Available online at www.sciencedirect.com
ScienceDirect
Talanta 74 (2008) 766–772
Talanta
www.elsevier.com/locate/talanta

Electrochemical pesticide sensitivity test using acetylcholinesterase biosensor based on colloidal gold nanoparticle modified sol–gel interface

Dan Du [a,*], Shizhen Chen [a], Jie Cai [b], Aidong Zhang [a,*]

[a] *Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, PR China*
[b] *The Technology Center of Wuhan Iron & Steel Company, Wuhan 430080, PR China*

Received 17 March 2007; received in revised form 6 July 2007; accepted 9 July 2007
Available online 24 July 2007

Abstract

Based on the change in electrochemical behavior of enzymatic activity induced by pesticide, a novel electrochemical method for investigation of pesticide sensitivity using acetylcholinesterase (AChE) biosensor was developed. The sol–gel-derived silicate network assembling gold nanoparticles (AuNPs-SiSG) provided a biocompatible microenvironment around the enzyme molecule to stabilize its biological activity and prevented them from leaking out of the interface. The composite was characterized using atomic force microscopy and proved to be chemically clean, porous and homogeneous. AuNPs promoted a conductive pathway for electron transfer and improved electrochemical reactions at a lower potential. Typical pesticides such as monocrotophos, methyl parathion and carbaryl were selected for pesticide sensitivity tests. Due to the inhibitions of pesticides, the electrochemical responses of substrate on AChE-sensors decreased greatly. The inhibition curves showed good correspondence with the results by UV spectrophotometry assay. The proposed electrochemical pesticide sensitivity test exhibited high sensitivity, desirable accuracy, low cost and simplified procedures. This method could be developed as a conventional method to select efficient enzyme inhibitors and investigate toxic compounds against to enzyme.

Keywords: Acetylcholinesterase; Biosensor; Electrochemical test; Gold nanoparticles; Pesticide sensitivity

1. Introduction

Pesticides (herbicides, fungicides, insecticides) are widely used in agriculture and industry due to their high insecticidal activity [1,2]. Many of them are highly toxic and their accumulation in living organisms can be a cause of serious diseases [3,4]. With exploration of many new pesticides, considerable current interest in food safety is not only to determine the trace content but also to select high efficient and low environmental existent pesticides [5]. Thus there is an essential need to develop a method for simple pesticide sensitivity test to select the most effective one.

The toxic actions of pesticides are based on their abilities to irreversibly modify the catalytic serine residue in acetylcholinesterases (AChE); subsequent inhibition of the AChE effectively prevents nerve transmission by blocking breakdown of the transmitter choline [6]. Traditional methods such as UV spectrophotometry and colorimetry are usually used to determine enzymatic activity based on pesticide inhibition [7,8]. However, these methods include complicated pretreatment procedure, professional operators and expensive instruments, which limit their applications for on-site screening of these compounds. In order to simplify the test procedure, decrease the test cost and enhance the test sensitivity, a novel electrochemical method for pesticide sensitivity test based on AChE biosensor was proposed.

In recent years, the use of AChE and thiocholine in biosensor technology has gained enormous attention, in particular with respect to pesticide detection. It has proven possible to use a screen-printed biosensor for the detection of pesticides in water miscible organic solvents based on the use of *p*-aminophenyl acetate as AChE substrate [9]. Solna et al. reported that the screen-printed four-electrode system could be used as an

* Corresponding author. Tel.: +86 27 67867953.
E-mail address: dudan@mail.ccnu.edu.cn (D. Du).

0039-9140/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2007.07.014

amperometric biosensor for determination of phenols and pesticides using immobilized AChE [10]. Electropolymerization of phenylenediamine monomers has been reported to produce thin nonconducting permselective polymers for immobilization of AChE with rapid response times [11].

When AChE was immobilized on working electrode surface, its interaction with substrate of acetylthiocholine obtained an electro-active product of thiocholine, which produced an irreversible oxidation peak. The inhibition of pesticide on AChE was monitored by measuring the oxidation current of thiocholine. Due to the notable change in electrochemical signal of AChE, a simple method for determination of pesticides and comparison of pesticide sensitivity was established.

