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Visual detection of mixed organophosphorous pesticide using QD-AChE aerogel based microfluidic arrays sensor

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ABSTRACT

In this paper, we present a simple strategy to fabricate a sensitive fluorescence microfluidic sensor based on quantum dots (QDs) aerogel and acetylcholinesterase enzyme (AChE) for organophosphate pesticides (OPs) detection The detection is based on the change of fluorescence intensity of QDs aerogel, which will be partly quenched as a consequence of the hydrolytic reaction of acetylthiocholine (ATCh) catalyzed by the AChE, and then the fluorescence of QDs aerogel is recovered due to decreasing of the enzymatic activity in the presence of OPs. The QDs-AChE aerogel based microfluidic arrays sensor provided good sensitivity for rapid detection of OPs with a detection limit of 0.38 pM, while the detection range is from 10^{-5} to 10^{-12} M. Due to the result of random orientations of AChE in the 3D porous aerogel nano-structure, the sensor presents similar calibration curves to difference pesticides, which promises the ability of the sensor to monitor total OPs of mixture. This determination sensor shows a low detection limit, wide linear range, and highly accurate determination of total OPs and carbamate content. Finally, we show the proposed sensor can be used to monitor of simple OPs and mixture in spiked fruit samples. This novel QDs-AChE aerogel sensor has an extremely high sensitivity and large detection range, it is a promising tool for accurate, rapid and cost-effective detection of various OP residues on agricultural products.

1. Introduction

Organophosphorous pesticides (OPs) are widely used in industrial agriculture to protect crops from pests (Hart, 1975; Mendoza et al., 1968). The residues have been frequently found in soil, atmosphere, groundwater, as well as agricultural products (Neufeld et al., 2000). Besides the negative effects on the environment and natural circulation, the residues have high toxicity, which holds great potential to cause neurological disorders in humans even at very low concentrations. Consequently, there is an increasing need to develop rapid, cheap and sensitive methods for reliable quantification of OPs with low concentrations in foods and drinking water. Conventional OPs test kits are able to detect various OPs and easy to use. However, they are limited in sensitivity. Due to OP compounds' defference in toxicity, these kits are also unable to detect total concentrations of a mixed OPs. High sensitive detection of OPs compounds are performed under laboratory conditions with the assistance of expensive instruments, such as capillary electrophoresis (Guidi et al., 2010; Qidan and Yingsing, 2010), gas chromatography (GC) (Gu, 1995), gas/liquid chromatography mass spectrometry (GC/LC-MS) (Qu et al., 2010) or high performance liquid chromatography (HPLC) (Pérez-Ruiz et al., 2005; Pinto et al., 1995). These conventional methods are sensitive and reliable, but they are time-consuming and require expensive instruments and complex operation. Thus they are not suitable for real-time detection, on-site application or home-use. To circumvent these issues, various simple and novel biosensors were developed to achieve rapid and portable detection of pesticides based on enzyme acetylcholinesterase (AChE) (Arun Prakash et al., 2009; Kumar et al., 2016; Min and Feng, 2017; Shu and Chung, 2017) or butyryl cholinesterase (Mathews et al., 2017). These enzyme-based biosensors were combined with different transduction schemes, such as potentiometric responses (Neufeld et al., 2000; Zejli et al., 2009), field effect transistors (Chang-Soo et al., 2009), optical

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methods (Luan et al., 2016; Shan et al., 2016; Zheng et al., 2011b), microcantilever (Karnati et al., 2007; Zuo et al., 2006) or colorimetric assays (Li et al. 2011, 2018), which offered remarkable advantages over conventional methods, mainly in terms of simplicity, rapidity, reliability, low detection limits and cost-effectiveness.

However, in these above methods, AChE immobilization methods are normally based on physical or chemical adsorption and covalent binding, which may cause uneven enzyme distribution, decrease of enzyme's stability, or the denaturing the activity of enzyme (Guodong and Yuehe, 2006). Another big challenge to prevent the practical application of these biosensors is inaccuracy when the real sample contains mixed OP pesticides or carbamate pesticides (Bachmann et al., 2000; Matuszczyk et al., 1993; Mulchandani et al., 2001). One possible approach to solve these obstacles contained to optimized the orientation of the immobilized enzyme and surrounding environment of enzyme, which affect affinity selectivity to OP compounds (Arun Prakash et al., 2009; Wei et al., 2009). Thus, the enhancement of sensor performance is expected by stable immobilization of enzyme, keeping the enzyme activity, and modulating their selectivity toward different types of pesticides.

