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Short Communication

A System for Pesticide Residues Detection and Agricultural Products Traceability Based on Acetylcholinesterase Biosensor and Internet of Things

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This study presents a system based on acetylcholinesterase (AChE) biosensor and internet of things (IoT) for pesticide residues detection and agricultural products traceability. The system we presented based on AChE biosensor and IoT aims to extend the benefits of the pesticide residues detection date—remote control ability, data processing and sharing, agricultural products traceability and so on—to detection devices (hypogynous computer) in detection locations. These detection data got from detection devices were further aggregated, processed and analyzed by Epigynous computer in order to extract useful information（detection time, production places, detection samples, pesticide residues values, detection inspector）which were effective in protecting the quality and safety of agricultural products. In this study, the code of useful information was used in form of QR code, tracing and retracing the safety of agricultural products was achieved efficiently and reliably. In view of above, we design and implement a system based on AChE biosensor and IoT for pesticide residues detection and agricultural products traceability. It provides safe fruit and vegetable information for consumer, and lay foundation for the traceability of agricultural product.

Keywords: acetylcholinesterase biosensor; pesticide residues rapid detection; QR barcode ; internet of things; agricultural products traceability.

1. INTRODUCTION

Pesticides are chemical substances applied to crops at various stages of cultivation and during the post-harvest storage of crops. The use of pesticides is intended to prevent the destruction of food

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crops by controlling agricultural pests or unwanted plants and to improve plant quality [1]. Pesticide use in commercial agriculture has led to an increase in farm productivity. Despite the wide ranging benefits of using pesticides in agriculture, several incorrect applications can result in high and undesirable levels of the compounds in the produce that reaches consumers. These include inappropriate selection of pesticides used on foodstuffs, over use of pesticides and harvesting the crops before the residues have washed off after application [2,3].

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].

With the gradual advance of urbanization construction, the procurements of vegetables and fruits are most in markets and supermarkets. However, these procurement locations almost have no pesticide residues detection devices. 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, laborious and not convenient to popularize and promote. Moreover, they don't have the ability of information sharing and remote control. Therefore, they are not suitable for rapid detection and agricultural products traceability. 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.

With the explosive growth of smart phones, wireless technologies and sensor technologies have become a fundamental tool for everyday life around the world. The coming wave of interconnected devices, appliances, sensors, meters and countless other "things" represents the next generation of a hyper-connected world, the IoT [12].

Interconnected entities can open a communication channel with each other based on the IoT. Many technologies serve as the building blocks of this new paradigm, such as QR barcode, cloud services, machine-to-machine interfaces (M2M), and so on. Also, this paradigm has a multitude of application domains [13].

The IoT we used in this pesticide residues detection system on the one hand allows epigynous computerto receive detection date from dispersed pesticide residues detection devices based on biosensor (hypogynous computer). The epigynous computer turn database links into barcode-like images that can be scanned using a mobile phone or a QR code reader. On the other hand, people can obtain database link after having read the QR code and then access data in the database. With this we can establish an interconnection between those heterogeneous objects as long as they have access to the Internet [14-16].

The information sharing platform will take care of the centralization of the data of each hypogynous computer, allowing them to interact and communicate with epigynous computer through the creation of a ubiquitous network by solving the interconnection problem [17].

Due to the above, the purpose of this investigation is to design a system for pesticide residues detection and agricultural products traceability. We intend to allow anyone to interconnect this system without any programming knowledge. This system can be used in supermarkets, markets and plantations. Moreover, this system also can be used in the areas of purchasing, storage and transportation. The consumers and purchaser just have to scan the QR code affixed to the commodity to get the useful information at any time, any place, and in any way [18-20].

The remainder of this paper is structured as following: In section 2, there is a description to the whole system including structure and function. In section 3 we describe the structure, function and detection principle of the detection device (Hypogynous computer). Furthermore, we present out research on the detection principle. Section 4 and section 5 show the study of Epigynous computer and information sharing platform, respectively. In section 6 we cover the evaluation and discussion of the data obtained from experimental and system testing. Finally, in Section 7 we give the conclusion of the whole paper and describe the performance, stability, application fields and promotion value of this system.

