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ENVIRONMENTAL ANALYSIS

A Novel Method for the Detection of Chlorpyrifos by Combining Quantum Dot-labeled Molecularly Imprinted Polymer with Flow Cytometry

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ABSTRACT

Uniform-sized fluorescent molecularly imprinted polymers were prepared by one-step swelling and suspension polymerization, while chlorpyrifos, methacrylic acid, ethylene glycol dimethacrylate, and oil-soluble CdSe/ZnS quantum dots were used as the carrier, template molecule, functional monomer, cross-linker, and fluorophor, respectively. The morphology, adsorption dynamics, binding ability, and selectivity of quantum dot-labeled molecularly imprinted polymers were evaluated. The dosage of quantum dots for labeling the molecularly imprinted polymers was optimized. The results showed that the optimized dose of quantum dots was 200 µL using a concentration of 8.0 µM. The microsphere size was approximately 10 µm with a honeycombed surface. The quantum dot-labeled molecularly imprinted polymers had an even brightness and a high selectivity. In the presence of different concentrations of chlorpyrifos, a decrease in the fluorescence intensity of the quantum dot-labeled molecularly imprinted polymer was clearly identified by flow cytometry. The whole detection process was accomplished within 2 h including pretreatment. This method was used for the determination of chlorpyrifos in tap water samples.

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Introduction

Chlorpyrifos, an acetylcholinesterase inhibitor, is a widely used organophosphate pesticide (Caughlan et al. 2004). As a pesticide with high efficacy and broad spectrum, it can be found as a residue in a large variety of foods, including vegetables, fruit, and crops. Because of its neurotoxicity and long half-life in the environment (Caughlan et al. 2004), it is harmful to the environment and to human health. Currently, it is usually detected by gas chromatography (GC) (Oliva et al. 1999), enzyme inhibition methods (Rodriguez, Carvajal, and Penuela 2013), and immunization assays (Richardson, Chambers, and Chambers 2001). However, chromatography is complicated and consumes a large amount of organic solvents (Xiong et al. 2012). Although enzyme inhibition method is of low cost and highly sensitive, this technology is only suitable for certain types of pesticides (Gu et al. 2013). Immunoassay antibodies are difficult to prepare and the biological activity is influenced by the environment although they offer the advantages of rapid and specific detection

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(Kim et al. 2011; Liu et al. 2013). Hence, the development of stable and highly selective methods for the determination of pesticide residues in environmental sample is of great significance.

Molecular imprinting is an increasingly applied method for preparing polymers with predetermined molecular recognition properties (Ye and Haupt 2004). The obtained molecularly imprinted polymers possess many advantages, such as chemical stability and predetermined selectivity. Therefore, molecularly imprinted polymers are widely used in pesticide sample pretreatment, such as solid-phase extraction (Barahona, Turiel, and Martín-Esteban 2011; Xie et al. 2013; Yang et al. 2014), solid-phase microextraction (Koster et al. 2001; Hu et al. 2008), and membrane separation (Ulbricht 2004; Kim et al. 2011). Although introducing molecularly imprinted polymers to sample pretreatment may save pretreatment solvents and time, the preconcentrated analytes must be eluted to be detected by GC, which is also a complicated and time-consuming task. Therefore, significant efforts have been devoted to develop direct techniques for pesticide determination, such as electrochemistry (Lin et al. 2014), quartz crystal microbalance (Tsuru et al. 2006), and spectrofluorimetry (Lei et al. 2014).

Among these methods, fluorometric methods are attractive due to their advantages such as easy readout, high sensitivity, wide linear range, and good selectivity. The limit of detection of aminated pesticides was between 0.45 and 3.48 μg/L (Navarrete-Casas et al. 2005). Fluorescent materials are also common. Among these fluorophors, semiconductor quantum dots, as a novel nanomaterial, have drawn attention because of the wide excitation wavelength, narrow emission peaks, strong fluorescence intensity, high stability against photobleaching, and symmetrical emission peaks (Caughlan et al. 2004). A large body of research is focused on quantum dots in bioimaging applications and analytical chemistry (Panagiotopoulou et al. 2016). Zhao et al. (2012) developed a facile method for the detection of diazinon in water based on the novel fluorescence quenching relying on energy transfer from quantum dots' excitation to diazinon. Bian, Liu, and Yu (2010) established a highly sensitive fluorometric method for the determination of paraquat using CdTe/CdS quantum dots as a sensor. The calibration curve was linear over the concentration range of 9.9×10^{-9}–1.50×10^{-6} mol/L with a correlation coefficient of 0.999 (Bian, Liu, and Yu 2010).