Herein, we combined QDs aerogel with a three-dimensional (3D) structure and microfluidic chips as new OPs sensor to achieve mixed OP compounds detection. The QDs aerogel was prepared by prepared by freeze-drying the CdTe hydrogel, which is composed of self-assembled CdTe QDs stabilized by natural peptide L-Glutathione (GSH). This 3D aerogel in microfluidic chips has relatively uniform morphology and strong red fluorescence, while the natural peptide in aerogel could provide the enzymes with near-physiological conditions that minimize the denaturation, and improve the stability of the sensor. Furthermore, the porous aerogel could improve the surface area and reduce the hindrance of the sensor.

The red fluorescence of QDs aerogel was quenched by the reaction of AChE-catalyzed hydrolysis of ATCh, through dissociation of photogenerated electron-hole pairs in QDs. OPs, as known inhibitors for AChE, could prohibit the hydrolysis of ATCh and recover the red fluorescence of QDs aerogel, which enable visual detection of OPs without the need of any expensive equipment. Even more, by analyzing the gray value of different channels in the absence or presence of OPs under UV light, we are able to determine the concentration of OPs accurately. In addition, the sensor reveals a strong ability to detect a total concentration of mixtures of different OPs. Furthermore, this QDs aerogel based microfluidic arrays sensor has been demonstrated to detect OP residues in fruits with high detection sensitivity and selectivity.

2. Material and methods

2.1. Reagents and materials

All chemicals were analytical grade. $CdCl_2{\cdot}2.5H_2O$, $NaBH_4$, L-Glutathione(L-GSH) and Na_2TeO_3 were purchased from Alfa Aesar (Shanghai, China). NaOH and isopropanol (C_3H_8O) were purchased from KESHI (Chengdu, China). ATCh and AChE were purchased from Sigma-Aldrich (St. Louis, USA). Ultrapure water ($\geq$18 MΩ cm^2) was produced by a Millipore water purification system.

Fluorescence spectra were recorded by Lengguang Tech F96 pro fluorescence spectrophotometer (Lengguang Tech, Shanghai, China). The morphology of the synthesized CdTe-AChE aerogel was analyzed by an FEI Sirion field emission scanning electron microscope (FEI, Hillsboro, America). Fluorescence images were taken with a Canon 7D® digital camera (Canon, Tokyo, Japan), using a Qinke ZF-7A UV lamp (Qinke, Shanghai, China) as a light source.

2.2. Fabrication of microfluidic arrays sensor

The microfluidic arrays sensor contains five identical flow channel reactors to enable parallel measurements (Supplementary Fig. S1). Each flow channel reactor consists of a 12 mm long, 0.5 mm wide and 0.05 mm deep channel with a pair of inlet and outlet ports. The sensor's outer dimensions are 30 mm in length, 26 mm in width, 1 mm in thickness.

The QDs-AChE structure was fabricated inside the flow channel according to our method published before with slight modification (Hu et al., 2018). First, 10 mL QDs were purified by precipitation with isopropanol and dispersed in 3 mL PBS with 150 U AChE. Second, the mixed solution was injected into the flow channel of microfluidic chip by a micro-injector, then the microfluidic chip was kept in the refrigerator at −20 °C for 48 h. Finally, the chip was freeze-dried to obtain the porous three-dimensional QDs-AChE structure.

2.3. Calibration of OPs sensors

The sensor was calibrated with various pesticide concentration ranging from 10^{-5} M to 10^{-12} M. For each test, first, 0.8 μL pesticide sample was injected into the flow channel. After 10 min, ATCh solution was injected into the microfluidic chips again, and incubated for 5 min. Then the microfluidic chip was placed at the same location inside an ultraviolet analyzer, and corresponding photos were captured under a 365 nm UV lamp. Subsequently, the gray value of the flow channel was analyzed using ImageJ software. I is the gray value of the flow channel filled with different concentrations of pesticide. I_0 is the gray value of the flow channel filled with PBS without any ATCh and pesticide. C is the concentration value of pesticide. We calculated the value of I/I_0 and $\log_{10}$ (C) and plotted the curve of I/I_0 with $\log_{10}$(C).

