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Layer-by-Layer self-assembled acetylcholinesterase/PAMAM-Au on CNTs modified electrode for sensing pesticides

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ABSTRACT

In this paper, an acetylcholinesterase (AChE)/dendrimers polyamidoamine (PAMAM)-Au/Carbon nanotubes (CNTs) multilayer modified electrode based on LbL self-assembled technique was employed in the detection of carbofuran in samples. The configuration of the nanostructure on the electrode provided a favorable environment to the immobilization of AChE. The modified films also improved the electrocatalytic characteristics and electron transfer speed between the films and the surface of electrode. The PAMAM-Au nanoparticles were characterized by SEM and UV–VIS methods. A set of experimental conditions were also optimized for the detection of the pesticides. A linear response over carbofuran concentration in the range of 4.8×10^{-9}M to 0.9×10^{-7}M was exhibited with a detection limit of 4.0×10^{-9}M. The biosensor showed high sensitivity, good stability and reproducibility with promising application.

1. Introduction

The increasing amount of potential harmful pesticides released in the environment calls for the development of fast and sensitive analytical techniques for their monitoring. In the past few years, traditional analytical methods which were costly and complex, e.g. high performance liquid chromatography (HPLC), limited their application on regular detection, especially in field test. In this respect, the bioelectrochemical sensors are considered to be a suitable candidate for the on-site determinations of pesticides released in the environment and residue in food [1,2].

The combination of electrochemistry and biology methods (enzymes) has been developed in the past two decades as the most promising alternative for direct monitoring of pesticides, which is commonly based on quantification of their inhibition in the presence of pesticides. The degree of the inhibition of the acetylcholinesterase (AChE) depends on the concentration of the pesticide, the exposure time and other parameters, which can be measured amperometrically. The sensitive and selective detection of the anticholinesterase pollutants were performed using different bioelectrochemical transducers [3,4].

The inhibition in the single and bienzyme system [5] is monitored by measuring the oxidation current of the product of the enzyme reaction. Acetylthiocholine is enzymatically converted to thiocholine which can be easily oxidized into an appropriate disulfide (Eq. (1)) [6].

$$\begin{aligned} &acethythiocholine + H_2O \xrightarrow{AChE} thiocholine + acetic\,acid \\ &2-thiocholine \xrightarrow{anodic\,oxidation} disulfide + 2H^+ \end{aligned} \quad (1)$$

In the following bienzyme system, (Eq. (2)) [7] AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine into acetate and choline. The choline is subsequently converted by ChO, producing hydrogen peroxide in the presence of oxygen. Hydrogen peroxide can be detected amperometrically with different electrochemical transducers.

$$\begin{aligned} &\text{acethycholine} + H_2O \xrightarrow{\text{AChE}} \text{choline} + \text{acetate} \\ &\text{choline} + O_2 \xrightarrow{\text{ChO}} \text{betanie aldehyde} + H_2O_2 \end{aligned} \quad (2)$$

Amperometric biosensors are considered as sensitive and accurate method in the substrate determination due to their liner relationship between the current and concentration of the substrate at relatively low substrate concentration. Despite many efforts to develop these biosensors, certain analytical characteristics of these systems require further improvement to meet the required specifications, such as higher sensitivity, selectivity, and stability.

One approach to improve the performance of characteristics of the AChE-based inhibitor biosensors would be to design and produce appropriate enzymes with characteristics more suitable for biosensor applications [8]. However, this approach has been hampered by the fact that AChE from various sources was not easily available because of

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difficulties in isolation and purification procedures [9]. Another possible approach for improving the performance characteristics of the AChE-based inhibitor biosensors is to improve the biosensor design and the electrochemical detection of the enzymatic product [10].

The LbL technique has been largely employed in the immobilization of proteins and other biomolecules following the pioneering works, where advantage was taken of the outstanding control in both film thickness and supramolecular architecture. Therefore, in our work, the Layer-by-Layer (LbL) self-assembled AChE/PAMAM-Au/CNTs multilayer electrode was employed for the detection of pesticides in samples by DPV and CV. Here, CNTs provided the interface between the bare electrode surface and enzyme layers because the nanotubes possess high electrical conductivity and enable individual tethering of the redox center of enzymes. Moreover, the dendrimers polyamidoamine (PAMAM) was used to provide a suitable microenvironment to retain the AChE activity in the films of the modified electrode. The Au nanoparticles (AuNPs) in the films played an important role similar to a conducting wire, which was easier for the electron transfer to take place.

