Food Chemistry 324 (2020) 126889

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Ultra-highly sensitive organophosphorus biosensor based on chitosan/tin disulfide and British housefly acetylcholinesterase

Xinke Liu[a], Rajalakshmi Sakthivel[b], Wai-Ching Liu[b], Ching-Wen Huang[b], Jian Li[a], Chuyu Xu[a], Yueqi Wu[a], Lijun Song[c], Wei He[d,*], Ren-Jei Chung[b,*]

[a] College of Materials Science and Engineering, Shenzhen University, Shenzhen 518060, People's Republic of China
[b] Department of Chemical Engineering and Biotechnology, National Taipei University of Technology (Taipei Tech), Taipei 10608, Taiwan
[c] Research Center of Guangdong Intelligent Charging and System Integration Engineering Technology, Shenzhen Winsemi Microelectronics Co., LTD., Shenzhen 518000, People's Republic of China
[d] College of Electronics and Information Engineering, Shenzhen University, Shenzhen 518060, People's Republic of China

ARTICLE INFO

Keywords:
British housefly acetylcholinesterase
Chitosan
Tin sulfide
Cyclic voltammetry
Organophosphorus pesticides

ABSTRACT

Pesticides have been extensively applied worldwide to protect crops from worms and insects; however, the continuous use of pesticides affects ecosystems, agricultural product safety, nontarget organisms, and human health. In this paper, we report a highly sensitive biosensor for the determination of pesticides based on tin sulfide (SnS_2) and chitosan (CHIT) nanocomposites decorated with a unique British housefly acetylcholinesterase (AChE). The hydrothermally synthesized nano-SnS_2 mixed with chitosan solution (CHIT-SnS_2) was drop-casted onto a glassy carbon electrode (GCE). Subsequently, the British housefly AChE was immobilized on the CHIT/SnS_2-coated GCE that was then employed for pesticide detection. The developed biosensor showed an ultra-high sensitivity and wide linear detection range from 0.02 nM to 20000 nM with a detection limit of 0.02 nM for the detection of chlorpyrifos as the model pesticide. Furthermore, the AChE/CHIT-SnS_2/GCE exhibited acceptable storage stability, good reproducibility, and selectivity.

1. Introduction

Pesticides are chemical substances that are widely utilized to prevent, control, or kill pests for crop protection. Generally, pesticides are categorized as either inorganic, biological, or synthetic. In particular, carbamates, organochlorines, triazine, and organophosphates are used frequently in agriculture (Vimala, Clarke, & Kaur, 2016). Among these, organophosphorus pesticides (OPs) are the most extensively used in commercial agriculture to control pests because of their high efficiency, virulence, and ease of degradation. However, the continuous usage of OPs in pesticides can cause environmental pollution of water, soil, and air, harming the environment and human health (Liu & Wei, 2014). As a result, numerous toxic agricultural products can become widely available and endanger the health of the population, making effective and accurate detection of pesticide residues in crops crucial. These OPs interfere with or inhibit the activity of acetylcholinesterase (AChE) that regulates the acetylcholine (ACh) neurotransmitter in the nervous system. As ACh accumulates in large quantities and cannot be decomposed, it will cause muscles to be unable to relax, resulting in adverse consequences such as dyspnea or even death (Costa, Giordano, Guizzetti, & Vitalone, 2008; Eddleston, Buckley, Eyer, & Dawson, 2008; Goel & Aggarwal, 2007; Jones, 2005; Lin, Lu, & Wang, 2004; Purves et al., 2004; Tadeo, 2008; Thundiyil, Stober, Besbelli, & Pronczuk, 2008). Hence, the sensitive and selective determination of OPs has received considerable attention. Numerous analytical techniques such as high-performance liquid chromatography, spectrophotometry, and gas chromatography have been employed for the investigation of pesticides (Lee, Kim, Cho, & Lee, 2002; Nesakumar, Sethuraman, Krishnan, & Rayappan, 2016). Nevertheless, electrochemical methods have been shown to have a smaller footprint, low cost, good stability, high sensitivity, and low detection limit compared to the general analytical methods. To date, electrochemical methods have been extensively applied for the determination of pharmaceutical drugs, biological molecules, and dyes (Ardakani et al., 2011; Beitollahi, Tajik, Asadi, & Biparva, 2014; Ganjali et al., 2017, 2018; Kazemipour, Ansari, Mohammadi, Beitollahi, & Ahmadi, 2009; Moghaddam, Tajik, & Beitollahi, 2019; Motaghi, Beitollahi, Tajik, & Hosseinzadeh, 2016; Tajik, Beitollahi, & Biparva, 2018; Tajik, Taher, Beitollahi, &

* Corresponding authors at: Department of Chemical Engineering and Biotechnology, National Taipei University of Technology (Taipei Tech), No. 1, Sec. 3, Zhongxiao E. Rd., Taipei 10608, Taiwan.
E-mail addresses: hewei@szu.edu.cn (W. He), rjchung@ntut.edu.tw (R.-J. Chung).

https://doi.org/10.1016/j.foodchem.2020.126889
Received 8 September 2019; Received in revised form 27 March 2020; Accepted 20 April 2020
Available online 21 April 2020
0308-8146/ © 2020 Elsevier Ltd. All rights reserved.

Torkzadeh-Mahani, 2015; Tajik, Taher, Beitollahi, Hosseinzadeh, & Ranjbar, 2016). The major advantage of the electrochemical detection method is its fast response and the lack of requirement for the use of sophisticated instruments for the analysis. Therefore, an AChE/CHIT-SnS_2 nanocomposite-based biosensor was developed for the determination of chlorpyrifos.

