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Development of a fluorescence-linked immunoassay based on quantum dots for fenvalerate

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The glutathione-coated CdTe quantum dots (QDs) were synthesized in a paper. After purification, the QDs were coupled with monoclonal antibody to fight against fenvalerate. The conjugates had the same emission wavelength as that of QDs. The excitation wavelength and emission wavelength of the conjugates were determined to obtain the highest signal-to-noise ratio. After the antibody concentration optimization, the fluorescence-linked immunoassay method was developed. The method used the QDs as the signal to quantify the fenvalerate. Compared with the enzyme-linked immunosorbent assay (ELISA), it saved more than 1 h and decreased the false-positive rate using the specified emission wavelength of QDs. The 50% inhibitory concentration (IC₅₀) of the method was 0.28 µg mL⁻¹. The detection limit was 25 ng mL⁻¹ and the linear range was 60 ng mL⁻¹–3.83 µg mL⁻¹. Via preliminary application, fenvalerate residues in spiked samples were determined. The recovery of fenvalerate in water samples ranged from 84.5% to 96.2% and that in vegetables ranged from 72.5% to 125.7%. It was a rapid detection of the fenvalerate residue in environment and vegetables.

Keywords: quantum dots; CdTe; fenvalerate; antibody; FLISA

Introduction

Due to their high bioefficiency, synthetic pyrethroids are widely used in agriculture especially for growing vegetables and fruits. The fenvalerate, one of the most commonly used synthetic pyrethroids, is classified as a type II pyrethroid (Arena et al., 2008) because it contained an alpha-cyano group. It is a nonsystemic insecticide used against a wide range of pests, especially those resistant to organochlorine, organophosphorus, and carbamate insecticides (Sharma, Gupta, & Shankara, 2008).

Its extensive use, however, does harm human and livestock, though it is less toxic to mammals. (Mak et al., 2005). The residues also cause great damage to the aquatic environment and organisms (Pieters & Liess, 2006), and most pyrethroids are highly toxic to aquatic organisms.

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Nowadays, governments and people pay increasing attention to food quality and safety. Pesticide residues (Bouwman, Sereda, & Meinhardt, 2006; Jiménez, Bernal, del Nozal, Bernal, & Toribio, 2007; Sharma, Satya, Kumar, & Tewary, 2005) are one of the major factors which affect the health of humans and livestock. Fenvalerate is one of the most commonly used pesticides in the cultivation of vegetables and fruits, which are usually eaten raw by humans. Therefore, the detection and monitoring of fenvalerate residues are extremely important for public health.

Many methods have been reported for the detection and monitoring of the fenvalerate residues, including the gas chromatography (GC) (Pang et al., 2006), high-performance liquid chromatography (HPLC) (Zhou, Zhang, & Xie, 2011), ion-trap mass spectrometry and electron-capture GC (Pang et al., 2006), and solid-phase extraction (SPE)-GC (Gong et al., 2004). Although these methods all have high sensitivity and stability, they all require expensive equipments and skilled personnel, and are time-consuming processes, which are not suitable for the detection of fenvalerate residues in vegetables and fruits.

Immunoassay methods such as enzyme-linked immunosorbent assay (ELISA) and fluorescence immune assay (FIA) have the advantages of sensitivity, speed, and simplicity of operation, which are desirable for rapid detection and monitoring of the pesticides residue. Several ELISA methods have been reported (Shi, Zhang, Ye, Zheng, & Wang, 2011; Song, Lu, Liu, Xu, & Wang, 2011;) for permethrin, deltamethrin, cypermethrin, and fenvalerate. Compared with polyclonal antibody, monoclonal antibody is more specific and sensitive and is widely used in fenvalerate residue detection.

Unlike ELISA, fluorescence-linked immunosorbent assay (FLISA) is a kind of immunoassay which uses fluorescence as a signal to quantify the residue. Besides being simple and sensitive, FLISA is more rapid and gives less false positive than ELISA.

