Research Article

Study on the Highly Sensitive AChE Electrode Based on Multiwalled Carbon Nanotubes

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Using chitosan (CS) as carrier, the method named layer-by-layer (LBL) self-assembly modification to modify the glassy carbon electrode (GCE) with multiwalled carbon nanotubes (MWNTs) and acetylcholine esterase (AChE) was proposed to prepare the acetylcholinesterase electrode with high sensitivity and stability. The modified electrode was used to detect pesticide of aldicarb, and the enzyme inhibition rate of the electrode showed good linearity with pesticide concentrations in the range of $10^{-10} \cdot \text{g L}^{-1}$ to $10^{-3} \cdot \text{g L}^{-1}$. The detection limit was $10^{-11} \cdot \text{g L}^{-1}$. The modified electrode was also used to detect the actual sample, and the recovery rate range was from 97.72% to 107.15%, which could meet the rapid testing need of the aldicarb residue. After being stored in the phosphate buffer solution (PBS) in 4°C for 30 days, the modified electrode showed good stability with the response current that was 80% of the original current.

1. Introduction

At present carbamate pesticide residue detecting technology mainly includes such following types: enzyme inhibition based on biological detection techniques [1], immunoassay [2], chromatographic detection [3–5], and GC-MS&LC-MS [6]. Carbamate pesticide residue in vegetables is usually determined by HPLC, but it has a long test cycle, high price of derivative reagents, and poor stability. Biosensor, through preparing enzyme electrode, has aroused worldwide researchers’ interest in its advantages, such as good specificity, no sample pretreatment, and fast analysis. Cai and Du [7], and so forth, adopted drop-coating by dispensing mixed processing objects of CS and MWNTs on prepared screen printing glassy carbon electrode, then coating AChE on the surface to make enzyme electrodes for rapid detection of carbaryl. The detection limit reached $10^{-6} \cdot \text{g L}^{-1}$. Due to potential mechanical, thermal, electrical, and electrochemical properties, graphene is usually regarded as a competitive candidate for new electronic and electrochemical applications, such as batteries [8, 9], catalysts [10, 11], fuel cells [12, 13], biosensors [14, 15], solar cells [16, 17], sensors [18, 19], and super-capacitors [20, 21]. Upadhyay et al. [22], and so forth, modified mixed nanoparticles of gold and platinum in glassy carbon electrode surface at first and then modified AChE on electrode surface to make enzyme electrode for organophosphate and carbamate pesticide detection. The detection limit reached $10^{-9} \cdot \text{g L}^{-1}$. In China, the research of this aspect was generally focused on the preparation of AChE membrane [23–25] and conducted by adopting screen printing electrode technology [26]. Indeed, the inhibition of acetylcholinesterase (AChE) activity by pesticides can result in a disturbance of normal neuronal function and possibly death [27, 28]. At present AChE electrode’s immobilized enzyme technology is typically used to use the crosslinking methods with glutaraldehyde as crosslinking agent [29–31]. The immobilized enzyme was firm, but violent reaction, poor mechanical performance, and low enzyme activity greatly affected the sensitivity and stability of obtained AChE. To improve the sensitivity and stability of obtained AChE, this paper adopted CS and MWNTs as modified materials to fix MWNTs and AChE by LBL technique and made highly efficient and stable AChE electrodes for the detection of pesticide aldicarb. The existence of CS can provide both very good sensitivity and stability of the biosensor and good precision of measurements. The principle of layer self-assembly method was to use the mutual attraction between positive and negative charge to realize material modification and enzyme
imobilization. It hardly destroys the enzyme molecule, and at the same time through multilayer modification it can prevent loss of enzyme and improve the activity of electrode. In recent years, biosensors based on AChE have become a promising technique for environmental monitoring, toxicity analysis, military investigations, and foodstuff quality [32, 33].

2. Materials and Methods

2.1. Main Reagent and Instrument. AChE (317 U/mg, Sigma Company), ATChCl (Sigma Company), DTNB (Shanghai Junchuang Biological Technology Co. Ltd.), CS (Molecular weight is 15000, Deacetylation was about 95%, Zhejiang Namigang Co. Ltd.), MWNTs (Diameter was 40–60 nm, length was 1–10 μm, purity > 95%, Shenzhen Namigang Co. Ltd.), CH₃OH (AR, Guoyao Group Chemistry Reagent Co. Ltd.), aldicarb (10⁻³ g·L⁻¹, Shanghai Pesticide Research Institute), other reagents were AR.