3. Results and discussion

3.1. Synthesis and characterization of QDs-AChE aerogel and fabrication of microfluidic arrays sensors

The sensing principle of our OPs sensor is based on the fact that the QDs aerogel-AChE nanohybrids act as antennae for signal recognition, amplification and optical readout, while the three-dimensional (3D) structure aerogel containing abundant natural peptide (L-GSH) in microfluidic chips provides near-physiological conditions against denaturation to improve the stability of the sensor and the porous 3D structure improves the sensitivity of the sensor due to its large surface area. As described in experiment section 2.2, a simple one-step method was utilized to fabricate the QDs aerogel-AChE structure inside microfluidic chips. Then the sensor was characterized by fluorescence spectra, SEM and photographs under sun light and UV light. As shown in Fig. 1a, the sensor displays a strong fluorescence emission peak at 634 nm and absorption peak at 550 nm, which corresponds to the fluorescence emission peak and UV–Vis absorption spectrum of QDs. The SEM image (Fig. 1b) clearly shows the 3D porous morphology feature of the aerogel, which enhances the stability and sensitivity. Fig. 1c and d are photo images of the microfluidic sensor under sun light and UV light, which indicates the QDs aerogel is uniformly filled in the flow channel and has a bright fluorescence under 365 nm UV excitation.

Scheme 1 illustrates the fluorescence switch mechanism for OPs detection based on QDs-AChE aerogel. When the ATCh is injected into the channel, the fluorescence of aerogel is quenched with time. After a certain period of time (ca. 5 min), the fluorescence of aerogel tends to be stable. Photoluminescence (PL) quenching of the QDs-AChE aerogel

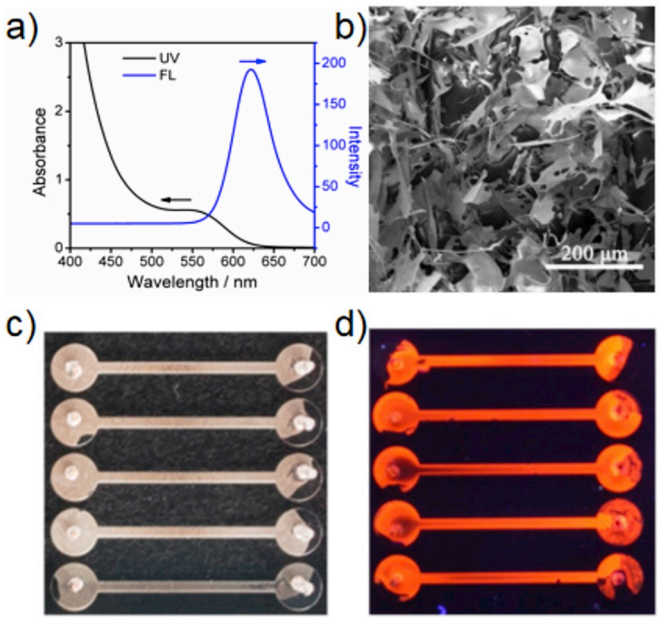


Fig. 1. (a) Fluorescent spectra and UV–Vis spectra of CdTe QDs aerogel. (b) SEM image of CdTe QDs aerogel. Photograph of the QDs-AChE aerogel microfluidic sensor under visible light (c) and UV light (d).

is attributed to the AChE-catalyzed hydrolysis of ATCh to acetate and thiocholine. Thiocholine acts as donors for the holes generated in the valence band of CdTe QDs upon photoexcitation and thus quenches PL of QDs aerogel. When OPs is injected into the flow channel, the OPs interact with the active centers of AChE and decrease the enzyme activity, which inhibits the production of thiocholine, and further recovers the fluorescence of QDs aerogel. Thus, based on the fluorescence intensity of QDs aerogel, the concentration of OPs can be detected.

3.2. Validation of sensing principle and optimization of the QDs-AChE aerogel biosensor

To prove the fluorescence quenching phenomenon of the QDs by ATCh, the image of the flow channel arrays were captured before and after injection of 4 mM ATCh, as shown in Fig. 2a and Fig. 2b. Before the injection of ATCh, the QDs aerogel sensor has a strong red fluorescence. After exposed to ATCh, the fluorescence decreases dramatically. The fluorescence quenching of the QDs aerogel based microfluidic sensor is attributed to the AChE-catalyzed hydrolysis of ATCh to acetate and thiocholine. In this process, thiocholine acts as donors for the holes generated in the valence band of CdTe QDs upon photoexcitation eliminates the electron-hole recombination (Vered et al., 2003; Willner and Willner, 2007).