2. SYSTEM DESCRIPTION

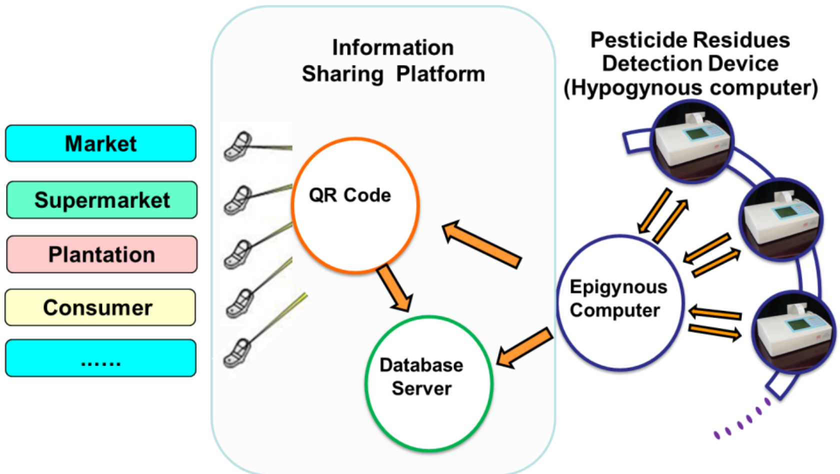


Figure 1. Architecture of the Detection System.

Figure 2. The prototype of the detection system.

The system's architecture can be divided in three parts, as it can be seen in Fig. 1: Hypogynous computer Based on AChE Biosensor, Epigynous computer based on LabVIEW platform and information sharing platform. AChE was immobilized on the working electrode and reacted with the substrate to produce the weak current signal. Hypogynous computer detection device collected weak current signal generated from AChE biosensor and transformed weak current signal into 0-5V standard voltage signal as an output signal. We could get the pesticide residues concentration based on the changed voltage signal. The detection information was transmitted to the epigynous computer through wireless transmission. Then epigynous computer processed detect information got from hypogynous computer detection devices placed in different locations and imported detection information into database. The QR code was printed by bar code printer at the same time and people can access the detection information in the database through scanning the QR code which encoded a date link. The prototype of this detection system was shown in Fig. 2.

2.1 Hypogynous computer detection devices

The hypogynous computer detection device of this detection system was made up of three-electrode module based on AChE biosensor, signal detecting and processing module, printing and storage module, power supply module and data transmission module. The hypogynous computer schematic based on the single-chip microcomputer was shown in Fig. 3. The hypogynous computer realized the real-time detection to the pesticide residues of real samples and sent the detection data to the epigynous computer. AChE was immobilized on the working electrode and reacted with the substrate to produce the weak current signal.

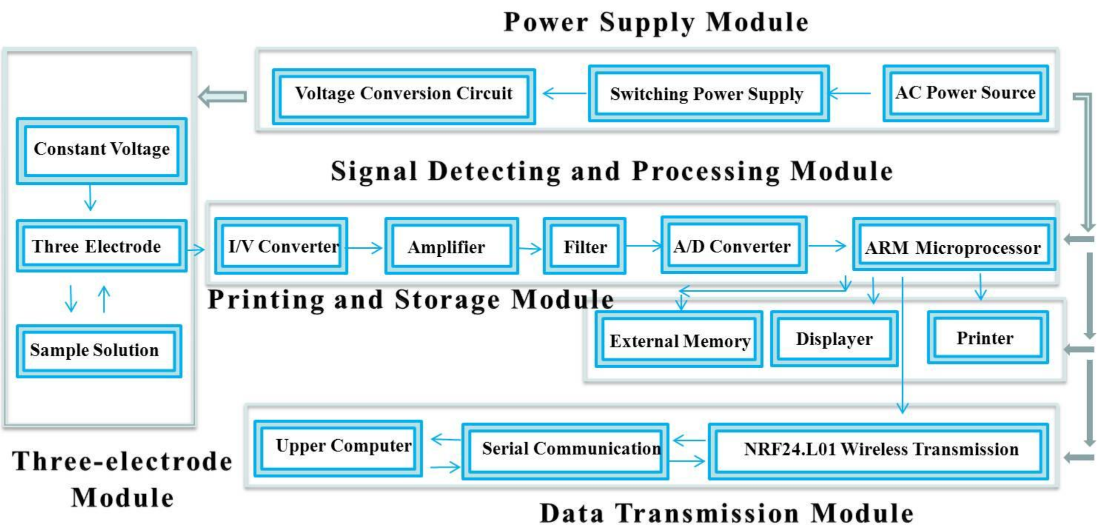


Figure 3. Detection device structure schematic drawing.