CHIs800 Electrochemical analyzer (Three electrode systems: Glassy carbon electrode was working electrode; Saturated calomel electrode was reference electrode; Platinum electrode as the counter electrode, current sensitivity range: 10⁻³–10⁻¹¹ A, Shanghai Chenhua Instrument Company), Electronic analytical balance (AB104-N, Mettle-Toledo Instr Ltd.), Ultrasonic Cleaner (KQ2B8CQ-250, Kunshan Ultrasonic Instruments Co. Ltd.), Collection hot type constant temperature magnetic blender (DF-101S, Gongyi Yingyu Yuhua Instrument factory); pH meter (PHB-3BW, Shanghai Lida Instrument factory).

2.2. MWNTs Modified Glassy Carbon Electrode. We polished carefully the glassy carbon electrode using Al₂O₃ turbid liquid with particle size of 0.3 and 0.05 μm successively on the shammry for 3 min until we got a bright mirror. Then we cleaned the electrode surface with deionized water to remove Al₂O₃. Afterwards, the electrode was ultrasonic-cleaned in acetone, ethanol, and deionized water for 3 min, respectively, in order to further clear the electrode surface dirt. Then we dried the electrode under the infrared lamp.

6 mL of deionized water dispersion of MWNTs with concentration of 10 g·L⁻¹ was put into reaction pool, and the pretreated glassy carbon electrode and platinum electrode were combined into two-electrode system with electrical deposition (ED) in 1.7 V voltage for 2 hours. In order to disperse it evenly, we set the magnetic stirring speed at 300 rpm (rev/sec) and the electricity deposition temperature was controlled for 30°C in water.

In order to make more MWNTs modified to the surface of electrodes, we dipped the probe into sodium borate solution of pH 9.18 for 15 min, carefully washed it with deionized water, and then immersed it into 0.5% concentration of CS solution (to adjust the PH value of chitosan solution with NaOH solution to 5.0) for 15 min. Then we rinsed the electrode surface with deionized water to get rid of redundant CS. Then it was immersed in 10 g·L⁻¹ of the MWNTs dispersion of sodium borate and removed after 15 min, after which the electrode surface was rinsed again with deionized water and nature-aird. Thus a layer of the electrostatic self-assembly had been completed. The steps above were repeated until the ideal number of modified layers was gained.

2.3. Layer Self-Assembly Method Modified AChE on the Electrode Surface. We carefully rinsed MWNTs/CS electrode which was well modified before with deionized water and immersed the electrode into 0.5% concentration of CS solution for 15 min. The electrode surface was rinsed with deionized water to get rid of redundant CS and then immersed in the enzyme liquid of 100 U (the isoelectric point of acetylcholinesterase Ip = 4.5, so when liquid pH > Ip, AChE was with a negative charge; the experiment was prepared for the AChE solution with the 7.4 pH PBS) for 15 min and then taken out and rinsed carefully with deionized water. Thus a layer of the AChE assembly was completed. Afterwards, this series of steps were repeated until the specified number of modified AChE electrodes was gotten.

2.4. AChE Electrode Detection of Aldicarb. 10⁻² g·L⁻¹ (100 ppm) of aldicarb standard samples progressively diluted to 10⁻⁷ g·L⁻¹ was prepared, and the enzyme electrode was immersed in the pesticide solution for 10 min. Then enzyme inhibition rate could be determined.

We weighed 5 g cabbage which was planted by the group and cut it into pieces properly. They were placed in a 25 mL centrifuge tube adding 1 mL concentration of 10⁻⁴ g·L⁻¹ of the standard sample aldicarb and then blended and sealed. 10 min later, 9 mL of methanol was added and shocked for 10 minutes. Then we poured out the extract and diluted it to 10⁻⁶ g·L⁻¹. Sample solution was obtained. Recovery rate should be detected with obtained acetylcholinesterase electrode.