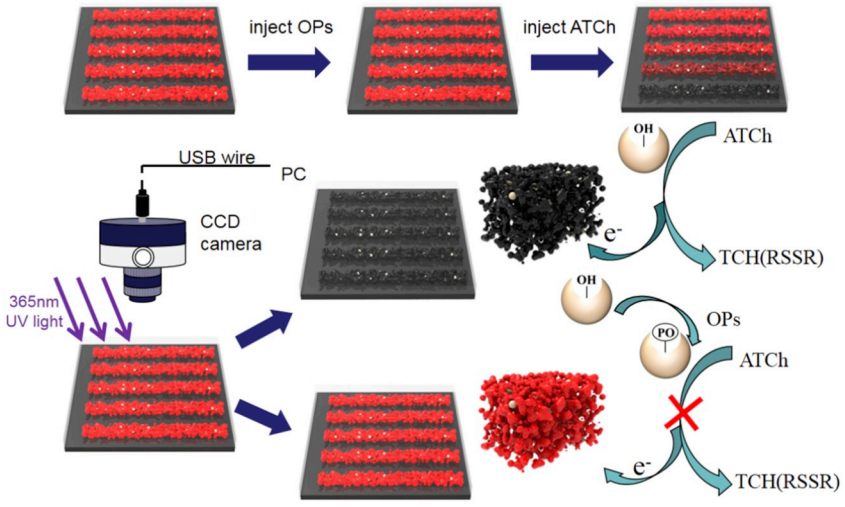
When the OPs are introduced into the sensor, they react with AChE and decrease its enzyme activity, inhibiting thiocholine production, and then recover the fluorescence of the QDs aerogel sensor.

Furthermore, to prove OPs are able to recover the fluorescence of QDs aerogel, four commonly-used OPs, Paraoxon, parathion, dichlorvos, and deltamethrin were tested to verify the proposed mechanism of the sensor. As shown in Fig. 2c, the first four flow channels (from the top) were loaded with paraoxon, parathion, dichlorvos, and deltamethrin samples, while the fifth flow channel was filled with PBS buffer as a reference experiment. Then ATCh was injected into the microfluidic chips. The microfluidic chips under UV light clearly displayed that the four commonly-used OPs absorbed on the AChE were able to decrease the thiocholine production and recover the fluorescence respectively.

The experimental conditions were further optimized to determinate the concentration of enzyme inhibitors. In order to expand the detection range and improve the detection sensitivity of the sensor, firstly, the concentration of ATCh was determined to make sure the fluorescent of the microfluidic sensor was quenched entirely. As shown in Fig. 3a, the fluorescent of the microfluidic sensor quenches gradually with the concentration of ATCh increasing from 5 mM–50 mM. As the concentration of ATCh increases to 50 mM, the fluorescent of the microfluidic sensor changes to dark. Then the incubation time of ATCh was optimized respectively. As shown in Fig. 3c, once ATCh solution is injected into the microfluidic sensor, the fluorescence of sensor decreases with the increase of incubation time, and the fluorescent quenches completely after 5 min incubation. Hence, the incubation time of OPs solution also influences the detection accuracy. In the following experiments, the ATCh concentration and incubation time were set to be 50 mM and 5 min, respectively.

3.3. Detection of four types of OP compounds

We used our sensor to measure the concentrations of calibration plots of four types of commonly-used OPs, including paraoxon, parathion, dichlorvos, and deltamethrin. As shown in Fig. 4, the calibration



Scheme 1. Schematic illustration of visual detection of OPs based on enzyme inhibition recovering the fluorescence of CdTe aerogel.

