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Short Communication

An Electrochemical Immunosensor Based on Microfluidic Chip for Detection of Chlorpyrifos

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With increasing of the abuse of overdose pesticides in agriculture, the threat to human health has become prominent, which leads to the urgent need for rapid detection of pesticide residues. In this article, an impedance immunosensor based on microfluidic chip was developed for rapid detection of pesticide residues in vegetable samples. This microfluidic chip consisted of a detection microchamber inlet and outlet microchannel. Gold interdigitated array microelectrode (IDAM) was embedded in the microchannel of microfluific chip. Using chlorpyrifos as the model compound, the methods of nanometer materials modified electrodes and protein A used for binding antibody's Fc fragment are used for the fabrication of the micro-fluidic chip immunosensors. The binding of chlorpyrifos target onto the antibody-modified IDAM surface produced a sensitive impedance change. And the impedance change was detected by the electrochemical impedance spectroscopy (EIS). The effect of experiment conditions of different pH value and the assembly time on the impedance sensor were also studied. Under the optimum operating conditions, this microfluidic chip immunosensor showed a broad linear response range, a high specificity and sensitivity and high stability. Moreover, the on-chip immunosensor can be used for direct detection of practical samples.

Keywords: Gold nanoparticles, microfluific chip, impedance immunosensor, chlorpyrifos

1. INTRODUCTION

Abuse of pesticides had posed a potential hazard to human health[1]. Among them, organophosphorus pesticides are principal cholinesterase inhibitors that inhibit acetylcholinesterase enzyme activity within nerve tissue[2-4]. The highly toxic of organophosphorus pesticides could cause serious diseases[5-7]. Therefore, the analysis of pesticide residues in food is very important for food safety.

Traditional analytical methods for pesticide residues detection have disadvantages such as extensive time consumption, expensive instrumentation and complicated pretreatment procedure, which limit their application for real-time detection[8-13]. Recently, because of biosensor technologies meet the demands for rapid and reliable testing of pesticides[14], it has been widely reserched,especially impedance immunosensors[15-18].. Compared with traditional gold electrode, the microelectrodes have been widely researched due to its various advantages, such as higher sensitivity, shorter detectde time and less sample dosage. Among microelectrodes, interdigitated array microelectrodes (IDAM) present promising advantages in terms of low ohmic drop, fast establishment of steady-state, rapid kinetics of reaction, and increased signal-to-noise ratio[19,20].

Because of the advantages of high performance, design flexibility, reagent economy, high throughput, miniaturization, and automation, microfluidic analysis systems has becoming powerful tools in chemical and biological assays[21]. Therefore,the combination of microfluidic chip and IDAM can bring greater benefits in biosensors[22,23].

Our team has reported that the application of microfluidic immunosensor chip by Protein A (PA) orientedly immobilizing antibody onto the embedded IDAM for pesticide residues detection[22]. Because the immobilization of biomolecules on the electrode have an important effect on improving the performance of immunosensors[24]. In the further research, we use PDDA and AuNPs to modify the IDAM surface for increasing the amount of immobilized biomolecules and improving the detection sensitivity.

To date, the application of microfluidic immunosensor chip by the layer-by-layer self-assembly technology and orientedly immobilizing antibody onto the embedded IDAM for pesticide residues detection has been still very less reported. In this study, we demonstrated an microfluific chip with embedded IDAM to reduce the amount of reagent, especially expensive antibody and improve the detection sensitivity based on impedance immunosensor as a new approach for the detection of pesticide residues in real samples. The methods of nanometer materials modified electrodes and protein A used for binding antibody's Fc fragment are used for the fabrication of the micro-fluidic chip immunosensors. Target chlorpyrifos with different concentration were captured by the immobilized antibody, resulting in a change of the impedance of microelectrode surface. Finally, the impedance change was record by electrochemical impedance spectroscopy (EIS).

