CdTe and CdSe quantum dots: synthesis, characterizations and applications in agriculture

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Abstract
This paper highlights the results of the whole work including the synthesis of highly luminescent quantum dots (QDs), characterizations and testing applications of them in different kinds of sensors. Concretely, it presents: (i) the successful synthesis of colloidal CdTe and CdSe QDs, their core/shell structures with single- and/or double-shell made by CdS, ZnS or ZnSe/ZnS; (ii) morphology, structural and optical characterizations of the synthesized QDs; and (iii) testing examples of QDs as the fluorescence labels for agricultural-bio-medical objects (for tracing residual pesticide in agricultural products, residual clenbuterol in meat/milk and for detection of H5N1 avian influenza virus in breeding farms). Overall, the results show that the synthesized QDs have very good crystallinity, spherical shape and strongly emit at the desired wavelengths between ~500 and 700 nm with the luminescence quantum yield (LQY) of 30–85%. These synthesized QDs were used in fabrication of the three testing fluorescence QD-based sensors for the detection of residual pesticides, clenbuterol and H5N1 avian influenza virus. The specific detection of parathion methyl (PM) pesticide at a content as low as 0.05 ppm has been realized with the biosensors made from CdTe/CdS and CdSe/ZnSe/ZnS QDs and the acetylcholinesterase (AChE) enzymes. Fluorescence resonance energy transfer (FRET)-based nanosensors using CdTe/CdS QDs conjugated with 2-amino-8-naphthol-6-sulfonic acid were fabricated that enable detection of diazotized clenbuterol at a content as low as 10 pg ml\(^{-1}\). For detection of H5N1 avian influenza virus, fluorescence biosensors using CdTe/CdS QDs bound on the surface of chromatophores extracted and purified from bacteria *Rhodospirillum rubrum* were prepared and characterized. The specific detection of H5N1 avian influenza virus in the range of 3–50 ng µL\(^{-1}\) with a detection limit of 3 ng µL\(^{-1}\) has been performed based on the antibody-antigen recognition.

Keywords: CdTe, CdSe, quantum dots, fluorescence labeling, pesticide, clenbuterol, virus detection

