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A Portable Instrument Based on Acetylcholinesterase Biosensor for the Rapid Detection of Pesticides Residues

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Abstract: In this paper, we integrated acetylcholinesterase (AChE) biosensor and detection system to fabricate pesticides residues portable detection instrument for an on-site use. The AChE biosensor was based on the inhibition of enzyme activity that was monitored by the change of oxidation current of the thiocholine using ordinary three electrodes and an own designed potentiostat. The current signal generated from AChE biosensor was very weak, thus, signal detecting and processing module was elaborately designed to reduce lots of the noise and system bias. The response signals of different concentrations cholorpyrifos pesticide were compared with electrochemical workstation to validate the instrument. This instrument could realize the rapid detection of pesticides residues in fruits and vegetables on-site with automatic data processing, display, print out and data storage. The results showed that the detection limit was found to be 2 μg/L for cholorpyrifos and the proposed instrument had a good precision and high stability which would be a new promising rapid detection instrument for pesticides residues in agricultural products.

Keywords: Biosensor, Acetylcholinesterase, Portable instrument, Pesticide residues, Rapid detection.

1. Introduction

Pesticides were the most important agricultural production materials which widely used in agricultural production for ensuring bumper harvest of agricultural high yield. They are designed to kill or repel pests but may be harmful and fatal to other organisms, including humans. Pesticides contribute significantly to overall cancer mortality [1-3].

Monitoring of pesticides in fruit and vegetable samples has increased in the last years since most countries have established maximum residue level (MRL) for pesticides in food products [4-6].

Gas chromatography (GC), liquid chromatography (LC) or combinations (GC-MS or LC-MS/MS) are traditional analytical techniques for identification and quantity determination of pesticides residues [7-9]. Although these methods offer quantitative analysis with sensitivity and selectivity, they are slow, expensive, and laborious. Therefore, they are not suitable for rapid detection and field applications. Biosensors account for an easy method to determine pesticides [10] in environmental and food matrices [11]. The use of biosensors as screening devices is cost effective and decreases the number of samples to be analyzed by traditional analytical techniques mentioned above.

Alain Hildebrandt, *et al.* have designed and developed a portable biosensor for the analysis of organophosphorus (OP) and carbamate insecticides in water and food [12]. Gilmo Yang, *et al.* have developed an opto-fluidic ring resonator biosensor for the detection of organophosphorus pesticides [13]. Vangelis G. Andreou, *et al.* have developed a portable fiber-optic pesticide biosensor based on immobilized cholinesterase and sol–gel entrapped bromcresol purple for in-field use [14]. All these methods are highly competitive with traditional analytical techniques in terms of shorter time response and lower cost, but they lack sufficient sensitivity and repeatability, and on the other hand, rather complex procedures make them unsuitable for industrial or commercial applications.

For food safety, rapid and accurate tests are essential to allow detection of contaminated foods before they are distributed to consumers. For these reasons, a portable instrument that can be applied for on-site rapid detection of OP compounds and other pesticides is of great practical interest for overcoming such problems.

This study of the portable instrument for the pesticide residues detection was based on the analysis of OP insecticides. OP blocks the normal nervous transmission impulse by reacting, irreversibly or reversibly with the active site of the acetylcholinesterase (AChE). AChE is used as biological recognition element which can be inhibited by a few μg/L of neurotoxic agent [15].

The detection principle of biosensor is based on the changes in the activity of AChE composure to the pesticides before and after. Following enzymatically catalysed hydrolysis of acetylthiocholine, the oxidation current of thiocholine is produced and monitored by instrument [16].

Considering the purpose of detecting pesticide residues rapidly, an instrument for pesticide residues detection based on AChE biosensor with the weak current signal detecting and processing circuit was designed for replacing large-scale analysis instruments mentioned above to develop a portable detection instrument to meet requirements of pesticide residues rapid detection [17-21].

2. System Description

The detection system of this portable instrument was made up of three-electrode module based on AChE biosensor, signal detecting and processing module, printing and storage module and power supply module. The schematic detection system based on the single-chip microcomputer was shown in Fig. 1. AChE was immobilized on the working electrode and reacted with the substrate to produce the weak current signal. Three-electrode module collected weak current signal generated from AChE biosensor. The detection of the weak current signal was realized by using the signal detecting and processing module. The weak current signal generated by the AChE biosensor was transformed into 0-5 V standard voltage signal as an output signal by this module. Microcontroller played a critical role during the signal detecting and processing process. The hardware circuit of detection system was shown in Fig. 2 and the prototype of this detection system was shown in Fig. 3.