Some researches have been reported for the fluorescence immunoassay based on quantum dots (QDs) for the detection of pesticide residues (Vinayaka, Basheer, & Thakur, 2009). But seldom has any research reported on the determination of the fenvalerate. QDs are a kind of nanomaterial which produces fluorescence signals of a specific wavelength. This material has advantages of continuous absorption spectrum within a wide range, narrow and symmetric emission spectrum, and a large Stokes shift. QDs with fluorescence at different wavelengths can be obtained by adjusting the sizes and components of the QDs (Hua et al., 2009). The peak wavelength of the emission fluorescence for a type of QD is stable. Therefore, QDs are more stable than the traditional fluorescein. As a result, the sensitivity of FLISA based on QDs is about 100 times that based on the traditional fluorescein (Resch-Genger, Grabolle, Cavaliere-Jaricot, Nitschke, & Nann, 2008; Wang, Li, & Slavik, 2007).

The objective of this paper is to explore the possible immunoassay based on QDs for the fenvalerate residue detection in vegetables and other samples. The FLISA method coupled with CdTe QDs as a fluorescent marker is described and applied to practical samples.

Experimental

Reagent

CdCl₂·2.5H₂O (99.0%), Na₂TeO₃, NaBH₄ (96.0%), trisodium citrate (99.0%), NaOH, CH₃OH, C₂H₅OH, β-C₂H₅SH, MgSO₄, acetone, acetonitrile, γ-L-glutamyl-

L-cysteinyl-glycine (GSH), and hexane are all purchased from Shanghai Guoyao chemical reagents corporation; OVA (agarose gel electrophoresis grade) were all purchased from Sigma corporation; white opaque plate with 96 wells was purchased from Coastar corporation; the antibody was from the hybridoma made in our experiment (Jiang et al., 2010); water was obtained from a MilliQ purification system (Millipore).

Instruments

UV spectrophotometer (500, Unicame corporation); fluorescence spectrophotometer (F-4500, Hitechi corporation); Vortex device (tongzheng corporation); enzyme-labeling instrument (spectramaxM2e, Molecular corporation); magnetic stirrer (MR 3001, Heidolph corporation); plate washer (Els-50, Biotek corporation); rotary evaporator (Laborota 4003 control, Heidolph corporation); gas chromatography with ECD detection (Aglient 6890); and nitrogen blowing instrument (BF-2000, Bafangshiji corporation).

Buffer and solution

The coating buffer was 0.025 M (pH 9.6) carbonate buffer. Washing buffer was phosphate buffer solution with tween (PBST), which was phosphate buffer solution (PBS) containing 0.05% (v/v) tween 20.

Synthesis of CdTe QDs

Colligating the reported methods (Sheng, Han, Hu, & Chen, 2010; Zhang et al., 2009), the CdTe QDs were synthesized by a modified method. $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (45.7 mg) was dissolved in 20 mL of ultrapure water kept bubbling with highly pure N_2 and the pH value of the mixed solution was adjusted to 10.5. The following reagents were added in sequence: GSH (73.8 mg), trisodium citrate (0.1 g), and Na_2TeO_3 (8 mg) at N_2 atmosphere after all the chemical reagents were dissolved. The mixture was kept in a microwave oven with a reflux device and the power of the oven was 600 W. The solution started to reflux when it became yellow. QDs of different size would be produced at different time intervals (Wang, Li, & Slavik, 2007). In this work, QDs with 560 nm fluorescence were accepted. About 30 min later, the reaction was finished.

Coupling of QDs with antibody (Dwarakanath, Bruno, & Shastry, 2004)

Ethanol was added dropwise into QDs solution under room temperature until the turbidity appeared. The mixed solution was centrifuged at 4000 rpm for 5 min and repeated again. The left Cd^{2+} , TeO_3^{2-} was eliminated. And then the precipitation was dissolved in PBS buffer. The fluorescence and ultraviolet absorption were measured to obtain the spectrum and concentration of QDs.

Active ester method (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and *N*-Hydroxysuccinimide) was used in the experiment because of GSH with the carboxyl and amino groups surrounding the surface of QDs. Mercaptoethanol was used as stabilizer in the method. Seventy-eight microliters of EDC solution (1 mg mL^{-1}) and 53 μL of NHS (1 mg mL^{-1}) were added to 2 mL of the above QDs

solution and the mixed solution was stirred for 1 h in dark. And then 10 μL β -mercaptoethanol was added. After that, 5 mg of anti-fenvalerate antibody dissolved in 500 μL PBS buffer was added, and the mixture was stirred for 3 h in dark. The final solution was dialyzed in PBS solution for about 2 days after the reaction was complete. Conjugate was collected by centrifugation for 3 min at 10,000 rpm and dissolved in PBST (3 mL) buffer.