2.5. Determination of Electrode Life. In order to validate the stability of the immobilized enzyme electrode, we saved prepared acetylcholinesterase electrode in the PBS at 4°C. We left it in the PBS of 0.10 mol·L⁻¹ to determine its current response to 80 μL of ATChCl with concentration of 0.10 mol·L⁻¹ every 5 days during 30 days.

3. Results and Discussion

3.1. [MWNTs/CS] Modified Layer Selection. After electrode-position it can be known that, with different {CS/MWNTs} layers, the background currents of the income of PBS scanning are different. As {CS/MWNTs} layer increased, the background currents of the modified electrodes’ CV scanning for PBS and the characteristic redox peaks of MWNTs also increased. When the number of layers was more than 5, the linear relationship began to become poor. The reason may be when the thickness of the modified layer became too large, it hindered the electron transfer between modified layer surface and electrodes. Thus, the number of this experiment’s {CS/MWNTs} self-assembly layer was five. At this time, the resulting modified layers were relatively dense and even. So far we obtained {MWNTs/CS}₅/ED/GCE.
3.2. Determination of the Effect of Activated Electrodes. The value of O/C on the surface of the electrode went up significantly after being activated by diluted sulphuric acid, which illustrated the great increase of oxygen-containing groups. Relevant research showed that those oxygen-containing groups, including carboxy groups, carbanyl groups, phenols and quinones, and so forth, played a crucial role in improving the electrochemical properties.

Figure 1 is GCE’s cyclic voltammograms scanogram with 10 cycles in diluted sulphuric acid. We can see that the electrochemical windows gradually decrease due to the small changes on the surface in the activation process, and it explains that GCE’s properties tend to be steady.

Figure 2: The electrochemical behavior of cysteine on the (A) GCE and (B) {MWNTs/CS}/ED/GCE.

3.3. Cysteine’s Electrochemical Behavior on {MWNTs/CS}/ED/GCE. Acetylthiocholine chloride generated thiococholine through the catalysis of acetylcholinesterase electrodes. It had the electrochemical activity because of the existence of sulfydryl. Under a certain potential difference it can produce significant oxidation current. But too high peak potential would bring negative effect to determination, such as interference of a large background current or other impurities [34]. So reducing the peak potential of thiococholine and increasing the peak current would be effective ways to improve the sensitivity of acetylcholinesterase electrodes. It was easier to get cysteine and it also had thiol. So this experiment used cysteine instead of thiococholine to detect its electrochemical behavior on {MWNTs/CS}/ED/GCE. For current configuration cysteine solution of 5 mmolL−1, cyclic voltammetry with polished bare glassy carbon electrode and modified {MWNTs/CS}/ED/GCE, scan rate was 0.1 V.S−1. According to Figure 2, it can be seen that, through the modification of MWNTs, the peak potential of cysteine reduced and the peak current obviously improved. The expected purpose was achieved.

3.4. {AChE/CS} Modified Layer Selection. To modify the different layers of {AChE/CS}n/{MWNTs/CS}/ED/GCE as the working electrode, the time-current method with the +0.3 V conditions was used. Add 60 μL, 0.1 mol.L−1 of ATChCl into PBS of pH 7.40 to get response current.

It can be seen from Figure 3 that when the number of self-assembled layers was less than 4, the immobilized enzyme activity improved rapidly with the number of layers increased. When the number of layers was more than or equal to 4, the enzyme activity no longer increased but had a downward trend. The reason may be that when there were too many layers of immobilized enzyme, enzyme molecule congestion would be caused by excessive supply of the enzyme, steric hindrance enhanced, and the substrate and the products spread too late. That is why the enzyme activity was no longer even greater [35]. In order to obtain the most significant experimental results, we chose immobilized layers for 4. At this time the response of the enzyme electrode to the substrate choline chloride acetyl sulfur generation was the biggest.