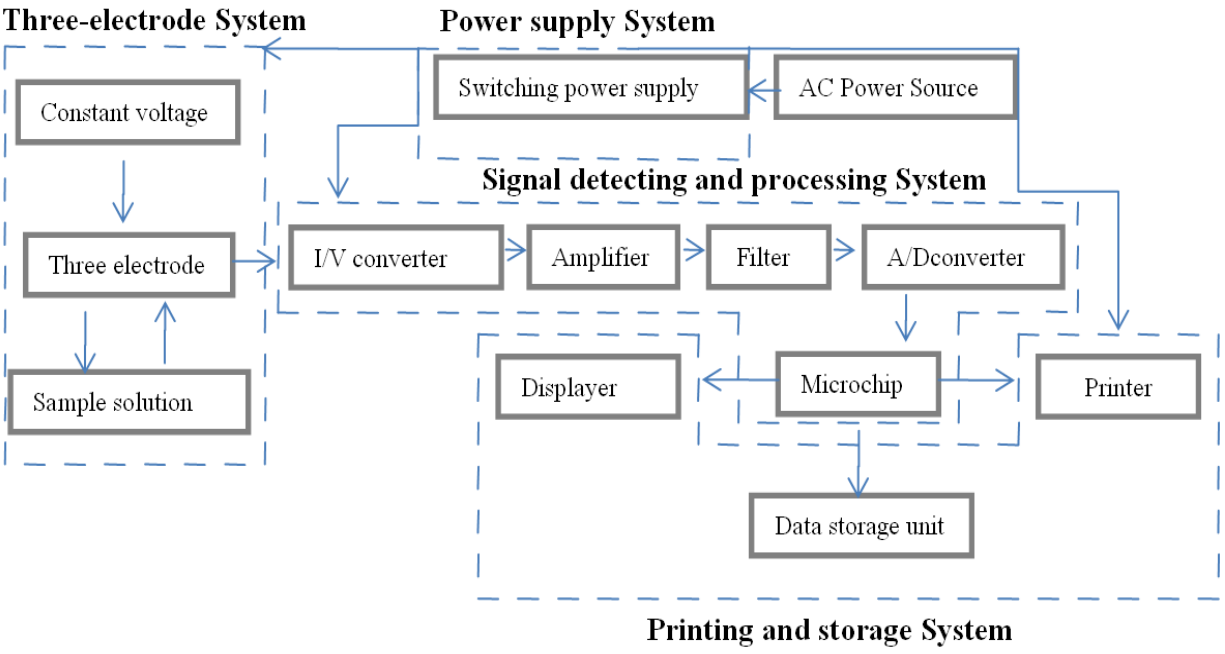

Fig. 1. Detection device structure schematic drawing.

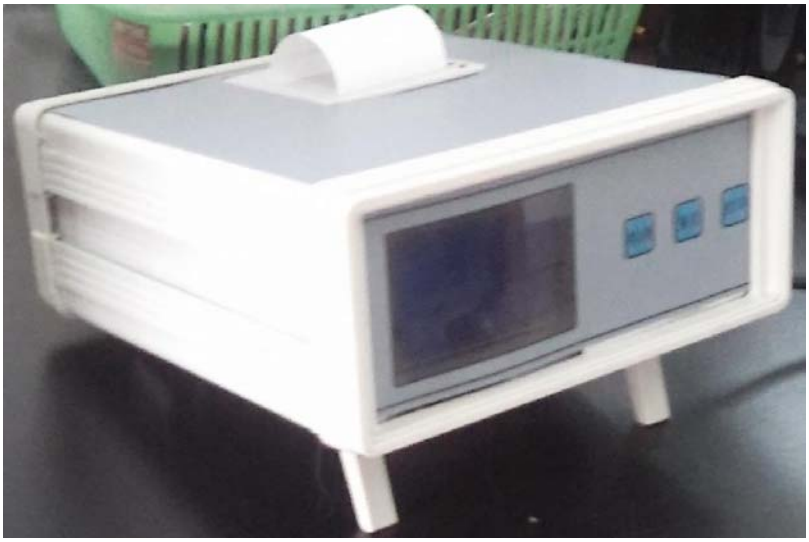

Fig. 2. The prototype of the detection system.