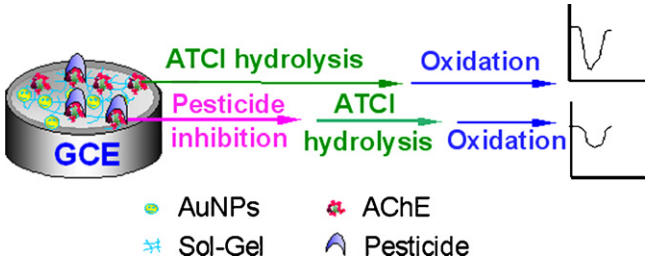
For fabrication of biosensor, immobilization of enzyme to solid electrode surface is a crucial step [12,13]. Sol–gel technology provides an attractive way for the immobilization of biological entitles including full cell, enzyme, protein and antibody or antigen due to the inert low-temperature process [14]. The class of sol–gel silicate matrix possesses attractive features including chemical inertness, physical rigidity, tenable porosity and thermal stability [15], which led to an intensive research in the fabrication of biosensors [16–18]. Recently, nanoparticles, particularly the gold nanoparticles (AuNPs) have received considerable attention in analytical electrochemistry since they have been appeared as highly conductive (fast electron transfer) nanomaterials and are of tremendous current interest [19]. Willner's group has extensively studied the electrochemical and optical applications of AuNPs and the ability to promote the electron transfer [20–22].

This work assembled AuNPs nanoparticles on a sol–gel-derived silicate network for immobilization of AChE, leading to a stable AChE biosensor for investigation of pesticide sensitivity (Scheme 1). Typical pesticides such as monocrotophos, methyl parathion and carbaryl were selected to verify this proposed method. The developed electrochemical method combining the advantages of electrochemical technique with AuNPs was sensitive and accurate for studying the sensitivity of different pesticide. The results obtained were in good agreement with those with UV method.

2. Materials and methods

2.1. Reagents

Acetylthiocholine chloride (ATCl), acetylcholinesterase (Type C3389, 500 U/mg from electric eel) was purchased from Sigma–Aldrich (St. Louis, USA) and used as received. Tetrathoxysilane (TEOS) was obtained from international laboratory (USA). Monocrotophos, carbaryl, methyl parathion and Gold (III) chloride hydrate (3.9 g/mL) were purchased from Treechem Co. (Shanghai, China). The solution of 17 nm-diameter AuNPs was prepared according to reference [23] and stored in a brown bottle at 4 °C. Chitosan (95% deacetylation), phosphate buffer solution (PBS, pH 7.0) and other reagents used were of analytical reagent grade. All the chemicals were of analytical grade and aqueous solutions were prepared with double distilled water.



Scheme 1. Principle of electrochemical pesticide sensitity test based on AChE biosensor.

2.2. Instruments

Electrochemical measurements were performed on a Bioanalytical System (BAS, cv-50w, USA) with a conventional three-electrode system comprising platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference and AChE combined to silica sol–gel (SiSG) assembling AuNPs composite modified glass carbon electrode (AChE–AuNPs-SiSG/GCE) as working electrode. The static water contact angle was measured at 25 °C by a contact angle system OCA 20 (Dataphysics, Germany).

UV spectra were recorded using a UV-2501 spectrophotometer (Shimadzu, Kyoto, Japan). Atomic force microscopy (AFM) experiments were performed on SPA-300 HV atomic force microscopy with a SPI 3800 controller (Seiko, Japan). It was carried out in liquid (PBS solution) using tapping mode. The SI-DF-3 cantilevers (Seiko Instruments Inc.) were used (spring constant was about 3 N/m, resonance frequency ranging from 90 to 150 kHz).

2.3. Preparation of silica sol–gel assembling AuNPs composite

A homogenous silica sol–gel assembling AuNPs composite was prepared by mixing 20 μL of TEOS, 60 μL of ethanol, 150 μL of AuNPs, 600 μL of 0.5 mg/mL chitosan solutions (final concentration 0.3%). This mixture was stirred for 1 h until a clear sol–gel composite was formed and its pH was adjusted to 4.0–6.0 using 170 μL 0.1 M NaOH solution. The obtained sol–gel composite can be stored for several months at 4 °C.

2.4. Preparation of the biosensor

GCE was polished to mirror finish using the BAS-polishing kit with 0.3 and 0.05 μm Al_2O_3 paste. After sonicated with ethanol and water, the electrode was applied a potential of +1.75 V under string in pH 5.0 PBS for 300 s and then the electrode was scanned from +0.3 to +1.25 V and +0.3 to −1.3 V until a steady-state current–voltage curve was obtained [24]. After this treatment, a homogeneous aqueous film can be observed on the activated electrode surface [25]. The electrode then allowed the conjugation of the SiSG film with high stability.

