Enzyme and Microbial Technology 58–59 (2014) 8–13

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

# An acetylcholinesterase biosensor based on graphene–gold nanocomposite and calcined layered double hydroxide

Chen Zhai [a], Yemin Guo [a], Xia Sun [a,*], Yuhe Zheng [b], Xiangyou Wang [a]

[a] School of Agriculture and Food Engineering, Shandong University of Technology, No. 12, Zhangzhou Road, Zibo 255049, Shandong Province, PR China
[b] Shandong Water Conservancy Technician Institute, No. 169, Shuiyuan Road, Zibo 255130, Shandong Province, PR China

ARTICLE INFO

*Article history:*
Received 8 July 2013
Received in revised form 6 February 2014
Accepted 8 February 2014
Available online 17 February 2014

*Keywords:*
Acetylcholinesterase biosensor
Cu–Mg–Al calcined layered double hydroxide
Graphene–gold nanocomposite
Pesticides

ABSTRACT

In this study, a novel acetylcholinesterase-based biosensor was fabricated. Acetylcholinesterase (AChE) was immobilized onto a glassy carbon electrode (GCE) with the aid of Cu–Mg–Al calcined layered double hydroxide (CLDH). CLDH can provide a bigger effective surface area for AChE loading, which could improve the precision and stability of AChE biosensor. However, the poor electroconductibility of CLDHs could lead to the low sensitivity of AChE biosensor. In order to effectively compensate the disadvantages of CLDHs, graphene–gold nanocomposites were used for improving the electron transfer rate. Thus, the graphene–gold nanocomposite (GN-AuNPs) was firstly modified onto the GCE, and then the prepared CLDH-AChE composite was immobilized onto the modified GCE to construct a sensitive AChE biosensor for pesticides detection. Relevant parameters were studied in detail and optimized, including the pH of the acetylthiocholine chloride (ATCl) solution, the amount of AChE immobilized on the biosensor and the inhibition time governing the analytical performance of the biosensor. The biosensor detected chlorpyrifos at concentrations ranging from 0.05 to 150 μg/L. The detection limit for chlorpyrifos was 0.05 μg/L.



## 1. Introduction

Pesticide residues in food, livestock and water pose severe threat to human health [1]. Therefore, rapid determination and reliable quantification of pesticide compounds have become increasingly important for public security and health protection [2,3]. Biosensor is an analytical device incorporating a biological material with a suitable transducer that converts the biochemical signal into quantifiable electric signals [4]. Enzyme-based amperometric biosensors are important tools to detect pesticides in healthcare measure, food industry and environmental analysis [5,6]. These devices are designed to complement or replace the existing reference analytical methods such as gas/liquid chromatographic and mass spectrometric by simplifying or eliminating sample preparation, thus decreasing the analysis time and cost [7].

Metal nanoparticles are considered to be one kind of attractive nanomaterials due to their extraordinary advantages which include stability, conductivity, biocompatibility, low cytotoxicity and catalytic property [8,9]. With the rapid development of nanotechnology, various nanomaterials have been synthesized, which open new way to amplify the signal of biosensor [10]. Graphene nanosheets (GNs), a perfect two-dimensional (2D) carbon nanophase material found in 2004, have attracted tremendous attention [11,12], due to their exceptional thermal and mechanical properties, good chemical stability, high surface areas (calculated value, 2630 $m^2/g$), and excellent electrical conductivity [13,14]. Wang et al. have reported a biosensor based on acetylcholinesterase (AChE) immobilized on CdS-decorated graphene nanocomposite [15]. Li et al. have reported a sensitive amperometric biosensor through immobilizing AChE on porous-reduced graphene oxide [16]. Here, we prepared a very stable graphene–gold nanocomposite as modification material to fabricate an amperometric biosensor for pesticides detection.

Searching for a simple and reliable scheme to immobilize enzyme is of great importance. Layered double hydroxides (LDHs), also known as the anionic clays or hydrotalcite clays, can be expressed with the following general formula: $[M^{II}_{1-x}M^{III}_{x}(OH)_2] \times [A^{n-}_{x/n} \times mH_2O]$ [17]. Where, $M^{II}_{1-x}$ are divalent cations ($Mg^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$); $M^{III}_{x}$ are trivalent cations ($Al^{3+}$, $Cr^{3+}$, $Fe^{3+}$), and $A^{n-}$ is an interlayer anion ($Cl^-$, $NO_3^-$, $CO_3^{2-}$, $SO_4^{2-}$) compensating for the charge on the layers [18,19]. LDHs are an important class of host-guest materials consisting of positively charged metal

* Corresponding author. Tel.: +86 533 2786367.
*E-mail address:* sunxia2151@sina.com (X. Sun).

http://dx.doi.org/10.1016/j.enzmictec.2014.02.004
0141-0229/

hydroxide sheets with charge-balancing intercalated anions and water molecules [20,21]. By heating LDHs above 400 °C, the interlayer $CO_3^{2-}$ can be removed. Therefore, the resulting calcination products of LDHs (CLDHs) can be used for removing inorganic anions by adsorption on the external surface of the layers, intercalation process or reconstruction behavior [22]. Nowadays, CLDHs have been paid more attention owing to their larger surface areas, higher metal dispersion, smaller crystallite size, better stability against sintering, higher thermal stability, better dispersion of the active species, and less diffusion resistance than LDHs [23]. In previous works, LDHs have been demonstrated as attractive enzyme immobilization matrix [24,25]. To date, however, the application of CLDHs in AChE biosensors has been still very less reported.