Tin sulfide (SnS_2) is an *n*-type semiconductor consisting of two layers of hexagonal close-packed sulfur anions with sandwiched tin cations (Chang et al., 2012; Xia et al., 2015a). The optical bandgap of SnS_2 is in the range of 2.18–2.44 eV (Yang et al., 2011). Owing to the advantages of SnS_2 such as good chemical stability, low cost (Rout et al., 2014; Tao, Wu, Wang, & Wang, 2015), and good optical properties (Bai, Zong, Yu, Chen, & Wang, 2014; Yang et al., 2015) it has become a popular material for use in light-sensitive photocatalysts. However, SnS_2 has been rarely used in biosensor construction. SnS_2 is easily available and easy to synthesize. Because of its high theoretical capacity, SnS_2 demonstrated better applicability and good biocompatibility compared to other metal sulfides in electrochemical sensor applications (Sakthivel, Kubendhiran, Chen, & Kumar, 2019). Hence, the SnS_2 nanomaterial was used for the preparation of the nanocomposite. On the other hand, chitosan (CHIT) is a membrane material that is suitable for the immobilization of enzymes on the electrode surface because of its ability to carry $–NH_2$ and –OH functional groups and maintain the biological activity of biomolecules (Khor & Lim, 2003; Meraz et al., 2016). CHIT has many interesting biological properties including biodegradability, biocompatibility, natural origin, regenerative effects on connective gum tissue, non-toxicity to normal body tissues, aggressive binding to mammalian and microbial cells, antitumor and anticholesterolemic behavior, acceleration of the formation of osteoblasts responsible for bone formation, and acting as a central nervous system depressant (Ohkawa, Minato, Kumagai, Hayashi, & Yamamoto, 2006). Furthermore, CHIT has been widely used as a stabilizing agent for enzyme immobilization because of its biocompatibility, mechanical strength, excellent film-forming ability, and cost effectiveness (Muthusankar & Ragupathy, 2018). Generally, OPs interfere or inhibit the activity of AChE. Hence, AChE was immobilized on the SnS_2/CHIT surface. The AChE enzyme was extracted from the heads of British houseflies that have been carefully fed in an indoor environment at the Taiwan Agricultural Research Institute that has been free of contamination for more than 50 years. Compared to wild flies that tend to show pesticide resistance, this housefly species is fed in an air-conditioned room without any external pollution. Therefore, the sensitivity of AChE in the detection of pesticides is much higher using AChE generated from the British housefly than the AChE obtained from *Electrophorus electricus* (electric eel) that is the most common source of the AChE used in biosensors and therefore serves as a universal standard. Acetylcholinesterase (AChE) contains carboxylesterase that is a serine protease that stabilizes the level of the acetylcholine neurotransmitter. Furthermore, AChE can be highly effectively immobilized on the electrode surface by forming a covalent bond of the $–NH_2$ of CHIT with the –COOH of AChE.

In this study, we demonstrate the hydrothermal synthesis of SnS_2 and the preparation of a composite with CHIT. Then, AChE was used for the immobilization on the surface of the CHIT-SnS_2/GCE. After careful evaluation, the fabricated electrode was used for the detection of chlorpyrifos. More specifically, the interference and real-time performance of this biosensor in vegetable samples were determined.

2. Experimental section

2.1. Materials

AChE (3.3 U/mg from British housefly) and acetylthiocholine iodide (ATCI) were purchased from Si Cheng Biological Technology Co. (Taiwan). Chlorpyrifos was purchased from Echo Chemical (Taiwan). Tin chloride pentahydrate ($SnCl_4{\cdot}5H_2O$) and thioacetamide (C_2H_5NS) were purchased from Showa (Japan) and Alfa Aesar (USA), respectively. CHIT was obtained from Cabco (Taiwan). Phosphate buffer saline (PBS; 0.2 M) was prepared by mixing stock solutions of Na_2HPO_4 and NaH_2PO_4. All chemical reagents were of analytical grade, and all solutions were prepared using deionized water.

2.2. Instruments

All electrochemical experiments were performed using a CHI6114E electrochemical workstation (CHI Instruments, USA) containing a three-electrode system. The unmodified or modified GCE was used as the working electrode, platinum wire as the auxiliary electrode, and $Hg/HgCl_2$ as the reference electrode. The crystal structure and morphologies of the SnS_2 were characterized using X-ray diffraction (XRD, PANalytical, Netherlands), field emission scanning electron microscopy, (FE-SEM, SIGMA, Germany), and field emission gun transmission electron microscopy (FEG-TEM, FEI, USA). The optical measurements of OPs were performed using spectrophotometry (Thermo scientific, USA).

2.3. Synthesis of SnS_2 nanoparticles

SnS_2 nanoparticles were synthesized using a simple hydrothermal method. In the classic synthesis of SnS_2, $SnCl_4{\cdot}5H_2O$ (0.351 g), C_2H_5NS (0.3 g), and deionized water (70 mL) were mixed and maintained at pH 10.5. Then, the mixed solution was poured into a hydrothermal synthesis reactor, placed in an oven, heated to 200 °C at a rate of $3°Cmin^{-1}$ for 1 h, and kept at 200 °C for another 11 h. The solution was extracted and centrifuged (6000 rpm, 30 min) with deionized water and ethanol to obtain the SnS_2 powders.

2.4. Preparation of the AChE/CHIT-SnS_2/GCE

The method used to prepare the biosensor is illustrated in Fig. 1. A glassy carbon electrode (GCE) was polished with alumina (0.05 μm) powder and rinsed with double-distilled water. Then, SnS_2 powder (0.02 g) was mixed with 0.2 M CHIT solution (4 mL) in order to prepare the CHIT-SnS_2 solution. Next, CHIT-SnS_2 solution (10 μL) and AChE solution (4 μL) were drop-casted onto the surface of the pre-cleaned GCE, and then placed into the vacuum dryer to dry for 10 min. The obtained AChE/CHIT-SnS_2/GCE was stored at 4 °C when not in use.

2.5. Electrochemical detection of chlorpyrifos

The electrochemical characteristics of the AChE/CHIT-SnS_2/GCE were investigated using cyclic voltammetry (CV). The experiment was conducted in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 1 mM ATCI containing 0.2 M PBS pH 7 at a scan rate of 0.01 V s^{-1}, and the applied potential scanning range was –0.4 V ~ 1.0 V. Herein, the chlorpyrifos were used as the reference pesticide and AChE/CHIT-SnS_2/GCE was employed for the determination of chlorpyrifos by the differential pulse voltammetry (DPV) method. The performance of the biosensor was evaluated using DPV.

