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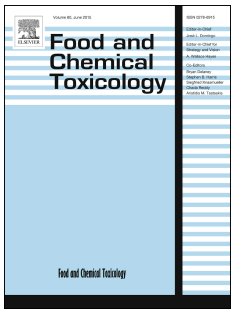

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Trace determination of carbamate pesticides in medicinal plants by a fluorescent technique

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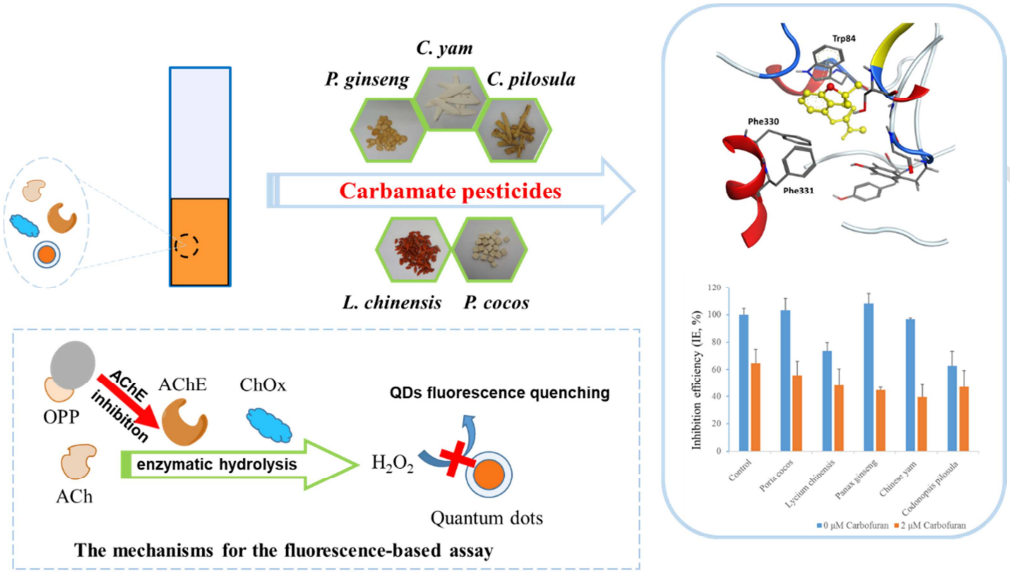
Graphical abstract



1. Introduction

Medicinal plants (MPs) as the common therapeutic agents and nutritional supplements have been widely used in developing countries because of their strong reputation for excellent beneficial efficacy (Huang et al., 2010; Zhang et al., 2015a; Zhu et al., 2014). According to the report from World Health Organization (WHO), the international market of MPs has expanded very fast in recent years and the annual sales is estimated to be USD 60 billion (WHO, 2002). However, pesticide residues have been detected in many MPs distributed in the market, and the levels of some samples even exceed the maximum residue limits (MRLs) regulated by the European Union (EU) (Kuang et al., 2013). Particularly, some of the pesticides are categorized as highly hazardous ones or extremely hazardous ones by the WHO, such as carbofuran, methomyl, omethoate and so on. It is well known that the pesticides residues could enter the human body via food chain, thus even trace amount of them could pose a huge threat to human health (Government of Canada; Kraft, 2015; Lei et al., 2015; United States Environmental Protection Agency). The increasing safety risk regarding MPs pesticides pollution has caused world-wide concerns.

Carbamate, organochlorine and organophosphorus pesticides are the three major insecticides that used in the process of MPs production. In comparison with the other two types of pesticides, carbamates are well-known for their low bio-accumulation, low mammalian toxicities and broad spectrum of pest control, resulting in a massive use for controlling pest so as to increase agricultural yields (Cesarino et al., 2012; Eddleston et al.,

2002; Wu et al., 2005). Recently, a variety of methods have been employed in the detection of carbamate pesticides. Featuring with low detection limits, high separation efficiency and high reproducibility, the chromatographic methods including gas chromatography, high performance liquid chromatography and capillary electrophoresis coupled with various kinds of detectors are commonly used (Chowdhury et al., 2013; Grimalt and Dehouck, 2016; Masia et al., 2016; Wei et al., 2016). However, these techniques require qualified personnel, expensive instruments and tedious sample preparation, which limit their usage in MPs production, storage, and distribution process. Therefore, it is very attractive for developing some cheap, rapid, user-friendly platforms for on-site monitoring, such as sensing and immunoassay techniques (Choi et al., 2015; Ma et al., 2015; Rodrigo et al., 2014; Ye et al., 2016).

Owing to great progress in the interdisciplinary field of material sciences, the fluorescence-based techniques have obtained rapid and remarkable development. Comparing with colorimetric detection methods, fluorescence-based techniques have become the powerful approaches for the contaminants analysis due to the higher sensitivity. Quantum dots (QDs) are the most dominant nanomaterial adopted in fluorescence-based techniques, because of the striking characteristics including high quantum yield, less environmentally sensitive, broad absorption spectra, as well as narrow and symmetric emission bands (Alivisatos, 2004; Lia and Zhu, 2013; Michalet et al., 2005). Some previous studies have highlighted the application of QDs-based biosensors on the trace pesticides detection due to their remarkable sensitivities, the limits of detection (LODs) of which were much lower than the regulatory

limits or guidelines (Nsibande and Forbes, 2016; Ren et al., 2015; Yi et al., 2013). Core/shell QDs, with the protection of an inorganic shell layer on the QD surface, outperform core-only QDs with the advantages of higher resistance to chemical degradation and higher quantum yield (Danek et al., 1996; Hines and Guyot-Sionnest, 1996). However, only one biosensor has been reported so far for the sensitive detection of carbamate pesticide on the basis of core-shell QDs (Zhang et al., 2015b).