Classification numbers: 4.01, 4.03, 5.04
1. Introduction

Semiconductor QDs have been intensively studied due to their interesting characteristics both from the views of fundamental research and practical application. In the past two decades, significant advances have been made towards the synthesis of colloidal semiconductor QDs, particularly II–VI compound such as CdSe, CdS and CdTe [1–10]. These highly visible-luminescent nanomaterials are very promising for various applications in optoelectronics and biological labeling [3–34]. For the former, phenomena related to the interface between QDs and surrounded polymers are under intensive study [4, 33–36]. For the latter, the optical properties of QDs themselves and QDs in conjugation with other entities have been extensively studied because of considerable requirements from current agricultural production. Pesticides/herbicides and growth-promoting hormones have been widely used in agricultural production that are residual in various agricultural products and also accumulated in cultural soils; in another area avian influenza virus has spread in breeding farms; all these prevent green agricultural development and make human society harmful. Among pesticides, organophosphorus (OP)-based chemicals have been used for a long time with a large amount in the field. Residual pesticides cause many side effects for humans. In breeding production, some farmers have illegally used clenbuterol, a stimulant drug that helps increase the metabolism of the body to boost muscle growth of animals. Residue of clenbuterol in meat (mainly in pork and beef) makes people who eat such meat experience restlessness, palpitations, trembling of fingers, headache, excessive sweating, muscle pains, etc. Avian influenza virus such as H5N1 clearly causes danger not only to the breeding farm but also to human health. Therefore, it is obvious that we need to develop fast but reliable methods for detecting such residual pesticides, clenbuterol and avian influenza viruses. One should clearly classify different types of biological labels corresponding to their sophisticated structure and sensitivity. The simplest label is just to bind luminescent QDs on the surface or inside the cells (e.g. yeast cells) for enough time and then observe their photoluminescence (PL) under microscope. This is usually used for counting cells without any specific identification. If a specific labeling is needed, a much more complicated label must be designed in that an antibody attached with the luminescent QDs would specifically recognize its corresponding antigen. The highest sensitive and selective detection is realized by some biosensor structures in which one can measure the change in fluorescence intensity or electrochemical signal with specific interaction of antibody with antigen. In other words, one needs to exploit the dependence of the PL intensity or electrochemical signal on the presence of antigen because of the change of pH or the energy transfer [7, 13–22]. For detection of the objects in agricultural products such as residual pesticide, growth-promoting hormone or virus, various kinds of sensors based on the PL intensity change have been realized with different structures. These are alternative approaches to avoid the time-consuming and expensive methods like gas and liquid chromatography in detection of organophosphorus-based pesticides/insecticides or clenbuterol [21, 37–46]. Based on the mechanism for antigen recognition by specific antibody, along with the production of proton from the synthesis of adenosine triphosphate (ATP) with the F$_6$F$_7$-ATPase universal enzyme during the antigen-antibody reaction, CdTe QDs attached on the chromophores have been proposed for detecting in early-stage of some diseases caused by virus [15, 16, 47, 48]. Among II–VI semiconductor compounds, CdTe has attracted much interest because it is a direct gap material with a bandgap energy of 1.52 eV which is suitable for emitting in the visible spectral range by controlling the size correspondingly with the quantum confinement of charge carriers. It is quite possible to distinguish different kinds of antigen with CdTe QDs at different sizes [26, 31]. Moreover, compared to CdSe, from the literature, it seems that one could more easily produce CdTe QDs in aqueous phase, which is very significant for biolabeling applications, including the labeling of tumor cells, sensing of drug delivery and the detection of residual pesticides and clenbuterol [15–22, 28–32, 37, 47]. For CdSe QDs synthesized in high-boiling point organic solvent, usually a ligand exchange must be done for the water-soluble CdSe QDs from which the biosensors could be realized. Note that because of the toxicity, the use of Cd-based QDs as fluorescent tags for biomedical applications is limited. However, for the detection of residual pesticides/herbicides, clenbuterol or avian influenza virus, these QDs are very reliable [14, 16, 21, 37, 47, 50].

In this paper, we present the work as a whole from the preparation of nanomaterials to the testing of their applications in agricultural production. The syntheses of CdTe QDs in aqueous phase and CdSe QDs in high-boiling organic solvents were successfully performed [2, 10, 27, 51–60]. By a systematic study of the variation of each technological parameter, namely the molar ratio of the precursors, ligands and the pH values in the reaction solution, we have determined the optimal technological parameters for getting the best quality CdTe and CdSe QDs which are of very good crystallinity, spherical shape and strongly emit in the green-to-red spectral region (~500–700 nm) with luminescence quantum yield (LQY) of 30–85%. By shellling such QDs with the wide-bandgap CdS, ZnSe and/or ZnS, the CdTe/CdS or CdSe/ZnS core/shell and CdSe/ZnSe/ZnS core/shell/shell structures were well formed to improve their performance. The morphology, structural phase and optical properties of the synthesized QDs were characterized by using scanning electron microscope (SEM), x-ray diffraction (XRD) and absorption, PL, including the PL dependent on temperature or the pH or the resonant energy transfer. Testings of CdTe and CdSe QDs as the fluorescence labels for agricultural-bio-medical objects (e.g. residual pesticides or clenbuterol in agricultural products, H5N1 virus, etc) were carried out showing that the QDs synthesized in our lab are applicable for the designs of most fluorescence biosensors.