However, the electroconductibility of CLDHs is not very good, which can lead to the low sensitivity of enzyme biosensor. The graphene–gold nanocomposites which have excellent conductivity and biocompatibility, could effectively compensate the disadvantages of CLDHs, and then improve the performance of the biosensor.

In this study, we described the application of CLDHs as enzyme immobilization matrix to construct highly performance-enhanced AChE biosensor. CLDH can result an increase of effective surface area for AChE loading, which could improve the precision and stability of the AChE biosensor. Graphene–gold nanocomposite was dropped on the surface of the glassy carbon electrode, which obviously improved the conductivity of the modified electrode. Compared with other kinds of electrochemical AChE biosensors, it was much better in sensitivity, reproducibility and stability for the determination of pesticide, and it could be applied in real samples measurement.

## 2. Experimental

### 2.1. Apparatus

Electrochemical measurements were performed with CHI660D electrochemical workstation (Shanghai Chenhua Co., China). The working electrode was glassy carbon electrode (GCE) ($d$ = 3 mm) or modified GCE. A saturated calomel electrode (SCE) and platinum wire electrode were used as reference electrode and auxiliary electrodes, respectively. Scanning electron micrographs (SEM) was studied by Sirion 200 SEM.

### 2.2. Reagents and materials

Acetylcholinesterase (Type C3389, 500 U/mg from electric eel), acetylthiocholine chloride (ATCl), chlorpyrifos were purchased from Sigma (USA). $HAuCl_4$ was obtained from National Chemical Pharmaceutical Co., China. Graphene was obtained from nanoon Co., China. The 0.1 M pH 7.5 phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of $NaH_2PO_4$ and $Na_2HPO_4$. Cu $(NO_3)_2{\cdot}3H_2O$, Mg $(NO_3)_2{\cdot}6H_2O$, Al $(NO_3)_3{\cdot}9H_2O$ and other reagents were of analytical grade. All solutions were prepared using double distilled water.

### 2.3. Synthesis graphene–gold nanocomposites

2.0 mg graphene was added into 105 μL of 0.01 M $HAuCl_4{\cdot}4H_2O$ by sonicating until GN disperse equably. Then 105 μL of 0.01 M sodium citrate, 10.0 mL ethanol and 20.0 mL deionized water were added to the above suspension in sequence. 125 μL of 0.05 M $NaBH_4$ ice-cold solution were added to the above mixture and stirred until the color of the solution did not change. After stirred for an additional 10 h, the suspending liquid was separated by centrifuging at a speed of 16,000 rpm, washed with deionized water for several cycles. Finally, the precipitates were redispersed in 5 mL pH 7.5 PBS, and then stored in a brown bottle at 4 °C for use [26,27].

### 2.4. Preparation of Cu–Mg–Al CLDH

The preparing method for Cu–Mg–Al LDH was similar to what reported in literature [23,28]. In brief, 20 mL solution containing 1.208 g of $Cu(NO_3)_2{\cdot}3H_2O$, 3.846 g $Mg(NO_3)_2{\cdot}6H_2O$ and 3.751 g $Al(NO_3)_3{\cdot}9H_2O$ was titrated with 20 mL mixture solution of 2.40 g NaOH and 5.30 g $Na_2CO_3$ under vigorous stirring. During the synthesis, the temperature was maintained at 25 °C. The resulting suspension was then maintained at 65 °C for 1 h with stirring. The obtained product was filtered and washed thoroughly with deionized water until a neutral pH was observed, then dried at 60 °C for 2 days in air. Thus, the Cu–Mg–Al LDH was obtained. The Cu–Mg–Al CLDH was prepared by heating Cu–Mg–Al LDH in a muffle furnace at 500 °C for 7 h.

### 2.5. Preparation of CLDH-AChE composite

The colloidal suspension of CLDH (2 mg/mL) was prepared by dispersing CLDH in deionized water stirring overnight. Then, a stock solution of AChE was mixed with the colloidal solution of LDHs (2 mg/mL) with volume ratio 1:1 to obtain the suspension of CLDH-AChE. The resulting suspension was stored at 4 °C for use.