In recent years, researchers have been working on the rapid detection of chlorpyrifos. In our previous work, a molecularly imprinted film sensor was constructed and applied for the determination of chlorpyrifos in water (Lin et al. 2014). It was shown that a reasonable linear response curve between potential and concentration was obtained from 1.0×10^{-12} to 2.0×10^{-8} mol/L, with a detection limit of 1.0×10^{-13} mol/L. Nevertheless, this detection method is not simple to operate. Ren, Liu, and Chen (2015) showed that molecularly imprinted polymer-coated quantum dots may act as a probe for the selective and sensitive detection of chlorpyrifos by fluorescence and gave recoveries in the range from 87.1 to 94.5%. However, the results are susceptible to the suspension of molecularly imprinted polymers in the sample, resulting in large differences when the polymers are unevenly distributed in the sample.

Currently, flow cytometry has been widely used in clinical research for the development of multiplexed assays in which analytes present in the same sample are simultaneously detected (Kim and Ligler 2010; Fraga et al. 2014). It is used to accomplish clinical tasks like cell counting, measurement of cell viability, antibody quantitation, and detection of cell

death. The design principle of flow cytometry is used to detect the cells with fluorescent properties. If molecularly imprinted polymers fluoresce, these materials may be used to detect chlorpyrifos using flow cytometry. According to the detection principles of flow cytometry, the fluorescence of a certain number of molecularly imprinted polymers (e.g., 10,000) is recorded as fluorescence intensity of the sample. Compared to other methods, this recording principle of fluorescence intensity is more accurate. It has been shown that this approach is suitable for the detecting of fluorescence intensity (Liu et al. 2013). In addition, the inherent advantages of this method, such as high sensitivity, easy readout, low sample volume, and simple operation, make it attractive for various applications (Goedken and Guise 2004).

In this work, we synthesized quantum dot-labeled chlorpyrifos molecularly imprinted microspheres by a method using one-step swelling and suspension polymerization. The quantum dot-labeled molecularly imprinted polymer was successfully used for the determination of chlorpyrifos in aqueous media using fluorescence quenching. Scanning electron microscopy (SEM), energy-dispersive X-ray analysis, and gas chromatography were applied to the characterization of the quantum dot-labeled chlorpyrifos molecularly imprinted microspheres. The combination of quantum dot-labeled chlorpyrifos molecularly imprinted polymer with flow cytometry is an original method which allows us to detect pesticide residues with high sensitivity, simple operation, and easy readout.

Experimental

Chemicals

Methacrylic acid and styrene were purchased from Tianjin Chemical Reagent (Tianjin, China) and were used after vacuum distillation. All other reagents were of analytical grade and used without further purification. Doubly distilled water was used throughout the study. Polyvinyl alcohol, azoisobutyronitrile, and butyl phthalate were obtained from Tianjin Bodi Chemical Reagent (Tianjin, China). Ethylene glycol dimethacrylate was obtained from Alfa Aesar Chemical (USA). Sodium dodecyl sulfate was purchased from Tianjin Kemiou Chemical Reagent (Tianjin, China). Polyvinyl pyrrolidone was obtained from the Shanghai Chemical Reagent Supply Station of the Chinese Medicine Pharmaceutical Company (Shanghai, China). Chlorpyrifos was obtained from Shenyang East Dick Biological Pharmaceutical (Shenyang, China). Oil-soluble CdSe/ZnS quantum dots (Figure 1) were obtained from Wuhan Jiayuan Quantum Dots Technology Development (Wuhan, China).

Instrumentation

In this study, SEM (FEI Quanta 250, USA) was used to examine the morphology of quantum dot-labeled molecularly imprinted polymers. Energy-dispersive X-ray analysis (Ametek, USA) was used to examine the quantum dot-labeled molecularly imprinted polymer. A microscope (Laica AF6000, Germany) equipped with a digital color camera was used to obtain the fluorescence images. A gas chromatograph (Thermo Scientific, USA) with a flame photometric detector was used to determine the concentration of chlorpyrifos. The fluorescence measurements were performed using the LSR Fortessa flow cytometer (BD, USA) with a 488-nm wavelength laser and a 625/20-nm channel.