2. Experimental

2.1. Chemicals and apparatus

PAMAM-NH_2 (G4) in methanol solution was offered by Dr. Dongmei Wu (East China Normal University). Carbofuran was purchased from Dr. Ehrenstorfer GmbH Company. Acetylcholinesterase (AChE, EC 3.1.1.7, 1100 units/mg) and Acetylthiocholine chloride (ATChCl) were purchased from Fluka and Sigma-Aldrich Co, respectively. All other reagents were of analytical reagent grade. All solutions were prepared with double-distilled water.

PAMAM-Au 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 AChE/PAMAM-Au/CNTs modified electrode ($d = 3$ mm, Bioanalytical System, Tokyo, Japan) serving as working electrode, while a saturated calomel electrode (SCE) and platinum wire served as reference and counter electrode, respectively. Electrochemical experiments were carried out in a 10.0 ml electrochemical cell at room temperature (25 °C ± 1). All potentials were referred to the SCE reference electrode.

2.2. Synthesis of PAMAM-Au nanocomposite

Our approach for the preparation of dendrimer-encapsulated Au metal particles is similar to the previously proposed solutions for Pt [11]. PAMAM-Au nanocomposites were prepared as follows: 2.5 ml $HAuCl_4$ solution (0.3 mM) was added to 2.5 ml PAMAM (0.1 mM) with vigorous stirring for 20 min. Then, 2.5 ml of formic acid (0.1 mM) was incrementally added (with at least 15 min between additions) into the previous solution. When zerovalent Au complex was formed, the color changed from yellow to fuchsia. This reaction took over 4 h. To obtain the different sizes of PAMAM-Au particles, various volumes of $HAuCl_4$ were added to the same volume of PAMAM together with an excess of reducing agent to make sure that most of the $HAuCl_4$ was reduced to Au. The nanocomposites growth kinetics was followed by UV–VIS spectroscopy. And the morphology and particle sizes of the nanocomposites were characterized using a scanning electron microscope (SEM).

2.3. Fabrication of AChE/PAMAM-Au/CNTs/GCE

AChE/PAMAM-Au/CNTs films were prepared on glass carbon electrode by Layer-by-Layer method as shown in Fig. 1. Firstly, the multiwall CNTs were pretreated by sonicating in a mixture of concentrated HNO_3 and H_2SO_4 (v/v 1:3) for 6 h followed by extensive washing in double-distilled water until the filtrate was neutral. After this procedure, the CNTs were better separated and more active centers were formed in the surface. The pH was adjusted to about 8.0. 5 μL of the prepared CNTs solution was dropped onto the clean glass carbon electrode and dried in room temperature. Then, the prepared CNTs/GC electrode was dipped in 1 M NaOH solution for 10 min to obtain negative charged CNTs. After washed by double-distilled water, the CNTs/GC electrode was dipped in PAMAM-Au solution for 30 min and rinsed with distilled water again. At last, the PAMAM-Au/CNTs/GC electrode was immersed in 0.3 unit/ml AChE for 30 min and rinsed with distilled water and dried in nitrogen.

2.4. Electrochemical experiments

AChE/PAMAM-Au/CNTs electrode was employed in the CV measurements with the potential range from −0.2 V to 0.8 V at different scan rates in $[Fe(CN)_6]^{4-/3-}$ solution (0.05 M). DPV measurements were performed to optimize the experimental conditions and determine the OPs content at room temperature in the potential range from 0 V to 0.8 V with the scan rate of 100 mV/s.

2.5. Preparation and determination of real samples

The onion, lettuce and cabbage were purchased from the supermarket and cleaned three times using double-distilled water. Different concentrations of parathion solution were sprinkled on the surface of the three kinds of vegetables [12,13]. After 24 h, all the samples weighing 10–20 g were chopped and meshed. Then a mixed solution of 1 ml acetone and 9 ml 0.1 M phosphate buffer (pH 7.4) was added to each sample. All the above experiments were maintained under the nitrogen atmosphere and the mixed solution was treated in ultrasonic