Three microliters of the above AuNPs-SiSG composite was coated on a pretreated GCE and allowed for reaction at 20 °C for 4 h. The obtained electrode was then coated with 4.0 μL AChE solutions, which was incubated at 25 °C for 30 min. After evaporation of water, the modified electrode was washed with PBS to remove the unbound AChE. The obtained AChE–AuNPs-SiSG/GCE was stored at 4 °C when not in use.

2.5. Measurement procedure

For measurements of pesticides, the obtained AChE–AuNPs-SiSG/GCE was first immersed in the PBS solution containing different concentrations of standard pesticides for 10 min, and then transferred to the electrochemical cell of 1.0 mL pH 7.0 PBS containing 1.0 mM ATCl to study the electrochemical response by cyclic voltammetry (CV). The inhibition of pesticide was calculated as follows:

$$\text{Inhibition } (\%) = \frac{i_{\text{P,control}} - i_{\text{P,exp}}}{i_{\text{P,control}}} \times 100$$

where $i_{\text{P,control}}$ is the peak current of ATCl on the AChE–AuNPs-SiSG/GCE and $i_{\text{P,exp}}$ is the peak current of ATCl on the AChE–AuNPs-SiSG/GCE with pesticide inhibition.

3. Results and discussion

3.1. Surface hydrophilicity

The hydrophilicity of the electrode surface after pretreatment was characterized by measuring the contact angles of untreated GCE and treated GCE. Their contact angles were 64.6° and 20.8°, respectively (Fig. 1). The treated GCE showed lower contact angle compared with the untreated electrode, indicating the better hydrophilic surface for film adhesion. According to the results of Kepley and Bard [26], the activation of GCE involved the formation of a new phase, which contained a significant amount of microcrystallinity and graphite oxide, thus increased the surface hydrophilicity [27].

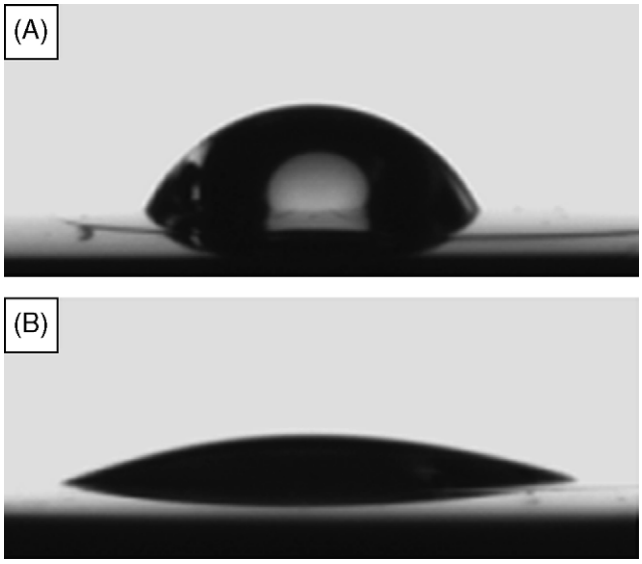


Fig. 1. Contact angle graphs: (A) untreated GCE and (B) treated GCE.

3.2. AFM characterization

AFM measurement is an effective method to provide surface topography and phase images. As seen from Fig. 2, the AuNPs-SiSG composite displayed a porous and netlike structure with uniform distribution (Fig. 2A). This structure provided a significant increase of effective electrode surface for immobilization of biomolecules. After AChE was immobilized on the AuNPs-SiSG composite, some bright particles of AChE were observed clearly (Fig. 2B). The AuNP embedded in SiSG film provided an environment similar to that of biomolecules in native system and efficiently prevented the leakage from electrode.