In this study, a simple, rapid, and sensitive method based on core-shell QDs has been developed for the detection of three carbamate pesticides, including carbofuran, aldicarb and methomyl. The proposed strategy of the assay is illustrated as follows (Scheme 1). Acetylcholine (ACh) can be hydrolyzed and oxidized by acetylcholinesterase (AChE) and choline oxidase (ChOx) along with the production of hydrogen peroxidase, which will cause the fluorescence quenching of QDs. In the presence of carbamate pesticides, the reaction of ACh with AChE could be inhibited, and the inhibition efficiency could be evaluated by measuring the fluorescence changes of QDs. As distinct from the biosensor with complex assembly process, the present method provides a simple approach to detect analytes with only a mixing process. It would largely reduce the cost and simplify the operation for the manufacture of this sensor. Furthermore, it shows potential to be a convenient tool for onsite analysis of carbamate pesticides by combing with a portable fluorescence spectrometer. Besides, molecular docking has been carried out to investigate the binding modes and interactions of the investigated carbamate pesticides with the active sites of AChE.

Since various kinds of substances in real sample may induce suppression or enhancement

of the detection signal, the sample matrix could have a strong influence on the quantification of the analytes (SANTE, 2015). Several studies have investigated the matrix effects of crops, vegetables, fruits, and MPs in the quantitative analysis of pesticide residues by using traditional chromatographic method (Guedes et al., 2016; Hajslova and Zrostlikova, 2003; Hernandez et al., 2005; Mastovska et al., 2005; Zuin and Vilegas, 2000). Particularly, a variety of active components in MPs, including saponin, alkaloid and phenolic acid may interfere with the analysis to a great extent. Therefore, in order to highlight the applicability of the developed method for the detection of pesticides in real samples, the matrix effects of eight common MPs (*Panax ginseng, Codonopsis pilosula, Poria cocos, Chinese yam, Lycium chinensis, Astragalus membranaceus, Glycyrrhiza uralensis,* and *Pericarpium citri reticulatae*) have been systematically explored in the present work.

2. Materials and methods

2.1 Reagents, chemicals and standards

Acetylcholine chloride (ACh-Cl), bovine serum albumin (BSA), hydrogen peroxide (H_2O_2), AChE (Type VI-S, ≥1000 U/mg from *Electrophorus electricus*, lyophilized powder) and ChOx (≥10 U/mg from *Alcaligenes* sp., lyophilized powder) were purchased from Sigma-Aldrich. Carbamate pesticide standards, including carbofuran, aldicarb and methomyl were purchased from Dr. Ehrensdorfer (Augsburg, Germany). Three different sizes water-soluble CdSe/ZnS core/shell QDs were supplied by Ocean Nanotech (Springdale, AR, USA), and the maximum fluorescence emissions of QD 1, 2 and 3 were 530 nm, 597 nm, and 650 nm,

respectively. Phosphate buffered solution (PBS) (pH 8.0, 10 mM) and Milli-Q ultrapure water (Millipore, ≥18 MΩ·cm) were used throughout. Acetonitrile was used to prepare stock solutions of the investigated pesticides, which were then stored at 4 °C in the refrigerator. A series of working solutions were prepared daily by diluting stock solutions with PBS (pH 8.0, 10 mM) containing 0.5 mg/mL of BSA. Dried MPs samples including *Panax ginseng* (*P. ginseng*), *Codonopsis pilosula* (*C. pilosula*), *Poria cocos* (*P. cocos*), *Chinese yam* (*C. yam*), *Lycium chinensis* (*L. chinensis*), *Astragalus membranaceus* (*A. membranaceus*), *Glycyrrhiza uralensis* (*G. uralensis*), and *Pericarpium citri reticulatae* (*P. citri reticulatae*) were purchased from NEAUTUS Traditional Chinese Medicine Co., Ltd. in Sichuan, China.

2.2 Apparatus

A Lumina Fluorescence Spectrometer (Thermo Fisher Scientific) was used to measure the fluorescence intensities of solutions. Both the excitation and emission slits were set at 5 nm. The samples for the fluorescence measurements were placed in a quartz cuvette with an optical path length of 10 mm. The sample treatment was carried out on an ultrasonic cleaner (SCIENTZ SB-300 DTY, Ningbo Scientz Biotechnology Co., Ltd., China).

2.3 Experimental procedure for pesticide determination

The MPs specimens were sliced into small pieces (2 mm in diameter) and then mixed together thoroughly. 1.0 g homogenized sample was transferred into a 15 mL glass centrifuge tube, and 10 mL acetonitrile was added. The sample was extracted by sonication for 10 minutes at 60 kHz, and then the tube was centrifuged for 5 min at 800×g at 25 °C. 1.0 mL of the supernatant was decanted into a 2.0 mL centrifuge tube before evaporating to dryness with

a gentle stream of nitrogen at room temperature. The residue with various concentrations of carbofuran was reconstituted in AChE (83 U/L, 300 μL) and incubated at 35 °C for 20 min. After step-wise addition of 425 μL ChOx (882 U/mL), 125 μL QDs (0.02 μM) and 50 μL ACh (6 mM), the fluorescence signal of the samples was recorded over time with emission wavelength range of 550–650 nm. The fluorescence intensity was measured with an emission of wavelength of 597 nm. All the data were presented from three independent experiments.

2.4 Molecular docking

Molecular Operating Environment (MOE, Chemical Computing Group, Canada) was used as docking software to mode the interactions of the pesticides and AChE. The X-ray crystal structure of AChE was taken from the Protein Data Bank (PDB code: 1ACJ), and water molecules were removed from the PDB files. The structures of pesticides were converted to 3D structures, and docked into the active site of the protein after energy being minimized with the Merck molecular force field 94x (MMFF94x) using MOE. The Dock scoring in MOE software was done by ASE scoring function.