The electrode was rinsed with PBS (pH 7.5) after incubation in pesticides with the desired concentration for 10 min, and then DPV measurements were carried out. The inhibition rate of the pesticides was calculated according to:

$$\text{Inhibition}(\%) = [(I_0 - I)/I_0] \times 100\% \quad (1)$$

where I_0 is the peak current of thiocholine obtained as the hydrolysis product from ATCI on the AChE/CHIT-SnS_2/GCE, and I is the peak current of AChE/CHIT-SnS_2/GCE inhibited by chlorpyrifos. Inhibition (%) was plotted against the concentration of chlorpyrifos to obtain linear calibration graphs. In addition, Ellman's method which is the standard method for the determination of AChE was used, and the performance of AChE/CHIT-SnS_2/GCE was compared to the previous

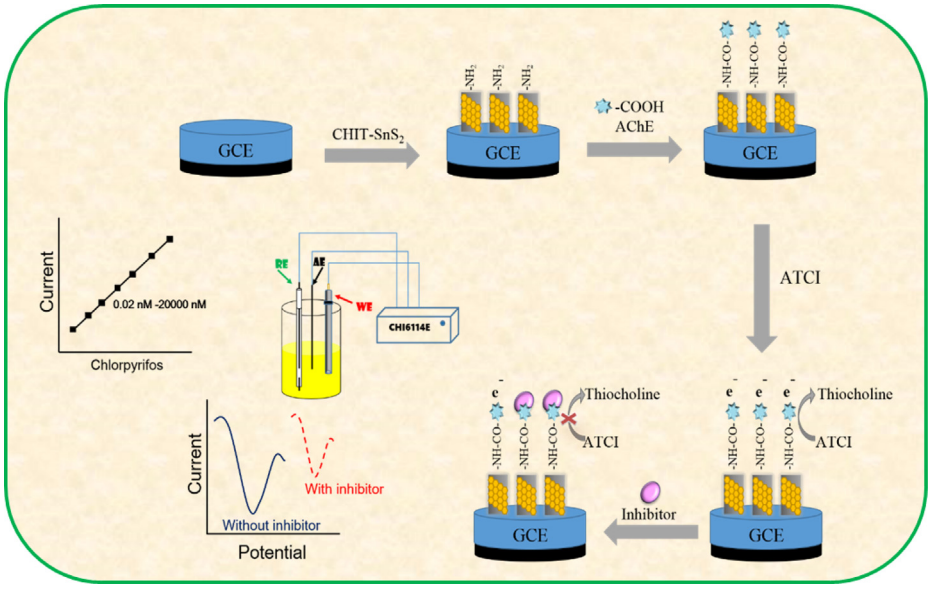

Fig. 1. Schematic of the fabrication process of the AChE/CHIT-SnS_2/GCE.

results. The experimental details for Ellman's method have been reported in the literature and are not discussed here (Pohanka, Hrabinova, Kuca, & Simonato, 2011).

2.6. Storage stability

The stability of AChE/CHIT-SnS_2/GCE stored under two conditions was examined to ensure the properties of the biosensor. The electrodes were stored either at room temperature or at 4 °C for a period of 7 days. The same electrode was taken out for the measurement of the response current every day, and the results were compared to the initial current response of the same electrode measured on the first day.

2.7. Interference study

The inhibitory effect of the potential interferences on AChE/CHIT-SnS_2/GCE was investigated in the presence of 0.2 nM of chlorpyrifos with different solutions such as 0, 0.1 mM of vitamin C, 0.1 mM of glucose, 0.1 mM of sodium chloride (NaCl), and 0.1 mM of sodium bicarbonate ($NaHCO_3$).

2.8. Detection of chlorpyrifos in real samples

The recovery test was performed by adding a certain amount of chlorpyrifos to vegetable samples. Fresh vegetables, cabbage, and Malabar spinach (*basella alba*) were purchased from a local market. Two grams of cabbage or *basella alba* were spiked with 2 mL of 0.02 nM chlorpyrifos. Afterwards, the samples were placed in a refrigerator at 4 °C for 24 h, and then, each sample was added to PBS (10 mL, 0.1 M, pH 7.5) followed by sonication. The solution mixture was centrifuged at 9000 rpm for 10 min. Finally, the supernatant was collected, and the recovery results were measured using the DPV technique.

3. Results and discussion

3.1. Material characterization

The diffraction pattern of the hydrothermally synthesized SnS_2 nanoparticles is shown in Fig. 2A. The XRD patterns show that the as-prepared SnS_2 nanoparticles have significant peaks matching those of pure hexagonal phase SnS_2 (JCPDS card 089-2358). Fig. 2B and C show the FE-SEM images use for the examination of the surface morphologies of the SnS_2 nanoparticles. Fig. 2B shows the uniform size distribution of the SnS_2 nanoparticles, with most of the particles observed to be the typical hexagonal crystals. The particle size of the SnS_2 nanoparticles was approximately 340.7 nm, as shown in Fig. 2C. Fig. 2D presents an FEG-TEM image showing the inner structure of SnS_2 in the (0 0 1) basal plane, and the diffraction pattern was identified by selected area electron diffraction (SAED), as shown in Fig. 2E. The diffraction pattern suggests that SnS_2 has a hexagonal crystal structure.

3.2. Electrochemical behavior of the AChE/CHIT-SnS_2/GCE

The CV measurement results showed that the modified electrodes had different performance characteristics. As shown in Fig. 3A, the CV curve (a) of the bare GCE demonstrates the lowest peak current and higher potential than those of the other, modified electrodes. The CHIT-SnS_2/GCE (curve b) exhibits the highest current density because the CHIT and SnS_2 nanoparticles increase the conductivity of the GCE. On the other hand, the AChE/CHIT-SnS_2/GCE (curve c) showed a lower current density than the bare GCE (curve a). AChE is an enzyme that hinders the electron transfer and current density. The decreased current density of AChE/CHIT-SnS_2/GCE indicated that AChE successfully modified the surface of the electrode. Furthermore, Nyquist plots (Fig. 3B) showed the same trend for the electron transfer resistance (Rct) values that were in the order of AChE/CHIT-SnS_2/GCE > GCE > CHIT-SnS_2/GCE. Thus, the higher Rct on the AChE/CHIT-SnS_2/GCE confirmed the successful attachment of AChE onto the electrode.

3.3. Optimization of the parameters

The amount of AChE affects the electrochemical performance of AChE/CHIT-SnS_2/GCE (Fig. 4A). It is observed that the current density first increased and then decreased with increasing amounts of AChE, reaching the maximum amount of approximately 0.04 U. This may be due to the hindering of electron transfer by the excessive loading of AChE. Thus, 0.04 U was chosen as the enzyme loading amount. The effect of the inhibition time on biosensor response was also investigated, and the corresponding results are displayed in Fig. 4B. The AChE/CHIT-SnS_2/GCE was incubated in 200 nM chlorpyrifos solution for different time durations such as 4, 6, 8, 10, and 12 min. As the incubation time increased, the current density decreased and no distinct change was observed for the incubation times longer than 10 min.

