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RESEARCH PAPER

Acetylcholinesterase biosensors based on ionic liquid functionalized carbon nanotubes and horseradish peroxidase for monocrotophos determination

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Abstract
Long-term and excessive use of monocrotophos (MPs) pesticide leads to an accumulation of MPs residues in agricultural products. Electrochemical biosensor technology was developed as a simple and efficient method for detecting MPs. However, commercial acetylcholinesterase (AChE) sensors are not applied in practical MPs detection due to poor stability and reliability. In this study, the advantages of functionalized carbon nanotubes (Cl/MWCNTs) and a bi-enzyme system (horseradish peroxidase (HRP)/AChE) were combined, a novel bi-enzyme electrode (Cl/MWCNTs/HRP/AChE/GCE) was constructed. Under optimal conditions, the bi-enzyme sensor had a wide detection range of 1.0×10^{-11} to 1.0×10^{-7} mol/L and low detection limit of 4.5×10^{-12} mol/L. The proposed AChE biosensor exhibited excellent stability and sensitivity for MPs determination and presented a promising tool for monitoring food safety.

Keywords Monocrotophos · Carbon nanotubes · Horseradish peroxidase · Acetylcholinesterase · Biosensor

Introduction

Monocrotophos (MPs) is a high-efficiency organophosphorus (OPs) pesticide that is widely used in agricultural products. However, long-term and excessive use leads to gradual increases in MPs residues in agricultural products, which pose a great threat to human health [1]. Its toxicological effects are mainly due to the active site phosphorylation of human acetylcholinesterase (AChE) serine, which not only inhibits the activity of acetylcholine but also causes AChE to gradually age and finally trigger the disorder of human nerve regulation function. The pathology of Alzheimer's disease is related to the acetylcholine content in the brain [2]. In view of the high toxicity of MPs, it is necessary to achieve accurate and rapid determination of MPs.

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There are many disadvantages to conventional OPs determination techniques (such as chromatography [3], and spectroscopy [4]) which include expensive equipment, high technical requirements for the operators, long determination times, and cumbersome testing procedures. Electrochemical biosensor technology is a simple, economical, and efficient method for determining the content of electroactive substances in a solution. Compared to the conventional determination methods, its advantages are apparent and include low cost, convenient operation, and accurate results [5–7].

The current literature has reported a variety of efficient and sensitive enzymatic electrode determination technologies for the analysis of trace phosphorus pesticides in agricultural products [8, 9]. The determination of MPs by an enzymatic electrode is based on the inhibition of AChE activity by MPs. The amount of MPs is determined by detecting the difference in activity before and after the action of AChE and MPs. Acetylcholinesterase is used as the recognition component and choline, the hydrolysis product of acetylcholine, is used as the signal detection molecule. The residual amount of MPs is indirectly obtained by monitoring the oxidation current of choline. This method is widely used for the detection of organic phosphorus due to its high sensitivity and low cost. However, commercial AChE electrode sensors are not available in practical applications, mainly

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due to the poor stability and reliability of the existing AChE biosensors. Moreover, the sensitivity of the biosensor is easily affected by pH, temperature, and electrode type. [10].

In the AChE electrode preparation process, the AChE immobilization technique used often requires a complicated matrix. Furthermore, AChE is easily deactivated and the detection stability is poor. With the introduction of nanomaterials, such as carbon nanotubes (CNT), and graphene, the determination sensitivity and stability of AChE sensors have been greatly improved. Specifically, CNTs are ideal biosensor materials because of their excellent electrical conductivity, large specific surface area, good biocompatibility, and simple surface functionalization. Vikas et al. immobilized AChE on CNT to prepare an AChE sensor to detect MPs. The linear working range of this biosensor was 1×10^{-7} M to 1.30×10^{-4} M with a lowest determination limit (LOD) of 2.3×10^{-9} M [11]. Cevik et al. prepared an AChE sensor using poly (allylamine hydrochloride)-functionalized CNT as the matrix to detect MPs with a detection limit of 1.3×10^{-11} M [12]. In our previous work, the effects of different functional groups on the surface of CNTs were studied. The prepared AChE sensor was applied to the detection of monocrotophos. Under the optimized conditions, the detection limit was 3.3×10^{-11} M. Storage testing indicated that the reactivity of the prepared AChE biosensor remained above 98.5% within a 2-week period [13].

Presently, researchers have widely focused on how to improve the electron transferability of the biosensor reaction through different structures or functional surfaces of nanomaterials. However, as a hydrolase, AChE is not oxidative and there have been few reports on how to improve the oxidation ability of the choline hydrolyzate and reduce oxidative overpotential of the sensor. The determination of MPs by biosensors is indirectly obtained by monitoring the oxidation current of choline. Choline oxidation is the controlling step of the overall reaction. Therefore, it is necessary to add a highly active oxidation catalyst to accelerate the oxidation of choline. Chauhan et al. used Fe_3O_4/Au/MWCNT as a carrier to prepare an AChE sensor for the determination of monocrotophos [14]. The detection range of their sensor was 1.0×10^{-9} to 5.0×10^{-8} M and the detection limit was 1.0×10^{-9} M. Fe_3O_4 were used as an oxidation catalyst to promote the oxidation of choline and finally improve the sensitivity of the sensor. However, as a biomimetic catalyst, the selectivity of Fe_3O_4 for choline is low. The selectivity of the AChE biosensor would be higher if oxidase was substituted for Fe_3O_4. Baker et al. immobilized HRP and AChE in peptide nanotubes simultaneously. The prepared biosensor was used to detect malathion vapor concentrations. The detection limit of the organic phosphorus vapor was 5.4×10^{-10} M [15].