3. Results and discussion

3.1 Fluorescence quenching effect of the CdSe/ZnS QDs

Three different sizes of CdSe/ZnS QDs were characterized with fluorescence emission spectra at first. As shown in Fig. 1, the narrow emission spectra of the QDs in aqueous solution were presented and indicated the uniform size of the QDs. It can be seen that the maximum fluorescence emissions of the QDs at 530, 597 and 650 nm when the excitation

wavelength at 360 nm. It is demonstrated from Fig. 1 that fluorescence intensity of QDs decreased with the addition of hydrogen peroxide. As described above, ACh could be hydrolyzed and oxidized with the generation of hydrogen peroxide in the presence of AChE and ChOx. Therefore, the fluorescence of QDs could be quenched by the enzyme-generated hydrogen peroxide.

3.2 Optimization of experimental parameters

In the present study, comprehensive investigations were performed on experimental parameters that influence the fluorescence quenching effect of the solution-based biosensor. Parameters such as QDs species, the concentrations of ChOx, pH value, detection time, incubation temperature and incubation time were optimized. The stronger quenching effects of H_2O_2 on QD 1 and QD 2 were observed in Fig. 1 while the concentration of QD 2 was less than that of QD 1. Thus, QD 2 with the maximum fluorescence emissions at 597 nm was selected in the following experiments.

To evaluate the fluorescence quenching effects of the experimental parameters in the absence of pesticide, quenching efficiency (ΔF_t) values were calculated via equation 1.

$$\Delta F_t = F_O - F_t \quad (1)$$

where F_0 and F_t are the fluorescence intensity recorded at 597 nm before and at different time after the addition of ACh in the detection system, respectively. The ChOx concentration could directly influence the generation of H_2O_2, and it was optimized at first. The effect of ChOx concentration within the range of 41.7−375 U/L was investigated. The ΔFt values gradually increased with the increase of ChOx concentrations, where a slight increase of fluorescence

intensity could be observed from 250 to 375 U/L. Considering sufficient amount of ChOx was very important to the production of H_2O_2, 375 U/L was selected as the optimum concentration in the following experiment. ACh hydrolysis reaction was performed under a kinetically controlled condition. In order to produce the sufficient H_2O_2 and get better reaction activity of AChE, a large amount of ACh is necessary. The results indicated that the ΔF_t values reach a balance when ACh concentration was more than 300 nM. And pH as another critical factor that would affects enzyme activity dramatically, and pH 8.0 was selected since the optimum pH values for AChE and ChOx were 8.0–9.0 and 7.0–8.0 accordingly. Furthermore, the ΔF_t reached a maximum value and tended to stable while incubation temperature and time for AChE were optimized as 35 °C and 20 min, which was similar with the previous study (Wu et al., 2017; Zheng et al., 2011). Finally, different detection times were studied to ensure the reproductivity, and 30 min was chosen in the further experiment because the fluorescence intensity changed slightly after 30 min.

3.3 Inhibition efficiency of carbamate pesticides

As the AChE inhibitors, carbamate pesticides could inhibit the enzyme activity thus caused the decrease of QDs fluorescence quenching. Consequently, the fluorescence variations could be used to evaluate the inhibition efficiency of pesticide. A proper AChE concentration was necessary for sensitive detection of pesticides, which would provide a satisfied linear range between the inhibition efficiency and the pesticide concentration. Hence, the effect of three AChE concentrations (12.5, 24.9, and 125 U/L) on the fluorescence variations was investigated while keeping other experimental conditions unchanged. In the

present work, carbofuran was selected as the representative pesticide to evaluate the inhibitory effect of the pesticide. The values of inhibition efficiency (IE, %) were calculated according to the following equation 2:

$$\text{IE}\ (\%) = \frac{\Delta F_{30\ without} - \Delta F_{30\ with}}{\Delta F_{30\ without}} \times 100 \quad (2)$$

where $\Delta F_{30\ \text{without}}$ and $\Delta F_{30\ \text{with}}$ are the absolute quenching efficiency for the first 30 min without and with inhibition at a certain concentration of pesticide, respectively.

In this work, the IE values significantly increased with the increase of carbofuran concentration from 2 to 50 nM when the concentration of AChE was 24.9 U/L. With the carbofuran concentration was further increased to 200 nM, the IE values was increased slightly (Fig. 2A). When the concentration values were transformed into logarithmic forms, the relationship between the IE values and the pesticide concentrations showed a good linearity (R^2 = 0.9921) (Fig. 2B). Conversely, the narrow linear range was obtained in the presence of the lower AChE concentration at 12.5 U/L, and the detectable limit was worse when the higher AChE concentration at 125 U/L. As a result, the AChE concentration of 24.9 U/L was chosen for the further experiment. In order to demonstrate the applicability of this method, aldicarb and methomyl were selected and investigated in this study. As shown in Fig. 3, the linearity for methomyl and aldicarb were observed in the range of 50-5000 nM. The correlation coefficient (R^2) for methomyl and aldicarb were 0.9768 and 0.9839. The limits of detection (LODs) for carbofuran, aldicarb and methomyl were 2, 50 and 50 nM, respectively.

Moreover, to compare the strength of inhibitory effects of the three investigated carbamate pesticides, the concentration that induces an inhibitory effect of 20% (IC_{20}) has

been calculated. And the IC_{20} values were determined using the linear regression equations (Fig. 2 and Fig. 3). According to the results, carbofuran showed strong inhibitory effect with IC_{20} of 8.9 nM, while aldicarb and methomyl exhibited moderate inhibitory effect with IC_{20} of 109.6 nM and 229.1 nM.