### 2.6. Preparation of CLDH-AChE/GN-AuNPs/GCE biosensor

A GCE was polished carefully to a mirrorlike surface with 0.3 μm and 0.05 μm $Al_2O_3$ paste and washed using sonication with ethanol, nitric acid and doubly distilled water. Before the modification of the electrode, a potential scan was applied from −0.6 to 1.0 V in 0.5 mol/L $H_2SO_4$ for 300 s until a steady-state curve was obtained. 5 μL GN-AuNPs solution was coated onto the pretreated GCE and dried in the air. The obtained GN-AuNPs/GCE was washed thoroughly with double distilled water. Then a 5.0 μL CLDH-AChE solution (100 mU) was dropped onto the GCE and dried in air at room temperature. After washing carefully with pH 7.5 phosphate buffer solutions, the CLDH-AChE/GN-AuNPs/GCE was obtained. The CLDH-AChE/GN-AuNPs/GCE was stored at 4 °C when not in use. The scheme of the preparation of CLDH-AChE/GN-AuNPs/GCE biosensor was shown in Fig. 1.

### 2.7. Electrochemical detection of pesticides

The CLDH-AChE/GN-AuNPs/GCE biosensor was employed for the determination of pesticide by differential pulse voltammetry (DPV) method. The performance of the biosensor was investigated by its DPV response in pH 7.5 PBS solution containing 1.0 mM ATCl. Then the electrode was rinsed with water and incubated in an aqueous solution containing a certain concentration of chlorpyrifos for 10 min. Finally, it was transferred into the 1.0 mM ATCl solution for DPV measurements at the same condition. The inhibition rate of pesticides was calculated as follows:

$$\text{inhibition } (\%) = \frac{\left(I_{\text{P,control}} - I_{\text{P,exp}}\right)}{I_{\text{P,control}}} \times 100\% \quad (1)$$

where, $I_{\text{P,control}}$ was the peak current of ATCl on CLDH-AChE/GN-AuNPs/GCE with pesticides inhibition, $I_{\text{P,exp}}$ was the peak current of ATCl on CLDH-AChE/GN-AuNPs/GCE with pesticides inhibition. Inhibition (%) was plotted against the concentrations of the pesticides to obtain linear calibration graphs.