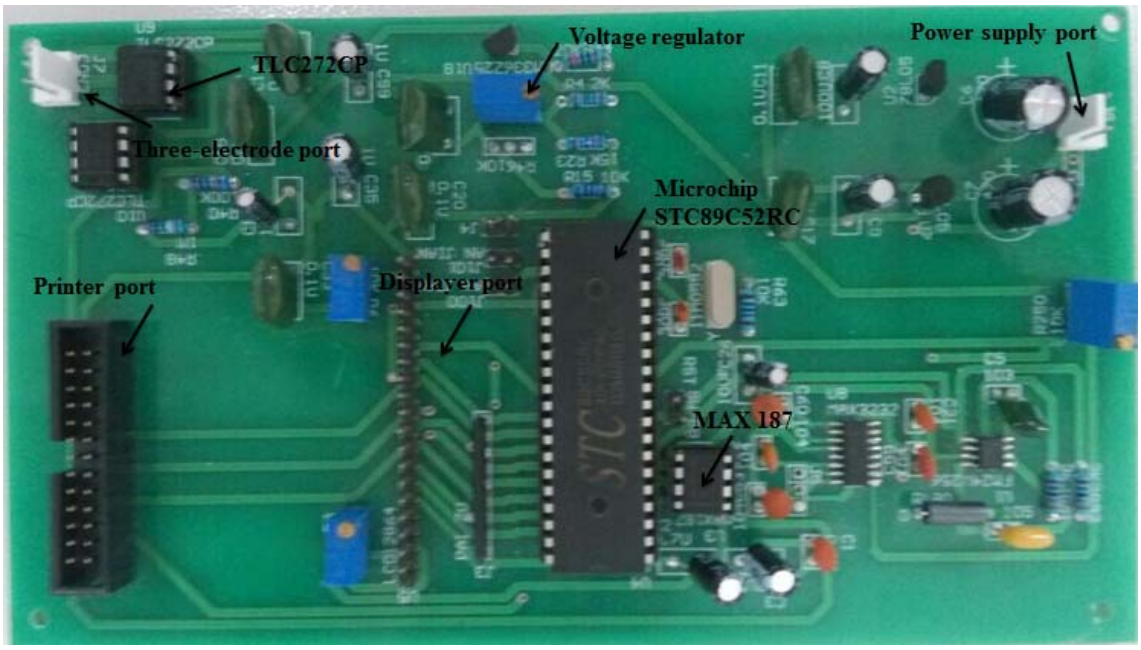

Fig. 3. Hardware circuit of pesticides residues detection instrument.

3. The Design of Main Hardware Circuit

3.1. Potentiostat

A 500 mV working potential between working electrode and reference electrode was applied by potentiostat in order to obtain higher oxidation current of thiocholine, as show in Fig. 4. A potentiostat was consisted of two TLC272CP (Analog devices) op amps due to its high input impedance, high common mode rejection ratio and low drift. Evidently, the stability of potentiostat played a crucial role in the accuracy of the detection system. The principle of the potentiostat was depended upon the equal electrical potential from the op amps (non-inverting input and inverting input) (Fig. 5a). The voltage divider circuit consisted of R4, R44 and R46. According to voltage divider equation:

$$+5V = U_4 + U_{44} + U_{46} = I \cdot (R_4 + R_{44} + R_{46}) \quad (1)$$

The potentiometer R_{46} can be seen as two resistances R_a and R_b, as shown in Fig. 6. The working potential between working electrode and reference electrode can be controlled by regulating the ratio of R_a to R_b.

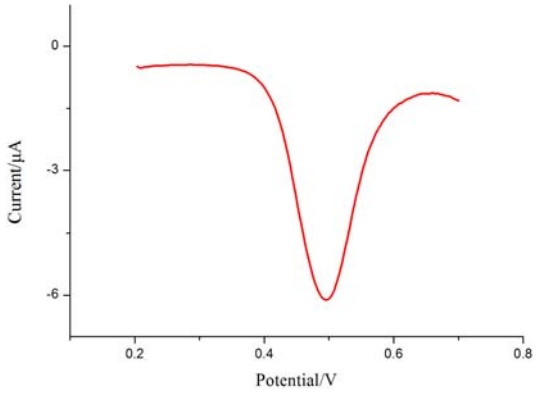

Fig. 4. The CV curve of three electrode.