3.4 Molecular docking results

In order to reveal the potential mechanism of the differences in inhibition strength among several pesticides on AChE, the binding modes of the selected pesticides have been investigated using the molecular docking method. There are two important properties required for the inhibitory effect. One is the steric properties to block access and form a stable enzyme-inhibitor complex. The other is high reactivity of the carbonyl carbon of carbamate pesticide to accept a nucleophilic attack from the serine hydroxyl in the active site of AChE. In this study, the steric properties of pesticides were investigated, since docking algorithms do not include the nucleophilic attack. It is well known that the catalytic anionic site (CAS) composed of Trp84, Phe330, and Phe331 is one of the active site of AChE while the other is esteratic site containing the catalytic triad Ser200-His440-Glu327 (Fig. S1) (Fang et al., 2008; Pan et al., 2014). According to the molecular docking results, the strong binding affinities were found between the selected carbamate pesticides and the CAS site of AChE. As shown in Fig. 4, the molecular docking results suggested that strong π–π interaction could be formed between Trp84 and the pesticides at the anionic site. As a result, the pesticides with aromatic group like carbofuran showed the strong inhibitory effect. Aldicarb and methomyl had aliphatic groups which can form H-π interaction with Trp84 and Phe330, respectively.

Therefore, both of them showed the moderate inhibitory effects.

3.5 Sample matrix effect

In order to investigate the applicability and reliability of the developed method in the real samples analysis, the matrix effects of eight MPs, including *P. ginseng, C. pilosula, P. cocos, C. yam, L. chinensis, A. membranaceus, G. uralensis,* and *P. citri reticulatae* on the detection system have been explored.

Firstly, the effects of the sample matrix on the QDs were investigated and evaluated by the fluorescence intensity ratio of QDs (F_{matrix} / F_{PBS}) prepared in sample matrix and PBS solution, respectively. As shown in Fig. 5A, most of the MPs matrices have not shown the significant influence on the fluorescence intensity of QDs except that of *G. uralensis* and *P. citri reticulatae*. It can be seen that some pigments adhered to the bottom of tube when the acetonitrile extractions of these two MPs were evaporated under a stream of nitrogen. This indicated that water-insoluble pigments in the MPs sample would bring the interference to the qualification and quantitation of analytes by using optical biosensor.

After that, the effects of sample matrix on the detection system were studied and compared in the selected MPs except *G. uralensis* and *P. citri reticulatae*. Fluorescence percentages ($\Delta F_{30\ \text{matrix}}$ / $\Delta F_{30\ \text{PBS}}$) were calculated to evaluate the matrix effect, where $\Delta F_{30\ \text{matrix}}$ and $\Delta F_{30\ \text{PBS}}$ are the absolute quenching efficiency for the first 30 min of biosensor prepared in sample matrix extract and PBS solution, respectively. Fig. 5B shows that the matrix effects in the range of 62.9% to 108.1%, and *C. pilosula* had highest suppression effect.

In this work, the fluorescence quenching effect of QDs was influenced by the production of enzyme generated H_2O_2. Therefore, the suppression effects of the MPs were associated with the consumption of H_2O_2 or the inhibitory effect of AChE. To investigate the effect of sample matrix on the consumption of H_2O_2, the QDs were prepared in the PBS solution and MPs extract with different sample matrix, respectively; and then mixed with a fixed amount of H_2O_2. $F_{10\ \text{matrix}}$ and $F_{10\ \text{PBS}}$ were the fluorescence intensity at the 10 min after the H_2O_2 added separately into the sample matrix extract and PBS solution, while fluorescence ratios ($F_{10\ \text{matrix}}$ / $F_{10\ \text{PBS}}$) were used to evaluate the effect of sample matrix on the consumption of H_2O_2. If the sample matrix can consume the generated H_2O_2, the fluorescence ratio would be greater than 1. Fig. 5C shows that all the sample matrices have no positive effects on the consumption of H_2O_2. In other words, the suppression effect in MPs was attributed to the inhibitory effect of sample matrix on AChE instead of the consumption of H_2O_2. According to the previous study on the screening of the AChE inhibitors from natural plants, alkaloids were considered important constituents with anticholinesterase properties (Konrath et al., 2013; Mukherjee et al., 2007). Alkaloids are one of the main chemical constituents of the plants from genus *Lycium* and *Codonopsis* (He et al., 2015; Qian et al., 2017) , which may be the good explanation for the inhibition effects of *C. pilosula* and *L. chinensis* on AChE activity.

To evaluate the practicality of the proposed method for the pesticide detection, the inhibition effects of pesticide on the detection system in the presence and absence of carbofuran have been compared. The differences of inhibition efficiency (ΔIE) between the concentration of carbofuran at 0 and 2 μM revealed the AChE sensitivity to the pesticide (Fig.

5D). In comparison with control group (PBS solution), stronger AChE sensitivities were observed in three MPs including *P. ginseng*, *C. yam* and *P. cocos*, which suggested that the pesticide analysis in these three MPs were as good as that in PBS solution. However, weaker AChE sensitivities were obtained in *A. membranaceus*, *L. chinensis* and *C. pilosula*. As we speculated in the above, the weaker AChE sensitivities in *L. chinensis* and *C. pilosula* may be resulted from the presence of alkaloids. But for *A. membranaceus* with high content of saponins, flavonoids and polysaccharides except alkaloids (Fu et al., 2014), pesticide degradation and dissipation in plants may be the reason for none AChE sensitivities (Fantke and Juraske, 2013; Zhou et al., 2015).