2.EXPERIMENTAL

2.1 Materials

Anti-chlorpyrifos monoclonal antibody, protein A and poly (diallydimethylammonium chloride) (PDDA) (5%, w/w in water) were all purchased from Sigma (Sigma Diagnostics Co., MI, USA). The deionized water (18.2 MΩ cm) produced by Zahner (Millipore Co., Hessen, Gemarny) was used throughout. $HAuCl_4$ was from Shanghai Sinopharm Chemical Reagent Co.Ltd. (China). 0.01 M phosphate buffer solution (PBS, pH 7.4, high-pressure sterilization) was used for dissolving the anti-chlorpyrifos monoclonal antibody and protein A. A PBS (0.1 M, pH 7.5) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl was used as the detection solution.

2.2 Preparation of AuNPs

The gold colloidal nanoparticles (AuNPs) were prepared as described in the literature[25]. All glasswares used were cleaned in a bath of reshly prepared aquaregia (3:1 HCl-HNO_3), thoroughly rinsed with distilled water and dried prior to use. 100 mL of 0.01% $HAuCl_4$ solution was taken in a flask, and 2.5 mL 1% sodium citrate solution of ice cold was quickly added to the boiling solution under vigorous stirring. The solution's color from blue changed to wine red. The solution was kept boiling for 15 min with vigorous stirring, and then cooled down to room temperature. Finally, the prepared solution, AuNPs solution, was stored at 4 °C in a dark bottle.

2.3 Microfluidic chips and Impedance measurements

Microfluidic chips were purchased from department of mechanical engineering in University of Arkansas. The microchannel consisted of a detection microchamber with the size of 6mm×0.5mm×0.02mm and volume of 60 nL. Impedance measurements were performed using an IM-6 impedance analyzer with IM-6/THALES-software. All impedance measurements were conducted in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) mixture in PBS (pH 7.5) as a redox probe. The tested frequency range was from 1 Hz to 1 MHz. Nyquist (imaginary impedance vs real impedance) diagrams was recorded. At the end of each test, the microchannel was washed with 0.1M sodium hydroxide for 15 min, deionized water for 10 min, 0.1M hydrochloric acid for 15 min, and a final rinse with deionized water for 10 min, espectively. The flow rate used for washing was 10 μL/min.

In order to determine frequency associated with the largest difference in absolute impedance between the vegetable sample and the control, a curve was drawn between normalized impedance change (NIC) and frequency. NIC was defined by the following equation:

$NIC=(Z_{sample}-Z_{control})/Z_{control}\times 100\%$

Where $Z_{control}$ is the absolute impedance of the microfluidic chip after immobilized antibody and Z_{sample} is the absolute impedance of the microfluidic chip after added the vegetables sample liquid.

2.4 Preparation and determination of real vegetable samples

Vegetable samples (cucumber, lettuce and pakchoi) for detection study were purchased from the local supermarket and cleaned thoroughly using deionized water. After dried in the air, the samples were chopped into 5×5 mm particles approximately. Then, different concentrations of chlorpyrifos solution were sprinkled on their surfaces. After equilibration for 3 h at room temperature to allow pesticide absorption onto the samples, a mixture of 1 mL acetone and 9 mL 0.1M phosphate buffer solution (pH 7.4) was added to each sample weighing 10 g. And then the suspensions, treated in ultrasonic for 15 min, were centrifuged for 10 min at 2000 rpm. Finaly, the clear supernatant extract was used to analyze the immunosensor detection method.

3. RESULTS AND DISCUSSION

3.1 Preparation of the microfluidic chip based immunosensor

PDDA solution was pumped into microfluidic chips with a syringe pump 10min. After washing and drying, AuNPS was injected 15 min. Then protein A (100 μg/mL, immersed in pH 7.5 PBS) solution was pumped into the microelectrodes and incubated for 45 min, which orientedly immobilized the anti-chlorpyrifos monoclonal antibody onto the IDAM surface. After washing and drying gently under a stream of nitrogen, 10 μg/mL of anti-chlorpyrifos monoclonal antibody solution was pumped into the microchannel and incubated for 90 min at 4 °C. After washing and drying thoroughly, samples with different concentrations of chlorpyrifos in PBS were dropped into the IDAMs. All impedance tests were performed in $[Fe(CN)_6]^{3-/4-}$ injected in the microchannel. The fabrication process of immunosensor was shown in Figure 1.