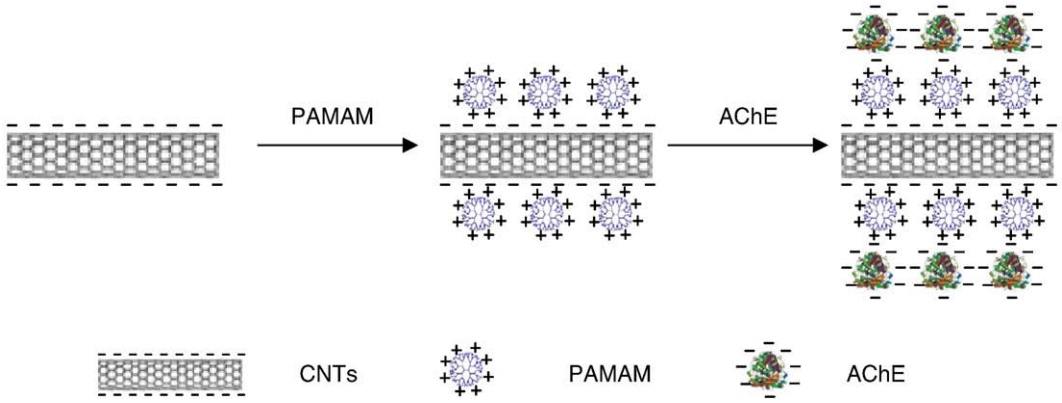

Fig. 1. Schematic representation of the fabrication of multilayer film assembly of CNTs PAMAM-Au and AChE.

for 15 min. The suspensions were centrifuged (10 min, 2000 rpm) and the supernatants were directly detected by DPV without extraction or preconcentration. The content of parathion in the samples can be achieved from the calibration curve.

3. Results and discussion

3.1. Characteristic of PAMAM-Au

Fig. 2A shows the absorption spectra of dendrimer-encapsulated AuNPs. Curves a and b in Fig. 2A show the absorption spectra of the PAMAM-$HAuCl_4$ solution and PAMAM-Au, respectively. As shown in Fig. 2, the solution of PAMAM-$HAuCl_4$ (curve a) has a ligand–metal charge-transfer band at 220 nm. After reduction of the composite, the spectrum changes significantly: there is now higher absorption intensity at low energy (curve b), which results from the interband transition of the encapsulated zero valent metal particles. As shown in Fig. 2B, SEM image also confirms that the chemical reduction of Au encapsulated within G4-OH and yields intra-dendrimer metal nanoparticles (the ratio of the Au particle to PAMAM is 3:1). The size of PAMAM-Au particle is about 13 nm and the size of the AuNPs in the PAMAM-Au complex is about 8 nm. Some free Au particles were found in the film which were possibly caused by the vigorous stirring and the incremental addition of the formic acid.

3.2. Characteristic of the electrode

The Layer-by-Layer (LBL) self-assembly technique is used to generate ultrathin film with molecular order and stability by electrostatic interaction. With this technique, it is possible to control, in a very precise manner, the thickness, composition, and surface structure of the multilayer building block [14–16]. The procedure of the construction of AChE/PAMAM-Au/CNTs electrode is outlined in Fig. 1. The electrostatic interaction among the oppositely charged species is a simple and powerful method for the construction of composites that are self-assembled on a nanometer scale. The negative charged CNTs were dropped onto the electrode surface. The PAMAM-Au in the solution which possesses positive charge (due to the protonation of the amino groups, the surface of the dendrimers should be positively charged at experimental conditions used [17]) could be adsorbed on the CNTs surface by electrostatic force. Because the isoelectric point I_p of AChE is 4.5, by the same procedure, a layer of negatively charged AChE was adsorbed on the CNTs film at PBS (pH = 8.0). Fig. 3 shows the cyclic voltammograms of the AChE/PAMAM-Au/CNTs electrode in $[Fe(CN)_6]^{4-/3-}$ solution (5 mM) at different scan rates from 10 to 300 mV/s. Each voltammogram in Fig. 3 is symmetric and has a pair of well-defined redox peaks. The cathodic and anodic peak currents are linearly proportional to the square root of the scan rates in the range of 10–300 mV/s (Fig. 3 inset), which shows a characteristic of a diffusion-controlled electrochemical process.

3.3. Optimum experimental parameters

Fig. 4 displays the response currents to the same concentration of ATCh (2.25 mM) by modified electrode with different proportions of AuNPs in dendrimers. We can see that with the decrease of the value of k ($k = C_{Au}:C_{PAMAM}$), the current responses increase until $k = 3$. It is because that the increase of the proportion of AuNPs in PAMAM-Au enlarges the metal surface, which may improve the electrocatalytic characteristic and the electron transfer speed. While k is bigger than 3, the current responses decrease slightly, which maybe because excessive AuNPs may affect the activity of the AChE. Furthermore, the decrease of the proportion of PAMAM in PAMAM-Au may affect the conformational changes of proteins, which keep the activity of the AChE [17]. Therefore in this work, we choose $k = 3$ as the optimum proportion of Au/PAMAM. The pH effect of the buffer was also investigated (figure not shown). As reported, the electrocatalytic oxidation of thiocholine undergoes two step reactions, as described below:

$$RSH \xrightarrow{-e^-} RSH^{+\bullet} \xrightarrow{-H^+} RS\bullet \rightarrow RSSR.$$