In view of the advantages of the functionalized surface and bi-enzyme system, this study was based on previous work and used a novel ionic liquid modifier to functionalize carbon nanotubes [13]. Combining the ionic conductivity and biocompatibility of ionic liquids was expected to enhance the electron transfer capability of the sensor and improve the catalytic environment of AChE. Second, the HRP was innovatively introduced to enhance the oxidation of choline and improve the sensitivity of the sensor. The prepared bi-enzyme electrode sensor Cl/MWCNTs/HRP/AChE/GCE was used to detect residual MPs in vegetables. This new type of Cl/MWCNTs/HRP/AChE/GCE biosensor was expected to achieve rapid and sensitive electrochemical determination of MPs.

Materials and methods

Reagents

Trimethoxy (3-chloropropyl) silane and 1-methylimidazole were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Hydroxyl modified MWCNTs (OH-MWCNTs, purity > 95%, length 0.5–2 μm, OD > 50 nm) were purchased from Beijing DK Nano Technology Co., Ltd. (China). Acetylcholinesterase (AChE, C3389, 500 U/mg), thioacetylcholine chloride (ATCl), and horseradish peroxidase (HRP) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were stored at − 20 °C. Monocrotophos (99.4%) was purchased from Putian Tongchuang Biotechnology Co., Ltd. (Beijing, China). Potassium ferricyanide, potassium ferrocyanide, anhydrous sodium sulfate, dichloromethane, and absolute ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Different pH phosphate buffers (PB) from 6.0 to 8.0 were prepared by mixing different ratios of NaH_2PO_4 and Na_2HPO_4. pH 8.5 and 9.0 were Tris–HCl Buffer. All reagents used in the experiments were analytical grade.

Preparation of ionic liquid and functionalized MWCNTs

Equimolar amounts of 1-methylimidazole and trimethoxy(3-chloropropyl)silane were stirred at 95 °C for 24 h to obtain a liquid chloride ion solution. Then, 1 g MWCNTs-OH black powder was weighed into another Erlenmeyer flask containing 100 mL chloroform and a uniform black MWCNTs-OH solution was obtained after sonication(40 kHz, 180 W, model: JP-031S.) for 15 min. Subsequently, 10 mmol chloride ion liquid was added to obtain a mixed solution [16]. Finally, the mixture was stirred at 25 °C for 5 days. The synthesized product was filtered with polytetrafluoroethylene organic membrane (0.22 μm), repeatedly washed with absolute ethanol and distilled water to remove un-synthesized impurities, and the precipitate was vacuum dried at 80 °C.

An ionic liquid-modified MWCNTs composite powder was obtained, which was abbreviated as Cl/MWCNTs [13].

Preparation of bi-enzyme biosensor based on AChE and HRP

The surface of the glassy carbon electrode was polished with alumina powder with particle sizes of 1 and 0.05 μm, then ultrasonically cleaned with deionized water and diluted HNO_3 (v/v 1:3), respectively. The electrode was used to perform a Cyclic Voltammetry (CV) scan in 5.0 mmol/L $K_3[Fe(CN)_6]_3$ solution. When the potential difference between the oxidation peak and the reduction peak was less than 68 mV, the electrode was clean. After that, the electrode was dried under an infrared lamp (230 W, model: TDP-T1).

Cl/MWCNTs (10 mg) were then weighed and dissolved in 1 mL PB buffer (pH 7.0) and sonicated for 15 min to prepare the carrier solution. A series of AChE (0–0.1 U/μL) and HRP (0.005–0.035 U/μL) concentrations were mixed in Tris–HCl (pH 7.0) and the bi-enzyme solution was obtained after 15 min sonication. Carrier solution (10 uL) was dropped onto the surface of the electrode and the electrode was dried with an infrared lamp for 20 min. After the electrode surface was dried, 10 μL bi-enzyme solution was dropped onto its surface. The bi-enzyme electrode sensor was obtained after the electrode surface was dried. The modified electrode was stored in a refrigerator at 4 °C for use. Figure 1 was a graphical representation of the synthesis of the Cl/MWCNTs/HRP/AChE/GCE biosensor.