2. Experimental

2.1. Synthesis of semiconductor QDs

2.1.1. Synthesis of CdTe core. Chemical substances for the synthesis of CdTe QDs are: sodium borohydride (NaBH$_4$, 

## Chemical formulas

\[ \text{NaBH}_4 \]
The synthesis of CdTe QDs follows the principle that the precursors (ion Cd$^{2+}$, Te$^{2-}$) react in aqueous solvent with the presence of ligand molecules (MPA or MSA) to nucelate the crystalline seeds. Then, these tiny seed crystallites are grown by the epitaxial process with the monomer provided from the solution. MPA or MSA surfactant plays an important role to make CdTe QDs existent in colloidal form. Concretely, a stock NaHTe (for the Te$^{2-}$ ions) solution must be prepared by putting 160 mg of Te powder and 100 mg of NaBH$_4$ into a two-neck flask and degassing for 30 min and then backfilling with nitrogen gas; and finally adding 2 ml of degassed distilled water into the flask. To promote the reaction mixture, an ultrasonic generator (40–50 kHz) was applied for 30 minutes at a temperature of ~50–60°C. As a result, a deep red transparent solution of NaHTe was formed. For producing typical CdTe QDs, 12.5 mM of CdBr$_2$ (for the Cd$^{2+}$ ions) solution was mixed with 18.75 mM of MPA solution at the ratio of Cd:MPA = 1 : 1.5 (mol/mol) and the pH of this solution was adjusted in the range of 7–12 by addition of 1.0 M NaOH solution. Then, the freshly prepared 6.25 mM NaHTe solution (corresponding to the mole ratio of Cd:Te = 2 : 1) was injected quickly into the Cd-containing flask at room temperature under nitrogen gas protection. The reaction mixture changes instantly to golden yellow indicating the nucleation of CdTe nanocrystals. The as-prepared CdTe QDs emit weak luminescence peaking at 510 nm.

We have used another method to synthesis high quality CdTe QDs from TeO$_2$ and CdBr$_2$ with MSA ligand/surfactant. In this synthesis, TeO$_2$ reacts with NaBH$_4$ to form NaHTe in the presence of CdBr$_2$, therefore to nucleate the CdTe seeds at the same time and in situ. This method is good to avoid the production of NaHTe as a separate step consequently avoids any requirement of the inert gas for protection during the synthesis.

The size of CdTe QDs was controlled by the growth time between a few minutes to several hours in an autoclave at 120°C. For detection of the OP pesticides we need to prepare CdTe QDs that emit strongly at 520 nm to overlap well to the absorption of dithizone (DZ) [14]. All the QDs synthesized in aqueous phase can be used directly in fluorescence bio labeling.

### 2.1.2. Synthesis of CdTe/CdS core/shell structure

To passivate surface dangling bonds and stabilize the photophysical characteristics of CdTe QDs, the CdS or ZnS shell was made over the CdTe core. The CdS shell was easily made by adding an excessive amount of thiourea (for the S precursor) into the solution containing CdTe QDs core (with the available excessive Cd$^{2+}$ ions) and then, the mixture was annealed at 120°C for certain periods of time depending on the required sizes of CdTe/CdS. Herein, thiourea was used as the sulfur source due to its decomposition and the S$^{2-}$ ions could easily combine with the excessive Cd$^{2+}$ ions in the initial solution to form CdTe/CdS core/shell structures. During the shelling from a few minutes to a few hours, not only did the CdS shell form but also the CdTe core developed to be bigger, giving emission at longer wavelengths.