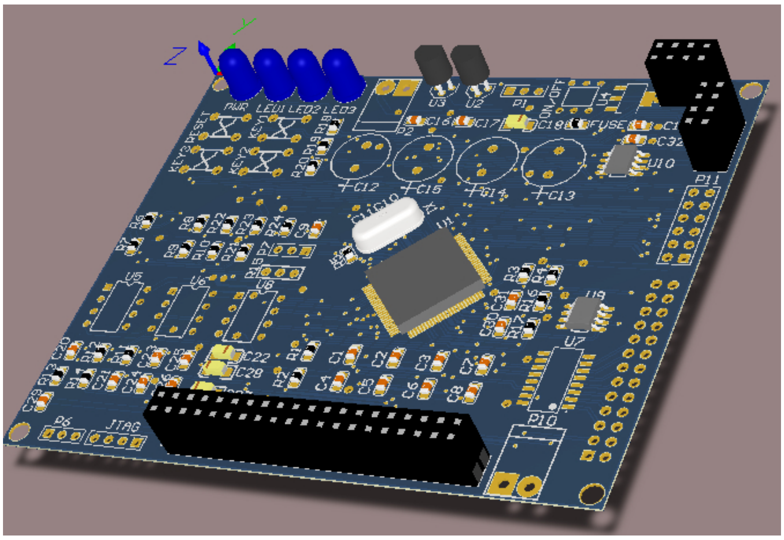


Figure 4. Hardware circuit of hypogynous computer detection device.

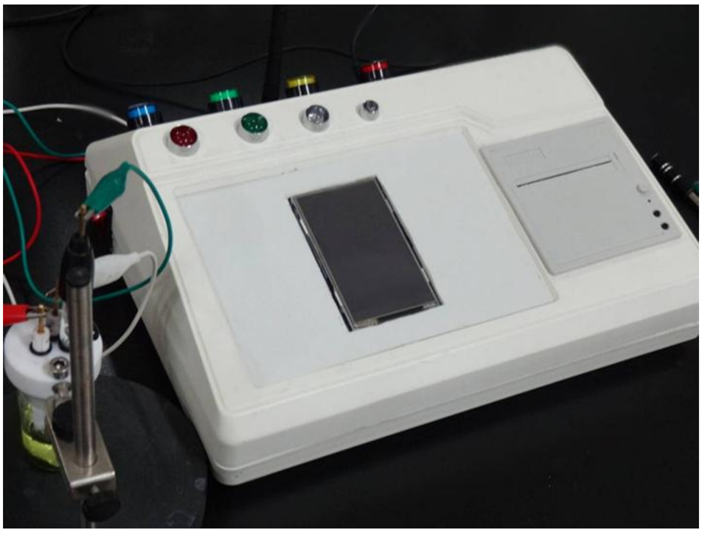


Figure 5. The prototype of hypogynous computer.

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 0-5V 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 hypogynous computer was shown in Fig. 4 and the prototype of hypogynous computer was shown in Fig. 5.

2.2 *Epigynous computer*

A rapid real-time epigynous computer system with the National Instruments' graphical programming language LabVIEW was designed to control and manage hypogynous computer detection devices. The system is centered round a computer running application developed for the purpose [21]. The previous detection devices can't realize multi-channel acquisition and detection, leading to low detection efficiency. The establishment of the epigynous computer on the one hand can realize the multi- channel data acquisition and detection, on the other hand, can realize the monitoring and management of the hypogynous computer detection devices. With this system, it is possible for the computer to receive data automatically and import the detection data into the database then the QR code was printed by bar code printer which encoded a date link at the same time. The functional flow diagram of epigynous computer system was shown in Fig. 6. The Epigynous computer system provided a convenient human-computer interaction means between the Hypogynous computer detection devices and operators. The front panel and block diagram of epigynous computer system were depicted in Fig.7a and Fig.7b respectively.