Optimization of the concentration of antibody linked with QDs

The concentration of antibody was determined via checkerboard method. An antigen at different concentrations (2 $\mu\text{g mL}^{-1}$, 4 $\mu\text{g mL}^{-1}$, and 8 $\mu\text{g mL}^{-1}$) was coated on the 96-well plate (100 μL per well) for 2 h at 37 °C. After a threefold washing, ovalbumin (OVA) solution (1%) was added to block the wells (200 μL per well) for 1 h at 37 °C. And then an antibody at different concentrations (from 1:1 to 1:16) was added to the wells to react with antigen, after which the plates were washed three times with PBST. The plates were incubated for 1 h at 37 °C. After the last threefold washing, PBST (100 μL) was added to each well. The fluorescence intensity of each well was measured.

Wavelengths of 400 nm and 564 nm were selected for excitation and emission, respectively, to lower the excitation light effect of the emission light and keep the emission strength at a high level. For the purpose of getting a high signal-to-noise ratio, proper concentration collocation of antigen and antibody should be determined.

Fluorescence-linked immunosorbent assay (FLISA)

100 μL per well of antigen solution (2 $\mu\text{g mL}^{-1}$) was coated on the plates and incubated for 2 h at 37 °C. The plates were washed and blocked as mentioned in above section. After another threefold washing, fenvalerate standards of various concentrations (from 5 ng mL^{-1} to 10.1 $\mu\text{g mL}^{-1}$ in PBS with 10% methanol) were added into the wells (50 μL per well) to compete with the antigen to react with the antibody solution (50 μL per well), and then the antibody solution was added (50 μL per well). The concentration of antibody solution was obtained in the above step. The reaction lasted for 1 h at 37 °C. After the last threefold washing, 100 μL of PBS was added into the wells to measure the fluorescence intensity.

In order to study the specificity of the method, three types of pyrethroid pesticides were used to measure the cross-reactivity (CR) between antibody and other pyrethroids. In the work, deltamethrin, cypermethrin, and bifenthrin were selected as related analytes. Deltamethrin and cypermethrin also had phenyl ether and alpha-cyano, which is similar to fenvalerate. Compared with that, there were more differences between bifenthrin and fenvalerate in respect of molecular structure. The CR value was calculated as reported previously (Ahn, Watanabe, Gee, & Hammock, 2004).

Sample test (FLISA and GC)

In order to study the recovery of the method, three types of samples were spiked and detected using the method. One sample was Yangtze River water, which represented

that the environment was polluted easily by the pesticide (Kong, Zhang, Zhang, Shirley, & Li, 2010); the other two were cabbage and rape, which were often under the protection of fenvalerate in agriculture. The samples were all free-fenvalerate.

The fenvalerate was dissolved in methanol and the water sample was mixed with 10% methanol. The fenvalate standard solution was spiked in water sample, which diluted the fenvalerate concentration to $0.08 \mu\text{g mL}^{-1}$, $0.24 \mu\text{g mL}^{-1}$, $0.72 \mu\text{g mL}^{-1}$, and $2.16 \mu\text{g mL}^{-1}$, respectively. And then the water samples were filtered to be detected because there were potential interferences in environmental water (Gui, Jin, Chen, Cheng, & Zhu, 2006).

Cabbage and rape samples were spiked and homogenized. After filtering, the samples' matrix solution was cleared up via a solid-phase extraction column (Florisil). The concentration of these spiked samples was the same as that in water.