3.5. Thiocholine’s Electrochemical Behavior on Modified Electrodes. Due to the sulfydryl structure, thiocholine (TCh) can react in a specific voltage as the following:

\[
2\left[H_3C\right]_3-N^+\text{-CH}_2\text{-CH}_2\text{-SH} \rightarrow -2e^- \left[H_3C\right]_3-N^+\text{-CH}_2\text{-CH}_2\text{-S-S-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}^+\text{-}[\text{CH}_3]_3 + 2H^+
\]

As shown in Figure 4, TCh would be oxidized on both bare electrode and modified electrode and formed anodic peak. On the surface of bare electrode, as the concentration of TCh came to 5 mmol.L−1, \(I_{pa} = -27 \mu A, E_{pa} = 0.65 V\). On the surface of modified electrode, as the concentration of TCh came to 5 mmol.L−1, \(I_{pa} = -54 \mu A, E_{pa} = 0.35 V\). It demonstrated that the electrocatalysis properties of modified electrode were improved apparently.
3.6. For the Detection of Pesticide Aldicarb by $\{\text{AChE/CS}\}/\{\text{MWNTs/CS}\}/\text{ED/GCE}$. With the increasing of the concentration of pesticides, the inhibition rate of the enzyme electrode also increased accordingly. The inhibition rate should be calculated according to the following formula:

$$ \text{Inhibition rate} \% = \frac{I_0 - I_1}{I_0} \times 100\% . \quad (2) $$

In the formula, $I_0$ was the steady current caused by acetylcholinesterase sensor which acted on a certain concentration of thio acetylcholine. $I_1$ was the steady current caused by acetylcholinesterase sensor which acted on the same concentration of thio acetylcholine after an inhibition by pesticides.

In the experiment, the enzyme electrode was put into pesticide solution for 10 min, and then we could determine the inhibition rate of enzyme.

It can be seen in Figures 5 and 6 that, in $10^{-4}$–$10^{-11}$ g L$^{-1}$ range of the aldicarb concentration, inhibition rate of the enzyme electrode had a good linear relationship with the negative logarithm of its concentration. The detection limit can achieve $10^{-11}$ g L$^{-1}$. Figure 6 showed the standard curve of aldicarb concentration detected by $\{\text{AChE/CS}\}/\{\text{MWNTs/CS}\}/\text{ED/GCE}$.

We detected the sample solution with three acetylcholinesterase electrodes which were prepared, respectively, and the determination results were showed as in Table 1. When the concentration of the sample solution was $10^{-6}$ g L$^{-1}$, the recovery rate was in the range of 97.72–107.15%, the average recovery rate was 103.19, and the standard deviation was 6.92. Considering the error of electrochemical detection method and the difference between the electrodes obtained, this result can be almost accurate.

![Figure 3: The response currents of the 0.1 mol L$^{-1}$ ATChCl by different number of [AChE/CS] layers.](image)

![Figure 4: Electrochemical behavior of TCh. (a) [CS/MWNTs]$_5$/ED/GCE; (b) bare electrode; (c) based current of bare electrode, 0.10 mol L$^{-1}$ of phosphate buffer with pH = 7.40 and scanning rate of 100 mV s$^{-1}$.](image)

![Figure 5: The curvilinear relation between the enzyme inhibition and the negative logarithm of aldicarb concentration.](image)
3.7. Determination Result of $\{AChE/CS\}_4/[MWNTs/CS]_5/ED/GCE$'s Age. According to Figure 7, the response current value of enzyme electrode was still 72% of the initial current after 30 days. This showed that acetylcholinesterase activity could be well preserved with chitosan and MWNTs as modified materials and layers self-assembly method used to immobilize acetylcholinesterase. The immobilized enzyme electrode may have longer life expectancy and provided the basis for its practical application of pesticide detection.

4. Conclusion and Outlook

The current-acetylcholinesterase electrode was prepared with layer self-assembly method and modified materials of carbon nanotubes and chitosan. Carbon nanotubes can effectively promote electronic relay and chitosan has good biological compatibility [36] that can fix acetylcholinesterase well. Experiments showed that modified cholinesterase sensor had speedy response, high sensitivity, and good stability. To take advantage of organophosphorus pesticides' inhibitory effect on acetylcholinesterase, with (ATChCl) as substrate, the limit of detection of organophosphorus pesticide parathion reached $10^{-11}$ g L$^{-1}$. The detection result of practical samples was favorable, so there would be a broad prospect for this method applied to detecting the organophosphorus pesticide residues.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