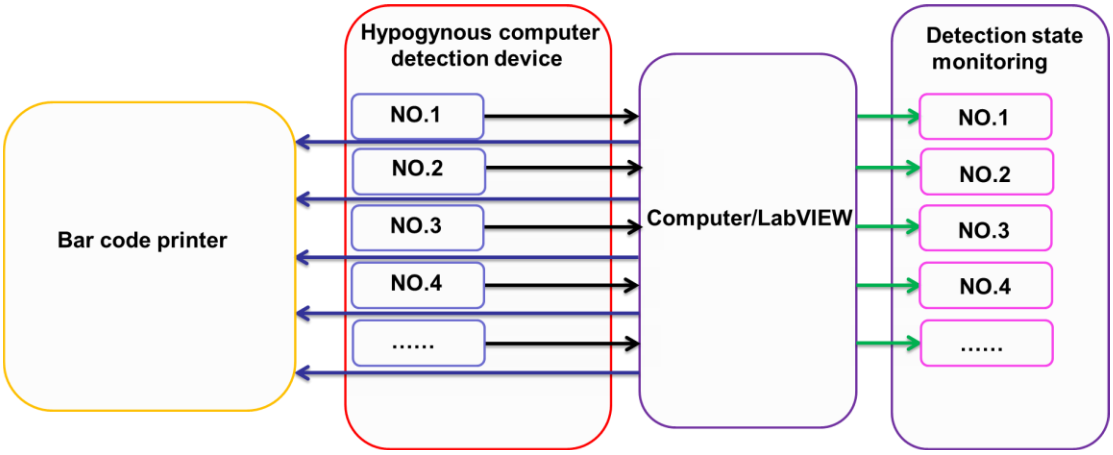
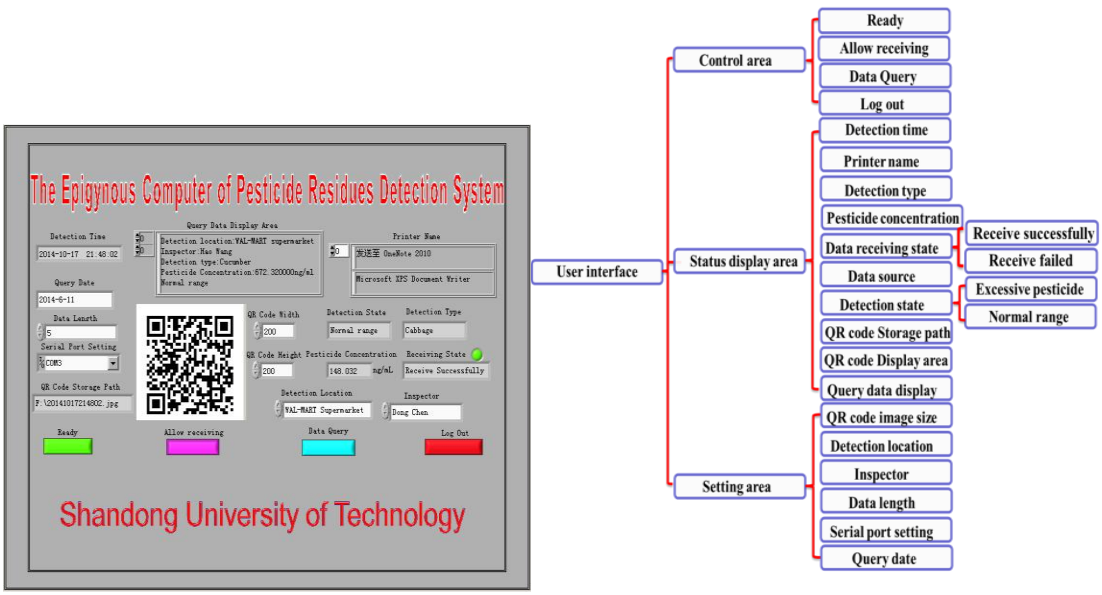


Figure 6. Functional flow diagram of epigynous computer system.



(A) **(B)**

Figure 7. The(a) front panel and (b)block diagram of epigynous computer system.

2.3 . Information sharing platform

Figure 8. The model of the proposed information sharing platform.

Aiming at the necessity to construct the information sharing platform of the pesticide residues system, this paper analyzed the function request of platform, integrated the technology of Access Database and ASP, at the end formed the system structure and design project of the system platform. The ASP program is executed on a network server and the generated HTML files from the execution results are applicable to the different browsers, people can access to the Access Database with ASP Webpage [22]. This information sharing platform has on the one hand provided a good platform for the co-construction and sharing of information resources, on the other hand greatly reduced the cost for information retrieving and knowledge acquiring, making it easy to look for information. People can access the detection information in the database through the information sharing platform by scanning the QR code which encoded a date link at any time, any place. The model of the proposed information sharing platform was depicted in Fig.8.

