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A Rapid Detection of Pesticide Residue Based on Piezoelectric Biosensor

Zhan-jiang Shang,[a, b] Yan-li Xu,[a, *] Yun-xu-zi Gu,[a, b] Yi Wang,[c] Dong-xu Wei,[b] Li-li Zhan[a]

[a] *Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China*

[b] *Graduate School, Northeast Agricultural University, Harbin 150036, China*

[c] *Rutgers University, NewJersey 08901, USA*

Abstract

A piezoelectric biosensor made of macromolecular polymer and carboxyl multi-wall carbon nanotubes (MWNTs-COOH) coated with acetylcholinesterases (AChE) on the Ag-coated crystal surfaces was used to determine pesticide residue in freshly picked radishes 4 and 8 days post application of phoxim and chlorpyrifos. The sensitivity of the method was compared with gas chromatography. The results show that there is no significant difference between the two methods, which indicates that our method can be used to perform the same task as gas chromatography in analyzing pesticide residue. The device is more portable, less expensive and simpler to operate, which can be easily used for pesticide residue detection.

Keywords: piezoelectric biosensor; carbon nanotubes; electrostatic assembly; pesticides; pollution

1. Introduction

Organophosphates pesticides, which are commonly used in agricultural practice, are highly neurotoxic to human beings and other animals in our ecosystems[1-3]. Gas chromatography (GC), liquid chromatography (LC) or combinations (GC-MS or LC-MS/MS) are the traditional analytical techniques for identification and quantity determination of pesticide residue. Nonetheless, the heavy and expensive equipment, the time-consuming and complex of the procedures and the requirement of highly skilled

* Corresponding author. Tel.: +86-451-86602919; fax: +86-451-86603736.
E-mail address: xyll@neigaehrb.ac.cn.

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technicians made these methods unsuitable for field usage. For the past few years, various inhibition and non-inhibition biosensor systems based on the immobilization of acetylcholinesterase (AChE) or organophosphorus hydrolase onto various electrochemical, piezoelectric or optical transducers, have been used to detect organic phosphorous residue[4]. Many studies have focused on two techniques, which are to enhance the sensitivity, stability, and reproducibility for the biosensor and to improve the efficiency of enzyme immobilization.

Since their discovery in 1991, carbon nanotubes (CNTs) have been investigated extensively due to their unique structural, electrical, chemical, and mechanical properties[5]. Carbon nanotubes, which posse sufficient thermal conductivity and enormous specific surface areas (SSA), have been applied in electrochemical biosensor in recent years[6]. Great progress in nanoscience and nanotechnology has opened new opportunities in domain of biosensor.

In this paper, we report a piezoelectric biosensor for detection of organic phosphorous pesticide residue based on the surface of silver electrodes absorption of macromoleclar polymer, MWNTs-COOH via the layer-by-layer (LbL) assembly technique. The changes of the quality of surface for silver electrodes were monitored by quartz crystal microbalance (QCM) for the whole test. The sensitivity was compared with GC system in detecting pesticide residue of phoxim and as well as chlorpyrifos.

2. Materials and Methods

2.1. Reagent

Acetylcholinesterases (AChE, EC Nmber: 3.1.1.7), poly (styrene sulfonate) (PSS, Typical Mw = 70000) and poly (diallyldimethylammonium chloride) (PDDA, Typical Mw = 200000–350000) were purchased from Simga-Aldrich, Inc., USA. Multi-walled carbon nanotubes (MWNTs) (30 – 50 nm in diameter; SSA ~300 $m^2 \cdot g^{-1}$ and 95% purity) were obtained from Shenzhen Nanotech Port Co., Ltd., China. Standard preparation of phoxim and chlorpyrifos were purchased from Institute of Environmental Protection Agriculture Ministry (AEPI), China.

2.2. Method

$(PDDA/PSS)_{3.5}$/MWNTs-COOH was alternatively assembled via the LbL electrostatic assembly technique on the surfaces of silver electrodes. Scanning electron microscopic (SEM) was used to characterize distribution for MWNTs-COOH. Then, AChE was immobilized on the surfaces of MWNTs-COOH by the cross-linking method as recognizer component to detect pesticides (Fig. 1). QCM as described below was used to monitor the vibration frequency of silver electrodes.