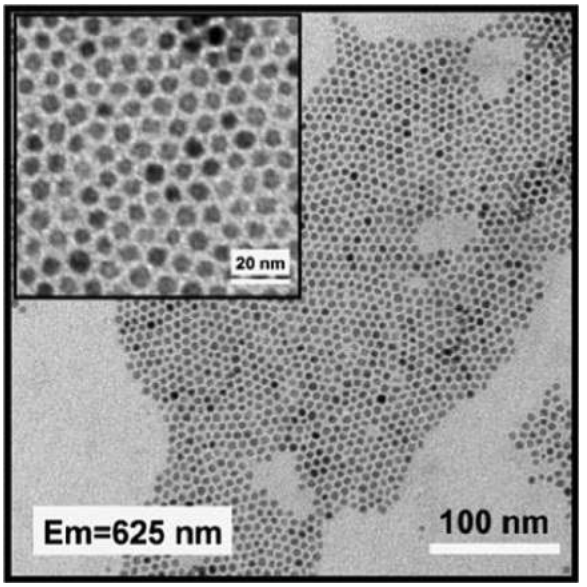

Figure 1. SEM images of the oil-soluble CdSe/ZnS quantum dots. *Note*: SEM, scanning electron microscopy.

Preparation of quantum dot-labeled molecularly imprinted polymeric microspheres

The synthesis of quantum dot-labeled chlorpyrifos-imprinted polymers was performed as follows (Chen et al. 2005). A 0.25 mmol chlorpyrifos was added into a three-necked flask equipped with a condenser tube, a nitrogen gas tube, and a thermometer. A total of 1.2 mL of chloroform containing 1 mmol methacrylic acid was added to 10–300 μL of an 8 μM solution of quantum dots. These solutions were subsequently mixed with 5 mmol of cross-linker. Then, 0.49 g butyl phthalate and 0.05 g azoisobutyronitrile, 42 mg polystyrene microspheres, and 12 mL 0.1% sodium dodecyl sulfate and 1.0% polyvinyl alcohol solution were added into this system and dispersed with ultrasound for 5 min. At room temperature, the solution was stirred at 125 rpm until the swollen droplets disappeared and then polymerized for 20 h at 60°C. The polymer particles were washed with methanol, 70–80°C water and tetrahydrofuran, respectively, followed by drying under vacuum at room temperature. For a comparison study, nonmolecularly imprinted polymeric microspheres were also synthesized without adding chlorpyrifos and used as a blank control material.

Procedure

Flow cytometry determination

First, 0.1 mg of 625-nm quantum dot-labeled molecularly imprinted polymers was added to 10 mL of chlorpyrifos aqueous solutions at concentrations of 0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.15, and 0.2 mg/L, respectively, and oscillated for 90 min. Second, the mixed solution was analyzed using the flow cyclometer. The side scattering count, forward scattering count, and the median fluorescent intensity were recorded. A total of 10,000 events was recorded at medium flow rate for all measurements.

Results and discussion

Morphology of the quantum dot-labeled molecularly imprinted polymer

Scanning electron microscopy was used to study the morphology of the molecularly imprinted polymers. A representative SEM image of the quantum dot-labeled molecularly imprinted polymers is shown in Figure 2a. The image clearly shows the particles' spherical shape and nearly uniform size. The size distribution was 9–11 μm. There are many honeycomb cavities on the surface of the molecularly imprinted polymer and the particle size of the quantum dot-labeled molecularly imprinted polymer is about 10 μm. Molecularly imprinted polymers prepared by one-step swelling polymerization have a large specific surface area (Zheng, Tu, and Fan 2015) that makes them suitable for binding template molecules. It can be seen from the true-color fluorescence images of quantum dot-labeled molecularly imprinted polymers (Figure 2b) that the fluorescent particles have good brightness and uniformity.