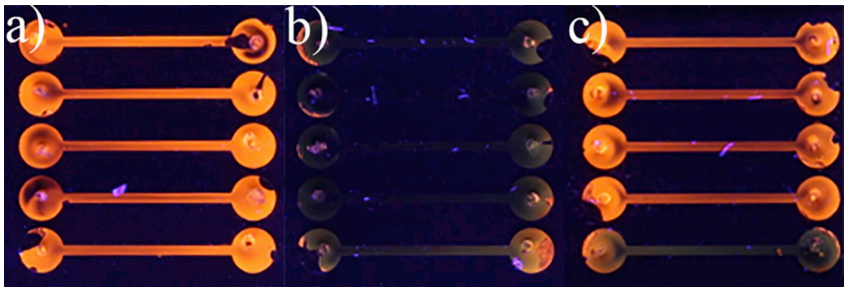


Fig. 2. (a) Fluorescence images of CdTe-AChE aerogel microfluidic sensor after injected PBS without ATCh. (b) Fluorescence images of CdTe-AChE aerogel microfluidic sensor after injected ATCh. (c) Fluorescence images of CdTe-AChE aerogel microfluidic sensor after injected PBS with four types of commonly-used OPs (first four lines) or without any OPs (last line) and injected ATCh.

curves are detected under the optimal conditions established in the above studies. The biosensors were firstly pre-incubated with pesticide solutions (paraoxon, parathion, dichlorvos, deltamethrin) for 10 min and then incubated with 50 mM ATCh for 5 min. The fluorescence response was recorded by a camera under UV light. Fig. 4a shows the fluorescence responses of our biosensor after incubation with various concentrations of paraoxon. As shown in Fig. 4a, the fluorescence of channels are recovered gradually with the increase of pesticide concentration. Furthermore, the fluorescence response is linear with the logarithm of OP concentration ranging from 10^{-5} M to 10^{-12} M as the following equation (1).

$$I/I_0 = 1.389 + 0.104 \log[\text{concentration of Paraoxon}] \quad (1)$$

Where, I is the fluorescence intensity of sensor with substrate ATCh and certain concentrations of OPs, and I_0 is the fluorescence intensity without substrate ATCh and OPs.

Remarkably, the relative fluorescence of the proposed biosensor is decreasing with the concentration of other three types of OPs in the same detection range, and is also linear with the logarithm of OP concentration as the following equations (2)–(4).

$$I/I_0 = 1.497 + 0.112 \log[\text{concentration of Dichlorvos}] \quad (2)$$

$$I/I_0 = 1.407 + 0.105 \log[\text{concentration of Parathion}] \quad (3)$$

$$I/I_0 = 1.329 + 0.095 \log[\text{concentration of Deltamethrin}] \quad (4)$$

The LODs for paraoxon, parathion, dichlorvos, deltamethrin are 1.2 pM, 0.94 pM, 11.7 pM and 0.38 pM, respectively. As our best knowledge, the detection limit of OPs is much lower than other enzyme based electrochemical or fluorescence methods listed in Table S1. According to the pesticide residue standard of the European Union (EC 149–2008), the LODs are enough to satisfy the detection requirements to the sensor.

The most remarkable advantage of the system is that similar response efficiencies are observed for three types of OPs (parathion, paraoxon and dichlorvos) and one carbamate pesticides (deltamethrin), as shown in Table 1. As previous report, AChE exhibits different binding properties for different types of OPs, therefore, the enzyme-based biosensor has a different response to different types of OPs at the same concentration. One plausible explanation of the similar response for different OPs is the orientation effect of AChE in the nanostructures. Compared with other film structures, AChE would adopt different orientations in our 3D porous aerogel, which means the active sites prefer a random arrangement. This orientation may increase the steric obstruction for attack fromOPs and carbamate compounds (Wei et al., 2009). In this case, the steric hindrance effect is dominant, and thus a similar response for different OPs and carbamate compounds is obtained with the proposed biosensor.

Furthermore, the mixtures consisted of different types of OP or carbamate compounds were detected by the sensor to evaluate its selectivity and the detectability to mixed OP compounds. Table 1 summarizes the detection results of the mixtures of paraoxon and parathion, paraoxon and dichlorvos, paraoxon and deltamethrin, paraoxon, parathion, dichlorvos and deltamethrin, respectively. These results confirm that the biosensor is a very promising analytical tool for the reliable detection of the total content of mixed OPs and carbamate pesticides at trace levels.