In this reaction, the $RS\bullet$ species undergoes rapid dimerzation to form a disulfide species. The increase of the pH to higher value may move the oxidation wave to higher potential and the decreasing of the pH to lower value may inhibit the formation of the $RS\bullet$[18]. Thus, in order to avoid the interference from other electroactive species and to keep the activity of AChE, pH 7.4 was adopted in the following experiments. The effect of the buffer concentration was also studied (figure not shown). The experimental results showed that the responses to the same

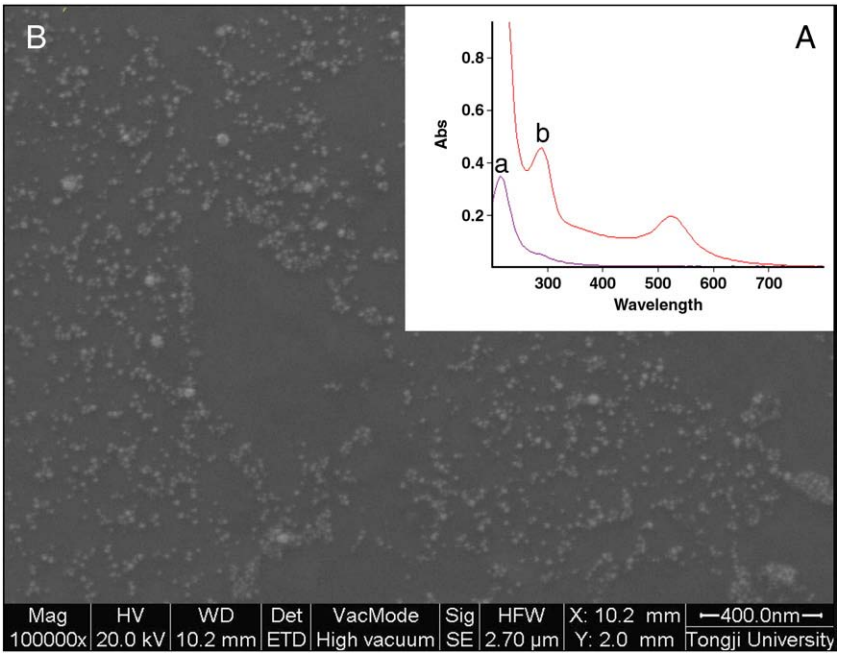

Fig. 2. (A) Absorption spectra of dendrimer solution. (a) PAMAM-$HAuCl_4$ solution; (b) PAMAM-Au solution. (B) SEM image of PAMAM-Au particles.

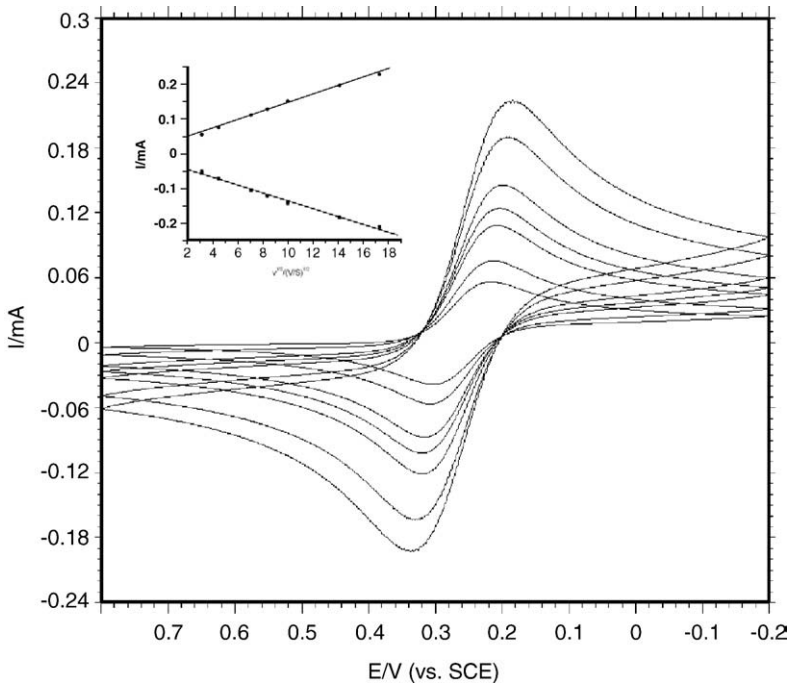

Fig. 3. The cyclic voltammograms of the AChE/PAMAM-Au/CNTs electrode in $[Fe(CN)_6]^{4-/3-}$ solution (5 mM) at different scan rates of 10–300 mV/s.

concentration of ATCh increase gradually from 0.01 to 0.1 M PBS (7.4) while the signal was decreased with the increasing of the buffer concentration at the range of 0.1–0.5 M. The decrease of the signal may be attributed to the leaking of AChE from the electrode surface. The successive layers of AChE/PAMAM-Au can be removed under conditions with higher salinity [9]. So 0.1 M PBS (pH 7.4) was employed in this work.