The samples were also determined by the GC method (Pang et al., 1999). For extraction of high-moisture samples, the chopped and spiked sample (15 g) was homogenized with 35 mL of acetone for 3 min at 18,000 rpm. After threefold washing with acetone (5 mL), all the mixture was filtered. Hexane (30 mL) was added and the mixture was shaken vigorously for 5 min. Sixty milliliters of 10% aqueous NaCl (w/v) was added and the contents were mixed vigorously for 5 min. The layers were separated and the aqueous one was discarded. MgSO_4 (10 g) was added to the hexane layer and mixed. After being washed and filtered, the hexane was evaporated nearly to dryness using rotary at 40°C . The residue was redissolved in hexane (10 mL), which was extracted by threefold extraction with 10 mL of acetonitrile-saturated hexane. The acetonitrile layer was obtained and evaporated nearly to dryness at 50°C . The residue was redissolved in 5 mL of hexane.

And then the solution was cleaned up by the Florisil which was pretreated with 5 mL of hexane and acetonitrile (9 + 1). At last, the solution was blown nearly to dryness with N_2 and then hexane (5 mL) was added to dissolve the residue for GC method.

Results and discussion

The appearance of the QDs

Figure 1 shows the appearance of QDs at different wavelengths (254 nm and 365 nm) of ultraviolet light. They emitted the same wavelength but different intensity light at different excited light. The QDs have a stable fluorescence characteristic (Kim, 2007; Kobayashi et al., 2007), which would be predicted in later measurement and verification.

The absorption and emission spectrum of QDs

The QDs have a excitation spectrum within a wide range and a single emission peak. UV absorption and emission spectra are shown in Figure 2. A wavelength of 564 nm was selected as the emission wavelength, which could supply a high fluorescence intensity value. It is known from the picture that the peak ranges from about 460 nm to 660 nm. In order to avoid the effect of excitation wavelength, 400 nm was selected as the wavelength to excite the QDs though the ultraviolet maximum absorption wavelength was about 500 nm.

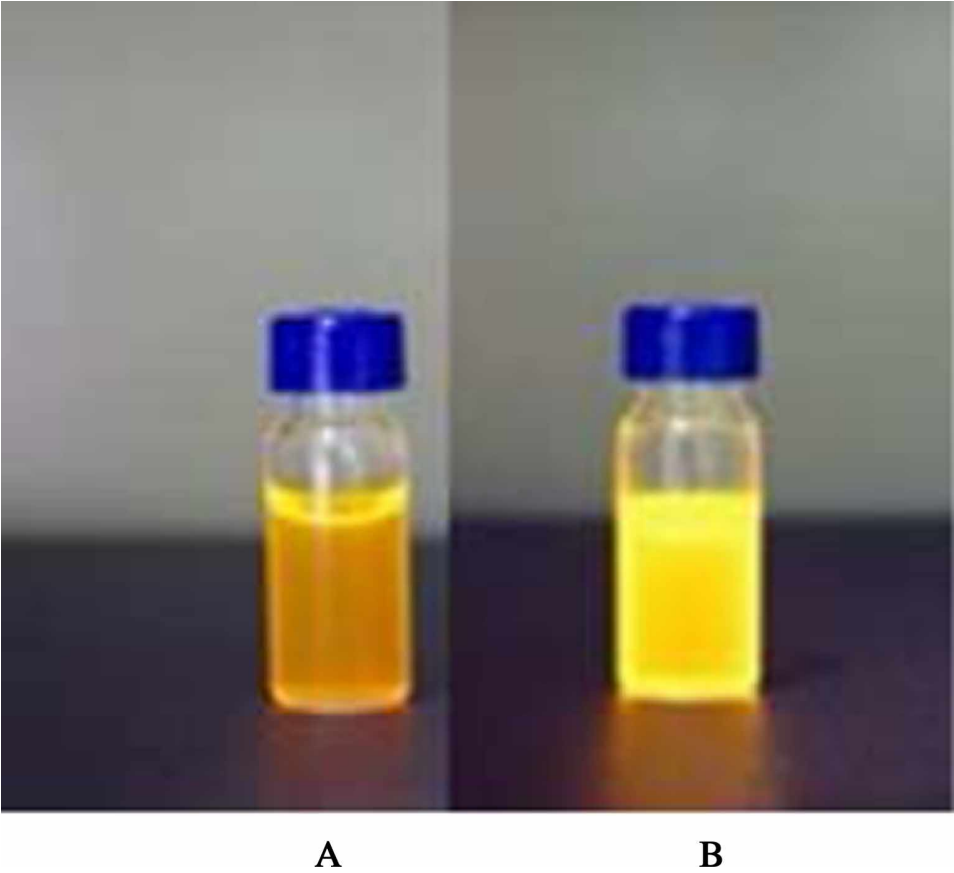


Figure 1. The fluorescence of QDs in the light of ultraviolet of different wavelengths (A: 254 nm, B: 365 nm).