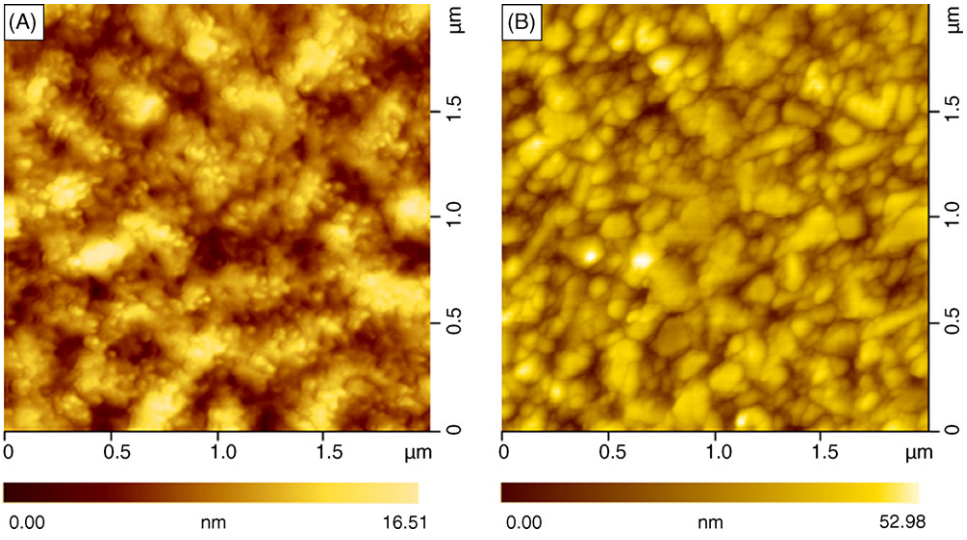


Fig. 2. AFM images: (A) AuNPs-SiSG surface and (B) AChE–AuNPs-SiSG surface.

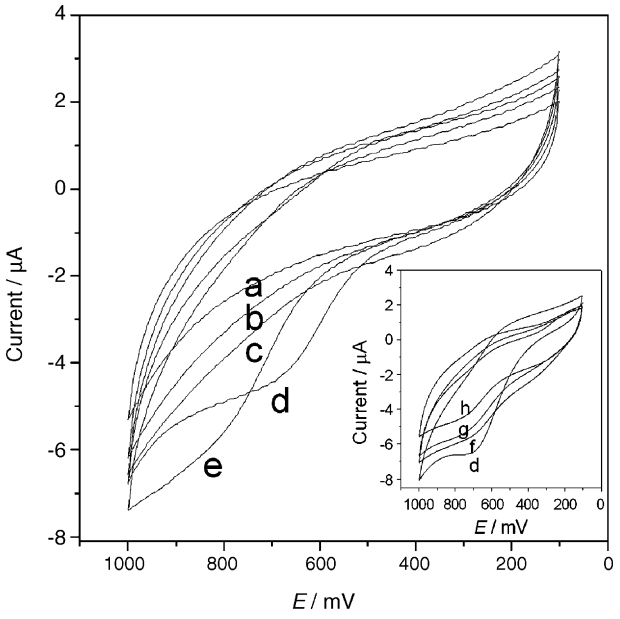


Fig. 3. Cyclic voltammograms: (a) GCE; (b) AChE–AuNPs-SiSG/GCE in pH 7.0 PBS; (c) AuNPs–SiSG/GCE; (d) AChE–AuNPs-SiSG/GC; (e) AChE–SiSG/GCE in pH 7.0 PBS containing 1.0 mM ATCl. (Inset) Cyclic voltammograms of AChE–AuNPs-SiSG/GCE in pH 7.0 PBS containing 1.0 mM ATCl after inhibition with 10 μg/ml pesticides for 10 min, (f) methyl parathion, (g) carbaryl and (h) monocrotophos.

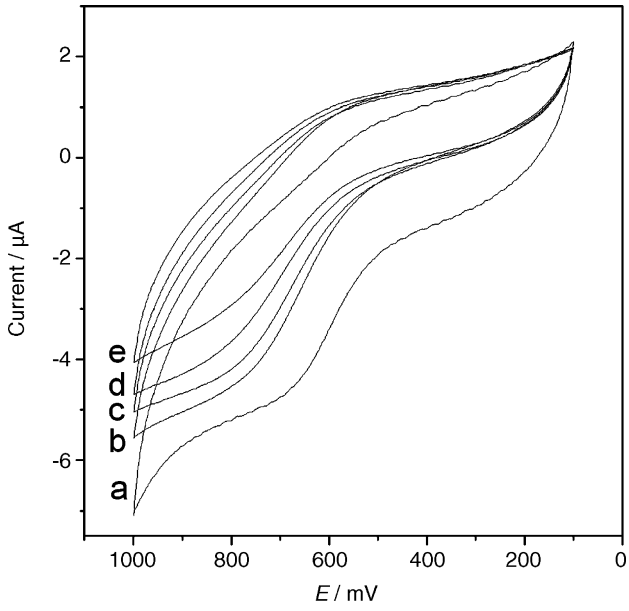


Fig. 4. Cyclic voltammograms of AChE–AuNPs-SiSG/GCE in pH 7.0 PBS containing 1.0 mM ATCl after inhibition with monocrotophos of different concentrations for 10 min: (a) 0 μg/mL, (b) 1 μg/mL, (c) 5 μg/mL, (d) 10 μg/mL and (e) 15 μg/mL.