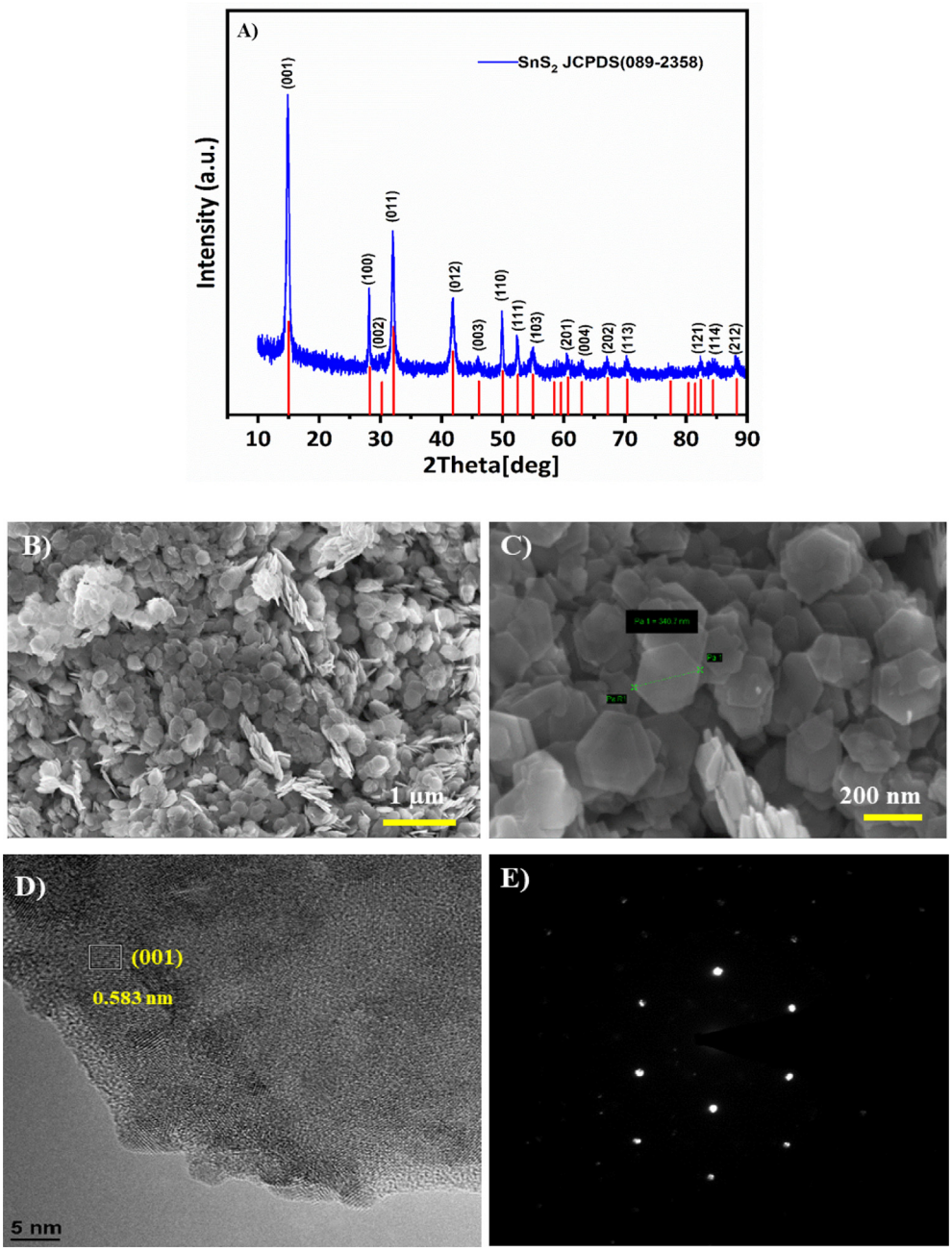

Fig. 2. (A) XRD pattern of the SnS_2 nanoparticles prepared by the hydrothermal method. FE-SEM image of the SnS_2 nanoflakes (B) at a magnification of 25000× (scale bar = 1 μm), and (C) at a magnification of 100000× (scale bar = 200 nm). (D) FEG-TEM image of the SnS_2 nanoflakes. (E) SAED image of the SnS_2 nanoflakes.

Therefore, 10 min was chosen as the working incubation time.

3.4. Determination of chlorpyrifos content

Fig. 4C shows the DPV curves for the different concentrations of chlorpyrifos varying from 0.02 nM to 20000 nM. It is observed that the current density decreased with increasing concentration of chlorpyrifos. The observed decrease in the current density may be due to the formation of covalent bonds between the –OH groups on the surface of AChE with the –OP surface of chlorpyrifos that reduced the surface charge. Fig. 4D shows the relationship between the inhibition rate and the chlorpyrifos concentration. The chlorpyrifos concentrations were spread over a wide linear range from 0.02 nM to 20000 nM, and the AChE/CHIT-SnS_2/GCE achieved a low detection limit (0.02 nM) and short incubation time (10 min). The obtained results were comparable to those obtained using the chlorpyrifos detection methods reported in previous studies, as shown in Table 1. (Chauhan, Narang, & Pundir, 2011; Chauhan, Narang, & Jain, 2016; Chauhan et al., 2011; Rotariu, Zamfir, & Bala, 2012; Sun, Zhai, & Wang, 2012, 2013; Viswanathan, Radecka, & Radecki, 2009; Wang et al., 2014; Xia et al., 2015b; Zamfir, Rotariu, & Bala, 2011; Zhai, Sun, Zhao, Gong, & Wang, 2013).