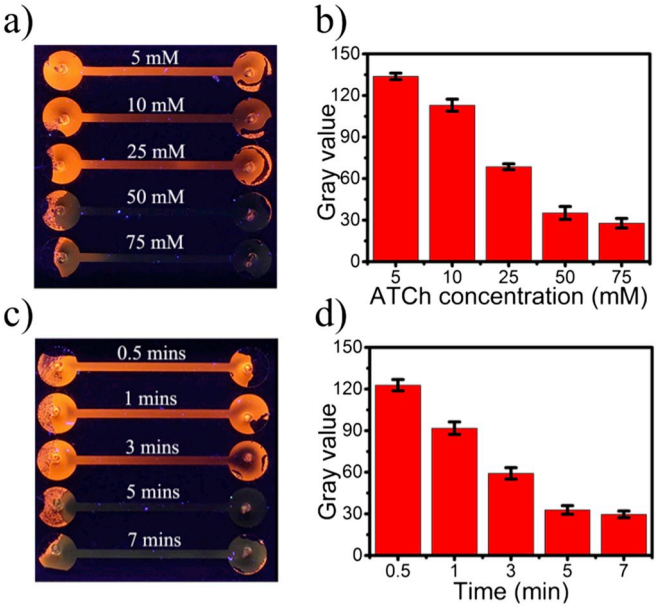


Fig. 3. (a) Fluorescence images of CdTe-AChE aerogel based microfluidic sensor after injected PBS after injected different concentration of ATCh. (b) Gray value histogram of CdTe-AChE aerogel based microfluidic sensor after injected PBS after injected different concentration of ATCh. (c) Fluorescence images of CdTe-AChE aerogel based microfluidic sensor at different time after injected 50 mM ATCh. (d) Gray value histogram of CdTe-AChE aerogel based microfluidic sensor at different time after injected 50 mM ATCh.

3.4. Analysis of pesticides in fruit by the biosensor

To evaluate the feasibility of the sensor's practical applications, different concentrations of pesticides in apple samples were determined. All apple samples were prepared through addition of different types of OP compounds onto the apple surface, and extracted using a commonly used method as described in other literature(Zheng et al., 2011a). The results are listed in Table 2. It can be seen that the recoveries of the samples are found to be in the range of 98–110%, and the RSDs are almost less than 10.0%. These results indicate the developed fluorimetric method has potential as a reliable and sensitive strategy for the detection of pesticide residues in real samples of fruits.

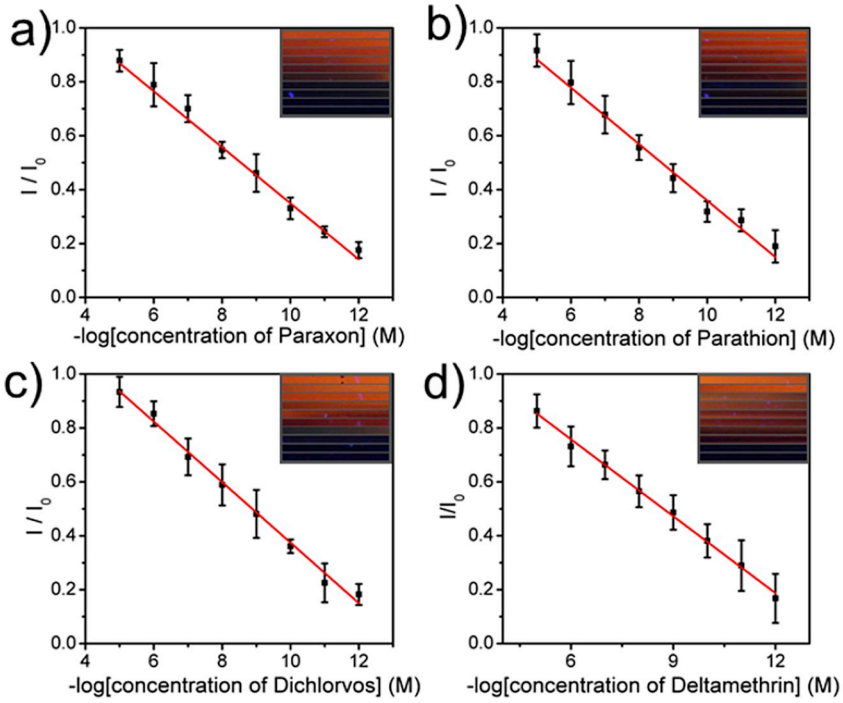


Fig. 4. Linear plots of the microfluidic biosensor to different concentrations of (a) paraoxon, (b) dichlorvos and (c) parathion. (d) Deltamethrin. Linear ranges are obtained from 10^{-5} to 10^{-12} M. (Each data point is an average of five experiments.)