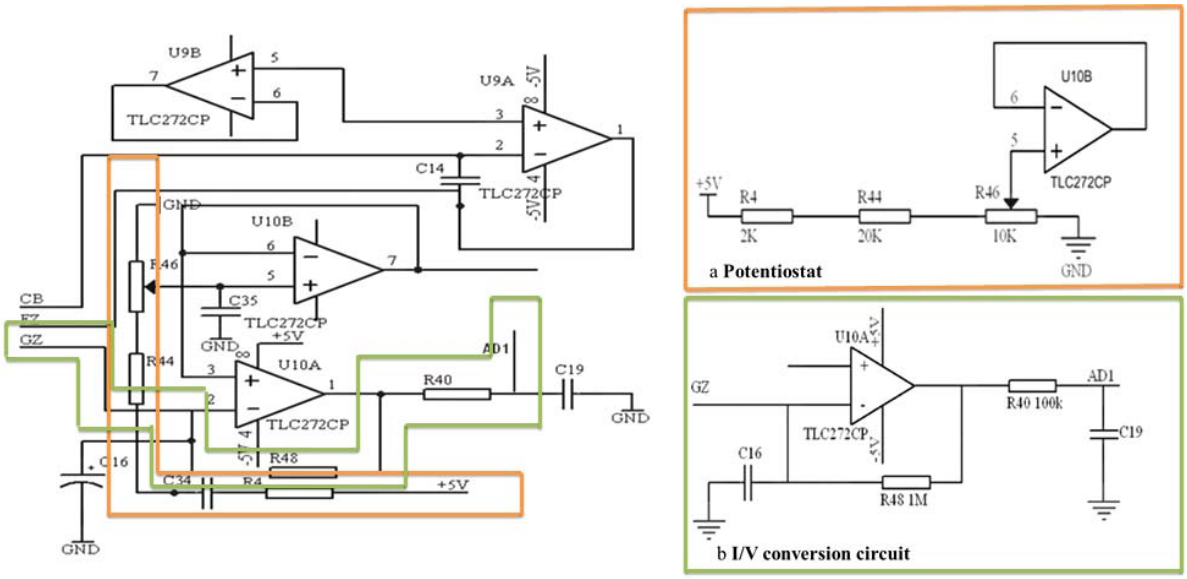

Fig. 5. Analog signal processing circuit (a Potentiostat, b I/V conversion circuit).

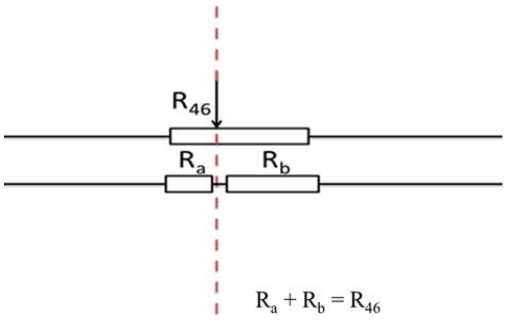

Fig. 6. Equivalent circuit of potentiometer R_{46}.

3.2. I/V Conversion Circuit

The voltage signal was detected depends upon I/V conversion circuit, the accuracy of detection was also rely on I/V conversion circuit. In fact, I/V conversion circuit changed weak current signal into standard voltage signal. I/V conversion circuit showed in Fig. 5b. The output voltage was calculated as follows:

$$V_{OUT} = -I_{in} \cdot R_f = -I_{in} \cdot R_{48}, \quad (2)$$

where I_{in} was the input weak current signal, R_f was the feedback resistance of I/V conversion circuit, V_{OUT} was the output voltage. The resistance of R_{40} was used for current-limiting and protecting amplifiers.

The weak current signal generated by enzyme electrode reaction was flowed into I/V conversion

circuit, amplifier circuit, at the same time, accompanied by different kinds of noises. To remove the 50 Hz power frequency noises or above in weak current detection was most important. For sake of collecting weak current signal, the only way was to restrain the influence of interference to the signal detection. So relevant capacitance such as C_{19} and C_{16} were designed to remove the 50 Hz power frequency noises or above.

3.3. A/D Conversion Circuit

This circuit was used for converting analog signal into digital signal and then transmitted digital signals into the microchip circuit which controlled every detection modules, as shown in Fig. 7. Op amp (MAX 187, Maxim, USA) was selected as the ADC converter which has high conversion accuracy. The design of A/D conversion circuit was shown in Fig. 7.

3.4. Digital Signal Processing Circuit

Microchip was used to control and respond all modules and the three buttons (Detect, Reset, and Print) were used to send commands to microchip. During the detection process, microchip was used as cores for the Digital signal processing circuit. Firmware was the program running on the microchip, which interacted with all other modules based on microchip:

1) Acquiring digital signals;
2) Processing the digital signals;
3) Encoding and transmitting the data to storage;
4) Controlling the displayer and printer.