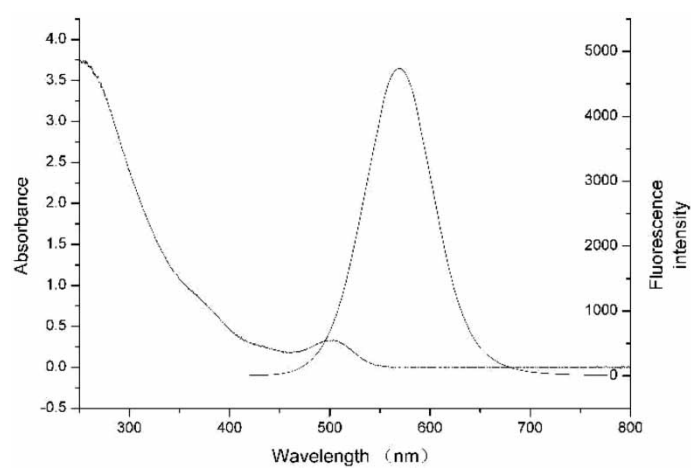


Figure 2. The UV absorption spectrum and fluorescence spectrum of QDs.

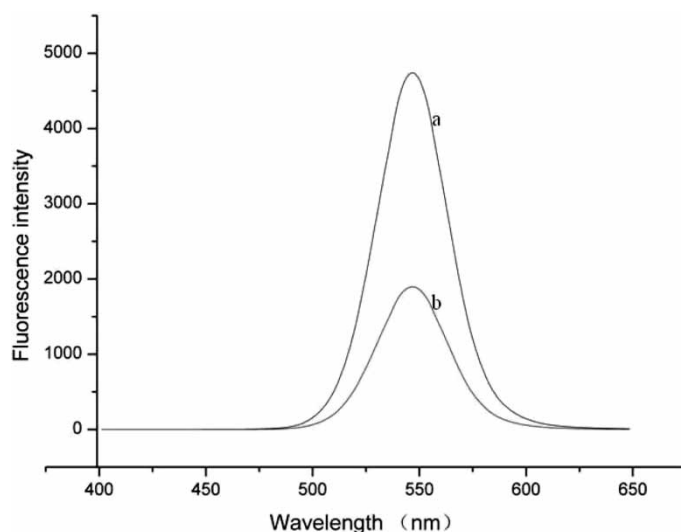


Figure 3. The comparison of fluorescence spectrum between QDs(a) and antibody linked QDs(b).

The QDs have a stable emission spectrum. Their wavelength of peak emission did not change after being linked with antibody as shown in Figure 3. Because the solution was diluted and the QDs were in excess, the fluorescence intensity decreased remarkably, as shown in Figure 3. According to the intensity, the QDs can also be used for detection after the free QDs have been separated. It also shows that the QDs linked with the antibody successfully.

The concentration and diameter of QDs

According to the formula (Zhang et al., 2009), the concentration of QDs was determined. The concentration of QDs was 5.8×10^{-6} M and the ratio among QDs, EDC, and NHS in the experiment was 1:35:40. Their average parameter was about 3.1 nm.

The optimization of antibody concentration

An antibody with a different concentration (1:1 to 1:16) which was linked with QDs was used in this work. The solution diluted eight times showed the highest value of signal-to-noise ratio (Figure 4). It was selected as the working fluid.