3.3. Cyclic voltammetric behavior of AChE–AuNPs-SiSG/GCE

Fig. 3 showed the cyclic voltammograms of different electrodes in absence and presence of ATCl in pH 7.0 PBS. No peak was observed at GCE (curve a) and AChE–AuNPs-SiSG/GCE (curve b) in pH 7.0 PBS. When 1.0 mM ATCl was added into PBS, the cyclic voltammograms of AChE–AuNPs-SiSG/GCE showed an irreversible oxidation peak at 689 mV (curve d), while no detectable signal was observed at AuNPs-SiSG/GCE without immobilization of AChE (curve c). Obviously this peak came from the oxidation of thiocholine, hydrolysis product of ATCl, catalyzed by immobilized AChE. The produced current by thiocholine was used as a quantitative measurement of the enzymatic activity, which reflected the biological effect of pesticide involved in the inhibition action. Furthermore, this peak current was much higher and the peak potential shifted negatively compared to that on the silica sol–gel modified electrode without AuNPs (AChE-SiSG/GCE) (curves e and d). This phenomena was due to the presence of AuNPs in the sol–gel film, which possessed inherent conductive properties and catalytic behavior, thus AuNPs-SiSG provided a conductive pathway to electron transfer and promote electron transfer reactions at a lower potential. If AuNPs were directly coated on the electrode surface instead of entrapment in cross-linked sol–gel, much higher background current was observed. Thus AuNPs-SiSG showed excellent conductivity and then AChE–AuNPs-SiSG/GCE was used in the following experiments. Furthermore, the effect of AuNPs content between 0 and 30% (V_{Au}/V) on response was studied in our previous work. The anodic current gradually increased with increasing amount of AuNPs and reached a maximum at 15% (V_{Au}/V) (Fig. S1). Further increase of the AuNPs led to the decrease of peak current. This phenomenon was possibly attributed to the increase of the resistance and double layer capacitance of the modified electrode. So 15% (V_{Au}/V) AuNPs was used for preparation of the biosensor.

As shown from inset of Fig. 3, the produced currents of 1.0 mM ATCl on AChE–AuNPs-SiSG/GCE decreased drastically (curve f: methyl parathion; curve g: carbaryl; curve h: monocrotophos) comparing with the control (curve d) after AChE–AuNPs-SiSG/GCE was immersed in standard solutions of three pesticides for 10 min, respectively. This was because pesticides exhibited fairly high acute toxicity and involved in the irreversible inhibition action to AChE, thus reduced the activity of enzyme to its substrate. However, their inhibition rates were related to their interaction between pesticide and enzyme. Due to the notable change in voltammetric signal of the AChE–AuNPs-SiSG/GCE, the simple electrochemical method for determination of pesticides and investigation of pesticide sensitivity was established.

3.4. Effect of monocrotophos on activity of immobilized AChE

Since the substrate concentration can affect the degree of inhibition, the absolute quantity of ATCl was first optimized. With the increasing concentration of ATCl the amperometric response increased linearly in the range of 10.0–1000 μM and then tended to a plateau value (Fig. S2), showing a typical Michaelis–Menten process. Thus 0.1 mM ATCl was selected for obtaining the maximum response.

Pure biological reagent monocrotophos was firstly selected to evaluate the effect on activity of immobilized AChE. As shown in Fig. 4, upon AChE–AuNPs-SiSG/GCE was immersed

in the standard solution of monocrotophos at a known concentration for 10 min, a notable change in voltammetric signal on AChE–AuNPs-SiSG/GCE was observed. The presence of monocrotophos resulted in a decrease of the peak current, indicating a decrease of the activity of immobilized AChE. This was because monocrotophos as one of the organophosphate pesticide exhibited fairly high acute toxicity and involved in the irreversible inhibition action on AChE, thus reduced enzymatic activity to its substrate. At high concentrations, the inhibition of monocrotophos trended to a maximum value, indicating the binding interaction with active target groups in enzyme could reach saturation, at which the maximum inhibition occurred.