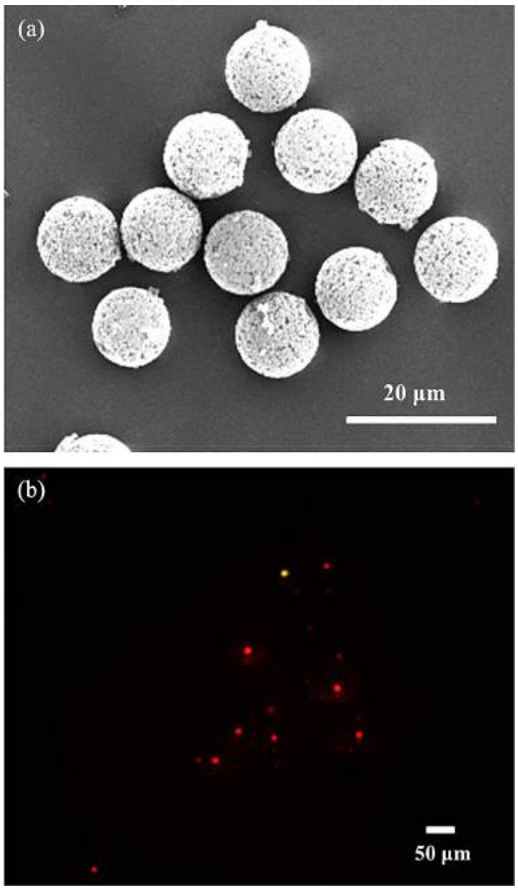

Figure 2. (a) SEM images of the quantum dot-labeled molecularly imprinted polymer and (b) true-color fluorescence image of the quantum dot-labeled molecularly imprinted polymer. *Note*: SEM, scanning electron microscopy.

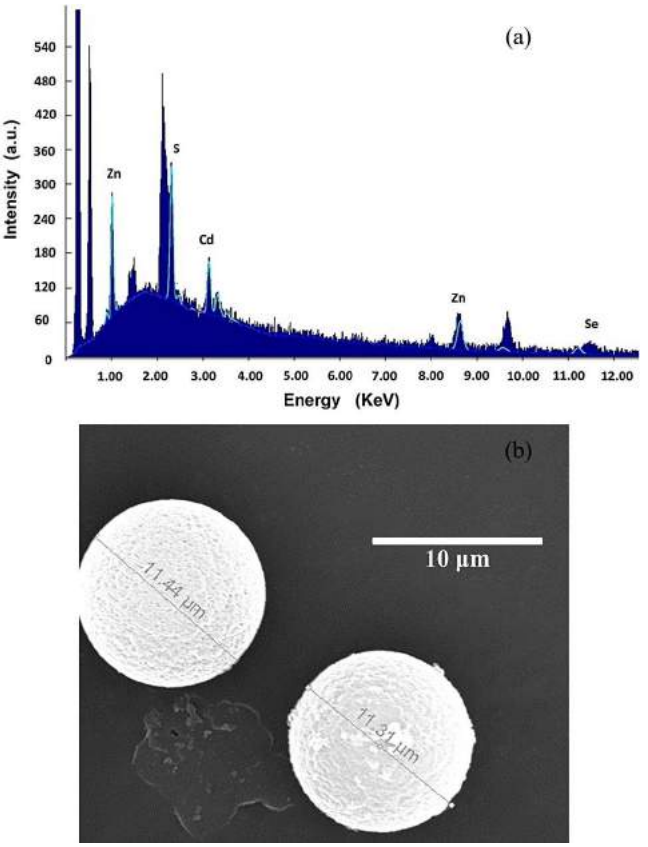

Figure 3. (a) Energy-dispersive X-ray analysis and (b) morphology of the quantum dot-labeled molecularly imprinted polymer.

Energy-dispersive X-ray analysis of the quantum dot-labeled molecularly imprinted polymer

Analysis of the energy-dispersive X-ray spectrum shows that Zn, S, Cd, and Se were present in the quantum dot-labeled molecularly imprinted polymer as shown in Figure 3. This illustrates that quantum dots have successfully labeled the molecularly imprinted polymer. The diameter of the quantum dot-labeled molecularly imprinted polymer is about 11 µm, approximately the same as the molecularly imprinted polymers. The results show that the quantum dot coding process does not change the diameter of molecularly imprinted polymers.