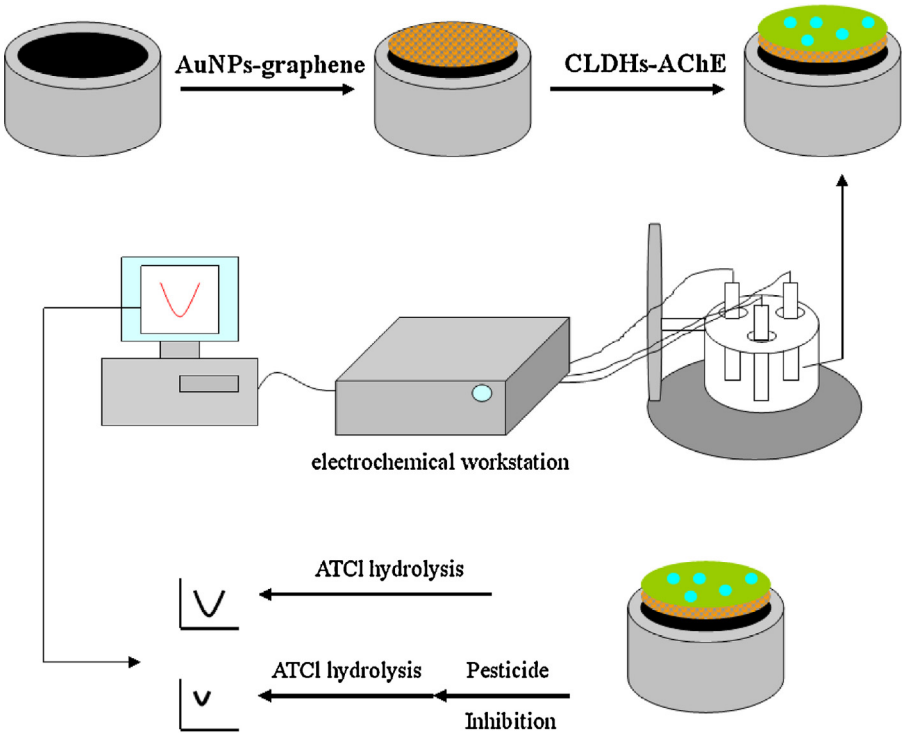


**Fig. 1.** The procedure of preparation of CLDH-AChE/GN-AuNPs/GCE.

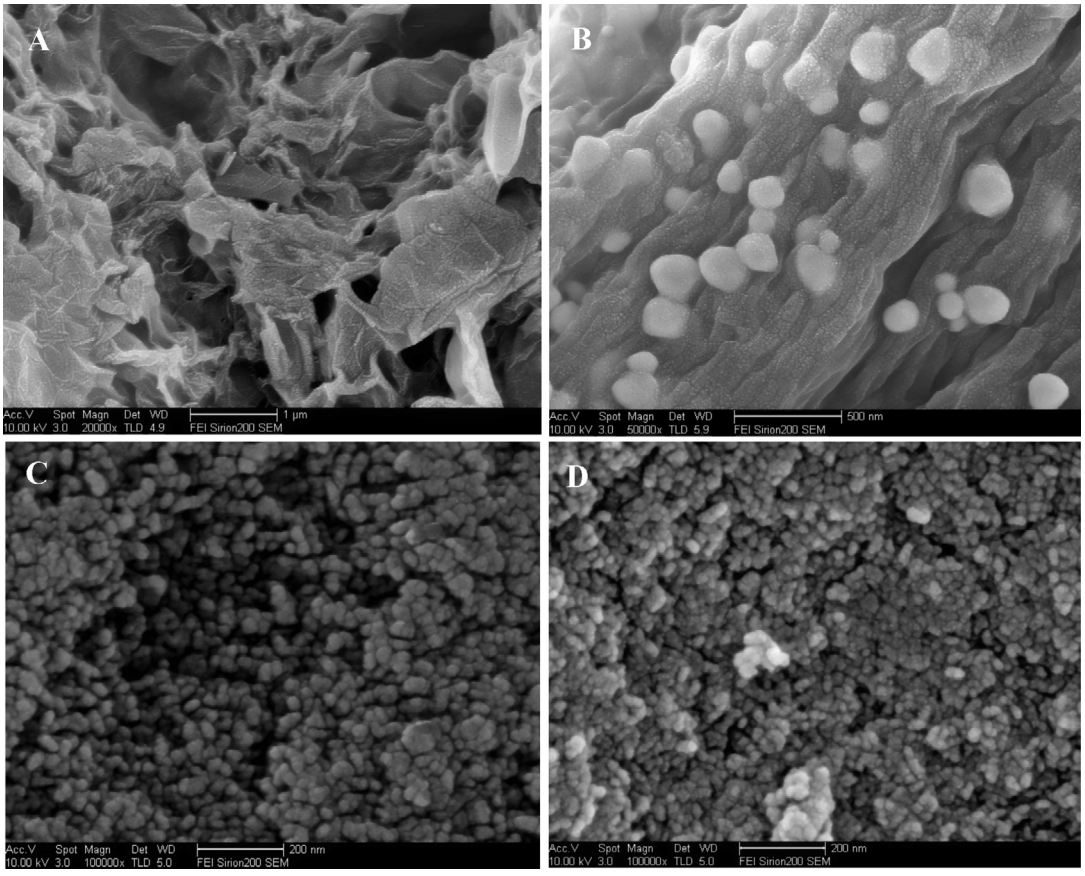


**Fig. 2.** SEM images of graphene (A); graphene–gold nanocomposite (B); LDH nanoparticles (C); CLDH nanoparticles (D).

### 2.8. Preparation and determination of real samples

The real samples were prepared according to the procedure described by Qu et al. [29]. The leek and pakchoi bought from a local supermarket were washed three times with double-distilled water and chopped. And then, 5 g of each sample was sprayed with different concentrations of chlorpyrifos. After 24 h of storage at 4 °C, each sample was mixed with the 10 mL mixed solution of 0.1 M pH 7.5 PBS, which was obtained through 15 min of ultrasonic treatment. After the suspensions were centrifuged (10 min, 10,000 rpm), the acquired supernatants were detected by DPV directly without extraction or preconcentration. The concentration of pesticides in the samples can be obtained from the calibration curve.

## 3. Results and discussion

### 3.1. SEM characterizations of graphene–gold nanocomposite and CLDH nanoparticles

In order to get an intuitionistic understanding about the conformation of graphene and graphene–gold nanocomposites, scanning electron microscopy (SEM) experiments were performed (Fig. 2A and B). Fig. 2A showed a typical wrinkled sheet texture of graphene. In Fig. 2B, it was clearly seen that Au particles were dispersed uniformly on the wrinkled graphene sheets, indicating that graphene–gold nanocomposite was successfully synthesized. Typical SEM image of the Cu–Mg–Al LDH and CLDH was displayed in Fig. 2C and D, respectively. As shown in Fig. 2C, there were a lot of uniform LDHs nanoparticles of 30–50 nm in average diameter. Compared with Fig. 2C, the diameter of CLDH nanoparticles were about 10–30 nm (Fig. 2D). Cu–Mg–Al CLDH film possesses larger specific surface area, higher surface reaction activity and more efficient transmission channel for the analyzed molecules to reach the active sites, which can help to improve the stability and sensitivity of biosensor [23].

### 3.2. Electrochemical reactivity of different electrodes GN-AuNPs/CLDH-AChE/GCE

EIS is a powerful tool for studying surface processes of electrode. In a typical Nyquist plot, the semicircle diameter at higher frequency range corresponds to the charge-transfer resistance ($R_{ct}$), and a linear part at lower frequency range represents the diffusion limited process [30,31]. In this report, EIS study of the modified electrodes was carried out in 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl with a frequency range of 0.01 Hz to 100 kHz. Fig. 3 showed the Nyquist plots of GCE modified with different modified materials. The $R_{ct}$ value of the bare GCE was about 680 Ω (curve a), by contrast, the $R_{ct}$ of AuNPs/GCE (curve b) and GN/GCE (curve c) was smaller than the $R_{ct}$ value of bare GCE, which were about 205 and 185 Ω, respectively. After the bare GCE was modified with GN-AuNPs nanocomposite, the $R_{ct}$ value was less than 50 Ω (curve d), suggesting that GN-AuNPs nanocomposite greatly improved the conductivity of the modified electrode. After CLDH-AChE composite was immobilized on GN-AuNPs/GCE, the $R_{ct}$ value of CLDH-AChE/GN-AuNPs/GCE (curve e) increased to about 550 Ω. This increase of $R_{ct}$ value is attributed to the fact that most biological molecules, including enzymes, are poor electrical conductors at low frequencies (at least <10 kHz) and could cause hindrance to the electron transfer. These results were supported by the cyclic voltammogram study.