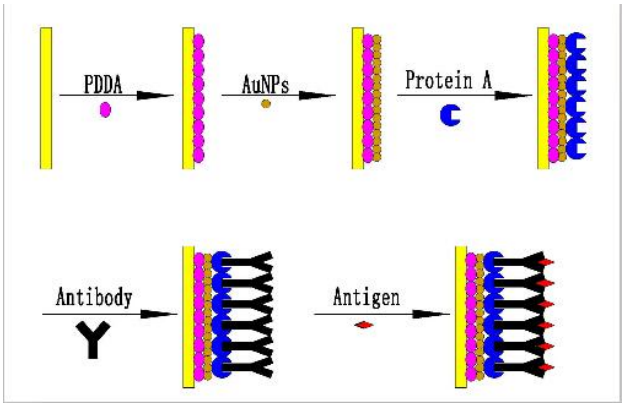

Figure 1. Schematic illustration of the stepwise procedures for the fabrication of the microfluidic immunosensor

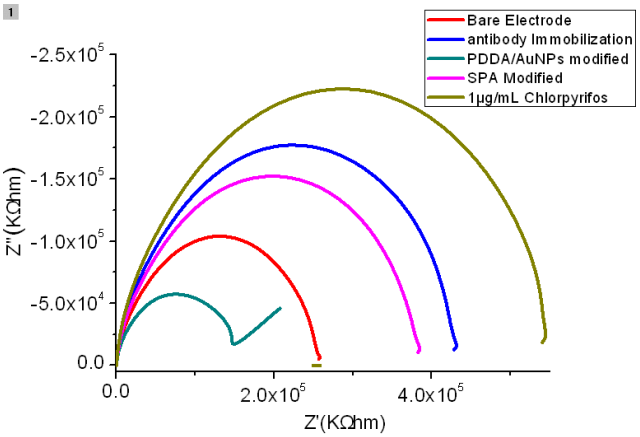

Figure 2. The typical Nyquist diagram of impedance spectra of the bare microelectrode, PDDA/AuNPs modification, protein A modification, antibody immobilization, chlorpyrifos binding with $[Fe(CN)_6]^{3-/4-}$ as a redox probe in the frequency range from 1Hz to 1MHz.

Figure 2. shows the typical Nyquist diagram of impedance spectra of the bare microfluidic chip, PDDA/AuNPs, protein A modification, antibody immobilization, chlorpyrifos binding with $[Fe(CN)_6]^{3-/4-}$ as a redox probe. As shown in Figure 3, in the frequency range from 1Hz to 1MHz, the impedance value at a given frequency have an obvious change upon the addition of PDDA/AuNPs, protein A, the antibody layer, and chlorpyrifos to the electrode surface compared to the bare electrode.

3.2 Optimization parameters of the experimental conditions

3.2.1 Influence of the applied working voltage on the impedance analyzer

We detected the impedance values of the voltages in the range of 10~100 mV. When the working voltage was changed, the impedance value had almost no change at all, but it tended to a stable value as shown in Figure 3. Considering that 100 mV can overcome the noise, 100 mV was set as external applied voltage in this experiment.