The design of digital signal processing circuit was shown in Fig. 8.

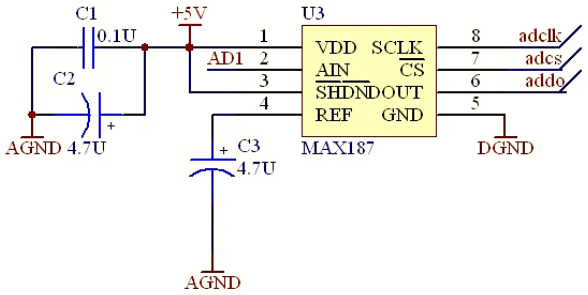

Fig. 7. A/D conversion circuit.

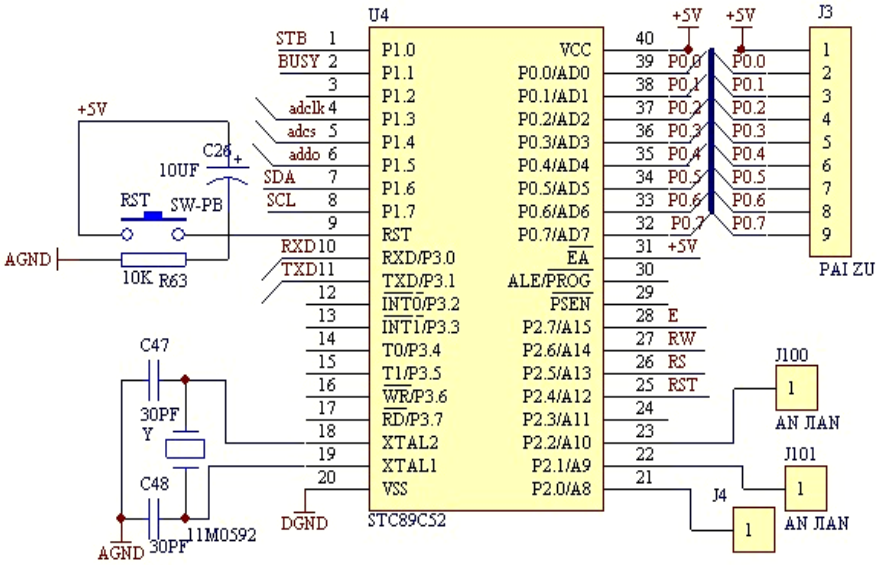

Fig. 8. Digital signal processing circuit.

3.5. Power Supply Circuit

The switching power supply with three outputs (+12V 1A, -12V 1A, +5V 5A) was used for the power supply module. The switching power supply was shown in Fig. 9. The output (+5V 5A) was used as the power source of micro-printer and the other two outputs were used for all circuits by voltage conversion circuit as shown in Fig. 10.

Fig. 9. Switching power supply.

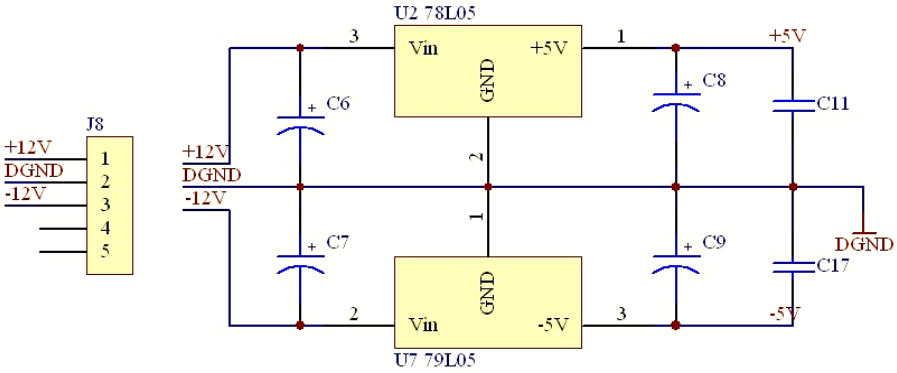

Fig. 10. Voltage conversion circuit.