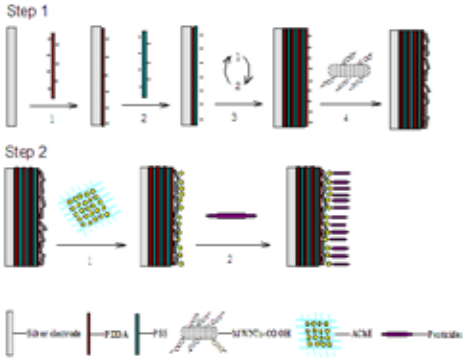

Fig. 1. The process of the electrode assembly and pesticide detection.

2.2.1. Quartz crystal microbalance (QCM) technique

Quartz crystals (9 mm diameter) were coated on both sides with silver electrodes 4.5 mm in diameter. The frequency was monitored by a Protek frequency counter (Model C3100). The amount of polyelectrolytes adsorbed, Δm, was calculated from the frequency decrease of the quartz crystal microbalance (QCM), ΔF, using Sauerbrey[7] equation:

$$-\Delta F = \frac{2F_0^2}{A\sqrt{\rho_q \mu_q}} \times \Delta m$$

F_0 is the parent frequency of QCM (9×10^6 Hz); A is the electrode area (0.16 cm^2); ρ_q is the density of the quartz (2.65 $g\cdot cm^{-3}$); and μ_q is the shear modulus (2.95×10^{11} $dyne\cdot m^2$). Simplified equation can be expressed as:

$$-\Delta F = 1.154 \times \Delta m$$

2.2.2. Preparation of carboxyl multi-wall carbon nanotubes

Multi-walled carbon nanotubes (MWNTs) were ultrasonicated in a 3:1 v/v solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) for 8 h at 35-40 °C followed by extensive washing and filtration in deionized water until the filtrate was neutral. The negatively charged MWNTs were centrifuged at 14000 rpm for 30 min to remove the supernatant and dried in a vacuum dryer at 50 °C. The nanotubes then were stored at room temperature for later usage[8].

2.2.3. Preparation of $(PDDA/PSS)_{3.5}$/MWNTs-COOH multilayer surfaces

The build-up processes of $(PDDA/PSS)_{3.5}$/MWNTs-COOH multilayer films on the Ag-coated crystal surfaces were described as follow: Before the deposition of the initial PDDA layer, the silver electrode was first rinsed with deionized water, dried with a stream of nitrogen gas, immersed in 1.0 M NaCl solution containing 1.0 mg/ml PDDA for 30 min, and dried under steady stream of nitrogen gas. The silver electrode with PDDA layer on surface was submerged in 1.0 M NaCl solution containing 1.0 mg/ml PSS for 30 min, rinsed, and dried as described above. The same procedure was repeated with the same electrode to produce the final layers of $(PDDA/PSS)_{3.5}$. Further, the Ag-coated crystal electrode was immersed in 0.3 mg/ml MWNTs-COON solution for 30 min to coat the surface with negatively charged MWNTs for the final binding of AChE (Fig. 1, Step 1).

2.2.4. AChE enzyme immobilization

As suggested by Sigma, 0.02M (pH 7.0) phosphate buffers (PBS) was used for 0.1 mg/ml AChE enzyme solution as optimum. First, 0.1 mg/ml bovine serum albumin (BSA) was mixed with 0.1 mg/ml of AChE in 3 ml PBS solution before 1 ml of 5% glutaraldehyde aqueous solution was added. Second, the modified Ag-coated quartz crystal electrodes were immersed in the above mixed solution for 30 min at room temperature to allow the binding of AChE to the surface of the electrodes. Third, the silver electrode was washed with 0.1M PBS (pH 7.0) to remove the free AChE. The finalized electrodes were stored at 4°C for later usage (Fig. 1, Step 2)[3, 9].

2.2.5. Pesticide residue analysis with QCM and GC

Radishes were planted in the flowerpot with inner diameter for 17 cm and depth for 13 cm at greenhouse. The radishes leaves were sprayed with chlorpyrifos (500 ng/L) or phoxim (200 ng/L) 20 days post-sowing. The whole plants including roots and leaves were sampled 4 and 8 days after the spray for residue analysis by QCM and GC. The procedure of sample preparation for the analysis was the same for QCM and GC. First, surface soil and dirt were removed from the whole pant by rinsing in deionized water. The plants then were chopped into pieces (about 1mm in length) and mixed before weighing. Five grams of the mixed sample were homogenized in 20ml ethyl acetate and centrifuged at 3500rpm for 5min.

The supernatant was transferred into a conical flask and pallet was extracted one more time with the same amount of ethyl acetate. The supernatant from the first and second extraction were mixed and dried with a rotary evaporator. The dried material in the treatment group was dissolved in 10 ml deionized water for QCM detection and the dried material in the control group was dissolved in 5ml ethyl acetate for GC analysis. Standard curve for chlorpyrifos or phoxim was established with different concentration (10, 30, 50, 100, 300 ng/L) of the active ingredient.