The development of FLISA method

The percentage of methanol in fenvalerate solution and the concentration of iron in the reaction system used in the work were optimal for the antibody, which had been reported (Jiang et al., 2010). The washing buffer used in FLISA was also PBST, which contains tween 20 that stabilizes the QDs (TalaPin et al., 2001; Wuister, Swart, Driel, Hickey, & Donegá, 2003; Wuister, Donegá, & Meijerink, 2004). Fenvalerate standards of eight concentrations (tripling dilution from $10.9 \mu\text{g mL}^{-1}$ to 5 ng mL^{-1}) were added to compete with antigen to react with antibody, and the result is shown in

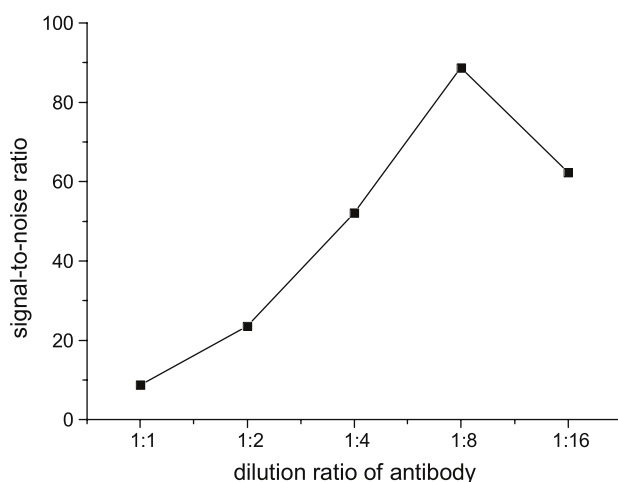


Figure 4. Signal-to-noise ratio of different antibody concentration.

Figure 5. The IC_{50} was $0.28 \mu\text{g mL}^{-1}$. The limit of detection (LOD) of the immunoassay was 25 ng mL^{-1} , and the linear range was from 60 ng mL^{-1} to $3.83 \mu\text{g mL}^{-1}$. The method can be used in the rapid detection and screening for the fenvalerate in samples like fruits and vegetables.

The CR from the reaction between antibody and other analytes represented the specificity of the method. The results are shown in Table 1. The method had CRs of 29.5% and 5.3% with cypermethrin and deltamethrin, respectively, which was much higher than that of bifenthrin ($<0.6\%$). Obviously, the difference in structure between fenvalerate and bifenthrin was much larger than that observed with the

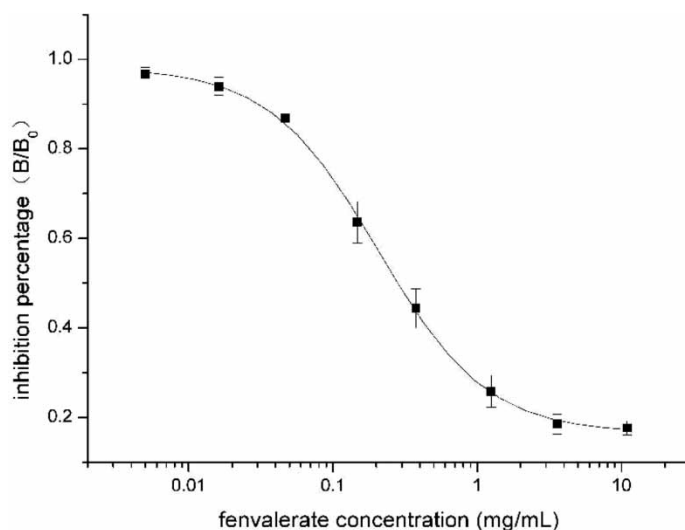
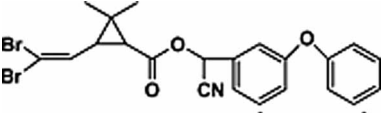
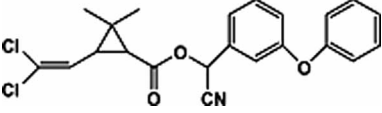
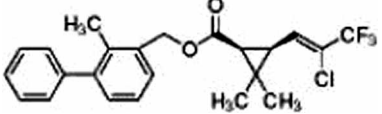
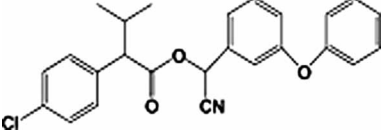


Figure 5. FLISA inhibitory curve for fenvalerate (B value was the fluorescence intensity of the well with fenvalerate deducted the background and the B_0 was the fluorescence intensity of the well without fenvalerate deducted the background.)