3.4. Assay of the AChE activity immobilized on the modified electrode

The responses of AChE sensor based on PAMAM-Au-CNTs films to repeating addition of the ATCh are presented in Fig. 5A. The incubation time was 3 min which was determined as the time necessary for the modified sensor to reach 95% of the steady-state signal. As shown in Fig. 5A, we can find that the current responses increase rapidly with the increase of the concentration of ATCh. While the concentration of ATCh reaches 2.25 mM or higher, the response current tends to reach a platform. To ensure that the electrode works under the kinetic-controlled conditions, the concentration of the substrate used must be near the upper limit of the system and within the liner range of the response. So 2.25 mM of ATCh was used as the substrate concentration in the detection of pesticides. The apparent value of Michaelis–Menten constant $K_M = 1.66$ mM calculated in accordance with Hanes equation is lower than the other AChE sensors [9]. This phenomenon may be another evidence of the conformational changes of proteins in the presence of dendrimers. To make sure the current was coming from the enzymatic product TCh instead of ATCh, a comparison experiment was performed with the PAMAM-Au/CNTs electrode and the AChE/PAMAM-Au/CNTs electrode in the presence of ATCh. The responses of 2.25 mM ATCh on the PAMAM-Au/CNTs electrode (a) and AChE/PAMAM-Au/CNTs (b) electrode were displayed in Fig. 5B. No response was obtained with the PAMAM-Au/CNTs electrode, while a sharp peak was obtained with the AChE/PAMAM-Au/CNTs after adding the same concentration of ATCh.

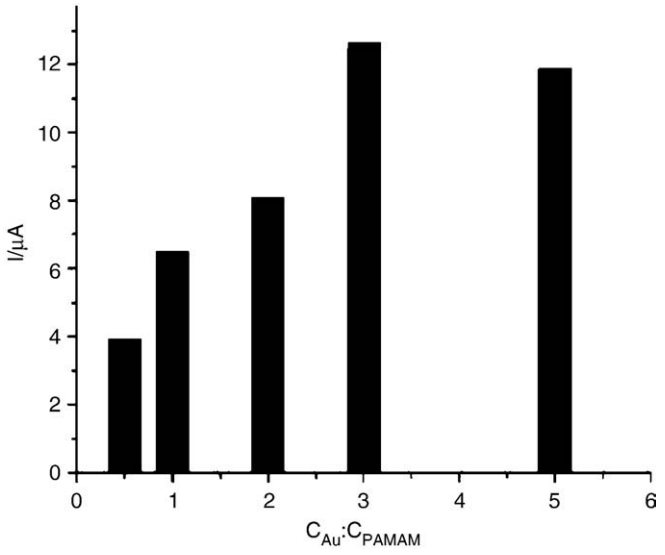

Fig. 4. The current responses to the same concentration substrate by modified electrode with different proportions of Au in dendrimers.

3.5. The detection of carbofuran in solution and real samples

The inhibition measurements were performed in conditions corresponded to the saturation of the enzymatic layer with the substrate at the ATCh concentration of 2.25 mM for the modified sensor. In saturation conditions, after contact with the pesticide solution, the change of the sensor response is only related to the enzyme–inhibitor interactions and does not depend on the mass transfer of the substrate or inhibitor from the solution to the enzyme layer, which makes it possible to reach the highest sensitivity of the pesticide determination. If the concentration of the substrate is lower, the decay in the enzyme activity due to the inhibition is partially compensated by the enzyme active sites which are free in the response measurement prior to the incubation in an inhibitor solution [1,9,19,20]. We also found that the residual activity of the enzyme was influenced by the exposure time of the AChE to carbofuran. Fig. 6 shows the dependence of the AChE activity on incubation time with different concentrations of carbofuran. The activity of AChE weakens as the incubation time grows. We can find that even a low concentration of 4.8 nM carbofuran could cause an apparent inhibition after a 9 min exposure. The higher the carbofuran concentration is, the greater stronger