2.2.6. Data Processing for QCM

The change of vibration frequency (ΔF) was calculated by $\Delta F = F_2 - F_1$, where F_1 represents before and F_2 represents after the absorption of chlorpyrifos or phoxim by the surfaces of silver electrodes. The standard curve established above was used to calculate the pesticide residue.

3. Results and discussion

3.1. Characterization of the surface of carboxylic MWNTs

Scanning electron microscopic (SEM) result showed that MWNTs was distributed on the entire surface of PDDA (Fig. 2), which indicated that MWNTs strongly bound to the surface of PDDA via electrostatic interaction. The diameter of MWNTs was 30-50 nm approximately, which was consistent with the previous observation for Shenzhen Nanotech Port Co., Ltd.

3.2. Comparison of different surfaces for AChE absorption

Four electrodes with different surfaces. Electrode: bare surface; PSS: the surface was coated with $(PDDA/PSS)_4$; PDDA: the surface was coated with $(PDDA/PSS)_{3.5}$; MWNTs-COOH: the surface was coated with $(PDDA/PSS)_{3.5}$/MWNTs-COOH. AChE was immobilized on the surface of the four electrodes using the procedure described in 2.2.4. Before and after the AChE was immobilized, frequency shift (ΔF) was measured by QCM. The results indicated that ΔF for each of the four electrodes declined by 125 Hz，320 Hz，1841 Hz，and 2483 Hz respectively (Fig. 3). The decrease of vibration frequency was caused by increased surface area of electrode.

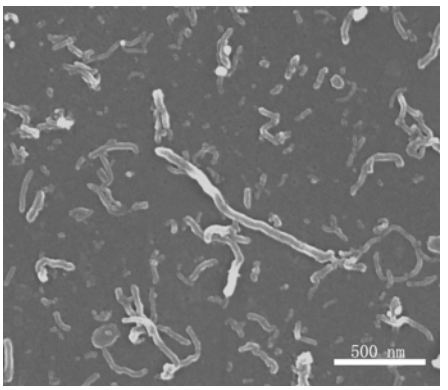

Fig. 2. SEM image silicon slide coated with $(PDDA/PSS)_{3.5}$/MWNTs-COOH via layer-by-layer assembly technique.

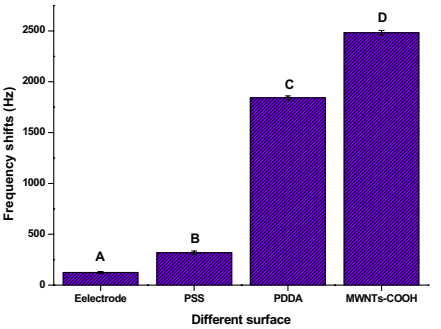

Fig. 3. Comparison of frequency shifts (ΔF) among four electrodes with different surface that fixed with AChE. Bars with the different letter were significantly different ($P < 0.01$).

3.3. Stability of $(PDDA/PSS)_{3.5}$/MWNTs/AChE

The enzyme coated electrodes were tested for frequency shift (ΔF) after different storage period (12, 24, 36, 48, 60, 72 and 84 h) at 4°C. The results show that there was no significant change after 84 hr of storage (Fig. 4). The electrodes were also tested after 7 days of storage at the same condition, where no significant change was observed (data not shown), which indicates that the fixed AChE is stable. We suggest the same procedure can be used for different enzymes to identify different substances.

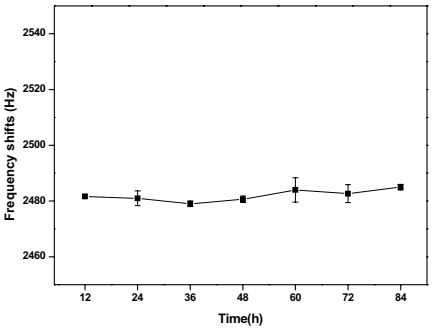

Fig. 4. The frequency shifts (ΔF) of the enzyme electrodes after different periods (12, 24, 36, 48, 60, 72 and 84 h) of storage at 4°C.