The various concentrations of chlorpyrifos (0.02 nM to 20000 nM) were measured by the conventional optical method known as the Ellman's test to validate the newly developed biosensor. The Ellman's test results (Fig. 4E) clearly showed (R^2 = 0.988) the similar tendency for the inhibition rate to that obtained using the electrochemical method (R^2 = 0.9965), with the higher concentration of chlorpyrifos exhibiting greater inhibition of AChE. Thus, using the conventional

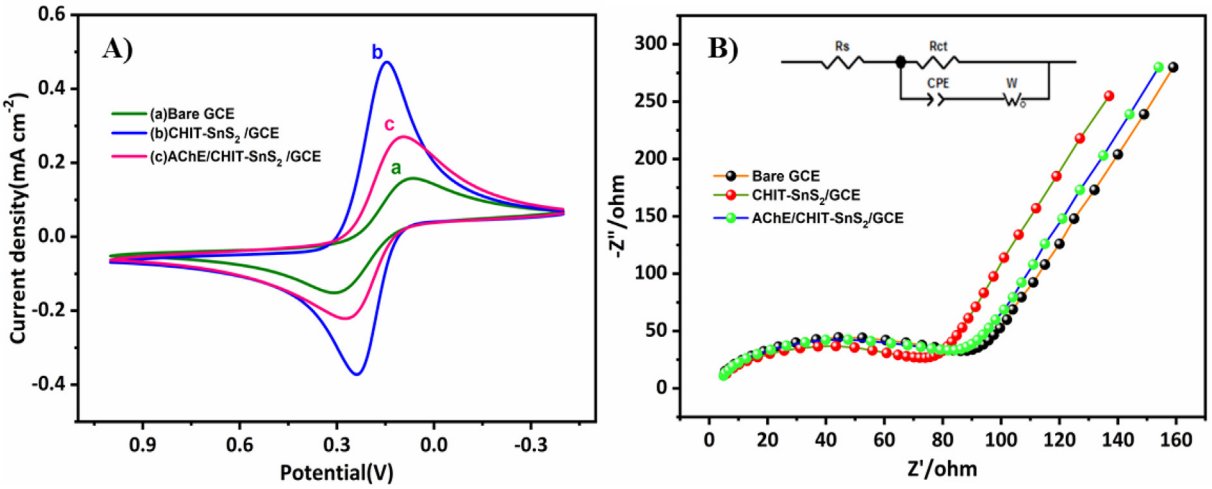

Fig. 3. (A) CV measured in 0.2 M PBS (pH 7.5) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ at 0.01 V s^{-1}. (B) Nyquist plot of EIS in 0.2 M PBS (pH 7.5) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$.

Ellman's method, it was confirmed that the fabricated AChE/CHIT-SnS_2/GCE is useful and accurate for the detection of AChE activity.

3.5. Storage stability

The AChE/CHIT-SnS_2/GCE biosensor was stored at room temperature and at the lower temperature of 4 °C, and the current density was measured once a day for one week to determine the stability of the electrodes. The current density retention was 64.7% (Fig. 5A) and 83.5% (Fig. 5B) after storage at room temperature and 4 °C, respectively, suggesting that the biosensor has is more stable in a low-temperature environment.

3.6. Interference study

As shown in Fig. 5C, it was found that the current values of vitamin C, glucose, NaCl, and $NaHCO_3$ were approximately 15% of those measured for chlorpyrifos. Since the concentrations of the interference species was much higher than the concentration of the chlorpyrifos, the interference should be negligible if they are at the same concentration. This proves that these possible interference species in the analysis will not interfere with the detection of chlorpyrifos.

3.7. Chlorpyrifos in real samples

The processed samples of cabbage and *basella alba* were tested for chlorpyrifos using DPV. As shown in Fig. 5D, the current densities of the

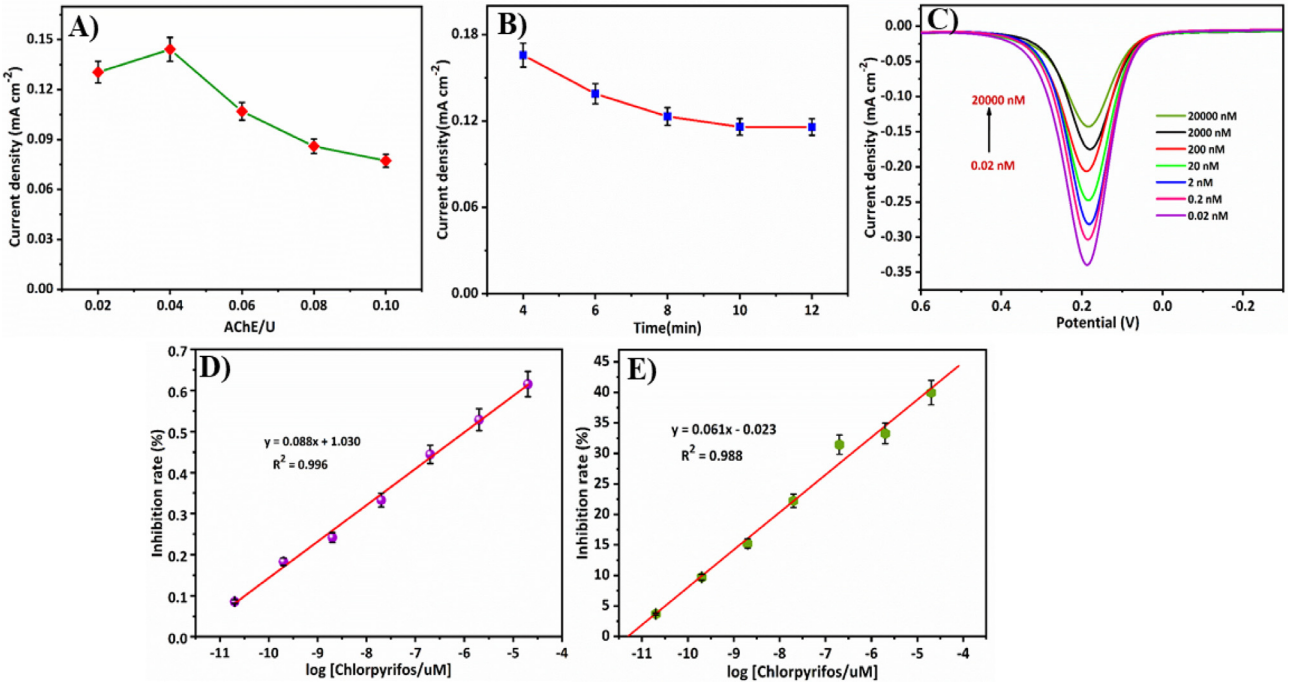

Fig. 4. (A) Effect of AChE loading on the response of AChE/CHIT-SnS_2/GCE. (B) Effect of incubation time on the response of AChE/CHIT-SnS_2/GCE. (C) DPV response of AChE/CHIT-SnS_2/GCE after inhibition in different chlorpyrifos concentrations for 10 min. (D) Inhibition rate of AChE/CHIT-SnS_2/GCE after inhibition in chlorpyrifos (0.02 nM–20000 nM) for 10 min versus the logarithm of the chlorpyrifos concentration. (E) Inhibition rate of different concentrations of chlorpyrifos (0.02 nM to 20000 nM) detection obtained by optical method versus the logarithm of chlorpyrifos concentration.