4.1. Apparatus

Electrochemical measurements were performed on a CHI660D electro-chemical workstation from Shanghai Chenhua Instrument Ltd. (Shanghai, China). Three electrodes were purchased from Aida technology Co. (Tianjin, China). The working electrode was gold electrode (d=1 mm). A saturated calomel electrode (SCE) and platinum electrode were used as reference and auxiliary electrodes, respectively. Pesticides residues detection instrument was made in our laboratory.

4.2. Reagents and Materials

Acetylcholinesterase (Type C3389, 500 U/mg from electric eel), acetylthiocholine chloride (ATCl) and chlorpyrifos were purchased from Sigma (USA). SnO_2 were obtained from Sinopharm Chemical Reagent Co., Lid. Multiwall carbon nanotubes (MWNTs) (purity>95 %) was purchased from Shenzhen Nanotech Port Company (China) and chitosan (CHIT) was from Shanghai Chemical Reagent Company (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. Other reagents were of analytical grade. All solutions were prepared using double distilled water.

4.3. Preparation of Nafion/AChE/MWNTs-SnO_2- CHIT/Au Biosensor

The Au electrode surface was freshly polished with 0.3 μm and 0.05 μm alumina powder, respectively, and then rinsed with ultrapure water after each polishing, finally cleaned ultrasonically with 95 % ethanol and acetone for 3 min, respectively. The SnO_2 nanoparticles and MWNTs with a mass ratio of 1:3 were dispersed in 0.2 % CHIT solution and stirred at room temperature for 3 h. The obtained highly dispersed black suspension would be named as MWNTs-SnO_2-CHIT. A 2.5 μm of MWNTs-SnO_2-CHIT suspension was coated on the Au electrode surface and air dried naturally to obtain MWNTs-SnO_2-CHIT/Au. Similarly, SnO_2-CHIT/Au and MWNTs-CHIT/Au were prepared under the same procedure as illustrated in MWNTs-SnO_2-CHIT/Au preparation just without MWNTs or SnO_2 existing, respectively. The obtained electrode (MWNTs- SnO_2-CHIT/Au) was washed thoroughly with ultrapure water and then dried in air at room temperature. After the water was evaporated, MWNTs-SnO_2-CHIT/Au was coated with 2.5 μL

AChE solution to obtain the AChE/MWNTs-SnO_2-CHIT/Au. Finally, the AChE/MWNTs-SnO_2-CHIT/Au electrode was coated with an extra 2.5 μL 0.5 % Nafion to maintain the stability of modified electrode.

4.4. Electrochemical Detection of Pesticides

For the measurement of pesticides, the obtained AChE/MWNTs-SnO_2-CHIT/Au was first immersed in pH 7.5 PBS containing different concentrations of standard pesticides solution for 10 min, and then transferred to the electrochemical cell of pH 7.5 PBS containing 2 mM ATCl to study the electrochemical response by cyclic voltammetry (CV) between 1.0 and 0 V. The inhibition rate of pesticides was calculated as follows:

$$\text{Inhibition } (\%) = (I_0 - I_1) / I_0 \times 100\ \%, \quad (3)$$

where I_0 and I_1 was the oxidation current, before and after biosensor exposure to pesticides, respectively. The pesticide residues concentration could be got from the linearity between inhibition rate and pesticide concentration.

5. Results and Discussion

5.1. The Detection of the Certain Concentration Pesticide

In order to evaluate the applicability and superiority of the proposed detection instrument, interference indictors were added into the pesticides solutions to investigate their effect on the current intensity. To further demonstrate the practicality of the proposed detection instrument, by using the mentioned detection instrument and electrochemical workshop at the same time, 50 data were acquired from one kind of standard pesticide in different concentrations which were 5 types: recorded with 2 μg/L, 5 μg/L, 10 μg/L, 100 μg/L and 200 μg/L standard pesticides, as shown in Table 1. The pesticide inhibition rate obtained from test results was 18 %-46 %.

Table 1. Inhibition rates of a standard pesticide in different concentrations detected by electrochemical workstation and pesticide residues detection instrument.

Detection method	No.	2 μg/L	5 μg/L	10 μg/L	100 μg/L	200 μg/L	Detection cycle
Electrochemical workstation (Inhibition ratio)	1	24.31	24.32	25.26	35.77	45.20	1 min
	2	24.45	24.03	24.87	34.56	44.98	
	3	24.64	25.00	25.20	34.7	45.12	
	4	24.37	24.9	25.27	34.82	45.21	
	5	23.22	25.33	25.25	35.07	45.19	
Pesticide residues detection instrument (Inhibition ratio)	1	19.96	20.49	21.39	29.97	41.20	25 s
	2	19.63	20.12	20.39	29.78	40.89	
	3	19.94	19.15	20.33	30.03	40.09	
	4	20.01	20.78	20.49	30.49	41.06	
	5	19.44	19.96	20.94	31.03	40.94	

Table 1 was showed the detection results in different concentrations by using mentioned instrument compared with electrochemical workshop. Electrochemical analysis instrument was not a specific rapid detection instrument but a universal instrument. Data from Table 1 was concerns rapid pesticides residues detection. It could be shown that the standard pesticides could be evaluated by the pesticides residues detection instrument.