MPs measurement process

To detect MPs, the modified electrode was immersed in a series of MPs solution concentrations (1×10^{-10}, 5×10^{-10}, 1×10^{-9}, 5×10^{-9}, 1×10^{-8}, 5×10^{-8}, 1×10^{-7}, 5×10^{-7} mol/L) and incubated for 10 min to allow the MPs to inhibit the activity of AChE by varying degrees. Then, the electrode surface was rinsed slowly using PB buffer (0.1 mmol/L, pH 7.0) to remove excess MPs. Finally, the treated electrode was placed in the tri-electrode system of the CHI 660D workstation and

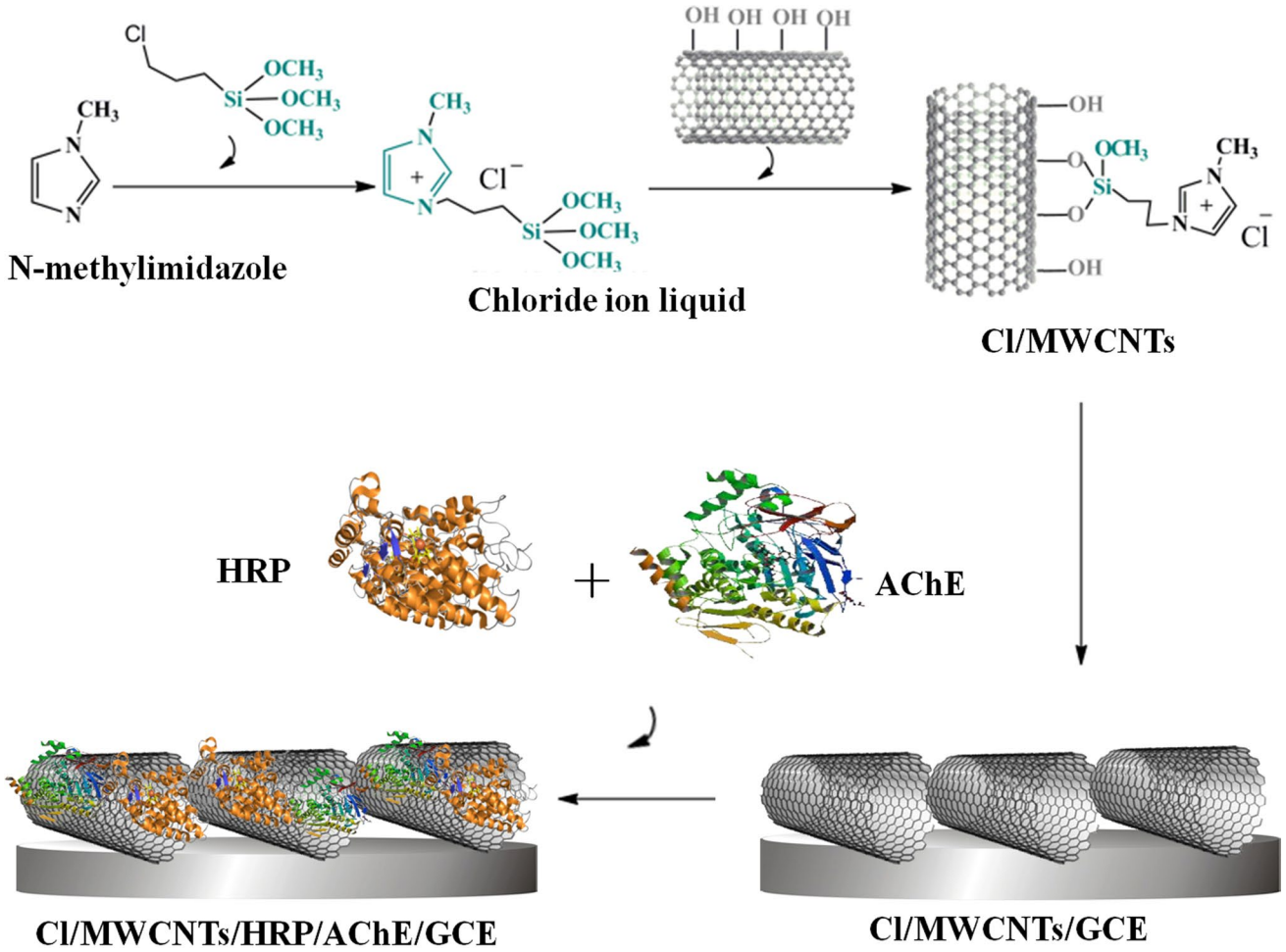

Fig. 1 Illustration of biosensor synthesis

scanned using the differential pulse voltammetry (DPV) method. The determination conditions were as follows: the test solution contained 1.0 mmol/L ATCl PB (0.1 mmol/L, pH 7.0), the DPV scan potential range was 0.3–0.9 V, and the step potential, pulse frequency, and pulse amplitude were 4 mV, 10 Hz and 25 mV, respectively [17].

The inhibition rate (%) of MPs was calculated as follows:

$$I(\%) = \frac{i_1 - i_2}{i_1} \times 100\%$$

where i_1 was the current peak of the biosensor without MPs treatment and i_2 was the current peak of the biosensor treated by MPs.

Sample analysis

Vegetable samples were purchased from the local supermarket. A 10.0 g chopped sample was placed in an Erlenmeyer flask and 35 g anhydrous Na_2SO_4 was added for dehydration and 0.4 g activated carbon was added for decolorization, followed by the addition of 70 mL CH_2Cl_2, shaking for 40 min, and filtering. A 35 mL aliquot of the filtrate was removed and dried at room temperature. The residue was rinsed three times with an appropriate amount of CH_2Cl_2 and diluted to 2.0 mL. Finally, a high concentration of MPs standard solution was added to the vegetable extract. The specific concentration of the MPs sample was finally obtained after dilution. The prepared working electrode was sequentially incubated in the sample to be tested containing different concentrations of MPs [18]. The content was detected using the DPV method under the optimal experimental conditions. The relative standard deviation (RSD) was calculated using Office Excel® 2017.