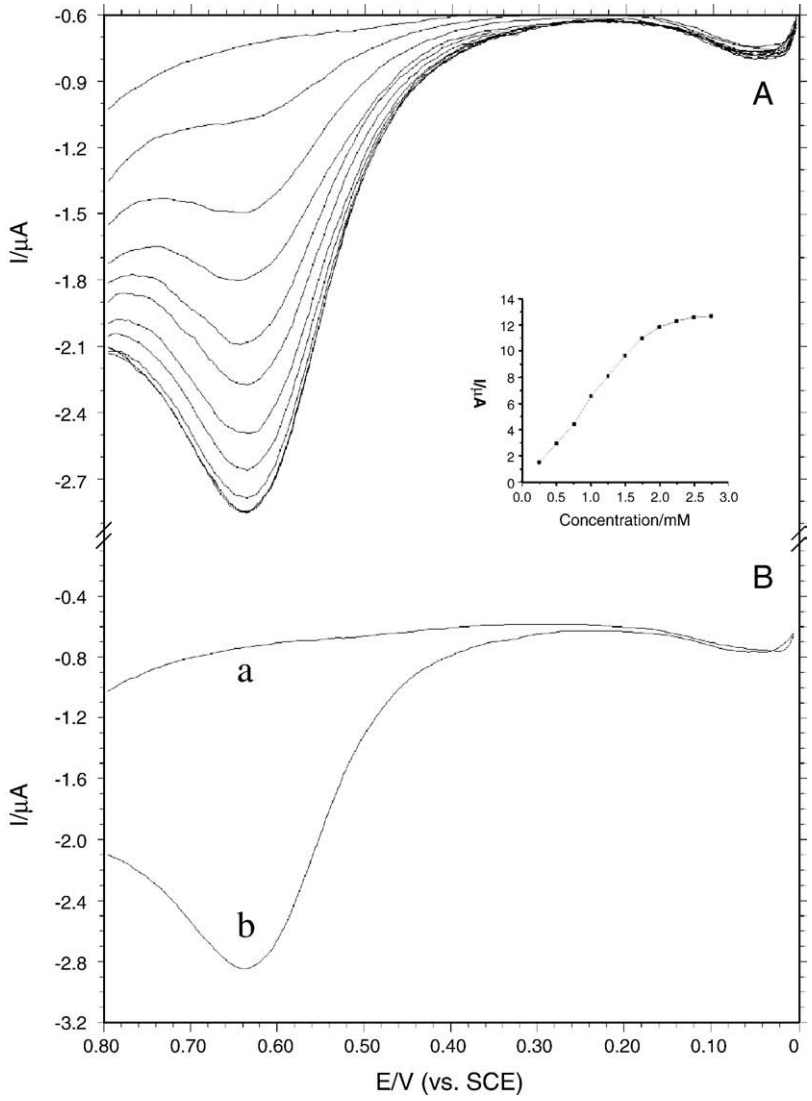

Fig. 5. (A) The current responses of AChE sensor based on PAMAM-Au-CNTs films electrode to repeating addition of the ATCh (0.25 mM). (B) The current responses of 2.25 mM ATCh on the PAMAM-Au/CNTs electrode (a) and AChE/PAMAM-Au/CNTs (b) electrode.

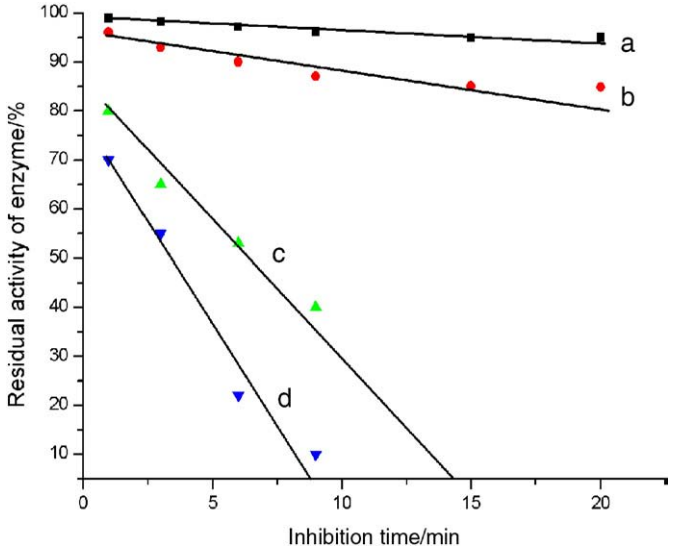

Fig. 6. The dependence of the AChE activity on incubation time with different concentrations of carbofuran.(a. 4.8 nM, b. 15 nM, c. 60 nM, d. 80 nM). Residual activity of enzyme $= 1-\frac{I_0-I_1}{I_0} \times 100\%$.

inhibition is. Considering the whole analytical detection time and the sensitivity of the detection system, we used 6 min as the incubation time of the carbofuran. Fig. 7 shows the calibration plots of the detection of