Table 1
Results of evaluation of different OPs and mixture analysis using the QDs-AChE aerogel biosensor.

Sample	Concentration	Detection Result	RSD
paraoxon	10 nM	10.15 ± 0.12	9.42%
parathion	10 nM	9.66 ± 0.09	8.46%
dichlorvos	10 nM	9.15 ± 0.13	7.49%
deltamethrin	10 nM	8.93 ± 0.18	10.91%
Paraoxon + parathion	5 + 5 nM	9.53 ± 0.18	11.56%
Paraoxon + dichlorvos	6 + 4 nM	9.21 ± 0.13	13.87%
Paraoxon + deltamethrin	7 + 3 nM	9.56 ± 0.29	8.74%
Paraoxon + parathion + dichlorvos + deltamethrin	3 + 3+2 + 2 nM	9.15 ± 0.48	14.80%

4. Conclusion

In summary, we demonstrated a novel QDs-AChE aerogel microfluidic sensor for high sensitive and rapid detection of organophosphorus pesticide (OP) residues. With this method, AChE-catalyzed hydrolysis of acetylthiocholine generates thiocholine, while OPs inhibit AChE's activity and recover the QDs' fluorescent intensity quenched by thiocholine. The recoveries of QDs' fluorescent intensity are correlated to OPs' concentrations. Our experiments proved QDs' fluorescent intensity increased gradually with the increase of OPs' concentrations. We also tested our sensor with four most commonly used pesticides. The limit of detection is lower than 1.2 pM, while the detection range is from 10^{-5} M to 10^{-12} M, which further prove our sensor has an extremely high sensitivity and broad detection range. Noteworthy, for the sensor had similar responses to the four types of pesticides, it is capable to detect the total OP concentration of a mixture with unknown OPs' type. We also evaluated our senor with fruit samples, the OPs' recovery is higher than 98%. Currently, the OPs' detection is based on single color fluorescence, the low contrast between red color and background color limits the application of visual detection. In the future, by adding

Table 2
Results of detection of different OPs and mixture in fruit samples by the QDs-AChE aerogel biosensor.

Sample	Added amount	Detection Result	RSD(%)	Recovery(%)
Apple	0	N/A		
Apple, add paraoxon	5	5.20 ± 0.53	10.15%	104.0 ± 10.6
Apple, add paraoxon	15	14.8 ± 1.29	9.82%	98.7 ± 8.6
Apple, added dichlorvos	5	5.29 ± 0.93	9.47%	105.8 ± 18.6
Apple, added deltamethrin	5	5.37 ± 0.86	5.46%	107.4 ± 17.2
Apple, add paraoxon and deltamethrin	5 + 5	10.62 ± 1.35	7.62%	106.2 ± 13.6
Apple, add paraoxon and dichlorvos	5 + 5	10.38 ± 1.90	6.23%	103.8 ± 19.0
Apple, add Paraoxon + parathion + dichlorvos + deltamethrin	3 + 3+2 + 2	10.92 ± 1.61	9.65%	109.2 ± 16.1

another reference color to improve the contrast, such as green fluorescence emitted by Carbon QDs, the visual detection sensitivity can be significantly improved. With simple construction and convenient operation, this novel QDs-AChE aerogel sensor enables high sensitive, rapid detection of various OPs residues and their mixtures on agricultural products.

Declaration of interests

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.

CRediT authorship contribution statement

Tao Hu: Conceptualization, Methodology, Investigation, Writing - original draft, Funding acquisition. **Jian Xu:** Data curation, Investigation, Writing - original draft, Validation. **Yi Ye:** Visualization, Investigation. **Yu Han:** Writing - review & editing, Investigation. **Xiao Li:** Investigation, Writing - review & editing, Validation. **Zhen Wang:** Investigation, Visualization. **Dongke Sun:** Writing - review & editing, Conceptualization, Validation. **Yunlong Zhou:** Conceptualization, Writing - review & editing, Validation. **Zhonghua Ni:** Methodology, Supervision, Project administration, Writing - review & editing, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.04.036.

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