CV of the ferricyanide system is a convenient and valuable tool to monitor the characteristics of the surface of each modified electrode [32]. In Fig. 4, after the bare GCE was modified by the AuNPs, GN, especially GN-AuNPs nanocomposite, an obvious increase of peak current (curve b–d) could be observed compared to bare GCE electrode (curve a). Finally, the immobilization of CLDH-AChE composite resulted in a decrease in the peak current (curve c), which was the direct evidence of successful binding of enzyme on the electrode surface.

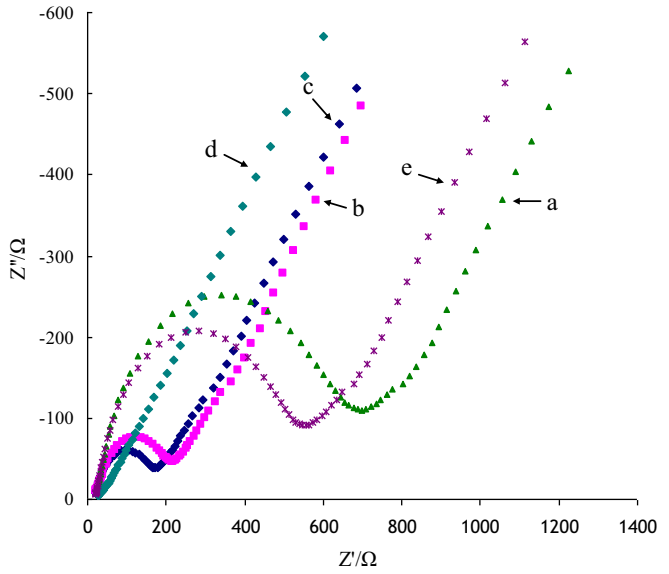


**Fig. 3.** Electrochemical impedance spectra of bare GCE (a), AuNPs/GCE (b), GN/GCE (c), GN-AuNPs/GCE (d), CLDH-AChE/GN-AuNPs/GCE (e) in pH 7.5 phosphate buffer solutions containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl.

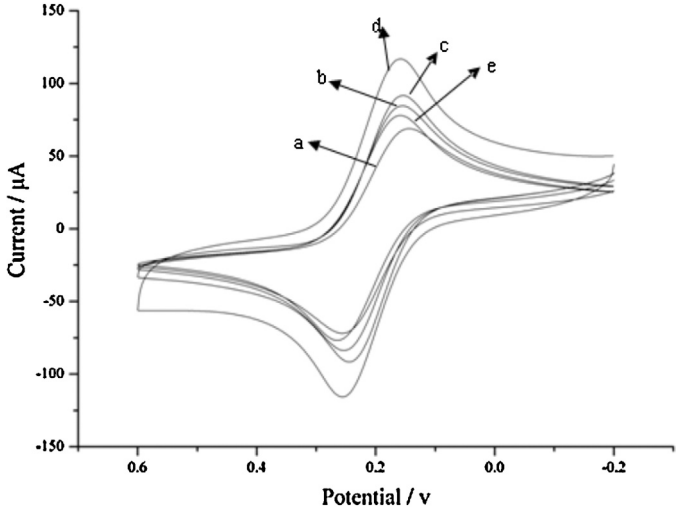


**Fig. 4.** Cyclic voltammograms of bare GCE (a), AuNPs/GCE (b), GN/GCE (c), GN-AuNPs/GCE (d), CLDH-AChE/GN-AuNPs/GCE (e)in pH 7.5 phosphate buffer solutions containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl.

### 3.3. Optimization parameters of the biosensor performance

In order to get high performance detection of pesticide, the experimental parameters were further optimized. The bioactivity of the immobilized AChE depends on the pH of the ATCl solution. The relationship between amperometric responses of AChE with the pH of the ATCl solution was showed in Fig. 5. In the pH range from 5 to 7.5, the amperometric response increased with the increase of pH and reached a maximum at 7.5. The further increase the pH of solution led to an obvious decrease of the amperometric response. Therefore, pH 7.5 was used in the detection solution.