Using this model, after successful registration, the remote user can reach a specific sensor node directly through the Internet and does not need to first connect with the gateway node, thereby ensuring a more straightforward approach.

3. EXPERIMENTAL

3.1 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=1mm). 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.

3.2 Reagents and materials

Acetylcholinesterase (Type C3389, 500 U/mg from electric eel), acetylthiocholine chloride (ATCl) and chlorpyrifos were purchased from Sigma (USA). SnO2 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 NaH2PO4 and Na2HPO4. Other reagents were of analytical grade. All solutions were prepared using double distilled water.

3.3 Preparation of Nafion/AChE/MWNTs-SnO2- 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 3h. The obtained highly dispersed black suspension would be named as MWNTs-SnO_2-CHIT. A 2.5μm 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 2.5μL AChE solution to obtain the AChE/MWNTs-SnO2-CHIT/Au. Finally, the AChE/ MWNTs-SnO_2-CHIT/Au electrode was coated with an extra 2.5μL 0.5% Nafion to maintain the stability of modified electrode.

4. RESULTS AND DISCUSSION

4.1 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 0.7 and 0.2V as shown in Fig. 9. The highest oxidation current of thiocholine could be obtained when the working potential was 500 mV between working electrode and reference electrode after the bare gold electrode was modified by the AChE/MWNTs-SnO_2-CHIT.

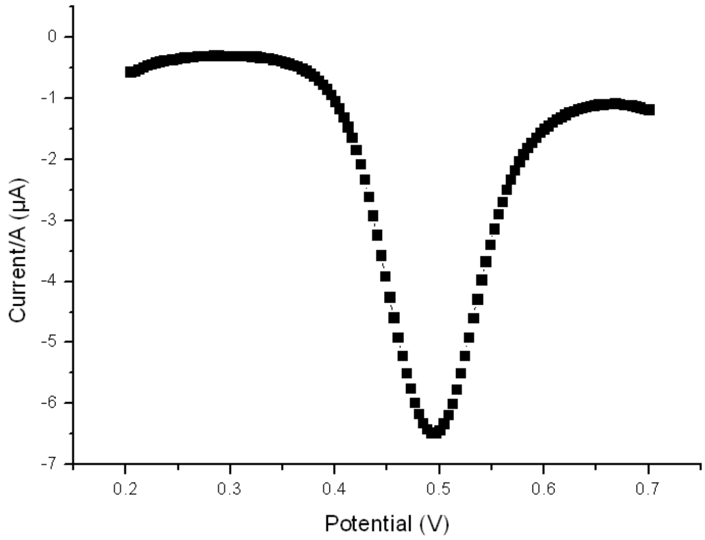


Figure 9. Cyclic voltammograms of AChE/MWNTs-SnO_2-CHIT/Au

The inhibition rate of pesticides was calculated as follows:

$$\Delta I\ (\%) = (I_0 - I_1)/\ I_0 \times 100\% \text{ or } \Delta U\ (\%) = (U_0 - U_1)\ /\ U_0 \times 100\% \qquad (3)$$

ΔI (%) is the inhibition rate, I_0 is the oxidation current obtained in the absence of pesticides and I_1 is oxidation current obtained after biosensor exposure to pesticides. The oxidation current signal generated by the AChE biosensor was transformed into 0-5V standard voltage signal as an output signal by current-to-voltage conversion circuit of hypogynous computer detection device based on the principle of U=I*R. Microcontroller played a critical role during the signal detecting and processing process. The resistance value is a constant, so the inhibition rate can be expressed by ΔI (%) and ΔU (%) as shown in formula (3). The pesticide residues concentration could be got from the linearity between inhibition rate and pesticide concentration.

4.2 The establishment of inhibition ratio curve

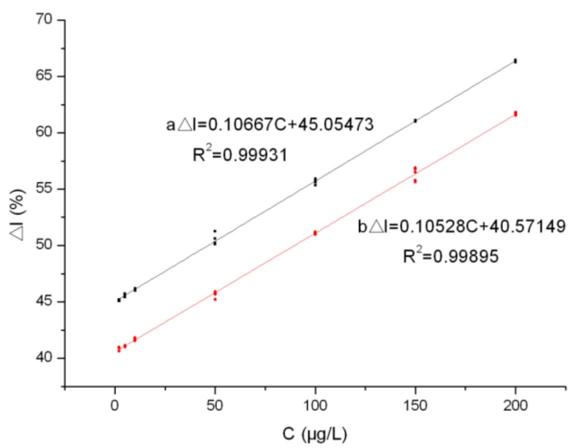


Figure 9. Detection of pesticides residues from the standard samples collected from biosensors laboratory. (a) Detecting the different concentrations of chlorpyrifos by using electrochemical workstation. (b) Detecting the different concentrations of chlorpyrifos by using hypogynous computer detection device.