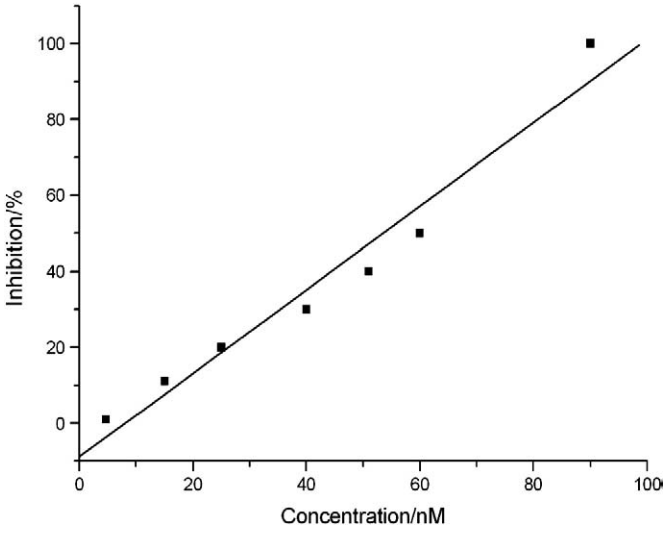

Fig. 7. The calibration plots of the detection of carbofuran *Inhibition* $= \frac{I_0-I_1}{I_0} \times 100\%$.

Table 1
The detection results of real samples.

Sample	The amount of parathion sprinkled on the vegetables (nM)	The amount of parathion detected by the modified electrode (nM)
Onion	80	72
Lettuce	80	70
Cabbage	80	74

carbofuran in the optimum conditions. As shown, the inhibition of the activity of the AChE is increasing with the concentration of carbofuran ranging from 4.8×10^{-9}M to 0.9×10^{-7}M with a detection limit of 4.0×10^{-9}M (calculated for 5% inhibition). The detection limit of the modified electrode was better than that achieved with Low-Temperature Cofired Ceramics Microsystem [21] and flow-injection determination of carbaryl and carbofuran based on $KMnO_4$–Na_2SO_3 chemiluminescence [22]. Further improvements for larger liner range can be achieved by increasing the incubation time of the electrode in the enzyme solution to introduce more enzymes to the surface of the electrode. Base on the inhibition of the AChE for the carbofuran ranging from 4.8×10^{-9}M to 0.9×10^{-7}M, a relative standard deviation of 5.4 ($n=7$) was obtained. The stability of the AChE/PAMAM-Au/CNTs sensor was examined 10 times by successive detection of carbofuran at room temperature (25 °C). When not in use, the sensor was kept in PBS (pH = 7.4) solution at 4 °C. There is no obvious decrease of the activity of the enzyme on the electrode after 1 week. There is about 10% activity lose of the enzyme after 3 weeks. The good stability is probably a consequence of the self-assembling AChE on the PAMAM-Au, which provides a favorable condition to maintain the activity of enzymes.

To evaluate the performance of the developed modified sensor, the contents of carbofuran in real samples of onion, lettuce and cabbage were detected by AChE/PAMAM-Au/CNTs modified electrode. Additionally, a blank sample was prepared from the noncontaminated vegetables and analyzed using the above method. From the data in Table 1, it can be seen that the amount of carbofuran in the vegetable samples was about 10% lower when analyzed by a modified sensor compared to the amount of carbofuran sprinkled on the vegetables. The lower result may be attributed to the insufficient inhibition of the carbofuran and the adsorption of the carbofuran on the vegetables.

4. Conclusion

An AChE/PAMAM-Au/CNTs multilayer modified electrode based on LbL self-assembly technique was employed in the detection of carbofuran in samples. The configuration of the nanostructure on the electrode provides a favorable environment to the AChE, electrocatalytic characteristics and fast electron transfer. The developed biosensor system has a low detection limit of 4.0×10^{-9}M carbofuran. According to our experiments, the biosensor shows high sensitivity, stability and reproducibility.

We believe that, the AChE/PAMAM-Au/CNTs modified sensor on the basis of electrochemical and biological methods may have promising application for OPs detection in environmental field.