Apparatus

Transmission electron microscopy (TEM) was performed on a JEOL JEM-2010 transmission electron microscope operating at 200 kV. In this experiment, the electrochemical properties of the carrier were measured by the CV method and the standard curve and various properties of the sensor were measured by the DPV method. All experimental tests were performed using a CHI660D electrochemical workstation (Shanghai Chenhua Co., China) under a tri-electrode system (glassy carbon electrode was the working electrode, Ag/AgCl electrode was the reference electrode, and platinum wire electrode was the counter electrode).

Results and discussion

Optimization of parameters

The effect of AChE and HRP loading was studied since it was one of the most important factor affecting the performance of the sensor. First, Cl/MWCNTs/AChE/GCE biosensors of different AChE loadings with 1 mg/mL carrier Cl/MWCNTs were prepared. DPV scans were performed in PB solution (0.1 mmol/L, pH 7.0) containing 1.0 mmol/L ATCl. The results are shown in Fig. 2a. As the AChE in the sensor increased, the peak current of DPV increased first, then decreased. At an AChE loading of 0.3 U, the peak current reached its maximum. This was because, at small enzyme amounts, the enzyme-catalyzed reaction did not reach saturation. The reaction rate increased with the addition of enzyme. When the amount of the enzyme was too high, the reaction rate did not rise further unless the amount of substrate was increased. Moreover, too much enzyme was unable to participate in the reaction and hindered electron transfer because AChE is a biomacromolecule [19]. Therefore, 0.3 U was chosen as the optimal AChE loading amount in this experiment.

Second, the HRP loading of the sensor was optimized by studying the effect of HRP loading on the electrochemical reaction. A Cl/MWCNTs/AChE/HRP/GCE bi-enzyme biosensor containing 0.3 U AChE and 1 mg/mL Cl/MWCNTs was prepared. A DPV scan was performed in PB solution (0.1 mmol/L, pH7.0) containing 1.0 mmol/L ATCl. As shown in Fig. 2b, the oxidation peak increased first, then decreased with the accretion of HRP. When the loading was 0.25 U, the peak current reached its maximum, the oxidation peak increased by 52% after addition of HRP. This was because small amounts of HRP enhanced the oxidation activity of acetylcholine due to its excellent oxidative action. Electron transfer was hindered and further increased the resistance of the electrode as the amount of enzyme increased [15]. Therefore, 0.25 U was chosen as the optimal HRP loading amount in this experiment.

The performance of the enzyme was greatly affected by the pH of the environment. In the case of bi-enzymes the effect of factors, such as pH, is more complicated. The pH of the reaction system was optimized by studying the effect of pH on the electrochemical reaction. Various buffers with different pH (6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0) containing 1.0 mmol/L ATCl were prepared and a DPV scan was performed by the Cl/MWCNTs/AChE/HRP/GCE bi-enzyme sensor. As shown in Fig. 2c, the peak current of the DPV first increased, then decreased with increasing pH of the reaction solution. When the pH was 7.0, the peak current reached its maximum. The sensor exhibited the highest catalytic activity in a neutral