3.5. Electrochemical pesticide sensitivity test

Based on the results obtained above, this work further explored pesticide sensitivity to AChE. Three pesticides, monocrotophos, carbaryl and methyl parathion, were used for this purpose. The treatment by pesticides resulted in the change in cyclic voltammetric response of ATCl at AChE–AuNPs-SiSG/GCE. Fig. 5 showed the inhibition curves of these three pesticides.

With an increase of immersing time in different pesticide solution, the peak currents of ATCl on AChE–AuNPs-SiSG/GCE decreased greatly. Although they played increased inhibition to AChE with increased time, the increase rate of monocrotophos (curve a) was much faster than those of carbaryl and methyl parathion (curves b and c). For monocrotophos, when the immersing time was longer than 10 min the peak current of ATCl on AChE–AuNPs-SiSG/GCE trended to a stable value, indicating the binding interaction with active target groups in enzyme reached saturation. However the inhibitions of carbaryl or methyl parathion trended to stable after 12 min and 18 min, respectively. Although the maximum values of inhibitions of three pesticides were not 100%, which was likely to attribute to the binding equilibrium between pesticide and binding sites in enzyme, the maximum inhibition of monocrotophos was about 90%. This value was much higher than those of carbaryl (~80%) and methyl parathion (~70%). The inhibition of these three pesticides was in the order of monocrotophos > carbaryl > methyl parathion. This conclusion is in agreement with model prediction [28] based on the reported V_{max}/K_m values for these compounds [29]. The inhibition rate was related to their interaction between pesticide and enzyme. Consequently, the pesticides changed the electrochemical response of substrate, from which a novel method for pesticide sensitivity test by voltammetric measurements could be evaluated.

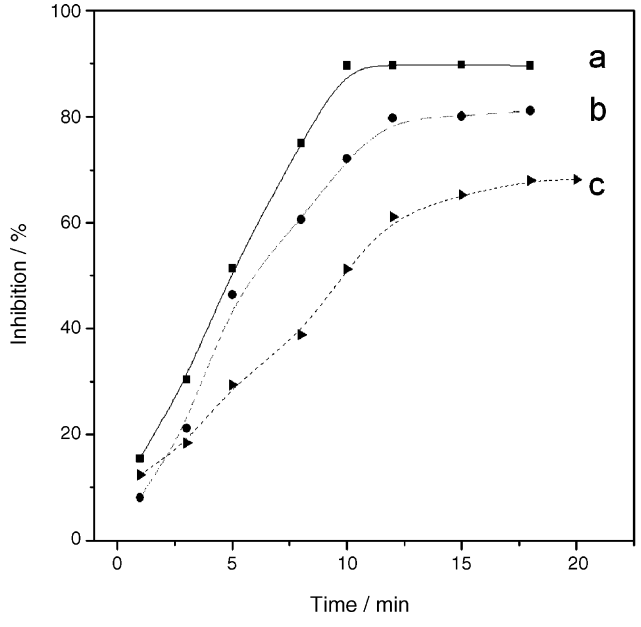


Fig. 5. Dependence of inhibition curves of three pesticides on inhibition time: (a) monocrotophos, (b) carbaryl and (c) methyl parathion.

3.6. Comparison of proposed electrochemical pesticide sensitivity with UV assay

As shown in Fig. 6, the proposed electrochemical pesticide sensitivity test and UV assay gave increasing inhibitions of these three pesticides with the increasing concentrations. These curves trended to the maximum values at high pesticide concentrations, indicating the binding interaction with active target groups in tumor cells could reach saturation when the pesticide concentration increased, at which the maximum incubation occurred. These curves were similar to the shape of Michaelis–Menten relationship. Obviously, the maximum values of inhibition of these three pesticides obtained with two assay methods were in good accordance. The slight difference might be attributed to the different conditions and assay procedures. Thus, the proposed electrochemical method was credible for pesticide sensitivity test.

3.7. Precision, reproducibility and stability of AChE–AuNPs-SiSG/GCE

The inter-assay precision was estimated by determining the response of 1.0 mM ATCl at six different electrodes, which were immersed in 0.1 μg/mL and 5.0 μg/mL monocrotophos for 10 min, respectively. The coefficients of variation were calculated to be 2.3% and 1.7%, respectively, indicating acceptable fabrication reproducibility. The intra-assay precision of the sensors was evaluated by assaying one enzyme electrode for six replicate determinations, and the R.S.D. was 0.8% at the ATCl concentration of 1.0 mM.