Acknowledgements

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References

[1] M. Snejdarkova, L. Svobodova, G. Evtugyn, H. Budnikov, A. Karyakin, D.P. Nikolelis, T. Hianik, Acetylcholinesterase sensors based on gold electrodes modified with dendrimer and polyaniline: a comparative research, Anal. Chim. Acta 514 (2004) 79–88.
[2] Sarıgül Tuba, Recai İnam, A direct method for the polarographic determination of herbicide triasulfuron and application to natural samples and agrochemical formulation, Bioelechemistry 75 (2009) 55–60.
[3] BenjaminJ. Privett, Shin Jae Ho, MarkH. Schoenfisch, Electrochemical sensors, Anal. Chem. 75 (2008) 4499–4517.
[4] N. Jaffrezic-Renault, New trends in biosensors for organophosphorus pesticides, Sensors 1 (2001) 60.
[5] Navrátilová Iva, Skládal Petr, The immunosensors for measurement of 2,4-dichlorophenoxyacetic acid based on electrochemical impedance spectroscopy, Bioelechemistry 62 (2004) 11–18.
[6] P. Skládal, M. Mascini, Sensitive detection of pesticides using amperometric sensors based on cobalt phthalocyanine-modified composite electrodes and immobilized cholinesterases, Biosens. Bioelectron. 7 (1992) 335–343.
[7] F.N. Kok, F. Boxoglu, V. Hasirci, Construction of an acetylcholinesterase-choline oxidase biosensor for aldicarb determination, Biosens. Bioelectron. 17 (2002) 531–539.
[8] S. Sotiropoulou, D. Fournier, N.A. Chaniotakis, Genetically engineered acetylcholinesterase-based biosensor for attomolar detection of dichlorvos, Biosens. Bioelectron. 20 (2005) 2347–2352.
[9] Liu Guodong, Lin Yuehe, Biosensor based on self-assembling acetylcholinesterase on carbon nanotubes for flow injection/amperometric detection of organophosphate pesticides and nerve agents, Anal. Chem. 78 (2006) 835–843.
[10] A.N. Ivanov, L.V. Lukachova, G.A. Evtugyn, E.E. Karyakina, S.G. Kiseleva, H.C. Budnikov, A.V. Orlov, G.P. Karpacheva, A.A. Karyakin, Polyaniline-modified cholinesterase sensor for pesticide determination, Bioelectrochemistry 55 (2002) 75–77.
[11] Zhao Mingqi, RichardM. Crooks, Homogeneous hydrogenation catalysis with monodisperse, dendrimer— encapsulated Pd and Pt nanoparticles, Angew. Chem. Int. Ed. 38 (1999) 364–366.
[12] J. Schurek, T. Portolés, J. Hajslova, K. Riddellova, F. Hernández, Application of headspace solid-phase microextraction coupled to comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for the determination of multiple pesticide residues in tea samples, Anal. Chim. Acta 611 (2008) 163–172.
[13] AnnaYu. Kolosova, Park Jung-Hyun, SergeiA. Eremin, Park Seon-Ja, Kang Sung-Jo, Shim Won-Bo, Lee Hye-Sung, Lee Yong-Tae, Chung Duck-Hwa, Comparative study of three immunoassays based on monoclonal antibodies for detection of the pesticide parathion-methyl in real samples, Anal. Chim. Acta 511 (2004) 323–331.
[14] Wang Xiaohui, Du Yumin, Ding Sha, Wang Ququan, Xiong Guiguang, Xie Min, Shen Xincheng, Pang Daiwen, Preparation and third-order optical nonlinearity of self-assembled chitosan/CdSe–ZnS core-shell quantum dots multilayer films, J. Phys. Chem. B 110 (2006) 1566–1570.
[15] G. Decher, Fuzzy nanoassemblies: toward layered polymeric multicomposites, Science 277 (1997) 1232–1237.
[16] C.A. Constantine, K.M. Gattas-Asfura, S.V. Mello, G. Crespo, V. Rastoqi, T.C. Cheng, J.J. DeFrank, R.M. Leblanc, Layer-by-Layer biosensor assembly incorporating functionalized quantum dots, Langmuir 19 (2003) 9863.
[17] B. klajnert, L. Stanislawska, M. Bryzewska, B. palecz, Interactions between PAMAM dendrimers and bovine serum albumin, Biochim. Biophys. Acta 1648 (2003) 115.
[18] R.R. Moore, C.E. Banks, R.G. Compton, Electrocatalytic detection of thiols using an edge plane pyrolytic graphite electrode, Analyst 129 (2004) 755–758.
[19] J.-L. Marty, D. Garcia, R. Rouillon, Biosensors: potential in pesticide detection, Trends Anal. Chem. 14 (1995) 329.
[20] S. Kumaran, C. Tran-Minh, Insecticide determination with enzyme electrodes using different enzyme immobilization techniques, Electroanlysis 4 (1992) 949.
[21] Llopis Xavier, Ibáñez-García Nuria, Alegret Salvador, Alonso Julián, Pesticide determination by enzymatic inhibition and amperometric detection in a low-temperature cofired ceramics microsystem, Anal. Chem. 79 (2007) 3662–3666.
[22] Waseem Amir, Yaqoob Mohammad, Nabi Abdul, Flow-injection determination of carbaryl and carbofuran based on $KMnO_4$–Na_2SO_3 chemiluminescence detection, Luminescence 22 (2007) 349–354.