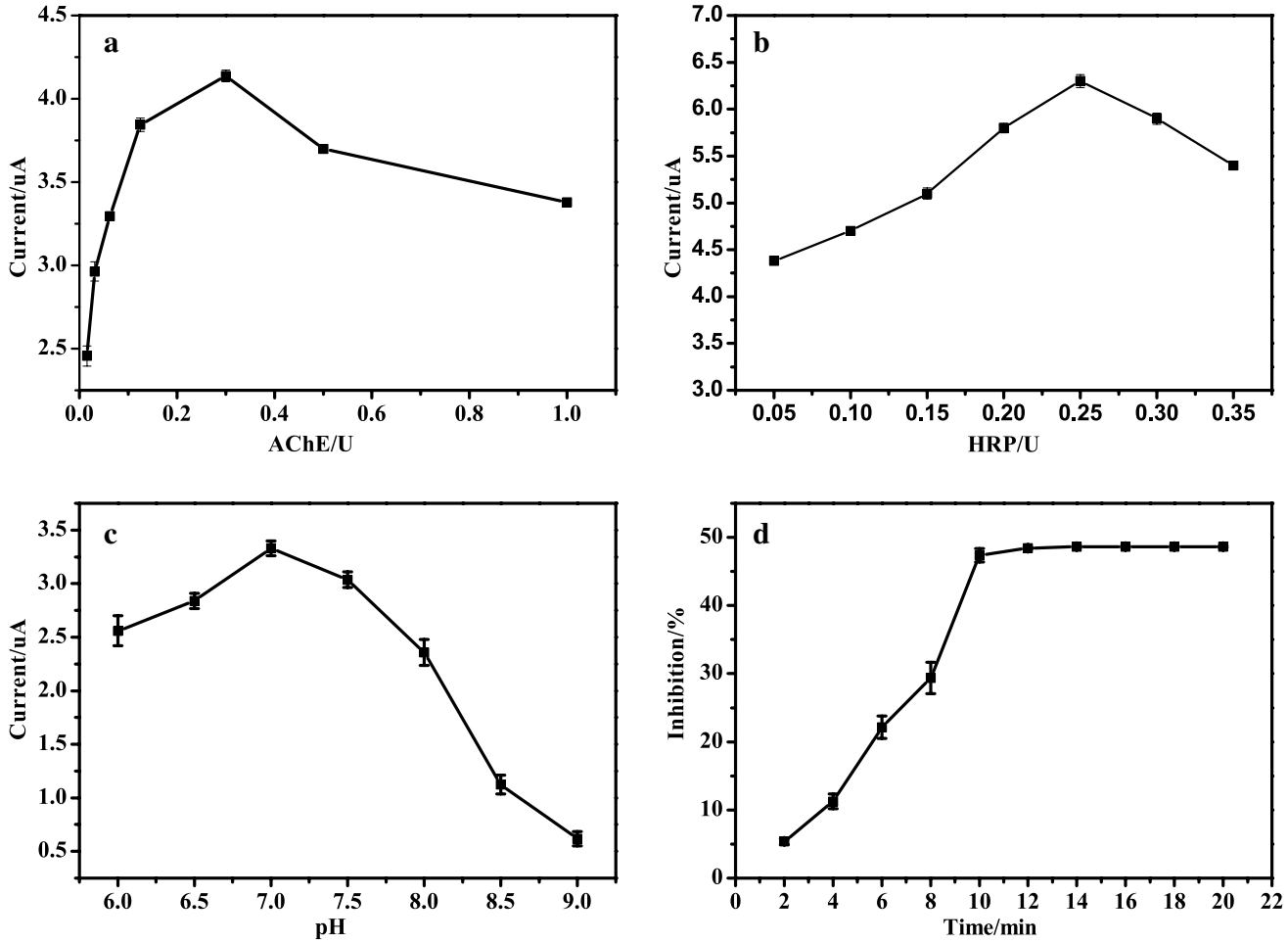

Fig. 2 Effect of solution AChE loading (**a**), HRP loading (**b**), pH (**c**), Inhibition time (**d**) on the amperometric response of Cl/MWCNTs/HRP/AChE/GCE biosensor in PB (0.1 mmol/L pH 7.0) containing 1.0 mmol/L ATCl

environment where the highest DPV peak was obtained [20]. Therefore, pH7.0 was chosen as the optimum pH of the reaction system.

As mentioned above, MPs inhibited the activity of AChE. After a certain period of time, the inhibition of AChE by MPs stabilized, so the inhibition time was optimized in this experiment [21]. A Cl/MWCNTs/AChE/HRP/GCE bi-enzyme biosensor containing 0.3 U AChE and 0.25 U HRP and 1 mg/mL Cl/MWCNTs carrier material was prepared. A DPV scan was performed in PB solution (pH7.0) containing 1.0 mmol/L ATCl after incubation in 1.0×10^{-9} mol/L MPs standard solution for different times (2–20 min). The results are shown in Fig. 2d. The inhibition rate increased sharply at inhibition times up to 10 min. However, the inhibition rate remained unchanged (~50%) when the inhibition time exceeded 10 min. Therefore, 10 min was chosen as the best time for the MPs to incubate with the sensor in this experiment.

Surface characterization of modified electrodes

The nanomaterials were characterized by TEM. The TEM images of OH-MWCNTs and Cl/MWCNTs are shown in Fig. 3, wherein Fig. 3a, c were OH-MWCNTs and Fig. 3b, d were Cl/MWCNTs. The scans showed that the OH-MWCNTs were evenly distributed and clear boundaries existed between the tube bundles and the background. After modification by the chloride ion liquid, the MWCNTs were dispersed more uniformly. Moreover, the surface and tube bundles of the MWCNTs were ambiguous or even showed signs of dissolution, indicating that the chloride ion liquid was successfully loaded onto the MWCNTs [22].