3.6 Pesticide detection in real sample

Subsequently, using carbofuran as a model, the applicability of the developed method for determining the investigated carbamate pesticides in real samples has been evaluated. The matrix-matched calibrations in MPs were prepared and studied. As shown in Fig. S2, the inhibition efficiency of carbofuran increased with the increase of pesticide concentrations. The regression analysis between the IE value and the logarithmic form of pesticide concentrations displayed satisfactory linearity (Fig. 6). It is demonstrated from Table 1 that linearity in the range of 2-200 nM and 5-500 nM were obtained in PBS solution and MPs, respectively. The results showed carbofuran exhibited satisfied calibration capacity with correlation coefficients (R^2) higher than 0.95 for all analytical curves except for that in *A. membranaceus*. And the LODs for carbofuran in MPs sample matrix were 5 nM, which were lower than the MRLs regulated by the United States Food and Drug Administration (FDA) as

well as European Union (EU). The slope ratios of calibration curves in the sample matrix and PBS solution were compared to investigate the matrix effect in the pesticide detection. The slope ratios of 1 indicates that the matrix does not significantly suppress or enhance the signal, otherwise denoting suppression (<1) or enhancement (>1) effect. It was demonstrated from Table 1 that *P. cocos* and *L. chinensis* with slope ratio at 0.77 and 0.84 showed the suppression effects. The strongest enhancement effect was observed in *C. pilosula*, followed by *C. yam* and *P. ginseng* with relatively less enhancement effects. And the slope ratios in the range of 0.77-1.24 illustrated the high occurrence of matrix effect. As indicated in Fig. 1, the standard deviations (SD) of repeated experiments in *P. cocos*, *C. yam* and *P. ginseng* were smaller than those in *C. pilosula*, and *L. chinensis*. It means the precise and accurate results for the sensitive detection of carbofuran could be easily obtained in *P. cocos*, *C. yam* and *P. ginseng*. The diverse SD values may attribute to the remarkable differences in the sensitivity of AChE to carbofuran in different sample matrices.

4. Conclusions

In the present study, a simple and sensitive fluorescent technique based on core-shell QDs has been developed for the detection of carbamate pesticides. The experimental parameters that influence the fluorescence quenching effect of the solution-based biosensor, including QDs species, the concentrations of ChOx, pH value, detection time, incubation temperature and incubation time were optimized. Under the optimum conditions, the lowest detectable concentrations for the investigated pesticides including carbofuran, aldicarb and

methomyl were no more than 0.05 μM, which were lower than the MRLs. The molecular docking study indicated that the selected carbamate pesticides bound to the catalytic active site of AChE via π–π or H-π interactions. Moreover, the matrix effects of eight MPs on the detection system have been explored to investigate the applicability and reliability of the developed method in the real samples analysis. The slope ratios ranged from 0.77 to 1.24 indicated high occurrence of matrix effect in MPs, and the necessity of matrix-matched calibration in real samples. In summary, we believe the contents reported in this paper are helpful to the researchers who focus on the rapid determination of some trace carbamate pesticides in real samples, especially those with complex matrices like MPs, vegetables, fruits, and other agricultural crops.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

Transparency document

Transparency document related to this article can be found online at

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Table 1 Analytical performance of the present work.

Control / MPs	Linearity range (nM)	Linear Equation	Correlation coefficient (R^2)	LODs (nM)	Slope ratio
Control	2-200	y = -21.045x + 63.114	0.9921	2	1.00
Astragalus membranaceus	N[a]	N	N	N	N
Poria cocos	5-500	y = -16.102x + 44.478	0.9554	5	0.77
Lycium chinensis	5-500	y = -17.665x + 51.171	0.9839	5	0.84
Panax ginseng	5-500	y = -22.031x + 72.228	0.9779	5	1.05
Chinese yam	5-500	y = -25.162x + 71.044	0.9901	5	1.20
Codonopsis pilosula	5-500	y = -26.165x + 70.260	0.9746	5	1.24

[a] N: not detected.

Figure legends

Scheme 1. Schematic illustration of the biosensor for the carbamate pesticides detection in medicinal plants based on core-shell QDs.

Fig. 1 The fluorescence spectrum of three kinds of QDs (Solid line; QD 1, 530 nm, green line; QD 2, 597 nm, red line; QD 3, 650 nm, crimson line) and the QDs after the addition of hydrogen peroxide at 10 min (Dashed line; QD 1, 530 nm, green line; QD 2, 597 nm, red line; QD 3, 650 nm, crimson line). The concentrations of QD 1, QD 2, QD 3 and hydrogen peroxide were 17.5 nM, 2.5 nM, 7.5 nM and 0.6 mM, respectively. (Excitation wavelength: 360 nm)

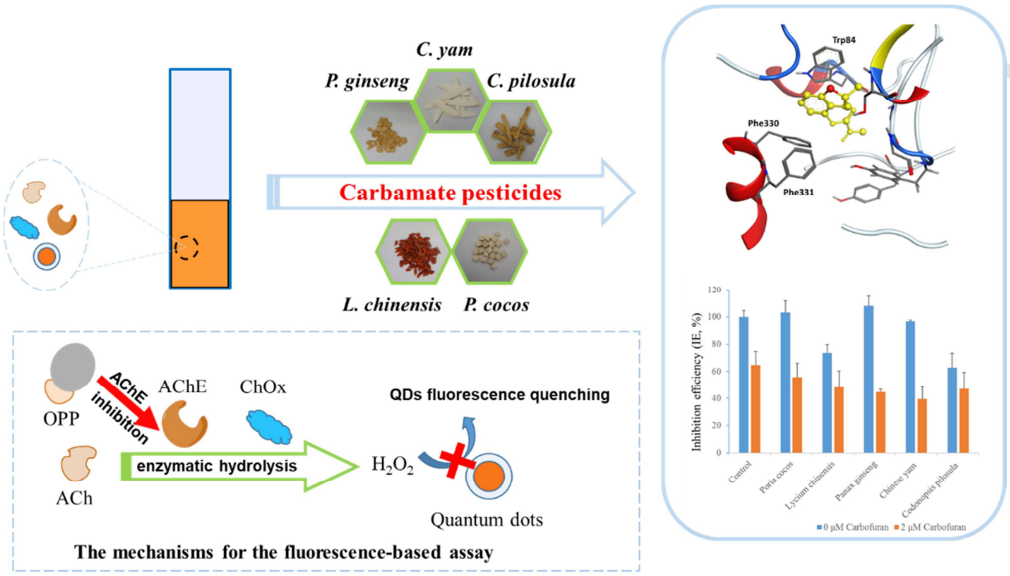
Fig. 2 The plots of inhibition efficiency vs Carbofuran concentration (A) and inhibition efficiency vs the logarithm of Carbofuran concentration (B) in PBS solution. The concentrations of QDs, AChE and ChOx in all experiments were 2.5 nM, 24.9 U/mL and 375 U/mL, respectively.