Optimization of quantum dot dosage

To obtain the stable fluorescent coding, the chlorpyrifos-imprinted microspheres were prepared using different volumes of the 0.8 µM solution of quantum dots. The minimum amount of quantum dots providing a bright fluorescence was determined in these experiments. As shown in Figure 4, the fluorescence intensities of the quantum dot-labeled molecularly imprinted polymer microspheres increased with the number of quantum dots

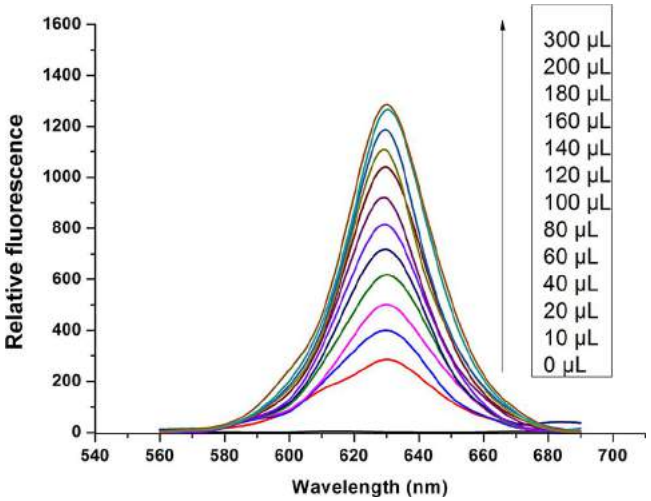

Figure 4. Relationship between the quantity of quantum dots and the fluorescence intensity of the quantum dot-labeled molecularly imprinted polymer.

up to 200 μL, while there is little additional increase at higher volumes. Therefore, 200 μL of quantum dots was selected to be the optimum volume of quantum dots.

Evaluation of the adsorption capacity of the quantum dot-labeled molecularly imprinted polymers

Langmuir–Freundlich isotherm

An adsorption isotherm is a measure of the relationship between the equilibrium concentration of bound and free guest over a certain concentration range and may be easily generated from equilibrium batch rebinding studies. Adsorption isotherm curves include Langmuir, Freundlich, and Langmuir–Freundlich isotherms, but the accuracy of Langmuir and Freundlich curve fitting are not as good as the Langmuir–Freundlich adsorption curve (Koster et al. 2001; Hu et al. 2008). Therefore, a Langmuir–Freundlich adsorption curve was used to analyze the data in this study. The formula is as follows:

$$B = \frac{N_t a F^m}{1 + a F^m} \tag{1}$$

where B is the concentration of bound guest, F is the concentration of the free guest, N_t is the total number of binding sites, m is the heterogeneity index, and a is related to the medium affinity constant.

To examine the adsorption performance of the quantum dot-labeled molecularly imprinted polymer, a series of standard solutions of chlorpyrifos of different concentrations was prepared. A total of 10 mg of the quantum dot-labeled molecularly imprinted polymer and quantum dot-labeled nonmolecularly imprinted polymer was mixed with 10.0 mL of solution containing a known concentration of 1, 5, 10, 20, 40, 60, 80, or 100 mg/L, respectively. After shaking for 90 min at room temperature and centrifuging at 2000 rpm for 3 min, the free concentration of chlorpyrifos in the suspension was detected by GC.

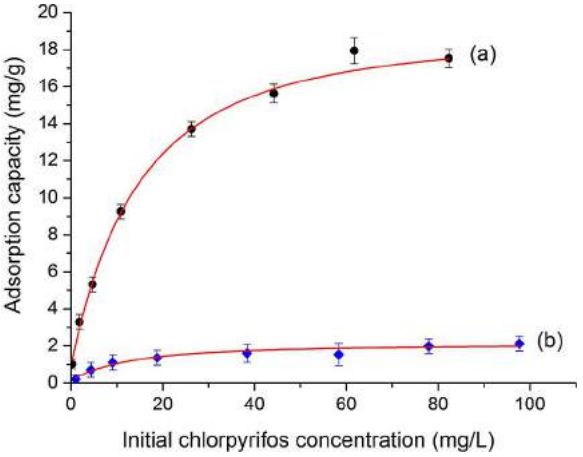

Figure 5. Plots of the adsorption capacity for the (a) quantum dot-labeled molecularly imprinted polymer and (b) quantum dot-labeled nonmolecularly imprinted polymer.

The experimental adsorption isotherms were fitted to determine the heterogeneity. It was found that the experimental values were well represented by the Langmuir–Freundlich model (Figure 5). The adsorption capacity is an important factor to evaluate the binding affinity of the polymers to chlorpyrifos. Adsorption isotherms were obtained to assess the binding affinity of the quantum dot-labeled molecularly imprinted polymer and the quantum dot-labeled nonmolecularly imprinted polymer.