5.2. Performance Evaluation

This instrument has been used to test standard pesticide and real sample to compare with electrochemical analysis method. The precision of the instrument was evaluated by analyzing five concentrations of standard chlorpyrifos samples.

The linear regression equations for the modified electrode with using different detection methods were $\Delta I\ \% = 0.10567C\ (\mu g/L) + 24.14213$(Fig. 11a) and $\Delta I\ \% = 0.10624C\ (\mu g/L) + 19.60392$ (Fig. 11b) in the range 2 to 200 μg/L. Clearly, the correlation between electrochemical analysis method and the mentioned instrument was observed to be linear with a similar slope detect for the biosensors with Nafion/AChE/MWNTs-SnO2-CHIT/Au composite film as shown in Fig. 11. This means the standard pesticides could be evaluated by the pesticides residues detection instrument and the results indicated that the instrument was suitable for direct analysis of pesticides.

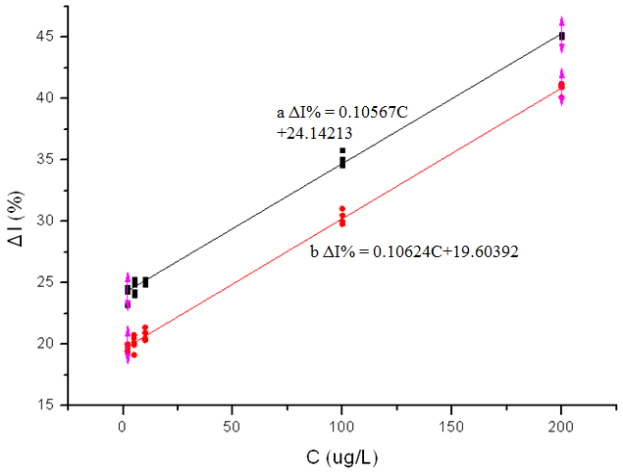

Fig. 11. Detection of pesticides residues from the standard samples collected from biosensors laboratory.
(a) Detecting the different concentrations of chlorpyrifos by using electrochemical workstation.
(b) Detecting the different concentrations of chlorpyrifos by using pesticides residues detection instrument.

The overall performance of the present instrument showed the capability of the pesticides residues detection with good sensitivity and high practical value. The limit of detection was 2 μg/L.

6. Conclusions

In this study, a portable instrument consisting of an AChE biosensor and a weak current signal detection system was developed for pesticide residues rapid detection. This instrument owned a rapid monitoring, cost effective, and can be used on-site. The system showed to be successful in screening OP pesticide in vegetables and fruits samples. For cholorpyrifos extracts, the biosensor instrument permitted to determine concentrations of 2 μg/L, thus indicating the performance of this instrument can satisfy the detection requirement of real vegetables and fruits samples. The instrument for determination of pesticide residues was also suitable for test in the field.