Fig. 3 The linear relationship between the inhibition efficiency with the logarithm of concentration of methomyl (A) and aldicarb (B) ranging from 50 to 5000 nM, and the plots of inhibition efficiency and the concentration of methomyl (C) and aldicarb (D) ranging from 50 to 5000 nM.

Fig. 4 Molecular docking of carbofuran (A and B), methomyl (C), and aldicarb (D) with AChE generated with MOE.

Fig. 5 The effect of sample matrix on the fluorescence intensity of QDs (A), biosensor without pesticide (B), the consumption of hydrogen peroxide (C) and the pesticide detection (D).

Fig. 6 The linear relationship between the inhibition efficiency with the logarithm of concentration of carbofuran ranging from 5 to 500 nM in *Panax ginseng* (**1**); *Poria cocos* (**2**); *Chinese yam* (**3**); *Codonopsis pilosula* (**4**) and *Lycium chinensis* (**5**).



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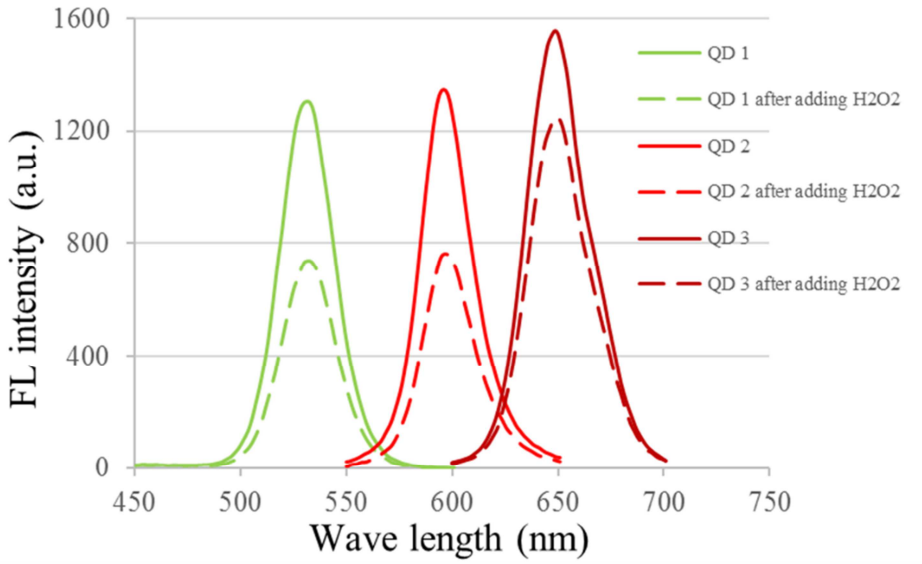

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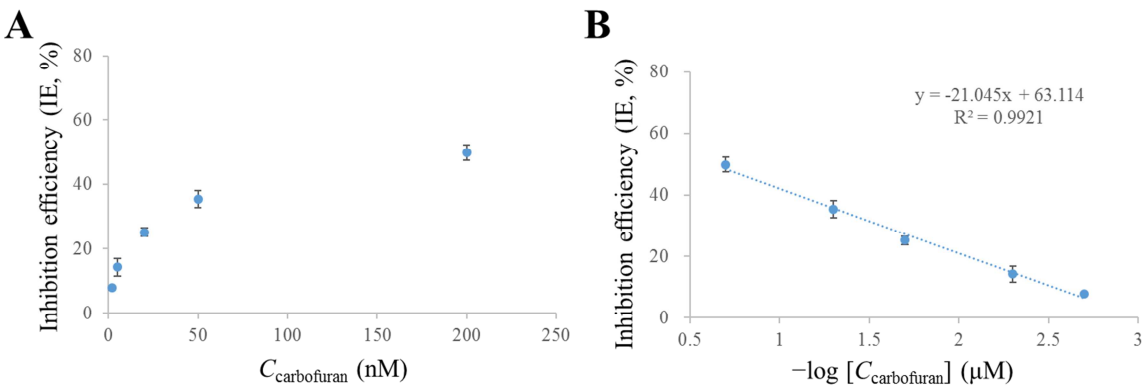

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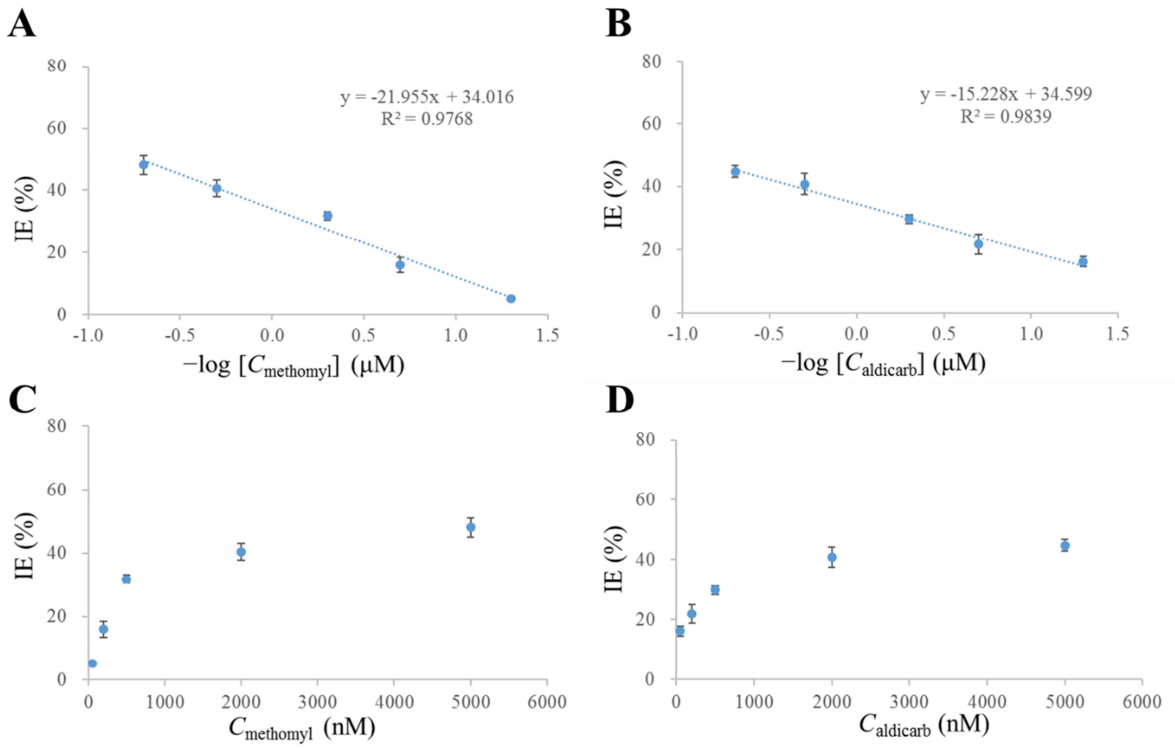