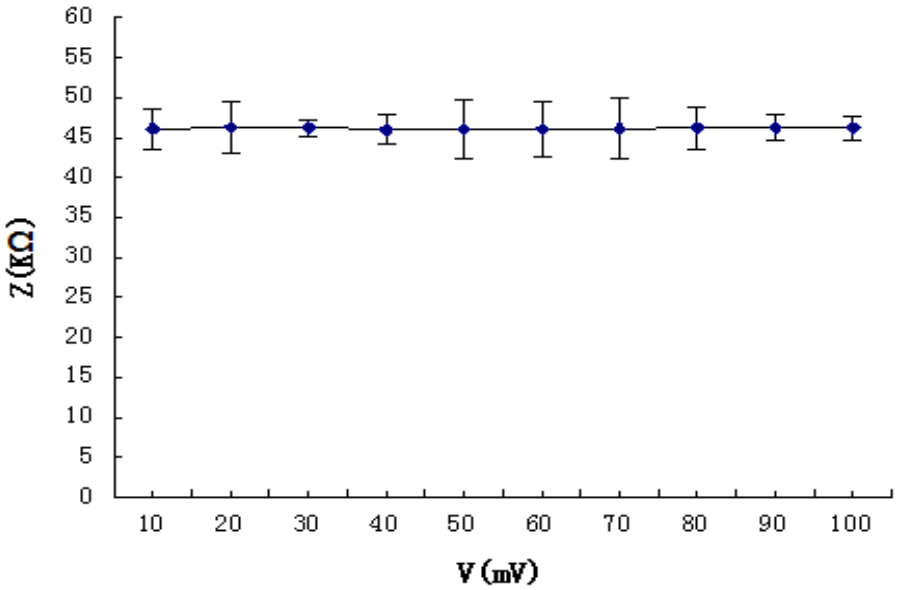

Figure 3. Influuence of the applied voltage on the impedance analyzer in PBS containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. Frequency range 1Hz to 1MHz

3.2.2 Influence of the pH on the detection solution

The pH of the detection solution plays an important factor in achieving good analytical performance. The anti-chlorpyrifos antibody modified electrodes were incubated in phosphate buffer solutions (PBS) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl with different pH of 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. As shown in Figure 5, the maximum impendance response of the immunosensor was obtainde at pH 7.5. Therepore,pH 7.5 was chosen as the optimal pH.

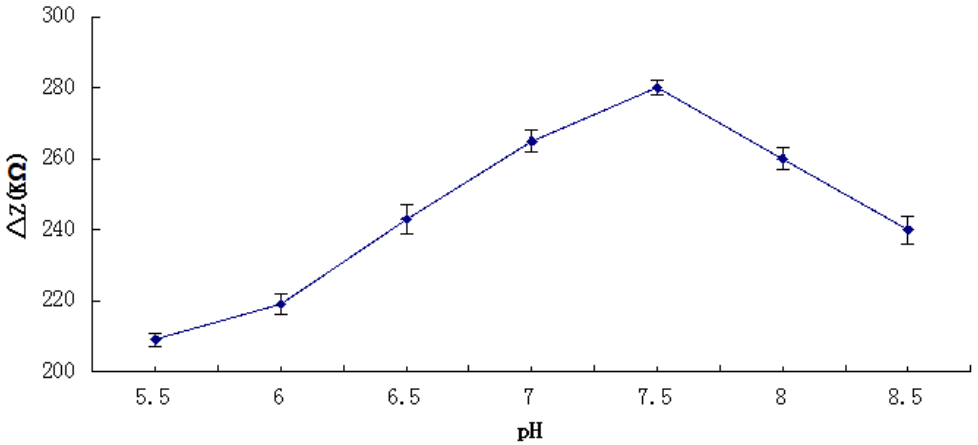

Figure 4. Influence of detection solution pH Frequency range 1Hz to 1MHz

3.2.3 Influence of the incubation time

Since it takes time for the chlorpyrifos immobilized on the electrode to react with the antibody molecules to reach the saturated equilibrium, we detected the impedance change of the incubation time in the range of 10~55 min. As shown in Figure 6, the impedance change reached the maximum in 40 min and were stable when the time was extended, indicated that the interaction between antibody and chlorpyrifos had reached saturation. Thus, 40 min of the incubation time was chosen as the optimal incubation time.

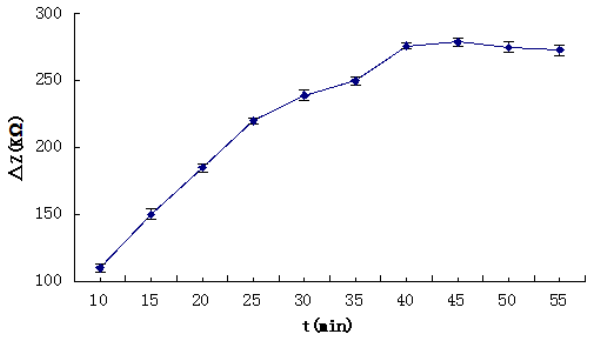

Figure 5. Influence of the incubation time on impedance change in PBS containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. Frequency range 1Hz to 1MHz.

