in equal amount to obtain a mixed standard solution. Immunosensors was incubated with the mixed standard solution of different concentrations (10^4–10^{-5} ng/mL), and the ΔI value was measured to obtain a competition inhibition curve (Fig. 4A). The obtained linear correlation coefficient (R^2) was 0.9856, the sensitivity (IC_{50}) was 2.45 ng/mL, and the detection range (IC_{20}–IC_{80}) was 1.82×10^{-3}–3.29×10^4 ng/mL. As the target concentration increases, more OPs-Ab complexes attached to the electrode surface increased the electrode surface impedance, hindered electron transfer, and thus increased ΔI. Figure 4b shown the linear relationship between the logarithm of organophosphorus pesticide concentration and ΔI. The obtained linear correlation coefficient (R^2) was 0.9918, the linear equation was $\Delta I = 53.59096 + 10.31913\text{LogC}$. After 3 measurements of negative samples, the mean value ($B0$) and standard deviation (SD) were obtained. The LOD could be obtained by substituting into the equation ($\text{LOD} = B0 - 3\times\text{SD}$). The LOD of OPs was 0.003 ng/mL.

These parameters were compared with results from other types of sensors (such as aptamers and enzymes), as shown in Table. 1. It was found that the detection limit of the electrochemical immunosensor proposed in this paper was lower and the detection linear range was wider, indicating that this work was meaningful and potential.

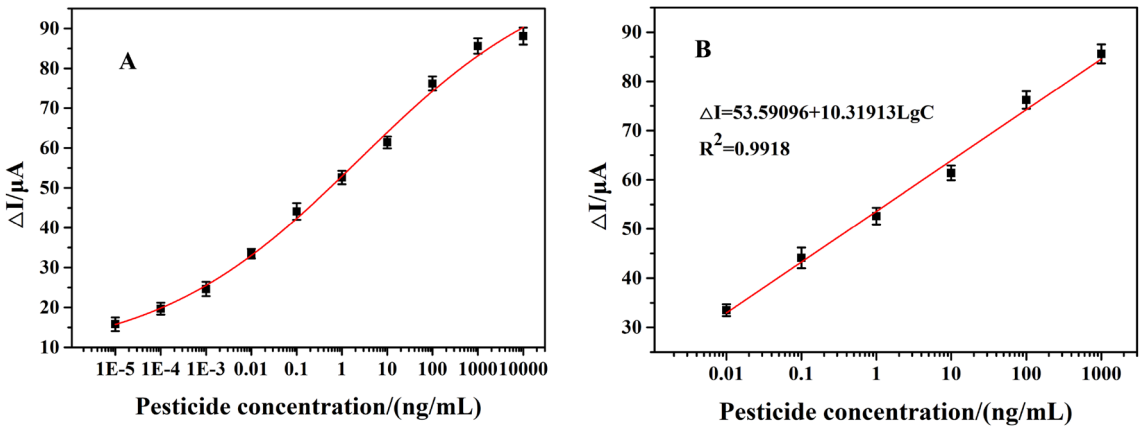

Fig. 4 **a** Competitive inhibition curve and **b** standard curve of the Ops

Table. 1 Comparison with other type of electrochemical sensors for OPs detection

Sensing layer	Modification methods	Target	Linear range	References
CuO-TiO_2/GCE	Drop	Methyl parathion	$0–2\times10^3$ ng/mL	[48]
AChE/Ce/UiO-66@MWCNTs/GCE	Drop	Paraoxon	$2.6–3.9\times10^4$ ng/mL	[49]
Aptamer-rGO-CuNPs/SPCE	Electrodeposition	Profenofos	$3.73–3.73\times10^4$ ng/mL	[43]
		Phorate	$2.64–2.64\times10^4$ ng/mL	
		Isocarbophos	$2.89\times10-2.89\times10^4$ ng/mL	
		Omethoate	$2.13\times10^2–1.07\times10^5$ ng/mL	
Chl-Ab/AuNPs /FTO	Drop	Chlorpyrifos	$3.50\times10^{-1}–3.29\times10^3$ ng/mL	[10]
AuNP–Abs–PB/SPCE	Electrodeposition	OPs (parathion, phoronidin, quetifosion, methyl parathion and so on)	$1.82\times10^{-3}–3.29\times10^4$ ng/mL	This work