The fitting parameters (Table 1) indicated that the quantum dot-labeled molecularly imprinted polymer had a much higher adsorption ($N_t = 22.49$) than the quantum dot-labeled nonmolecularly imprinted polymer ($N_t = 2.41$). The maximum adsorption capacity of the molecularly imprinted polymer was 10 times the maximum adsorption capacity of the nonmolecularly imprinted polymer. The specificity coefficient of the molecularly imprinted polymer indicated that the chlorpyrifos molecularly imprinted polymer had one type of site for binding for chlorpyrifos, while the nonmolecularly imprinted polymer had multiple types of adsorption sites that did not specifically bind chlorpyrifos.

Specificity of the chlorpyrifos quantum dot-labeled molecularly imprinted polymer

To confirm the high selectivity of the quantum dot-labeled molecularly imprinted polymer to chlorpyrifos in aqueous media, two related organophosphorus herbicides were evaluated (Liu et al. 2007). The selectivity of the polymers was evaluated by comparing chlorpyrifos

Table 1. Langmuir–Freundlich fitting parameters for quantum dot-labeled molecularly imprinted polymers and quantum dot-labeled nonmolecularly imprinted polymers.

	Total binding sites (mg/g)	Medium affinity constant (mg/L)	Heterogeneity index	Goodness of fit (R^2)
Quantum dot-labeled molecularly imprinted polymers	22.49	0.089	0.87	0.989
Quantum dot-labeled nonmolecularly imprinted polymers	2.41	0.12	0.83	0.944

with methamidophos and chlorpyrifos-methyl. A total of 1 mg of polymer was mixed with 10.0 mL of 1 mg/L chlorpyrifos, methamidophos, and chlorpyrifos-methyl, respectively. The mixtures were shaken for 90 min at room temperature and centrifuged at 2000 rpm for 3 min. The supernatant was withdrawn and the free concentrations of chlorpyrifos, methamidophos, and chlorpyrifos-methyl were detected by GC. The distribution coefficient K_d, selective coefficient k, and relative selectivity coefficient k' were calculated by the following formulas:

$$K_d = B/F \tag{2}$$

where K_d (mL/g) is the distribution coefficient constant; B (mg/g) is the adsorption quantity at the equilibrium; and F (mg/L), the concentration of the material in the supernatant; and

$$K = \frac{K_d(\text{imprinted molecule})}{K_d(\text{competition molecule})} \tag{3}$$

$$k' = \frac{k_{\text{molecularly imprinted polymers}}}{k_{\text{non - molecularly imprinted polymers}}} \tag{4}$$

where k reflects the selectivity ability of the molecularly imprinted polymers. As shown in Table 2, the absorption for chlorpyrifos of the quantum dot-labeled molecularly imprinted polymer is 87 and 11 times higher than for methamidophos and chlorpyrifos-methyl which suggests that the quantum dot-labeled molecularly imprinted polymer possesses high selectivity.

Fluorescence quenching and dynamic response of the chlorpyrifos quantum dot-labeled molecularly imprinted polymer

As a powerful technique for analysis, flow cytometry can provide information regarding the quantum dot-labeled molecularly imprinted polymer including the physical and spectral characteristics through multiple parameters such as forward scattering count, side scattering count, and multichannel fluorescence signals. The forward scattering count is mainly determined by the size and shape of the quantum dot-labeled molecularly imprinted polymer. The larger quantum dot-labeled molecularly imprinted polymer provides stronger light scattering. The side scattering count is primarily determined by the fluorescent intensity of the quantum dot-labeled molecularly imprinted polymer. It was observed that the 625/20-nm channel signal is proportional to the fluorescent intensity

Table 2. Binding specificity of quantum dot-labeled molecularly imprinted polymers and quantum dot-labeled non-molecularly imprinted polymers.

	Chlorpyrifos			Methamidophos			Chlorpyrifos-methyl		
Adsorb material	K_d	k	k'	K_d	k	k'	K_d	k	k'
Quantum dot-labeled molecularly imprinted polymer	246.67	8.61	69.76	31.31	8.61	69.76	28.67	7.88	53.35
Quantum dot-labeled nonmolecularly imprinted polymer	2.90	0.16		15.22	0.16		25.64	0.11	

K_d, distribution coefficient; k, selective coefficient; k', relative selectivity coefficient.
Initial concentration of analytes: 10 mg/L; volume: 10 mL; polymer quantity: 1 mg.

of the quantum dot-labeled molecularly imprinted polymer which is related to the analyte concentrations.