This hypogynous computer detection device of the pesticide residues detection system has been used to test standard pesticides and real samples to compare with electrochemical analysis method. The linear regression equations for the modified electrode with using different detection methods were ΔI% =0.10667C (μg/L) + 45.05473(Fig. 11a) and ΔI% = 0.10528C (μg/L) + 40.57149 (Fig. 11b) in the range 2 to 500μg/L, the limit of detection was 2μg/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- SnO_2-CHIT/Au composite film as shown in Fig. 9. This means the standard pesticides could be evaluated by the pesticides residues detection instrument and the results indicated that the hypogynous computer detection device was suitable for direct analysis of pesticides.

4.3 Detection of real samples

The precision of the detection device was evaluated by analyzing eight kinds of vegetables and fruits samples. The real samples test was studied by using fresh cucumber, long bean, spinach, cabbage, kidney bean, apple and tomato, lettuce. Table 1 was showed the actual detection results of different kinds of vegetables and fruits by using mentioned hypogynous computer detection device of the pesticide residues detection system, the pesticide residues concentration of real samples were all lower than the detection limit, so pesticide residue concentration displayed as a vacancy concentration. The detection results show that the pesticide residues did not exceed the standard. The detection results show that the pesticide residue did not exceed the standard.

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 [23-25]. 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.

Table 1. Pesticide residues concentration of real samples.

Samples	cucumber	long bean	spinach	cabbage	kidney bean	apple	tomato	lettuce
U_0(mV)	1581	1373	1583	1544	1559	1599	1501	1373
U_1(mV)	1572	1367	1588	1544	1558	1560	1502	1371
ΔU（%)	0.5693	0.437	0	0	0.0641	2.439	0	0.1457
Concentration	--	--	--	--	--	--	--	--

Alain Hildebrandt et al. have designed and developed a portable biosensor for the analysis of organophosphorus (OP) and carbamate insecticides in water and food [26]. Gilmo Yang et al. have developed an opto-fluidic ring resonator biosensor for the detection of organophosphorus pesticides [27]. 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 [28]. All these methods are highly competitive with traditional analytical techniques in terms of shorter time response and lower cost, but they are not enough instrumented, and on the other hand, rather complex procedures make them unsuitable for industrial or commercial applications. The pesticide residues detection system mentioned in this paper integrated the function of pesticides residues rapid detection, detection devices supervising and detection information sharing based on the AChE biosensor and IoT.

The detection performance of the hypogynous computer detection device showed the capability of the pesticides residues detection with good sensitivity and high practical value. The limit of detection was 2μg/L and the accuracy of measurement could meet rapid detection of pesticides residues requirements.

4.4 Performance evaluation

The working process of the mentioned pesticide residues detection system can be divided into 4 parts: ⑴Hypogynous computer detection device detect the pesticide residues and get the concentration of the pesticide residues. ⑵Epigynous computer system received detection information from hypogynous computer based on the wireless data transceiver module and the detection information would be stored in the database at the same time. ⑶Epigynous computer integrated detection information, output QR code and supervise the hypogynous computer detection devices. ⑷Customers got the detection information by scanning the QR code which encoded a date link. In order to evaluate the stability and reliability of the proposed pesticide residues detection system during the working process, interference factors were added into the detection and transfer process to investigate their effect on the detection results. The delivery result of original pesticide residues detection data in each part was acquired from eight kinds of vegetables and fruits samples, as shown in Table 2. The equal pesticide residues concentrations show that the system was stable and reliable. The pesticide residues detection data which users obtained is accurate and original. The overall performance of the present system showed the capabilities of the pesticide residues rapid detection, detection information wireless transmission, detection information management, detection devices remote dynamic supervision and detection information sharing, which lay the foundation for the traceability of agricultural products.