Table 1
Comparison of the biosensors for the detection of chlorpyrifos.

Study of AChE biosensor	Linear ranges	Detection Limit	Incubation Time (min)	Ref.
AChE/Fc-F/GCE	0.005–1 μM	3 nM	20	(Xia et al., 2015b)
AChE/Pin5COOH/Fe_3O_4NP/GCE	1.5–70 nM	1.5 nM	12	(Chauhan et al., 2016)
AChE/sol gel–TCNQ–MWCNT/SPE	1–100 nM	0.4 nM	30	(Rotariu et al., 2012)
AChE/[BMIM][BF4]-MWCNT/CPE	0.01–1 μM	4 nM	15	(Zamfir et al., 2011)
AChE/SWCNT–PANI/AuE	10 pM–1 μM	1 pM	15	(Viswanathan et al., 2009)
Nafion/AChE/chitosan–PB–MWCNTs–HGNs/AuE	0.05–75 nM	0.05 nM	10	(Zhai et al., 2013)
AChE/L-Cys/HGNs/chitosan/GCE	0.1–150 μg/L	0.06 μg/L	10	(Sun, Zhai, & Wang, 2013)
AChE/Fe_3O_4–MWCNT/AuE	0.1–50 nM	0.1 nM	15	(Chauhan and Pundir, 2011)
AChE/CMCS–AuNPs/GCE	0.1–100 μg/L	0.07 μg/L	10	(Sun, Zhai, & Wang, 2012)
AChE/ZnO–CGR/GCE	0.1 pM–10 nM	0.05 pM	12	(Wang et al., 2014)
AChE–Pin5COOH/ZnS/AuE	1.5–40 nM	1.5 nM	10	(Chauhan et al., 2011)
AChE/CHIT-SnS_2/GCE	0.02 nM – 20000 nM	0.02 nM	10	This work

sample measurements were similar to those of the pesticide-loaded samples, and the recoveries of chlorpyrifos from cabbage and *basella alba* were 98.5% and 98.4%, respectively. The results indicate that the electrochemical detection with AChE/CHIT-SnS_2/GCE is a precise and suitable method for the detection of pesticides in real samples.

4. Conclusion

In this study, tin sulfide was synthesized by a hydrothermal reaction and analyzed using XRD, FE-SEM, and FEG-TEM. A highly sensitive biosensor for the determination of pesticides was developed based on the nanocomposite of SnS_2 and chitosan decorated with a unique British housefly acetylcholinesterase. This biosensor is favorable because of its low-cost production and brief preparation method. Under optimized conditions, this AChE/CHIT-SnS_2/GCE biosensor showed ultra-high sensitivity with a wide linear detection range from 0.02 nM to 20000 nM with chlorpyrifos as the model pesticide (R^2 = 0.9965) and a detection limit of 0.02 nM. The results demonstrated that the developed biosensor has excellent storage stability at different temperatures. Furthermore, the results suggest that the developed biosensor exhibits a wide range of responses and good specificity for the detection of organophosphorus pesticides.

CRediT authorship contribution statement

Xinke Liu: Conceptualization, Methodology, Project administration, Funding acquisition, Writing - review & editing. **Rajalakshmi Sakthivel:** Writing - review & editing, Methodology, Formal analysis, Data curation, Visualization. **Wai-Ching Liu:** Writing - original draft, Methodology, Formal analysis, Data curation, Visualization. **Ching-Wen Huang:** Investigation, Formal analysis, Data curation, Validation. **Jian Li:** Investigation, Formal analysis, Data curation. **Chuyu Xu:** Investigation, Formal analysis, Data curation. **Yueqi Wu:** Investigation, Formal analysis, Data curation. **Lijun Song:** Resources, Formal analysis,

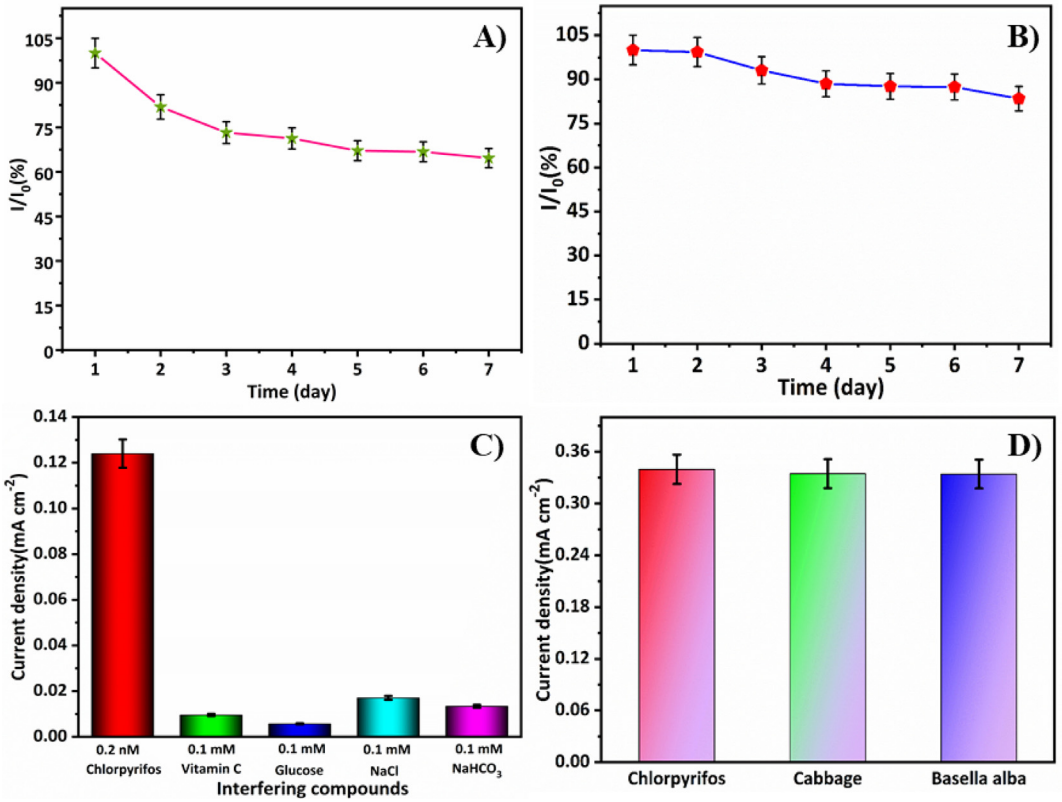

Fig. 5. (A) Stability tests of AChE/CHIT-SnS_2/GCE at (A) room temperature and (B) 4 °C. (C) Effects of possible interference species in analytes (0.1 mM) on the inhibition efficiency of chlorpyrifos (0.2 nM) in the AChE/CHIT-SnS_2/GCE system. (D) Current of 0.02 nM chlorpyrifos in real samples.