Figure 6 shows scatter plots of the quantum dot-labeled molecularly imprinted polymer in the presence of different concentrations of chlorpyrifos. The quantum dot-labeled molecularly imprinted polymer populations are concentrated in a specific region and the forward scattering count of the quantum dot-labeled molecularly imprinted polymer is

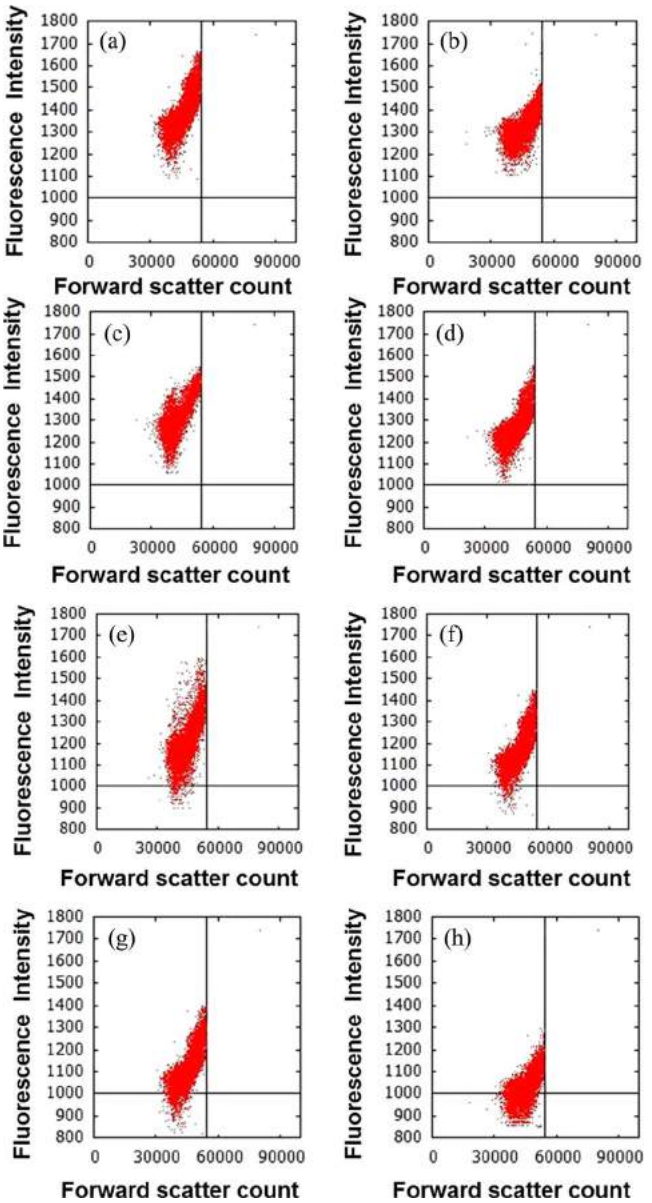

Figure 6. Scatter plot of the quantum dot-labeled molecularly imprinted polymers in the presence of varying concentrations of chlorpyrifos: (a) 0, (b) 0.02, (c) 0.04, (d) 0.06, (e) 0.08, (f) 0.1, (g) 0.15, and (h) 0.2 mg/L.

almost constant, indicating that the size of the quantum dot-labeled molecularly imprinted polymer is uniform and changes little after absorbing chlorpyrifos. The stability of the forward scattering count is independent of the fluorescence signals of the quantum dot-labeled molecularly imprinted polymer. As shown in Figure 6, the population of free binding sites on the quantum dot-labeled molecularly imprinted polymer decreases with the chlorpyrifos concentration, which indicates that the fluorescence quenching of the quantum dot-labeled molecularly imprinted polymer is due to the adsorption of more chlorpyrifos on the quantum dots. It is proposed that if the absorption band of the analyte overlaps with the excitation band of the fluorescent donor, the excitation energy transfers to the analyte and reduces the donor fluorescence which provides a facile and sensitive strategy for the direct fluorescence quenching detection of the analyte.