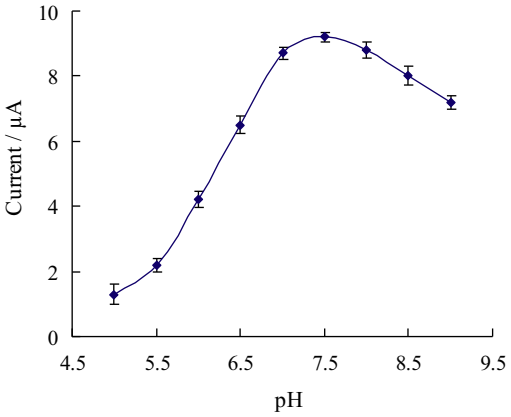


**Fig. 5.** Effect of pH on response current of the CLDH-AChE/GN-AuNPs/GCE biosensor.

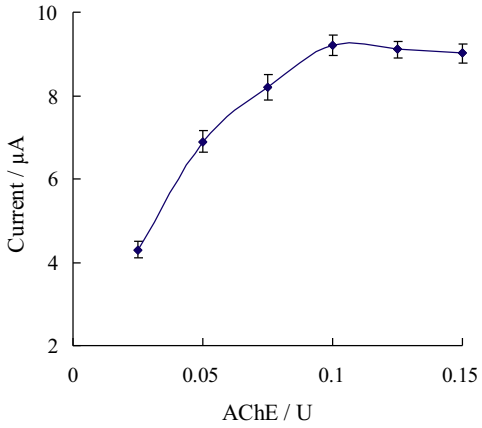


**Fig. 6.** Effect of AChE amount on response current of the CLDH-AChE/GN-AuNPs/GCE biosensor.

The influence of AChE amount on the response of biosensor was also investigated (Fig. 6). While the amount of AChE changed from 0.025 to 0.15 U, with the increasing of the AChE amount, the amperometric response of the biosensor increased gradually and reached a maximum at 0.1 U. A small quantity of AChE led to catalysis insufficiency of the biosensor. Further increasing the amount of AChE led to resistance increase and obvious decrease of the amperometric response of the biosensor. Thus, 0.1 U of AChE was used in these experiments.

The effect of inhibition time on the precision and stability of the AChE biosensor was investigated. As shown in Fig. 7, the inhibition rate increased rapidly with the increase of inhibition time, and then tended to be stable at 10 min. Thus, the inhibition time of 10 min was used in these experiments.

### 3.4. Pesticides determination

The DPV responses were examined before and after exposure to different concentrations of pesticides (Fig. 8). The DPV response of CLDH-AChE/GN-AuNPs/GCE showed an obvious peak at about 580 mV (curve a) in pH 7.5 PBS containing 1.0 mM ATCl. This peak came from the oxidation of thiocholine, hydrolysis product of ATCl, which was catalyzed by immobilized AChE. The DPV responses were associated with the inhibition of AChE activity based on the amount of pesticide added (curve b–m). The decrease in redox peak current likely occured as a result of blocking serine hydroxyl groups of AChE by covalently bound of pesticides, which invariably reduced the overall charge of the catalytic active site. As shown in

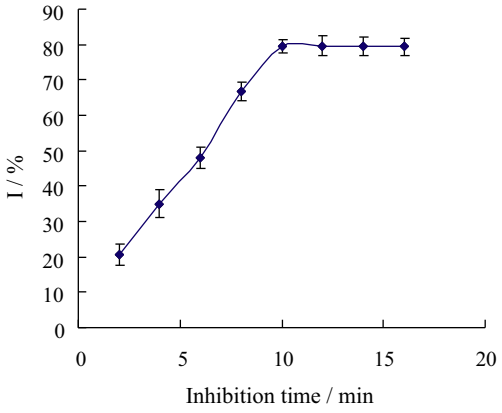


**Fig. 7.** Effect of incubation time on the inhibition rate of the CLDH-AChE/GN-AuNPs/GCE biosensor.

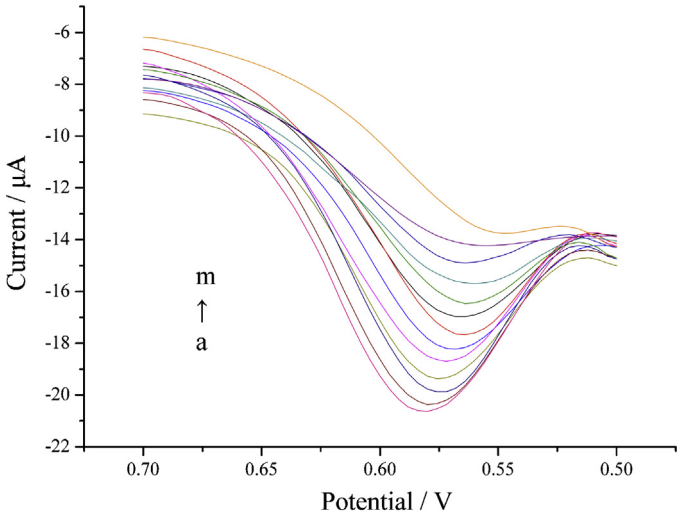


**Fig. 8.** DPV of CLDH-AChE/GN-AuNPs/GCE in pH 7.5 PBS solution containing 1.0 mM ATCl after inhibition with chlorpyrifos for 10 min. Malathion concentration (a)–(m): 0, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 150, 180 μg/L.

Fig. 9. The biosensor detected chlorpyrifos at concentrations ranging from 0.05 to 150 μg/L and the detection limit was 0.05 μg/L.