Data curation. **Wei He:** Methodology, Project administration, Funding acquisition, Writing - review & editing. **Ren-Jei Chung:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors appreciate the supply of high quality AChE of British housefly from Si Cheng Biological Technology Co. (Taiwan). The authors are grateful for the financial support received from the Ministry of Science and Technology of Taiwan (MOST 104-2622-E-027-027-CC3; MOST 104-2221-E-027-061, MOST 105-2221-E-027-028, MOST 106-2221-E-027-034), the National Taipei University of Technology-Shenzhen University Joint Research Program (NTUT-SZU-107-01 (2018001); NTUT-SZU-108-05 (2019005); NTUT-SZU-109-02 (2020011)); and in part from the Guangdong Province Key Research and Development Plan (2019B010138002). The first and second authors contributed equally to this work.

References

Ardakani, M. M., Beitollahi, H., Amini, M. K., Mirkhalaf, F., Mirjalili, B. F., & Akbari, A. (2011). Application of 2-(3, 4-dihydroxyphenyl)-1, 3-dithialone self-assembled monolayer on gold electrode as a nanosensor for electrocatalytic determination of dopamine and uric acid. *Analyst, 136*, 1965–1970.

Bai, Y., Zong, X., Yu, H., Chen, Z., & Wang, L. (2014). Scalable low-cost $SnS_{(2)}$ nanosheets as counter electrode building blocks for dye-sensitized solar cells. *Chemistry, 20*, 8670–8676.

Beitollahi, H., Tajik, S., Asadi, M. H., & Biparva, P. (2014). Application of a modified graphene nanosheet paste electrode for voltammetric determination of methyldopa in urine and pharmaceutical formulation. *Journal of Analytical Science and Technology, 5*, 1–9.

Chang, K., Wang, Z., Huang, G., Li, H., Chen, W., & Lee, J. (2012). Few-layer SnS_2/graphene hybrid with exceptional electrochemical performance as lithium-ion battery anode. *Journal of Power Sources, 201*, 259–266.

Chauhan, N., & Pundir, C. S. (2011). An amperometric biosensor based on acetylcholinesterase immobilized onto iron oxide nanoparticles/multi-walled carbon nanotubes modified gold electrode for measurement of organophosphorus insecticides. *Analytica Chimica Acta, 701*, 66–74.

Chauhan, N., Narang, J., & Jain, U. (2016). Amperometric acetylcholinesterase biosensor for pesticides monitoring utilising iron oxide nanoparticles and poly (indole-5-carboxylic acid). *Journal of Experimental Nanoscience, 11*, 111–122.

Chauhan, N., Narang, J., & Pundir, C. S. (2011). Immobilization of rat brain acetylcholinesterase on ZnS and poly (indole-5-carboxylic acid) modified Au electrode for detection of organophosphorus insecticides. *Biosensors & Bioelectronics, 29*, 82–88.

Costa, L., Giordano, G., Guizzetti, M., & Vitalone, A. (2008). Neurotoxicity of pesticides: A brief review. *Front Biosci, 13*, 1240–1249.

Eddleston, M., Buckley, N., Eyer, P., & Dawson, A. (2008). Management of acute organophosphorus pesticide poisoning. *Lancet, 371*, 597–607.

Ganjali, M. R., Dourandish, Z., Beitollahi, H., Tajik, S., Hajiaghababaei, L., & Larijani, B. (2018). Highly sensitive determination of theophylline based on graphene quantum dots modified electrode. *International Journal of Electrochemical Science, 13*, 2448–2461.

Ganjali, M. R., Salimi, H., Tajik, S., Beitollahi, H., Rezapour, M., & Larijani, B. (2017). Application of Fe_3O_4@ SiO_2/MWCNT film on glassy carbon electrode for the sensitive electroanalysis of levodopa. *International Journal of Electrochemical Science, 12*, 5243–5253.

Goel, A., & Aggarwal, P. (2007). Pesticide poisoning. *National Medical Journal of India, 20*, 182–191.

Jones, B. (2005). From waking to sleeping: Neuronal and chemical substrates. *Trends in Pharmacological Sciences, 26*, 578–586.

Kazemipour, M., Ansari, M., Mohammadi, A., Beitollahi, H., & Ahmadi, R. (2009). Use of adsorptive square-wave anodic stripping voltammetry at carbon paste electrode for the determination of amlodipine besylate in pharmaceutical preparations. *Journal of Analytical Chemistry, 64*, 65–70.

Khor, E., & Lim, L. (2003). Implantable applications of chitin and chitosan. *Biomaterials, 24*, 2339–2349.

Lee, H., Kim, Y., Cho, Y., & Lee, Y. (2002). Oxidation of organophosphorus pesticides for the sensitive detection by a cholinesterase-based biosensor. *Chemosphere, 46*, 571–576.

Lin, Y., Lu, F., & Wang, J. (2004). Disposable Carbon Nanotube Modified Screen-Printed Biosensor for Amperometric Detection of Organophosphorus Pesticides and Nerve Agents. *Electroanalysis, 16*, 145–149.

Liu, Y., & Wei, M. (2014). Development of acetylcholinesterase biosensor based on platinum–carbon aerogels composite for determination of organophosphorus pesticides. *Food Control, 36*, 49–54.

Meraz, K., Vargas, S., Maldonado, J., Bravo, J., Guzman, M., & Maldonado, E. (2016). Eco-friendly innovation for nejayote coagulation–flocculation process using chitosan: Evaluation through zeta potential measurements. *Chemical Engineering Journal, 284*, 536–542.

Moghaddam, H. M., Tajik, S., & Beitollahi, H. (2019). Highly sensitive electrochemical sensor based on La^{3+}-doped Co_3O_4 nanocubes for determination of sudan I content in food samples. *Food Chemistry, 286*, 191–196.

Motaghi, M. M., Beitollahi, H., Tajik, S., & Hosseinzadeh, R. (2016). Nanostructure electrochemical sensor for voltammetric determination of vitamin C in the presence of vitamin B6: Application to real sample analysis. *International Journal of Electrochemical Science, 11*, 7849–7860.