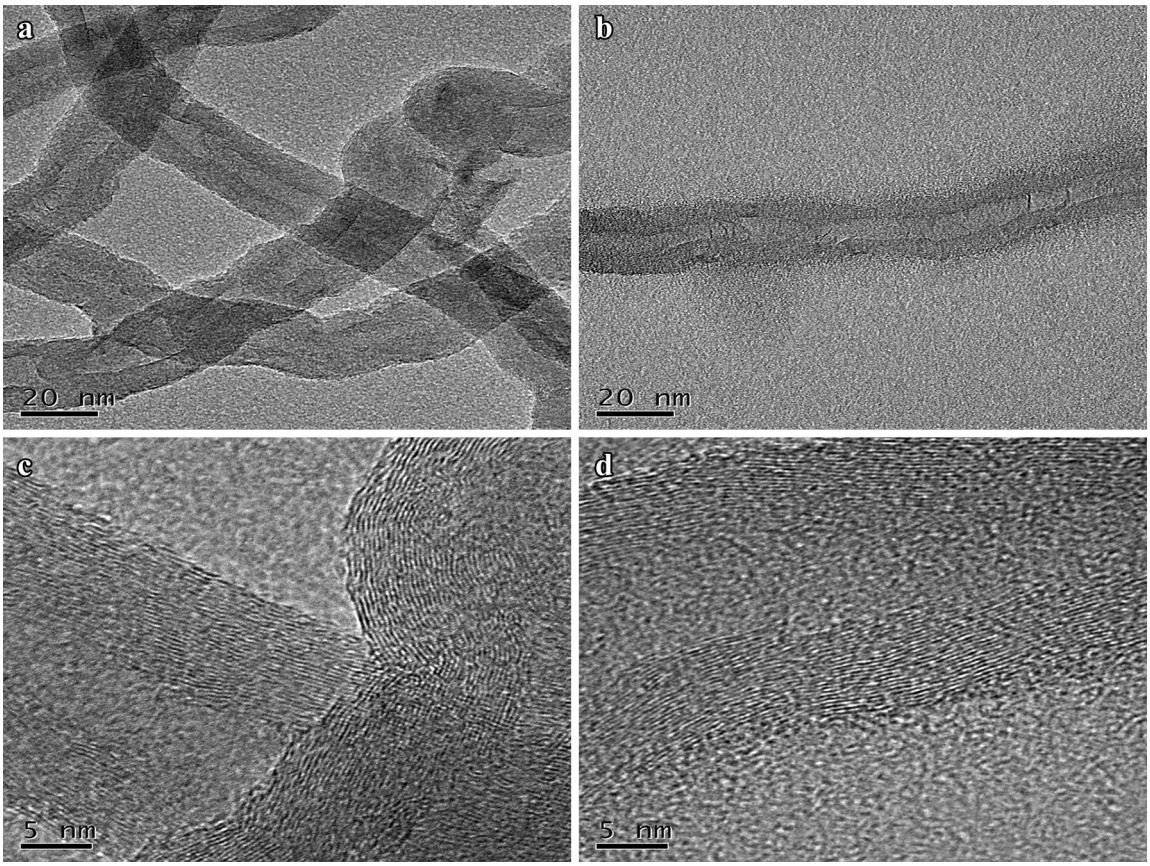

Fig. 3 TEM of MWCNTs (**a**, **c**) and Cl/MWCNTs (**b**, **d**)

Electrochemical behavior of the proposed bi-enzyme biosensor

The electrochemical properties of the different modified materials were tested by the CV method. Bare GCE, OH-MWCNTs/GCE, Cl/MWCNTs/GCE, and Cl/MWCNTs/HRP/AChE/GCE sensors were sequentially subjected to CV scans in PB solution (0.1 mmol/L, pH7.0) containing 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl. The results are shown in Fig. 4. A pair of well-defined redox peaks were observed for all modified electrodes in the range of − 0.6 to 0.2 V. Compared to the bare electrode, the electrode current modified by OH-MWCNTs was increased by 28 μA (Fig. 4, curve a, b), while the current value was increased by 46 μA (Fig. 4, curve a, c) after modification by Cl/MWCNTs, indicating that the electrochemical properties of the sensor were significantly enhanced due to ionic liquid modification. The combination of Cl/MWCNTs and bi-enzyme not only improved the sensitivity of the biosensor but also enhanced the stability of the sensor due to the wide electrochemical stability window, high electron transfer rate, and excellent solubility and adhesion of the ionic liquid. When the surface of the Cl/MWCNTs/GCE electrode was modified

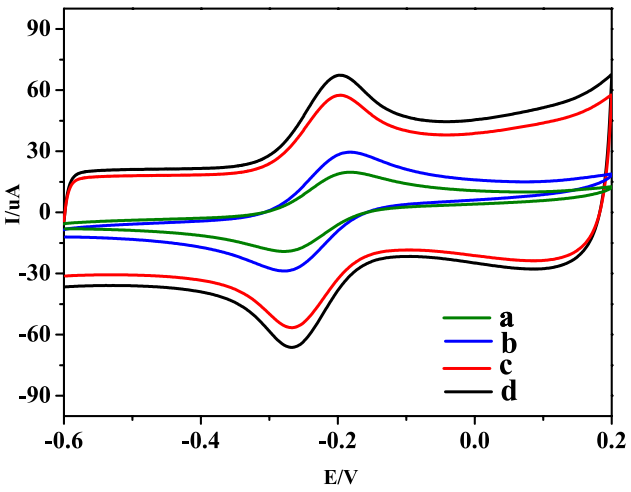

Fig. 4 CV of (a) Bare/GCE (b) OH-MWCNTs/GCE (c) Cl/MWCNTs/GCE (d) Cl/MWCNTs/AChE/HRP/GCE in the PB (0.1 mmol/L pH 7.0) containing 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl

with AChE and HRP (Fig. 4, curve e), the peak value of the sensor current dropped sharply (Fig. 4, curve d). This was because AChE and HRP are protein biomacromolecules with