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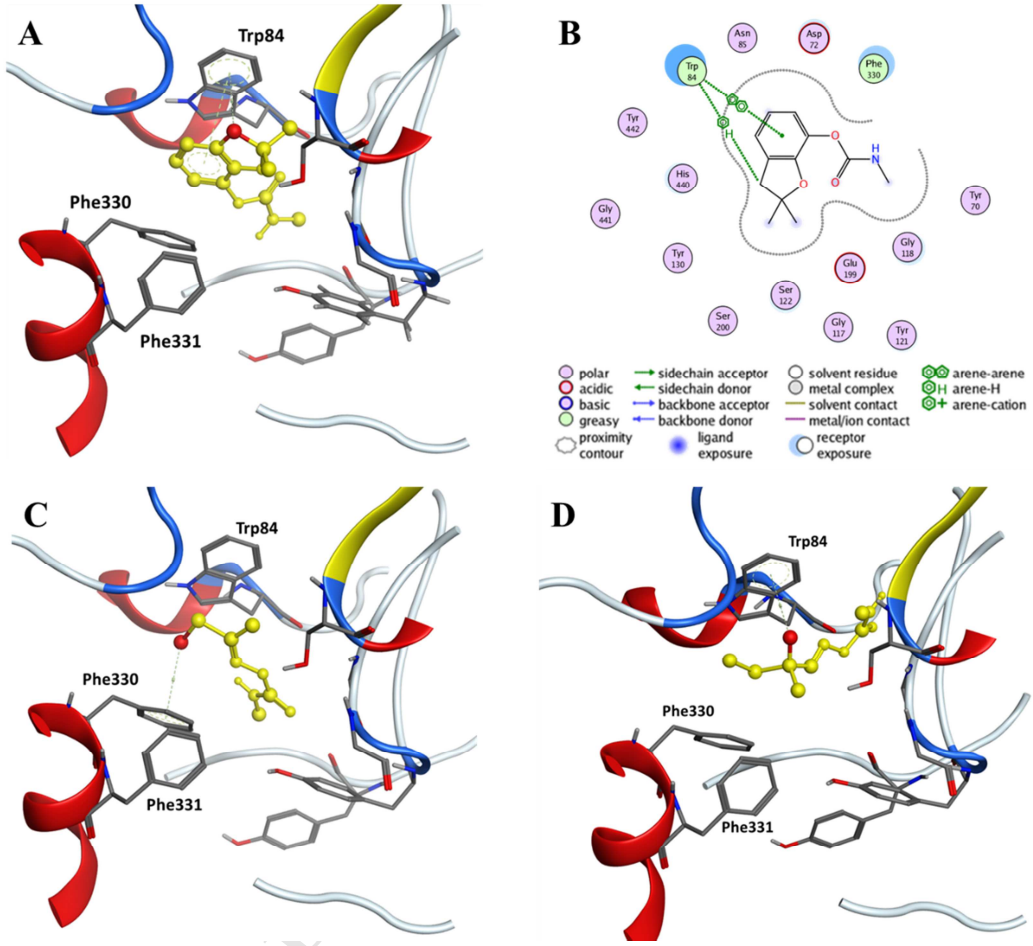