Table 2. Delivery results of detection data during the system working process

Samples	cucumber	Long bean	spinach	cabbage	kidney bean	apple	tomato	lettuce
Actual detection results (μg/L)	2.061	0	0	1.996	2.030	0	2.173	2.602
Epigynous computer receiving results	2.061	0	0	1.996	2.030	0	2.173	2.602
Database storage results	2.061	0	0	1.996	2.030	0	2.173	2.602
User accessing results	2.061	0	0	1.996	2.030	0	2.173	2.602

5. CONCLUSION

In this study, a system consisting of hypogynous computer detection devices, epigynous computer and information sharing platform was developed for pesticide residues detection and agricultural products traceability. The hypogynous computer in this system has rapid detection and wireless transmission function and can be used on-side. In order to achieve hypogynous computer management and multi-channel detection, epigynous computer has been proposed and carefully designed for both the dynamic supervision and QR code printouts.

The application of this pesticides residues detection instrument has been performed on real samples. The system showed to be successful in pesticide residues detection and agricultural products traceability. For cholorpyrifos extracts, the detection system based on biosensor permitted to determine concentrations of 2μg/L, thus indicating the performance of this system can satisfy the pesticide residues detection and information sharing requirement of real vegetables and fruits samples. The detection system based on AChE biosensor and IoT for pesticide residues detection can be used in every link in the agricultural products traceability.

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References

1. G. T. Bakirci, Y. Hisil, *Food Chem*, 135(2011) 1901
2. C. Chen, Y. Qian, Q. Chen, C. Tao, C. Li, Y. Li, *Food Control*, 22(2011)1114
3. T. Cserháti, E. Forgács, Z. Deyl, I. Miksik, E. Eckhardt, *Biomed Chromatogr*, 18(2004) 350
4. G. O. Guler, Y. S. Cakmak, Z. Dagli, *Food Chem Toxicol*, 48(2010)1218
5. A. R. Boobis, B. C. Ossendorp, U. Banasiak, *Toxicology letters*, 180(2008)137
6. A. N. Ivanov, L. V. Lukachova, G. A. Evtugyn, *J. Bioelectrochemistry*, 55(2002) 75
7. A. Agüera, M. Contreras, A. R. Fernandez-Alba, *J. Chromatogr A*, 655(1993)293
8. J. Ye, J. Wu, W. Liu, *Trac-Trend Anal Chem*, 28(2009) 1148
9. H. Guan, W. E. Brewer, S. T. Garris, *J. Chromatogr A*, 1217(2010)1867
10. M. P. Marco, D. Barceló, Meas Sci Technol, 7(1996)1547
11. M. Albareda-Sirvent, A. Merkoçi, S. Alegret, *Anal Chim Acta*, 422(2001)35
12. D. Shin, *J. Telematics and Inform*, 31(2014) 519
13. R. Roman, J. Zhou, J. Lopez, *J. Comput Netw*, 57(2013)2266

14. G. C. González, G.Pelayo, B. C. Bustelo, *J. Comput Netw*, 64(2014)143
15. L.Atzori, A.Iera, G.Morabito, *J. Comput Netw*, 54(2010)2787
16. L.Tarjan, I.Šenk, S.Tegeltija, *J. Comput Electron Agr*, 109(2014)1
17. L.Zhao, J.Qian, L.Chang, *J. Data Knowl Eng*, 69 (2010)737
18. D.Lorenzi, J.Vaidya, S.Chun, *J. Government Information Quarterly*, 31(2014)6
19. L.Bai, F.Wang, M.Zhang, *J. Procedia Soc Behav Sci*, 138(2014) 350
20. Y.Qin, Q.Gong, *J. Opt Commun*, 310 (2014)69
21. Y.Yu, Y.Zhang, X.Yuan, *J. Optik-International Journal for Light and Electron Optics*, 125(2014)2256
22. Y.S.Li, L.F.Hong, *J. Agric Sci China*, 10(2011) 805
23. A.Agüera, M.Contreras, A.R.Fernandez-Alba, *J* Chromatogr A, 665(1993)293
24. J. Ye, J. Wu, W. Liu, Trac-Trend Anal Chem, 28 (2009) 1148
25. H. Guan, W. E. Brewer, S. T. Garris, *J* Chromatogr A, 1217 (2010)1867
26. A. Hildebrandt, R. Bragos, S. Lacorte, Sensor Actuat B-Chem, 133 (2008)195
27. G. Yang, I. M. White, X. Fan, Sensor Actuat B-Chem, 133 (2008) 105
28. V. G. Andreou, Y. D. Clonis, Biosens Bioelectron, 17(2002) 61