Muthusankar, E., & Ragupathy, D. (2018). Chitosan Based Nanocomposite Biosensors: A Recent Review. *Sensor Lett. 16*, 81–91.

Nesakumar, N., Sethuraman, S., Krishnan, U., & Rayappan, J. (2016). Electrochemical acetylcholinesterase biosensor based on ZnO nanocuboids modified platinum electrode for the detection of carbosulfan in rice. *Biosensors & Bioelectronics, 77*, 1070–1077.

Ohkawa, K., Minato, K. I., Kumagai, G., Hayashi, S., & Yamamoto, H. (2006). Chitosan nanofiber. *Biomacromolecules, 7*, 3291–3294.

Pohanka, M., Hrabinova, M., Kuca, K., & Simonato, J. (2011). Assessment of acetylcholinesterase activity using indoxylacetate and comparison with the standard Ellman's method. *International Journal of Molecular Sciences, 12*, 2631–2640.

Purves, D., Auguestine, G., Fitzpatrick, D., Hall, W., LaMantia, A., McNamara, J., & Williams, S. (2004). *Neuroscience* (3rd ed.). Sunderland, MA: Sinauer Associates Inc.

Rotariu, L., Zamfir, L. G., & Bala, C. (2012). A rational design of the multiwalled carbon nanotube–7, 7, 8, 8-tetracyanoquinodimethan sensor for sensitive detection of acetylcholinesterase inhibitors. *Analytica Chimica Acta, 748*, 81–88.

Rout, C., Joshi, P., Kashid, R., Joag, D., More, M., Simbeck, A., ... Late, D. (2014). Enhanced field emission properties of doped graphene nanosheets with layered SnS_2. *Applied Physics Letters, 105*, 043109.

Sakthivel, R., Kubendhiran, S., Chen, S. M., & Kumar, J. V. (2019). Rational design and facile synthesis of binary metal sulfides VS_2-SnS_2 hybrid with functionalized multiwalled carbon nanotube for the selective detection of neurotransmitter dopamine. *Analytica Chimica Acta, 1071*, 98–108.

Sun, X., Zhai, C., & Wang, X. (2013). A novel and highly sensitive acetyl-cholinesterase biosensor modified with hollow gold nanospheres. *Bioprocess and Biosystems Engineering, 36*, 273–283.

Sun, X., Zhai, C., & Wang, X. (2012). Amperometric acetylcholinesterase biosensor based on O-carboxymethyl chitosan-gold nanoparticle nanocomposite by in-situ synthesis method. *IEEE Sensors Journal, 13*, 172–179.

Tadeo, J. (2008). *Analysis of pesticides in food and environmental samples.* Boca Raton, FL: CRC Press.

Tajik, S., Beitollahi, H., & Biparva, P. (2018). Methyldopa electrochemical sensor based on a glassy carbon electrode modified with Cu/TiO_2 nanocomposite. *Journal of the Serbian Chemical Society, 83*, 863–874.

Tajik, S., Taher, M. A., Beitollahi, H., & Torkzadeh-Mahani, M. (2015). Electrochemical determination of the anticancer drug taxol at a ds-DNA modified pencil-graphite electrode and its application as a label-free electrochemical biosensor. *Talanta, 134*, 60–64.

Tajik, S., Taher, M. A., Beitollahi, H., Hosseinzadeh, R., & Ranjbar, M. (2016). Preparation, characterization and electrochemical application of ZnS/$ZnAl_2S_4$ nanocomposite for voltammetric determination of methionine and tryptophan using modified carbon paste electrode. *Electroanal, 28*, 656–662.

Tao, Y., Wu, X., Wang, W., & Wang, J. (2015). Flexible photodetector from ultraviolet to near infrared based on a SnS_2 nanosheet microsphere film. *Journal of Materials Chemistry C, 3*, 1347–1353.

Thundiyil, J., Stober, J., Besbelli, N., & Pronczuk, J. (2008). Acute pesticide poisoning: A proposed classification tool. *Bulletin of the World Health Organization, 86*, 161–240.

Vimala, V., Clarke, S. K., & Kaur, S. U. (2016). Pesticides detection using acetylcholinesterase nanobiosensor. *Biosens J, 5*, 2.

Viswanathan, S., Radecka, H., & Radecki, J. (2009). Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA. *Biosensors & Bioelectronics, 24*, 2772–2777.

Wang, G., Tan, X., Zhou, Q., Liu, Y., Wang, M., & Yang, L. (2014). Synthesis of highly dispersed zinc oxide nanoparticles on carboxylic graphene for development a sensitive acetylcholinesterase biosensor. *Sens. Actuators B Chem. 190*, 730–736.

Xia, J., Zhu, D., Wang, L., Huang, B., Huang, X., & Meng, X. (2015a). Large-Scale growth of two-dimensional SnS_2 crystals driven by screw dislocations and application to photodetectors. *Advanced Functional Materials, 25*, 4255–4261.

Xia, N., Zhang, Y., Chang, K., Gai, X., Jing, Y., Li, S., ... Qu, G. (2015b). Ferrocene-phenylalanine hydrogels for immobilization of acetylcholinesterase and detection of chlorpyrifos. *Journal of Electroanalytical Chemistry, 746*, 68–74.

Yang, B., Zuo, X., Chen, P., Zhou, L., Yang, X., Zhang, H., ... Chen, X. (2015). Nanocomposite of tin sulfide nanoparticles with reduced graphene oxide in high-efficiency dye-sensitized solar cells. *ACS Applied Materials & Interfaces, 7*, 137–143.

Yang, Z., Ren, Y., Zhang, Y., Li, J., Li, H., Hu, X., & Xu, Q. (2011). Nanoflake-like SnS_2 matrix for glucose biosensing based on direct electrochemistry of glucose oxidase. *Biosensors & Bioelectronics, 26*, 4337–4341.

Zamfir, L. G., Rotariu, L., & Bala, C. (2011). A novel, sensitive, reusable and low potential acetylcholinesterase biosensor for chlorpyrifos based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwalled carbon nanotubes gel. *Biosensors & Bioelectronics, 26*, 3692–3695.

Zhai, C., Sun, X., Zhao, W., Gong, Z., & Wang, X. (2013). Acetylcholinesterase biosensor based on chitosan/prussian blue/multiwall carbon nanotubes/hollow gold nanospheres nanocomposite film by one-stepelectrodeposition. *Biosensors & Bioelectronics, 42*, 124–130.