3.3 Detection of chlorpyrifos

Under optimal experimental conditions, the calibration plot for detecting chlorpyrifos with the proposed immunosensor is shown in Figure 6. The linear relationship between the relative impedance change and logarithm of chlorpyrifos concentration was obtained in the range of 0.5 -500 ng/ml. The detection limit was achieved as 0.5ng/ml. The linear regression equation is $\Delta Z = 163.0 + 70.000 \lg C$ (ng/mL) with the correlation coefficients of 0.97645. The detection limit was estimated to be 0.5 ng/ml.

The performance of the anti-chlorpyrifos/PA/AuNPs/PDDA/Au impedance immunosensor was compared with other reported immunosensor. As showed in table 1, compared with other biosensors ,the immunosensor has a relative large linear range and lower detection limit.

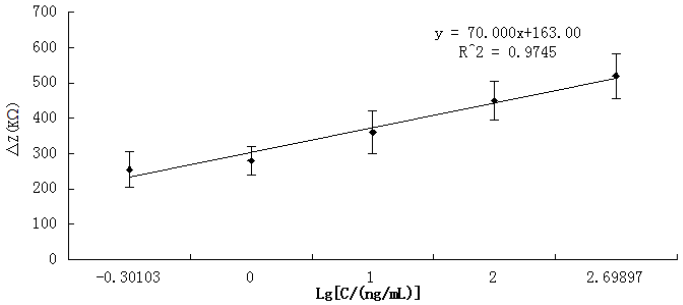

Figure 6. The calibration curve of the relative current changes (ΔZ) of the proposed microfluidic immunosensor versus the logarithm of chlorpyrifos concentration in PBS containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. Frequency range 1Hz to 1MHz.

Table 1 Comparison with other reported immunosensors for the detection of chlorpyrifos

electrode	Linear range (ng/mL)	detection limit (ng/mL)	References
PPy-PVS/ITO(Electrochemical entrapment)	1.6-20	1.6	[26]
AChE/[BMIM][BF_4]/MWCNT/CP	3.5-350	1.4	[27]
anti-chlorpyrifos/PA/Au	1.0-1.0 × 10^5	1.0	[22]
Anti-chlorpyrifos/PA/AuNPs/ PDDA/Au	0.5-500	0.5	This work

3.4 Determination of chlorpyrifos in vegetable samples

Table 2. Determination of chlorpyrifos in vegetable samples

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%)
Cucumber	10	7.52	75.2
	1.0×10^2	0.862×10^2	86.2
	1.0×10^3	0.817×10^3	81.7
lettuce	10	9.13	91.3
	1.0×10^2	0.886×10^2	88.6
	1.0×10^3	0.897×10^3	89.7
pakchoi	10	9.65	96.5
	1.0×10^2	0.924×10^2	92.4
	1.0×10^3	0.951×10^3	95.1

Vegetable samples (Cucumber, lettuce and pakchoi) were processing as described of 2.5. As shown in Table 2, the recovery tests were performed using 3 replicates with the microfluidic chip immunosensor that prepared in the same way. The recovery of 75.2~96.5% indicated that the microfluidic immunosensor was available for the analysis of chlorpyrifos in vegetable samples.

4. CONCLUSIONS

In this paper, we took advantage of microfluidic chip with embedded IDAM to fabricate immunosensor for rapid detection of pesticide residues in vegetable samples. This impedance immunosensor use chlorpyrifos as the model compound, after PDDA and gold nanoparticles modifing the microelectrode surface, anti-chlorpyrifos monoclonal antibody was orientedly immobilized onto the IDAM surface through protein A. Under the optimum condition, this microfluidic immunosensor showed a wide range and good specificity.. Owing to the wider applications of the rapid detection of pesticide residues in many fields, miniaturized immunosensors will make a contribution to fast, direct, and secure analysis of pesticide residues in real samples.

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