Table 1. Cross-reactivity (CR) of antibody with related pesticides and fenvalerate.

Pesticide	Structure	IC ₅₀ (μg mL ⁻¹)	CR(%) ^a
Deltamethrin		5.28	5.3
Cypermethrin		0.95	29.5
Bifenthrin		> 50	< 0.6
Fenvalerate		0.28	100

^aCR (%) = [IC₅₀ (fenvalerate) = IC₅₀ (related pesticide)]100%.

previous two pesticides. It can be speculated that fenvalerate and the two types of pesticides shared the same molecular structure, phenyl ether and alpha-cyano, which caused the recognition from the antibody.

Recovery of fenvalerate from spiked samples

To study the spiked recovery of the method, water samples from Yangtze River (Wuhan, Hubei) and vegetables from supermarkets were spiked with fenvalerate standards. The results are shown in Table 2. The recoveries range from 72.5%

Table 2. Recovery of fenvalerate from spiked samples.

Samples (n = 4)	Spiked (μg mL ⁻¹)	Measured (μg mL ⁻¹)	Recovery (%)	C*V (%)	Recovery(%) (measured by GC)
Water	0.08	0.071	88.8	2.5	89
	0.24	0.223	92.9	2.1	96
	1.5	1.443	96.2	1.8	94
	3	2.535	84.5	3.7	99.7
Cabbage	0.08	0.061	76.3	4.2	88.7
	0.24	0.200	83.3	3.6	90.6
	1.5	1.300	86.7	2.9	92.7
	3	3.381	112.7	5.1	105.6
Rape	0.08	0.058	72.5	5.3	91.5
	0.24	0.207	86.2	4.6	103.6
	1.5	1.219	81.3	3.7	98.5
	3	3.771	125.7	5.7	97.7

to 125.7%. It was evident that the average recovery of water samples (84.5% to 96.2%) was higher than that in vegetables (72.5% to 125.7%), which was caused by the matrix effects. It was speculated that the matrix in vegetable samples was much more complex than that in the water sample. Compared with GC method, the recovery of the FLISA method was not as well. The FLISA method was not only free of complex pretreatment but also saved a lot of time and reagent, which might have polluted the environment. Considering the acceptable recovery close to that obtained by the GC method, FLISA was regarded as a rapid, accurate, and relatively sensitive method, especially for the agro-food like vegetables and fruits.

Conclusion

In this work, a type of CdTe QDs was synthesized and coupled with monoclonal antibody fight against fenvalerate made in lab. The absorption spectrum and emission spectrum were obtained. Moreover, the emission wavelength of free QDs was the same as that of the conjugate, which showed the stable fluorescence characteristic. Proper excitation and emission wavelengths were selected and the concentration of the antibody was optimized. After these optimizations, a FLISA method was developed. The standard inhibitory curve was also developed. The LOD was 25 ng mL⁻¹ and the linear range was from 60 ng mL⁻¹ to 3.83 µg mL⁻¹. In the method, the CRs of antibody with deltamethrin and cypermethrin were 29.5% and 5.3%, respectively, which were much higher than the CR (<0.6%) between antibody and bifenthrin. Fenvalerate shared the same molecular structure, phenyl ether and α-cyano, which caused the recognition from the antibody. The result of the spiked recovery work was also obtained, including those for water samples and vegetables samples. The recoveries ranged from 72.5% to 125.7%, which was close to those obtained by the GC method. The average recovery of water samples was evidently higher than that of vegetables, which was caused by the complex matrix in vegetables. On the basis of the specific emission wavelength of QDs, the FLISA method reduced the false-positive ratio in the detection. The method also saved 1 h compared to ELISA, which can be used in rapid detection of fenvalerate residue in vegetables and other related samples.

Agro-food has a short shelf life. Vegetables and fruits are often eaten raw by people, which makes the pesticide residue become one of the major factors which pose threats to the health of human. Because of this, rapid detection of the residue of fenvalerate, which is widely used as a useful pesticide in the producing these agro-foods, is crucial. Rapid detection methods will play an increasingly important role in environment protection and the food quality and safety.

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