The performance of this CLDH-AChE/GN-AuNPs/GCE compared with other reported AChE biosensors was summarized in Table 1. It showed that the present CLDH-AChE/GN-AuNPs/GCE exhibited a wider range (Spanning four orders of magnitude) and lower detection limit. This indicated that the GN-AuNPs film improved the electron transfer rate and the CLDH provided an increase of effective surface area for protein loading and effective substrate diffusion, which was an important issue for improving the

**Table 1**
Comparison of the response of the fabricated biosensor for detection of chlorpyrifos with other biosensors based on immobilized AChE.

| Electrode | Linear range (μg/L) | Detection limit (μg/L) | Ref. |
|---|---|---|---|
| CLDH-AChE/GN-AuNPs/GCE | 0.05–150 | 0.05 | This work |
| AChE/L-Cys/HGNs/Chits/GCE | 0.1–150 | 0.06 | [31] |
| AChE-[BMIM][$BF_4$]-MWCNT/CP | 3.5–350 | 1.4 | [33] |
| AChE/CPBA/GR-AuNPs/GCE | 0.5–100 | 0.1 | [34] |
| AChE-Pin5COOH/ZnS/AuE | 0.5–14 | 0.5 | [35] |
| AChE/CMCS-AuNPs/GCE | 0.1–100 | 0.07 | [36] |

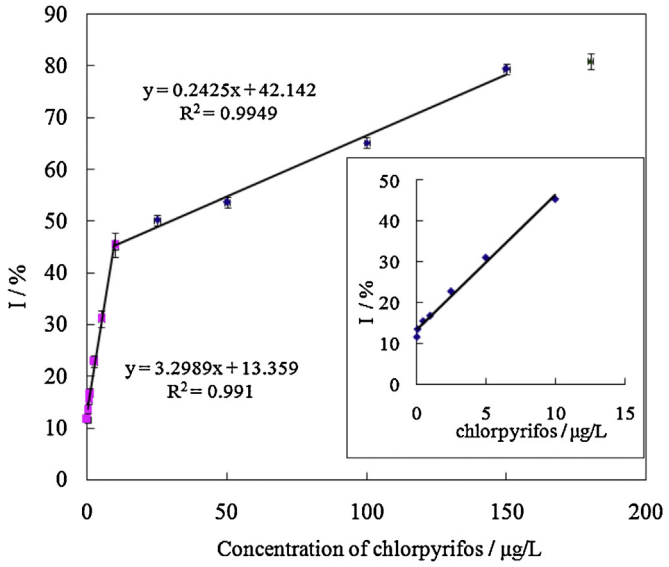


**Fig. 9.** The relationship between inhibition rate and chlorpyrifos concentrations. (Error bars are the standard deviation of three repetitive experiments).

**Table 2**
Recovery studies of chlorpyrifos in leek and pakchoi samples.

| Sample | Concentration | | Recovery (%) |
|---|---|---|---|
| | Taken (μg/L) | Found (μg/L) | |
| Leek | 10.0 | 10.71 | 107.1 |
| | 50.0 | 52.4 | 104.8 |
| Pakchoi | 10.0 | 10.59 | 105.9 |
| | 50.0 | 48.25 | 96.5 |

response properties of the biosensors. Therefore, CLDH-AChE/GN-AuNPs/GCE biosensor would be an excellent platform for the detection of pesticides.

### 3.5. Precision of measurements

The inter-assay precision was estimated at five different electrodes for the determinations in 1.0 mM ATCl after being treated with 10 μg/L chlorpyrifos solution for 10 min. Similarly, the intra-assay precision was estimated at five replicate electrodes. The RSD of intra-assay and inter-assay were found to be 4.1% and 6.9%, respectively, indicating acceptable reproducibility.

### 3.6. Storage stability

The stability of the CLDH-AChE/GN-AuNPs/GCE electrode could be maintained by being stored at 4 °C in a dry condition. No obvious decrease in the response of current was observed in a week. After a 30-day storage period, the sensor retained about 89% of its initial current response.

### 3.7. The detection of the real samples

To further demonstrate the practicality of the proposed method, the recovery tests were carried out by adding different amounts of chlorpyrifos into leek and pakchoi samples (summarized in Table 2). The recoveries were from 96.5% to 107.1%. The results indicated that the proposed method was highly accurate, precise and reproducible. It can be used for direct analysis of vegetable samples.

## 4. Conclusions

In this paper, combining the advantageous characteristics of Grapheme, AuNPs and Cu–Mg–Al calcined layered double hydroxide were prepared. The graphene–gold nanocomposites showed excellent conductivity and biocompatibility. Cu–Mg–Al calcined layered double hydroxide (CLDH) had larger surface areas that were favorable for AChE adhesion and improve stability of the AChE biosensor. The constructed biosensor exhibited many merits such as high sensitivity, wide linear response range, good fabrication reproducibility, acceptable stability and short response time. Moreover, it can also be used for direct analysis of practical samples, which would be a new promising tool for pesticides analysis.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 30972055, 31101286), Agricultural Science and Technology Achievements Transformation Fund Projects of the Ministry of Science and Technology of China (No. 2011GB2C60020) and Shandong Provincial Natural Science Foundation, China (No. Q2008D03).