### 2.1.3. Synthesis of CdSe core

Monodisperse CdSe QDs were prepared following the procedure described elsewhere [51–53, 61]. Chemical substances used for the synthesis of CdSe QDs are: cadmium acetate Cd(CH$_3$COO)$_2$, 2H$_2$O, cadmium oxide (CdO) 99.5%; cadmium dimethyl Cd(Me)$_2$; tri-n-octylphosphine oxide (TOPO, C$_{24}$H$_{43}$O$_2$, Merck) 98%; tri-n-octylphosphine (TOP, C$_{24}$H$_{43}$P, Fluka) 90%; hexadecylamine (HDA, C$_{16}$H$_ {35}$N, Merck) 92%; dodecylphosphonic acid (DDPA, C$_{12}$H$_{27}$O$_3$P, polycarbon Inc.); oleic acid (OA,C$_{18}$H$_{34}$O$_2$) 98%; 1-octadecene (ODE, Sigma-Aldrich) 98%; oleylamine (OLA, Merck) 98%; selenium (Pooe England) 99%; zinc stearate (C$_{36}$H$_{70}$O$_2$Zn, Aldrich); diesel; toluene and methanol (Merck). Depending on the synthesis, different high-boiling point organic solvents were used as the reaction medium. Both homogeneous and heterogeneous nucleation of the CdSe seed crystallites by using the heating-up and hot-injection methods were performed [1]. In the latter, the meeting of Cd$^{2+}$ and Se$^{2-}$ ions must be as fast as possible to nucleate the uniform CdSe seed crystallites. For this purpose, a special syringe with multi-injection holes was designed to introduce the TOPSe precursor simultaneously in many small squirts within a very short lapse of time. For typical synthesis using the heating-up method, the well mixed Cd(Me)$_2$ and TOPSe (the molar ratio of Cd:Se = 1–1.5:8) solution was injected into the TOP/OHA solvent at 250–300°C under the N$_2$ gas protection atmosphere. For the typical 120-mg CdSe QDs synthesis using the hot-injection method, CdO (0.8 mmol) was usually used as the Cd precursor to make the Cd-complex in hot (~210–270°C) TOPO/HDA (3.5 ml/6.5 ml) or ODE/OLA or diesel; and then the stock TOPSe (5 ml of the 0.4 M solution prepared from 1 mmol of selenium powder in 0.4 ml TOP under the argon or nitrogen gas protection) solution was injected swiftly into the reaction vessel at high temperature to nucleate the CdSe seed crystallites. The use of diesel as the reaction solvent instead of TOPO/HDA or ODE has some advantages, such as that the synthesis could be carried out at not very high temperature, around 210°C, which is near the boiling point of diesel, making the system very dynamic to promote the chemical reaction between the precursors. The CdSe QDs obtained are quite high quality to emit at the required spectral range.

The size of CdSe QDs was controlled by the growth/heating time, typically in minutes to hours [1, 27, 51–53, 61, 62]. However, in our opinion, we see that the size distribution of QDs is much narrower if the growth time is short, i.e. a few minutes. Thus, the temperature should be another technological factor to control the average size, namely to be higher if one needs to get bigger QDs. All the QDs synthesized in the organic solvents have to have experienced the ligand exchange to be water-soluble for applications in the bio labeling.

### 2.1.4. Synthesis of CdSe/ZnS, CdSe/CdS/ZnS and CdSe/ZnSe/ZnS core/shell(s) structures

To passivate surface dangling bonds and stabilize the photophysical characteristics of CdSe QDs, we made the ZnSe shell over the...
core (to form the core/shell (CS) structure) or ZnSe/ZnS or CdS/ZnS double-shell (the core/shell/shell (CSS) structure) [2, 10, 24, 27, 62]. Different sulfur precursors could be used but in our experiments the exclusively air-stable monomolecular precursors cadmium and zinc ethylxanthate are the sources of both Cd2+/Zn2+ ions and S2- ions were used to make the CdS or ZnS shell. Details of the shelling process for only ZnSe or ZnS were described elsewhere [2, 10, 24, 27, 63, 64]. Here, an example in the synthesis of CdSe/CdS/ZnS CSS QDs is presented using 0.06 µmol of CdSe core QDs dispersed in a mixture of 5 ml of ODE and 5 ml of OLA, heated to 220 °C under argon or nitrogen flow. For shelling with CdS, 0.01 mmol of Cd-ethylxanthate dissolved in 0.125 ml of TOP and 0.03 mmol of Cd-stearate dispersed in 0.375 ml of ODE are injected within 20 min into the reaction flask by means of a syringe pump. After the optical properties of the CdSe/CdS core/shell QDs had been stabilized, the ZnS shell was grown by injection of a mixture of 0.06 mmol of Zn-ethylxanthate in 0.75 ml of TOP and 0.17 mmol of Zn-stearate in 2.125 ml of ODE within 1 h.

We have used diesel as the solvent instead of ODE or TOPO for shelling too. The solvent at nearly the boiling point is very active to promote the chemical reaction between the precursors, making the shelling process more efficient.

2.2. Fabrications of fluorescence biosensors based on QDs

In this paragraph we describe the fabrications of various kinds of fluorescence sensors for detection of residual pesticides, clenbuterol or H5N1 avian influenza virus. All these biosensors are based on the change of the PL intensity as a function of the amount of pesticides, clenbuterol or H5N1 avian influenza virus.