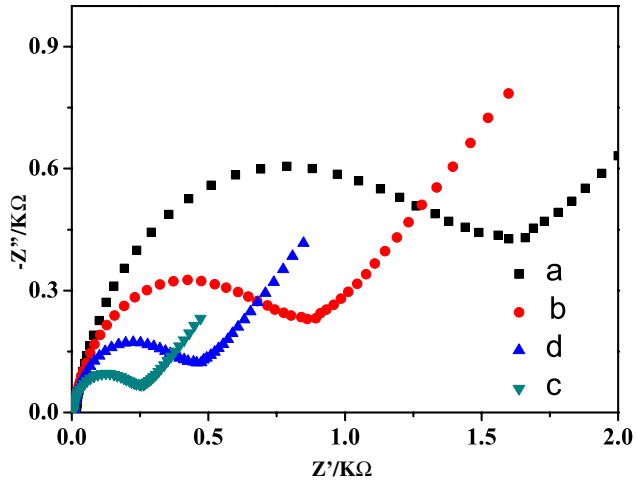

Fig. 5 EIS of (a) Bare GCE (b) OH-MWCNTs/GCE (c) Cl/MWCNTs/GCE (d) Cl/MWCNTs/HRP/AChE/GCE in the PB (0.1 mmol/L pH 7.0) containing 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl

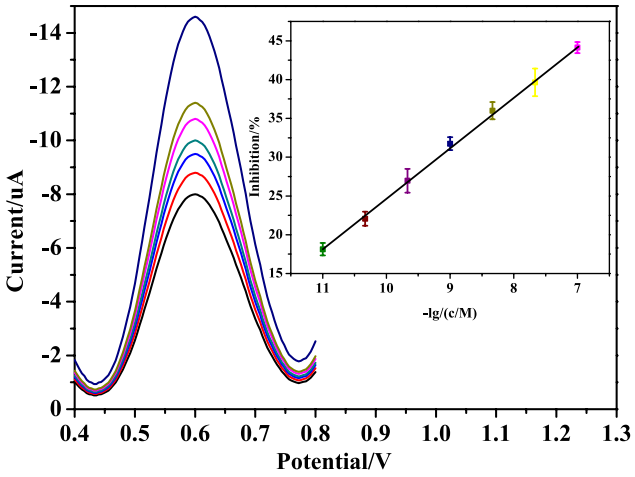

Fig. 6 DPV of the Cl/MWCNTs/HRP/AChE/GCE biosensor in PB (0.1 mmol/L pH 7.0) containing 1.0 mmol/L ATCl after incubation with MPs for 10 min. Inset: Relationship between biosensor inhibition rate and MPs

relatively poor conductivity and large loading dose (0.3 U AChE, 0.25 U HRP) but the final peak current of the sensor still remained higher (30 μA).

The interface characteristics during the charge transfer process of the sensor were investigated using electrochemical impedance spectroscopy (EIS). Figure 5 shows the impedance spectra of bare GCE, OH-MWCNTs/GCE, Cl/MWCNTs/GCE, and Cl/MWCNTs/AChE/HRP/GCE in PB solution (0.1 mol/L, pH 7.0) containing 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl. The bare electrode showed the largest semicircle and its impedance value was about 1875 Ω (Fig. 5, curve a). The impedance values of OH-MWCNTs/GCE (Fig. 5, curve b) and Cl/MWCNTs/GCE (Fig. 5, curve c) were about 977 Ω and 107 Ω, respectively. The impedance value increased sharply after the AChE/HRP bi-enzyme was added. This was ascribed to the poor conductivity of the biomolecule enzyme that inhibited the electron transfer. However, the final impedance of the Cl/MWCNTs/AChE/HRP/GCE sensor remained relatively low (583 Ω). The results of CV and EIS scans indicated the successful preparation of Cl/MWCNTs/AChE/HRP/GCE biosensors. Furthermore, the use of Cl/MWCNTs as carrier materials significantly improved the electrochemical performance of the sensor.

Under the optimal experimental conditions, the prepared Cl/MWCNTs/HRP/AChE/GCE sensors were used to test different concentrations of the MPs in a standard curve. Different modified electrodes were subjected to DPV scans in PB solution (0.1 mmol/L, pH 7.0) containing 1.0 mmol/L ATC1. The oxidation peaks were all observed at 0.6 V and the peaks were inhibited to some extent when the sensor was incubated for 10 min in different concentrations of MPs solution. As shown in Fig. 6, the inhibition rate of the sensor increased as the concentration of MPs increased. The figure inset shows the relationship between the concentration of MPs and the inhibition rate. The inhibition rate of the sensor and the negative logarithm of the MPs concentration were linear in a concentration range from 1.0×10^{-11} to 1.0×10^{-7} mol/L. The linear equation was $y(\%)=1.857–7.7857$ lgC ($R^2=0.9987$). According to the detection limit calculation formula (LOD = 3 × SD/slope, where SD was standard deviation), the detection limit was 4.5×10^{-12} mol/L. Compared to the values in Table 1 [11, 13, 23–25], the Cl/MWCNTs/HRP/AChE/GCE sensor in this test exhibited a wider detection range and a lower detection limit, which were mainly attributed to the excellent electrical properties of the Cl/MWCNTs carrier material and oxidative properties of HRP.