3.4. Detection of pesticide residues

Specimens which were treated in 2.2.5 were absorbed on enzyme electrodes, three repetitions each sample. Frequency shift (ΔF) of enzyme electrodes was monitored by QCM. The concentration of chlorpyrifos or phoxim was calculated by standard curve (Fig. 5). The QCM and GC results shown that there was no significant difference between the two testing system for either phoxim or chlorpyrifos residue in the sample collected from the same day (Tab. 1), which indicates that our QCM system has the same sensitivity with GC system. Considering its cost efficiency, simplicity, portability, and sensitivity to pesticide residue analysis, QCM possess a great potential in the market where onsite testing is needed.

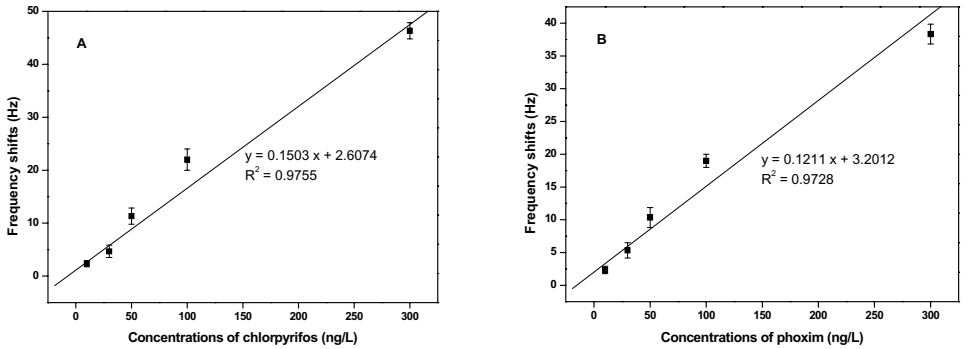

Fig. 5. Standard curve of frequency shifts (Δf) established with different concentrations (10, 30, 50, 100, 300 ng/L) of active ingredient for chlorpyrifos (A) and phoxim (B) in pH 7.0 orthophosphoric acid buffer solutions using the electrodes coated with $(PDDA/PSS)_{3.5}$/MWNTs-COOH/AChE.

Tab. 1. The results of residue analysis with Quartz Crystal Microbalance (QCM) and Gas Chromatography (GC) 4 and 8 days post-application of chlorpyrifos (500 ng/L) or phoxim (200 ng/L).

Pesticides name	Pesticide residues (ng/L)			
	4 days post application		8 days post application	
	GC	QCM	GC	QCM
chlorpyrifos	90.20 ±0.09	86.88±3.84	49.73±0.03	51.40±3.84
phoxim	100.74±0.05	97.43±8.26	67.72±0.03	67.15±4.76

4. Conclusions

We have developed a procedure to make a novel piezoelectric biosensor for detection of pesticide residues in soil, water, or food using multi-walled carbon nanotubes. Our test result has shown that the biosensor possesses similar sensitivity and accuracy with Gas Chromatography (GC), and has a great potential to be used in rapid detection of pesticide residues.

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References

[1] Zourob M, Ong KG, Zeng KF, Mouffouk F, Grimes CA. A wireless magnetoelastic biosensor for the direct detection of organophosphorus pesticides. *Analyst* 2007; **132**: 338-343.

[2] USEPA. *Preliminary Cumulative Risk Assessment of the Organophosphorus Pesticides*. Washington: DC; 2001.

[3] Viswanathan S, Radecka H, Radecki J. Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA. *Biosens. Bioelectron.* 2009; **24**: 2772-2777.

[4] Halamek J, Pribyl J, Makower A, Skladal P, Scheller FW. Sensitive detection of organophosphates in river water by means of a piezoelectric biosensor. *Anal. Bioanal. Chem.* 2005; **382**: 1904-1911.

[5] Iijima S. Helical microtubules of graphitic carbon. *Nature* 1991; **354**: 56-58.

[6] Komathi S, Gopalana AI, Lee KP. Covalently linked silica-multiwall carbon nanotube-polyaniline network: An electroactive matrix for ultrasensitive biosensor. *Biosens. Bioelectron.* 2009; **25**: 944-947.

[7] Sauerbrey GZ. The use of quartz oscillators for weighing thin layers and for microweighing. *Phys.* 1959; **155**: 206-222.

[8] Liu LJ, Zhang F, Xi FN, Lin XF. Highly sensitive biosensor based on bionanomultilayer with water-soluble multiwall carbon nanotubes for determination of phenolics. *Biosens. Bioelectron.* 2008; **24**: 306-312.

[9] Jie GF, Li LL, Chen C, Xuan J, Zhu JJ. Enhanced electrochemiluminescence of Cd Se quantum dots composited with CNTs and PDDA for sensitive immunoassay. *Biosens. Bioelectron.* 2009; **24**: 3352-3358.