After AChE–AuNPs-SiSG/GCE was stored in a refrigerator at 4 °C for 3 weeks, no obvious decrease of ATCl was observed. After a 30-day storage period, the sensor retained 90% of its initial current response. This indicated that silica sol–gel provided a biocompatible microenvironment around the enzyme to stabilize its biological activity to a large extent. The large quantities of hydroxyl groups in the sol–gel hybrid material were able to form strong hydrogen bonds. These hydrogen bonds and the intermolecular interactions between enzyme molecule and specific sites of the silica sol–gel prevented the immobilized enzyme from leaking out of the film.

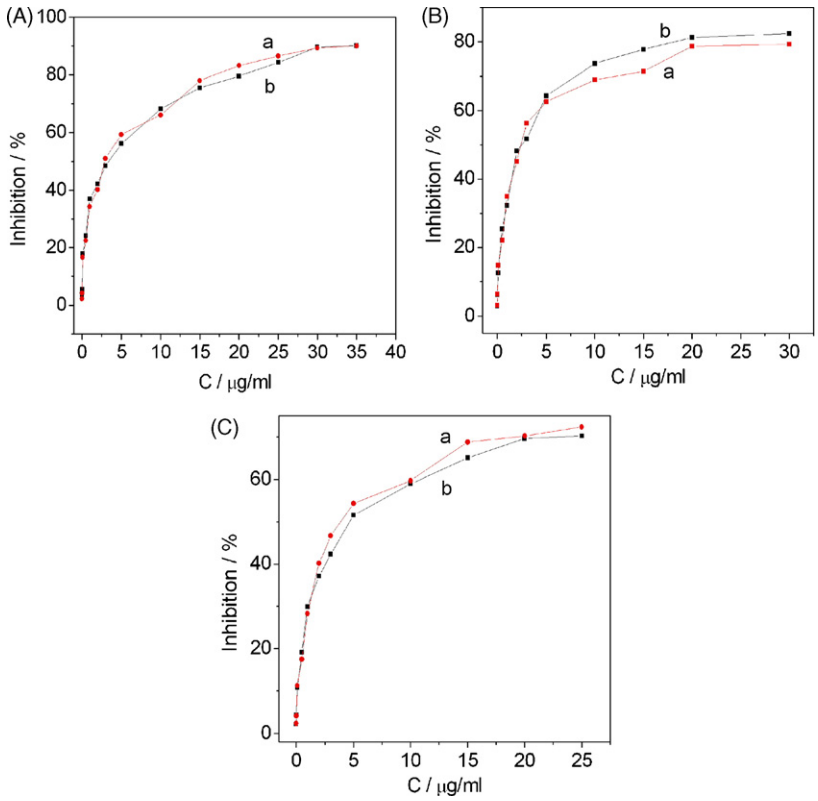


Fig. 6. (A) Inhibition curves of monocrotophos with different methods: (a) proposed electrochemical method and (b) UV assay. (B) Inhibition curves of carbaryl with different methods: (a) proposed electrochemical method and (b) UV assay. (C) Inhibition curves of methyl parathion with different methods: (a) proposed electrochemical method and (b) UV assay.

4. Conclusions

This work proposed an electrochemical pesticide sensitivity test combining with nanoparticles. The large quantities of hydroxyl groups in the sol–gel hybrid material formed strong hydrogen bonds, which interacted with the immobilized enzyme and prevented them from leaking out of the film. The assembled AuNPs nanoparticles on a sol–gel-derived silicate network exhibits superior character of conductibility and favors the interface enzymatic hydrolysis reaction to form electroactive substance, amplificating the sensitivity and amperometric response of the biosensor. The proposed method shows good accuracy with other conventional assays, which has potential application in the selection of enzyme inhibitors and investigation of toxic compounds against to enzyme. To sum up, the proposed electrochemical approach for pesticide sensitivity test with AChE biosensor is a sensitive, accurate and low-cost technique. We are currently facing the challenges of semi-automated procedures, which could be achieved through further improvement in miniaturization of electrochemical system.

Acknowledgements

The authors are gratefully acknowledging the financial support of the Natural Science Foundation of Hubei Province (No. 2006ABA183) and the National Natural Science Foundation of China (No. 20672043).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2007.07.014.