## References

[1] Ivanov Y, Marinov I, Gabrovska K, Dimcheva N, Godjevargova T. J Mol Catal B: Enzym 2010;63:141–8.
[2] Gong JM, Guan ZQ, Song DD. Biosens Bioelectron 2013;39:320–3.
[3] Sun X, Wang XY. Biosens Bioelectron 2010;25:2611–4.
[4] Alacam S, Timur S, Telefoncu A. J Mol Catal B: Enzym 2007;49:55–60.
[5] Gong JM, Wang LY, Song DD, Zhu XL, Zhang LZ. Biosens Bioelectron 2009;25:493–6.
[6] Luo LQ, Li QX, Xu YH, Ding YP, Wang X, Deng DM, Xu YJ. Sens Actuators B 2010;145:293–8.
[7] Yang L, Wang GC, Liu YJ, Wang M. Talanta 2013;13:135–41.
[8] Yang Z, Tjiu WW, Fan W, Liu TX. Electrochim Acta 2013;90:400–7.
[9] Wang CY, Chen SH, Xiang Y, Li WJ, Zhong X, Che X, Li JJ. J Mol Catal B: Enzym 2011;69:1–7.
[10] Song ZJ, Yuan R, Chai YQ, Jiang W, Su HL, Che X, Ran XQ. Biosens Bioelectron 2011;26:2776–80.
[11] Allen MJ, Tun VC, Kaner RB. Chem Rev 2010;110:132.
[12] Novoselov KS, Geim AK, Morozov SV, Jang D, Zhang Y, Dubonos SV, Grigorieva IV, Firsov AA. Science 2004;306:666.
[13] Lee HU, Yoo HY, Lkhagvasuren T, Song YS, Park C, Kim J, Kim SW. Biosens Bioelectron 2013;42:342–8.
[14] Liang H, Miao XJ, Gong JM. Electrochem Commun 2012;20:149–52.
[15] Wang K, Liu Q, Dai L, Yan JJ, Ju C, Qiu BJ, Wu XY. Anal Chim Acta 2011;695:84–8.
[16] Li YP, Bai YF, Han GY, Li MY. Sens Actuators, B 2013; 185: 706-12.
[17] Xu YH, Liu XJ, Ding YP, Luo LQ, Wang YL, Zhang Y, Xu YJ. Appl Clay Sci 2011;52:322–7.
[18] Beaudot P, De Roy ME, Besse JP. Chem Mater 2004;16:935.
[19] Baccar ZM, Caballero D, Eritja R, Errachid A. Electrochim Acta 2012;74:123–9.
[20] Wang ZH, Liu F, Lu C. Biosens Bioelectron 2012;38:284–8.
[21] Bastianini M, Costenaro D, Bisio C, Marchese L, Costantino U, Vivani R, Nocchetti M. Inorg Chem 2012;51:2560–8.
[22] Tezuka S, Chitrakar R, Sonoda A, Sakane K, Ooi K, Tomida T. Bull Chem Soc Jpn 2004;77:2101–7.
[23] Cui L, Yin HH, Dong J, Fan H, Liu T, Ju P, Ai SY. Biosens Bioelectron 2011;26:3278–83.
[24] Hidouri S, Baccara ZM, Abdelmelek H, Noguer T, Marty J-L, Campàs M. Talanta 2011;85:1882–7.
[25] Shan D, Yao WJ, Xue HG. Electroanalysis 2006;18:1485–91.
[26] Li YJ, Gao W, Ci LJ, Wang CM, Ajayan PM. Carbon 2010;48:1124–30.
[27] Yang L, Wang GC, Liu YJ. Anal Biochem 2013;437:144–9.
[28] Ai HH, Huang XT, Zhu ZH, Liu JP, Chi QB, Li YY, Zi LK, Ji XX. Biosens Bioelectron 2008;24:1048–52.
[29] Qu YH, Sun Q, Xiao F, Shi GY, Jin LT. Bioelectrochemistry 2010;77:139–44.
[30] Liu T, Su HC, Qu XJ, Ju P, Cui L, Ai SY. Sens Actuators B 2011;160(1):1255–61.
[31] Sun X, Zhai C, Wang XY. Bioprocess Biosyst Eng 2013;36:273–83.
[32] Wang Y, Yuan R, Chaia YQ, Li WJ, Zhuo Y, Yuan YL, Li JJ. J Mol Catal B: Enzym 2011;71:146–51.
[33] Zamfir L-G, Rotariu L, Bala C. Biosens Bioelectron 2011;26:3692–5.
[34] Liu T, Su HC, Qu XJ, Ju P, Cui L, Ai SY. Sens Actuators B 2011;160:1255–61.
[35] Chauhan N, Narang J, Pundir CS. Biosens Bioelectron 2011;29:82–8.
[36] Sun X, Zhai C, Wang XY. IEEE Sens J 2013;13:172–9.