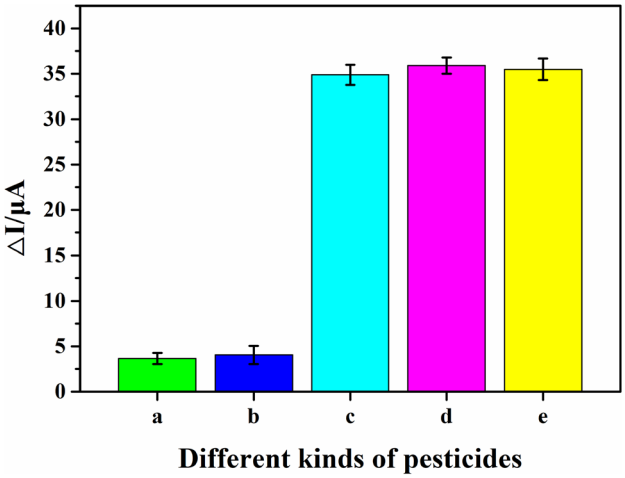

Fig. 5 Selectivity evaluation of the immunosensor (**a** carbofuran; **b** deltamethrin; **c** isocarbophos; **d** parathion, phoronidin, quetiophosphorus and methyl parathion; **e** parathion, phoronidin, quetiophosphorus, methyl parathion, carbofuran and deltamethrin)

Performance test of electrochemical immunosensor

Selectivity and anti-interference were important indicators of immunosensors, which were investigated by means of changes in electrochemical response signals of sensors before and after incubation with different interfering substances. Carbamate pesticides—carbofuran (Fig. 5a) and pyrethroid pesticides—deltamethrin (Fig. 5b) were selected as interfering pesticides. Figure 5c was isocarbophos, an organophosphorus pesticide, but not included in the mixed standard solution. Figure 5d was a mixture of standard solutions (parathion, phoronidin, quetiophosphorus, methyl parathion), while Fig. 5e was a mixture of OPs (parathion, phoronidin, quetiophosphorus, methyl parathion) and non-OPs (carbofuran, deltamethrin). As could be seen from the figure, the ΔI value was very small in the presence of no specific target, indicating that it was difficult for antibodies to bind these interfering pesticide molecules. Figure 5c–e all had large ΔI values, and the results were similar, indicating that the immunosensor had good selectivity and anti-interference ability.

At the same time, we checked the stability of the immunosensor. The prepared immunosensor was stored at 4 ℃ and used for detection on first, third and seventh days, respectively. The electrochemical signal was 94.39–113.62% of the initial signal, indicating that the immunosensor had good stability.

Detection of organophosphorus pesticides in vegetable samples

In order to evaluate the practicability of the proposed electrochemical immunosensor, baby cabbages and spinaches purchased from supermarkets were used to prepare sample matrix solutions for practical sample experiments. The standard addition method was used to add the mixed standard solution into methanol-PBS (10%, m/v) and two sample matrix solutions. Made the final spiked sample solution three different concentrations (0, 100, 1000 ng/mL) each. The immunosensor prepared in this experiment was used to detect and analyze the solution of the spiked sample. The experimental results were shown in Table. 2. The spiked sample solution prepared with methanol-PBS (10%, m/v) had a pesticide recovery rate between 99.57–100.46% and relative standard deviation (RSD) of 1.24–9.33%. The spiked sample solution prepared using the vegetable sample solution had a pesticide recovery rate between 98.05–102.05% and an RSD of 1.35–16.54%. The above test results shown a higher recovery rate and a lower RSD. And these results were all within acceptable limits. We thought them shown that the electrochemical immunosensor constructed in this study could be effectively applied to the detection of vegetable samples.

Table 2 Recovery of the sensor in samples ($n = 3$)

Sample	Spiked (ng/mL)	Detected (ng/mL)	RSD (%)	Recovery (%)
10% methyl alcohol-PBS	0	0	0	–
	100	99.57	1.24	99.57
	1000	1004.59	9.33	100.46
Baby cabbage	0	0	0	–
	100	102.05	1.35	102.05
	1000	980.4789	13.33	98.05
Spinach	0	0	0	–
	100	100.91	1.96	100.91
	1000	1011.84	16.54	101.18

Conclusion

In summary, we had prepared a new electrochemical immunosensor by electrodeposition technology and applied it to the detection of OPs. Firstly, the AuNPs were coupled with broad-spectrum antibodies to form AuNP–Abs. Then, the AuNP–Abs and PB were modified on the SPCE surface by one-step electrodeposition. It effectively simplified the preparation process of the immunosensor, improved the immobilized effect of the antibody, and enhanced the stability of the immunosensor. Under the optimal experimental conditions, the concentration of OPs had a good linear relationship with its corresponding electrochemical response, and the linear correlation coefficient was up to 0.9918. The immunosensor had a wide detection range (IC_{20}–IC_{80}: 1.82×10^{-3}–3.29×10^{4} ng/mL) and high sensitivity, and had good broad-spectrum, selectivity and stability. In addition, the immunosensor had a high recovery rate in the spiked sample recovery experiment. This work not only provided a new method for rapid pesticide detection in field, but also provided a new idea and reference for constructing immunosensor detection method for other small molecular targets. Admittedly, OPs are only one of many pesticide types, and this study was not enough to deal with the complex situation of pesticide abuse. If we apply this work to microarray electrodes with broad-spectrum antibodies of other pesticides, it may be possible to achieve the synchronous rapid detection of multiple pesticides.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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