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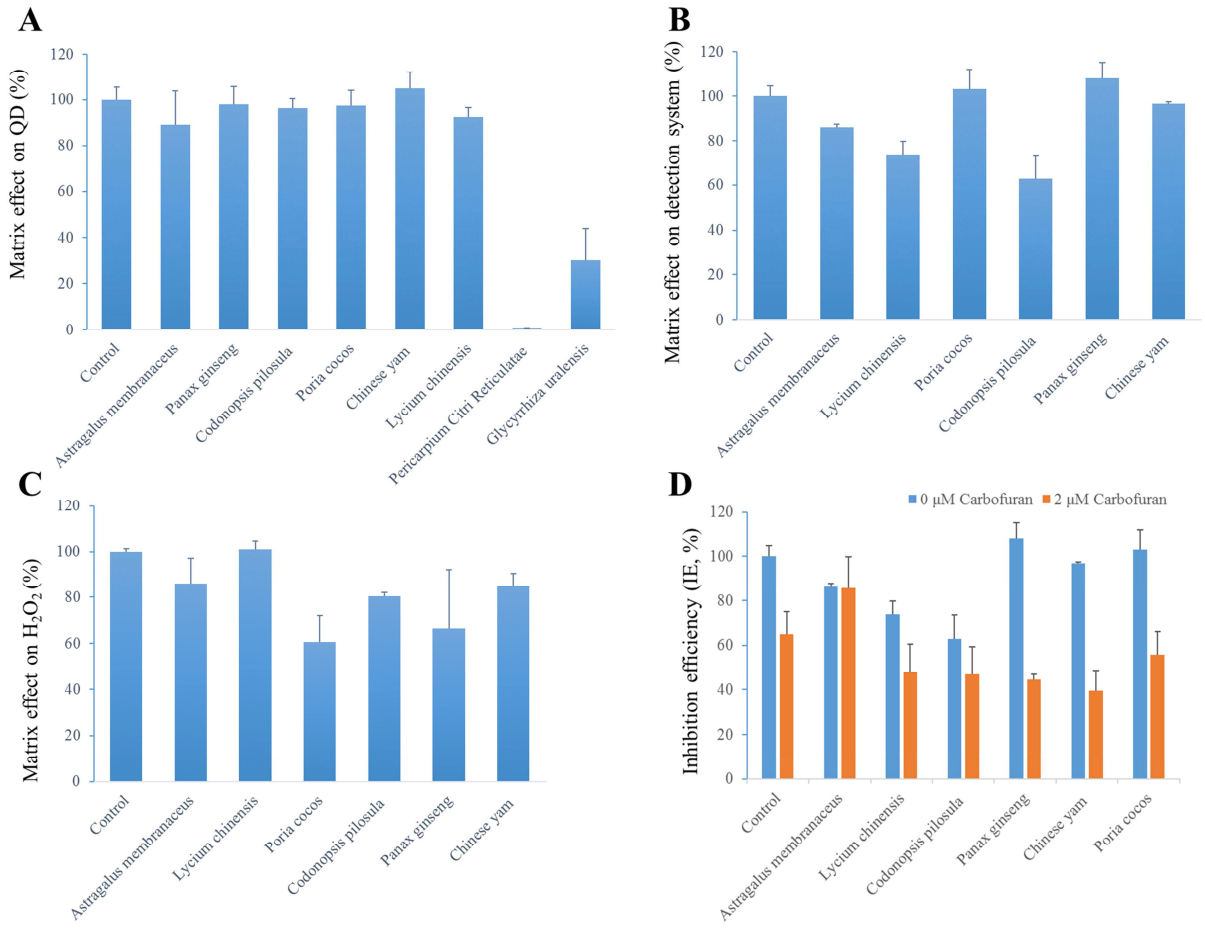

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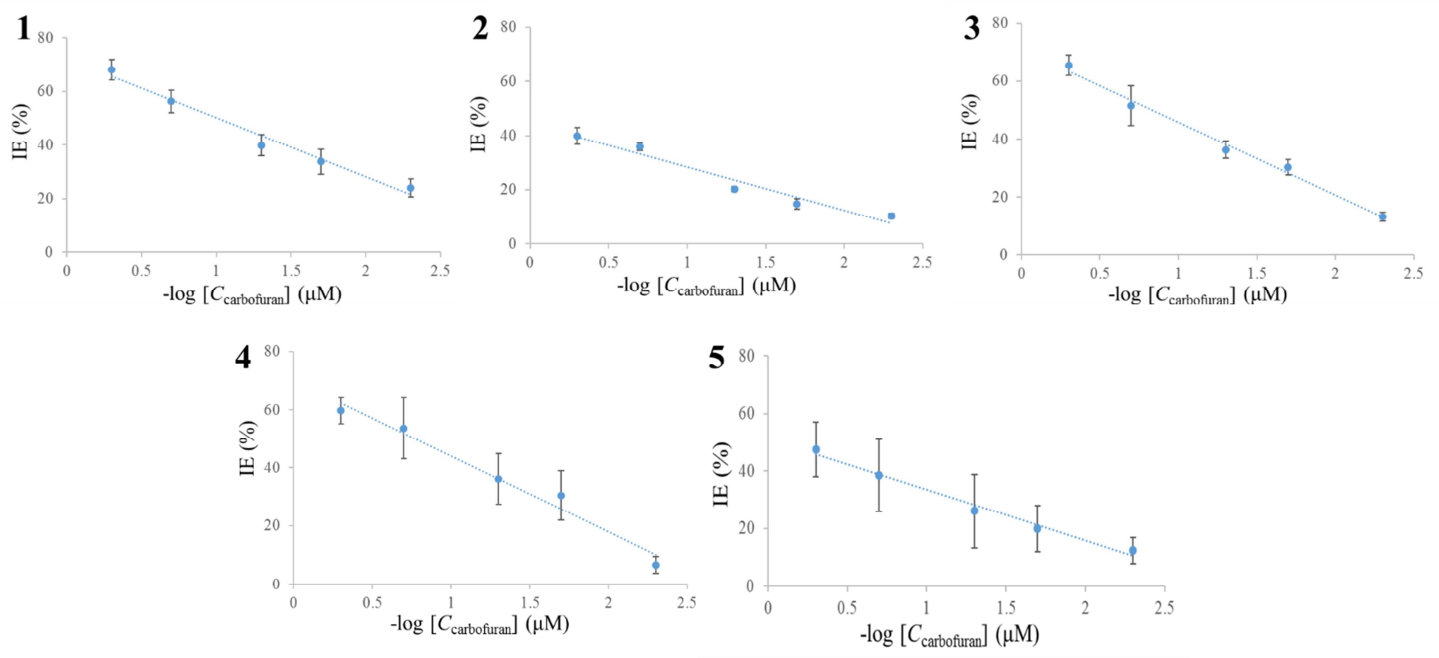

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Highlights

- Detection of carbamate pesticides using a fluorescence-based technique.
- The LDC for the investigated carbamates was no more than 0.05 μM.
- Carbamates bound to the CAS of AChE via π–π or H-π interactions.
- Matrix effects of eight MPs on the detection system were explored.