Acknowledgements

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References

[1]. C. W. Heath, Pesticides and cancer risk, *Cancer*, Vol. 80, Issue 10, 1997, pp. 1887-1888.
[2]. H. J. Stan, Pesticide residue analysis in foodstuffs applying capillary gas chromatography with mass spectrometric detection: State-of-the-art use of modified DFG-multimethod S19 and automated data evaluation, *Journal of Chromatography A*, Vol. 892, Issue 1, 2000, pp. 347-377.
[3]. M. Zourob, K. G. Ong, K. Zeng, *et al*., A wireless magnetoelastic biosensor for the direct detection of organophosphorus pesticides, *Analyst*, Vol. 132, Issue 4, 2007, pp. 338-343.
[4]. G. O. Guler, Y. S. Cakmak, Z. Dagli, *et al*., Organochlorine pesticide residues in wheat from Konya region, Turkey, *Food and Chemical Toxicology*, Vol. 48, Issue 5, 2010, pp. 1218-1221.
[5]. A. R. Boobis, B. C. Ossendorp, U. Banasiak, *et al*., Cumulative risk assessment of pesticide residues in food, *Toxicology Letters*, Vol. 180, Issue 2, 2008, pp. 137-150.
[6]. A. N. Ivanov, L. V. Lukachova, G. A. Evtugyn, *et al.*, Polyaniline-modified cholinesterase sensor for pesticide determination, *Bioelectrochemistry*, Vol. 55, Issue 1, 2002, pp. 75-77.
[7]. A. Agüera, M. Contreras, A. R. Fernandez-Alba, Gas chromatographic analysis of organophosphorus pesticides of horticultural concern, *Journal of Chromatography A*, Vol. 655, Issue 2, 1993, pp. 293-300.
[8]. J. Ye, J. Wu, W. Liu, Enantioselective separation and analysis of chiral pesticides by high-performance liquid chromatography, *TrAC Trends in Analytical Chemistry*, Vol. 28, Issue 10, 2009, pp. 1148-1163.
[9]. H. Guan, W. E. Brewer, S. T. Garris, *et al*., Disposable pipette extraction for the analysis of pesticides in fruit and vegetables using gas chromatography/mass spectrometry, *Journal of Chromatography A*, Vol. 1217, Issue 12, 2010, pp. 1867-1874.
[10]. M. P. Marco, D. Barceló, Environmental applications of analytical biosensors, *Measurement Science and Technology*, Vol. 7, Issue 11, 1996, pp. 1547.
[11]. M. Albareda-Sirvent, A. Merkoçi, S. Alegret, Pesticide determination in tap water and juice samples using disposable amperometric biosensors made using thick-film technology, *Analytica Chimica Acta*, Vol. 422, Issue 1, 2001, pp. 35-44.
[12]. A. Hildebrandt, R. Bragos, S. Lacorte, *et al*., Performance of a portable biosensor for the analysis of organophosphorus and carbamate insecticides in

water and food, *Sensors and Actuators B: Chemical*, Vol. 133, Issue 1, 2008, pp. 195-201.
[13]. G. Yang, I. M. White, X. Fan, An opto-fluidic ring resonator biosensor for the detection of organophosphorus pesticides, *Sensors and Actuators B: Chemical*, Vol. 133, Issue 1, 2008, pp. 105-112.
[14]. V. G. Andreou, Y. D. Clonis, A portable fiber-optic pesticide biosensor based on immobilized cholinesterase and sol–gel entrapped bromcresol purple for in-field use, *Biosensors and Bioelectronics*, Vol. 17, Issue 1, 2002, pp. 61-69.
[15]. Z. Yu, G. Zhao, M. Liu, *et al.*, Fabrication of a novel atrazine biosensor and its subpart-per-trillion levels sensitive performance, *Environmental Science & Technology*, Vol. 44, Issue 20, 2010, pp. 7878-7883.
[16]. M. H. El-Saeid, S. A. AL-Dosari, Monitoring of pesticide residues in Riyadh dates by SFE, MSE, SFC and GC techniques, *Arabian Journal of Chemistry*, Vol. 3, Issue 3, 2010, pp. 179-186.
[17]. L. M. Ravelo-Pérez, J. Hernández-Borges, A. V. Herrera-Herrera, *et al.*, Pesticide extraction from table grapes and plums using ionic liquid based dispersive liquid–liquid microextraction, *Analytical and Bioanalytical Chemistry*, Vol. 395, Issue 7, 2009, pp. 2387-2395.
[18]. M. Arienzo, D. Cataldo, L. Ferrara, Pesticide residues in fresh-cut vegetables from integrated pest management by ultra performance liquid chromatography coupled to tandem mass spectrometry, *Food Control*, Vol. 31, Issue 1, 2013, pp. 108-115.
[19]. R. Buiculescu, N. A. Chaniotakis, The stabilization of Au NP–AChE nanocomposites by biosilica encapsulation for the development of a thiocholine biosensor, *Bioelectrochemistry*, Vol. 86, Issue 1, 2012, pp. 72-77.
[20]. D. J. Monk, D. R. Walt, Optical fiber-based biosensors, *Analytical and Bioanalytical Chemistry*, Vol. 379, Issue 7, 2004, pp. 931-945.
[21]. M. Grossi, M. Lanzoni, A. Pompei, *et al.*, An embedded portable biosensor system for bacterial concentration detection, *Biosensors and Bioelectronics*, Vol. 26, Issue 3, 2010, pp. 983-990.