References

[1] F. Arduini, F. Ricci, C.S. Tuta, D. Moscone, A. Amine, G. Palleschi, Anal. Chim. Acta 580 (2006) 155–162.
[2] B.X. Li, Y.Z. He, C.L. Xu, Talanta 72 (2007) 223–230.
[3] J.R. Garbin, D.M.B.P. Milori, M.L. Simoes, W.T.L. da Silva, L.M. Neto, Chemosphere 66 (2007) 1692–1698.
[4] M. Khanmohammadia, M.A. Karimi, K. Ghasemi, M. Jabbari, A.B. Garmarudi, Talanta 72 (2007) 620–625.
[5] K. Fytianos, N. Raikos, G. Theodoridis, Z. Velinova, H. Tsoukali, Chemosphere 65 (2006) 2090–2095.
[6] M.H. Shi, J.J. Xu, S. Zhang, B.H. Liu, J.L. Kong, Talanta 68 (2006) 1089–1095.
[7] S.S. Soares, H. Martins, R.O. Duarte, J.J.G. Moura, J. Coucelo, C. Gutierrez-Merino, M. Aureliano, J. Inorg. Biochem. 101 (2007) 80–88.
[8] W. Kielan, J. Suzanowicz, M. Siewinski, Y. Saleh, A. Janocha, A. Skalski, R. Tarnawa, Gastric Cancer 7 (2004) 17–23.
[9] S. Andreescu, T. Nogue, V. Magearu, J.L. Marty, Talanta 57 (2002) 169–176.
[10] R. Solna, S. Sapelnikova, P. Skladal, M. Winther-Nielsen, C. Carlsson, J. Emneus, T. Ruzgas, Talanta 65 (2005) 349–357.
[11] H. Yang, T.D. Chung, Y.T. Kim, C.A. Choi, C.H. Jun, H.C. Kim, Biosens. Bioelectron. 17 (2002) 251–259.
[12] E. Casero, M. Darder, F. Pariente, E. Lorenzo, J. Martin-Benito, L. Vazquez, Nano Lett. 2 (2006) 577–582.
[13] C.A. Marquette, M. Cretich, L.J. Blum, M. Chiari, Talanta 71 (2007) 1312–1318.

[14] D. Du, F. Yan, S.L. Liu, H.X. Ju, J. Immunol. Methods 283 (2003) 67–75.
[15] Y. Wang, Z. Tang, M.A. Correa-Duarte, L.M. Liz-Marzan, N.A. Kotov, J. Am. Chem. Soc. 125 (2003) 2830–2831.
[16] Y.Z. Xian, F. Liu, L.F. Feng, F.H. Wu, L.W. Wang, L.T. Jin, Electrochem. Commun. 9 (2007) 773–780.
[17] B.K. Jena, C.R. Raj, Anal. Chem. 78 (2006) 6332–6339.
[18] G.H. Wang, L.M. Zhang, J. Phys. Chem. B 110 (2006) 24864–24868.
[19] M.C. Daniel, D. Astruc, Chem. Rev. 104 (2004) 293–346.
[20] I. Willner, V. Pavlov, Y. Xiao, Nano Lett. 5 (2005) 649–653.
[21] I. Willner, R. Baron, B. Willner, Adv. Mater. 18 (2006) 1109–1120.
[22] Y. Xiao, V. Pavlov, B. Shlyahovsky, I. Willner, Chem. Eur. J. 11 (2005) 2698–2704.
[23] Y. Xiao, H.X. Ju, H.Y. Chen, Anal. Chim. Acta 391 (1999) 73–82.
[24] D. Du, H.X. Ju, X.J. Zhang, J. Chen, J. Cai, H.Y. Chen, Biochemistry 44 (2005) 11539–11545.
[25] D. Du, X. Huang, J. Cai, A.D. Zhang, J.W. Ding, S.Z. Chen, Anal. Bioanal. Chem. 387 (2007) 1059–1065.
[26] L.J. Kepley, A.J. Bard, Anal. Chem. 60 (1988) 1459–1467.
[27] Z. Dai, F. Yan, J. Chen, H.X. Ju, Anal. Chem. 75 (2003) 5429–5434.
[28] M.J. Eddowes, Theoretical methods for analysing biosensor performance, in: A.E.G. Cass (Ed.), Biosensors: A Practical Approach, IRL Press, Oxford University Press, Oxford, 1990, pp. 211–263.
[29] J.R. Mason, F. Briganti, J.R. Wild, in: J.R. Wild, S.D. Varfolomeyev, A. Scozzafava (Eds.), Perspectives in Bioremediation: Technologies for Environmental Improvement, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1997, pp. 107–118.