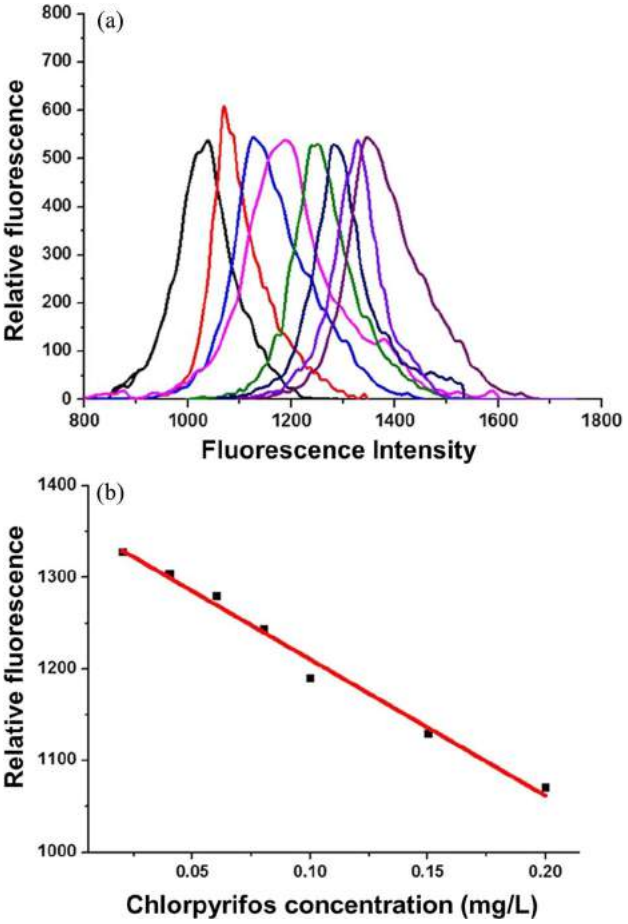

Figure 7. (a) Histograms of fluorescence signal of the quantum dot-labeled molecularly imprinted polymer as a function of the concentration of chlorpyrifos. The peaks from right to left in the histogram corresponds to the fluorescence signal triggered by 0 (blank control), 0.02, 0.04, 0.06, 0.08, 0.1, 0.15, and 0.2 mg/L chlorpyrifos, respectively. (b) Linear relationship between the relative fluorescence and chlorpyrifos concentration in the range from 0 to 0.2 mg/L.

Analytical performance of the quantum dot-labeled molecularly imprinted polymers for the determination of chlorpyrifos

Linear response range, limit of detection, and precision

Based on this fluorescence quenching phenomenon, a facile and direct fluorometric quantitative method was successfully developed for the chlorpyrifos in aqueous media without a preconcentration process. Figure 7a shows the corresponding fluorescence histogram of the quantum dot-labeled molecularly imprinted polymer in the presence of various concentrations of chlorpyrifos. A decrease in the peak fluorescence may be clearly identified. A linear range of 0.02–0.2 mg/L was obtained. The calibration relationship is $F = -1489.6\ C + 1359.5$ where C is the concentration, F is the fluorescence intensity, and R^2 is 0.988. The precision of the method is 1.5–6.8% ($n = 7$) and the limit of detection was 0.01 mg/L.

Flow cytometry analysis of tap water samples

This method was used to determine chlorpyrifos in tap water samples. After the water samples were collected and filtered, the determination was performed using the linear calibration curve method. There was no chlorpyrifos detected in the water samples. After adding 0.03 mg/L of chlorpyrifos to the tap water sample and repetitively measuring thrice, the detected chlorpyrifos concentration was 0.028 mg/L and the recovery was $92.8 \pm 4.8\%$.

Conclusion

An analytical system was established for the rapid detection of chlorpyrifos in water samples using a quantum dot-labeled molecularly imprinted polymer with flow cytometry. Compared to conventional analytical methods, this technique provides improvements with respect to reduced analysis time and simplified manipulation. In the presence of different concentrations of chlorpyrifos, a decrease in the fluorescence intensity of the quantum dot-labeled molecularly imprinted polymer was clearly observed by flow cytometry. This method may be used for the detection of chlorpyrifos in tap water sample. The whole detection process may be accomplished within 2 h including pretreatment. There are several advantages of this method, including rapid readout, high throughput, quantum dot-labeled molecularly imprinted polymer amplification of the optical signal using a single excitation source, low cost, and low toxicity. These distinct features make this proposed format a promising prescreening tool for monitoring environmental contamination in samples. A follow-up study will focus on optimizing the performance of the procedure.

Acknowledgments

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