Stability analysis of the proposed bi-enzyme biosensor for MPs

First, the reproducibility of the sensor measurements was determined in 1.0 mmol/L ATCl solution. The same sensor was continuously tested five times in the same concentration of ATCl solution. The relative standard deviation (RSD) remained within 3.0%, indicating that the Cl/MWCNTs/HRP/AChE/GCE sensor had good reproducibility. In general, the enzyme tended to fall off from the modified material when the electrode changed from one to another solution due to weak surface tension [26]. In the present study, the ionic liquid-modified MWCNTs provided excellent adsorption of the enzymes, thereby improving the repeatability of the sensor.

Second, six identical Cl/MWCNTs/HRP/AChE/GCE sensors were prepared under the same conditions as the

Table 1 Comparison with other reported biosensors for monocrotophos detection

Biosensors	Detection methods	Detection limit (mol/L)	Refs
SBA-15@AChE	Electrochemical	2.5×10^{-9}	[23]
Nafion/AChE-cMWCNT/ MWCNT/Au	Electrochemical	2.3×10^{-9}	[11]
MISPE-FICL	Chemiluminescence	4.5×10^{-6}	[24]
Cl/MWCNTs/AChE/GCE	Electrochemical	3.3×10^{-11}	[13]
Cl/FePP-MWCNTs/AChE/GCE	Electrochemical	3.2×10^{-11}	[25]
Cl/MWCNTs/AChE/HRP/GCE	Electrochemical	4.5×10^{-12}	This work

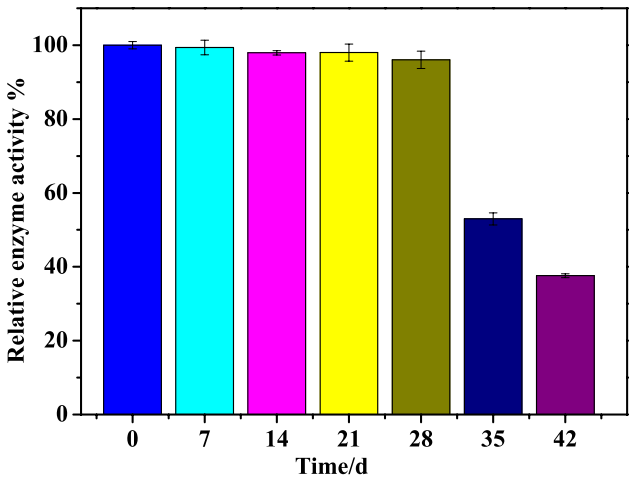

Fig. 7 Enzyme activity rate of Cl/MWCNTs/HRP/AChE/GCE compared with the initial peak current in the PB (0.1 mmol/L pH 7.0) containing 1.0 mmol/L ATCl at 4 °C

reproducibility test. The RSD of the electrode detection value was 5.6%, indicating that the sensor performed than other AChE sensors.

Finally, the storage stability of the sensor at 4℃ was studied. As shown in Fig. 7, the oxidation current slightly decreased after one week. Specifically, the current remained at 96.5% of its original value after four weeks. The results showed that the Cl/MWCNTs/HRP/AChE/GCE was rather stable.

Analysis of MPs in cabbage sample

The practical application of the prepared sensor was investigated using spike and recovery experiments. Green vegetable samples were pretreated according to the method in the section "Sample analysis". A certain amount of high concentration MPs standard solution was added to the green vegetable extract to obtain concentrations of 1.0×10^{-8}, 1.0×10^{-9}, 1.0×10^{-10} mol/L MPs test solution. Electrochemical DPV was carried out after the modified electrode was incubated for 10 min in the prepared MPs solution. The MPs content was calculated according to the pesticide standard curve corresponding to the obtained current peak. As shown in Table 2, the recovery rate of MPs was 83.0–104.0%. The sensor measurements were close to the actual value, which proved that the sensor was a good prospect for practical application.

Conclusion

In this experiment, a novel Cl/MWCNTs/HRP/AChE/GCE electrode constructed combining HRP and Cl/MWCNTs was used to detect MPs residues in agricultural products. The bi-enzyme sensor had a wide detection range of 1.0×10^{-11} to 1.0×10^{-7} mol/L and low detection limit of 4.5×10^{-12} mol/L. Storage stability experiments showed that the activity of the sensor remained above 96.5% after four weeks. The recovery of Cl/MWCNTs/AChE/HRP/GCE was between 83.0% and 104.0% in actual sample testing. The sensors prepared in this experiment were simple, rapid, and sensitive. The Cl/MWCNTs not only provided a large surface area on which to immobilize protease but also accelerated electron

Table 2 Determination of MPs in the vegetable sample

Biosensor	Sample	Added (mol/L)	Found (mol/L)	Mean recovery (%)	RSD (%) ($n=3$)
Cl/MWCNTs/HRP/AChE/GCE	1	1.0×10^{-8}	1.04×10^{-8}	104.0	4.4
	2	1.0×10^{-9}	0.83×10^{-9}	83.0	3.9
	3	1.0×10^{-10}	0.92×10^{-10}	92.0	3.2

transfer. In addition, the oxidation performance of HRP greatly enhanced the sensitivity of the entire sensor. Based on the above advantages, the Cl/MWCNTs/HRP/AChE/GCE biosensor had great application prospects as an actual MPs detection system in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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