For the purpose of development of biosensors for the detection of OP-based pesticides, two different kinds of sensors have been realized: (i) to measure the change in the PL intensity of the QDs which have been functionalized with the AChE enzymes to catalyze the hydrolysis of acetylthiocholine (ATCh); and (ii) to turn-on luminescence with the AChE enzymes to catalyze the hydrolysis of ATCh. The amounts and ratios of each constituent above were designed depending on the sensitivity and dynamic range of the pesticide detection. In normal biosensor fabrication, we used directly the CdTe/CdS CS or CdSe/ZnSe/ZnS QDs, while the CdTe or CdS QDs act as the luminescence indicators for the hydrolysis of ATCh. Eventually, the luminescence from QDs can indicate the AChE enzymatic activity, while the CdTe or CdS QDs act as the luminescence indicators for the hydrolysis of ATCh.

However, after the surface-modification with MPA, these CS and CSS QDs would be negatively charged, consequently causing difficulty for the AChE molecules to be bound on their surface because AChE molecules are also negatively charged in the environment with pH = 7. Thus, these CS and CSS QDs need to change their electrical status by coating/exchanging MPA with streptavidin (SA) molecules. Normal 1 × 1 × 4.5 cm3 organic glassy cuvettes were used to contain the solutions prepared by mixing well the enzymes AChE, ATCh and CdTe/CdS or CdSe/ZnSe/ZnS QDs. In the mixed solution, AChE catalyzes the hydrolysis of ATCh to produce TCh, which bears an additional thiol group (–SH) to make the possible increase of the pH surrounding the CdTe/CdS or CdSe/ZnSe/ZnS QDs. In this biosensor structure, ATCh acts as an indicator for the presence of the AChE enzymatic activity, while the CdTe or CdS QDs act as the luminescence indicators for the hydrolysis of ATCh. Eventually, the luminescence from QDs can indicate the AChE enzymatic activity or correspondingly the pesticide content because when adding pesticide into the mixed solution (biosensor) the pesticide would bind to AChE to inhibit the AChE enzymatic activity. The amounts and ratios of each constituent above were designed depending on the sensitivity and dynamic range of the pesticide detection. In normal biosensor fabrication, we used directly the CdTe/CdS CS or CdSe/ZnSe/ZnS CSS QDs dispersed in phosphate buffered saline (PBS) solution (pH = 7.4), mixed with 16.4 units ml−1 AChE dissolved in 1 ml of the QDs solution (typically absorbance of 10−4 corresponding to about 1012 QDs per ml) containing 25 µl ATCh (0.12 mM). The constituent’s solution was incubated at 37 °C for 30 min and well mixed with the assistance of an ultrasonic machine (45 kHz). The PL measurements were carried out 5 min after adding pesticide into biosensor at room temperature. PM pesticides in distilled water of various concentrations from 0.05 ng ml−1 (0.05 ppm) to 3 ng ml−1 (3 ppm) were prepared to check the function of the fabricated biosensors.

For detection of clenbuterol, FRET-based nanosensors were developed and tested [21]. In the sensor structure, CdTe/CdS QDs conjugated with 2-amino-8-naphthol-6-sulfonic acid (I) for detection of diazotized clenbuterol (II) by the specifically coupling reaction.

Figure 1. Nanosensor using CdTe/CdS QDs conjugated with 2-amino-8-naphthol-6-sulfonic acid (I) for detection of diazotized clenbuterol (II) by the specifically coupling reaction.
react specifically with the 2-amino-8-naphthol-6-sulfonic acid conjugated CdTe/CdS QDs by the coupling reaction. Then, by measuring the PL intensity of QDs in the nanosensor as a function of diazotized clenbuterol, we could calibrate the PL intensity change with the clenbuterol concentration. Note that diazotization of clenbuterol can happen only with the amino group at the benzene ring but not with the amino group at branch. This is a proper characteristic, important to avoid any diazotization with other amino groups available in bio-specimens. On the other hand, QDs act as the energy donor that must emit at spectral range matching the acceptor absorption.

For the purpose of developing a biosensor for detection of the H5N1 avian influenza virus and that can be modified to fit other viruses, we have prepared the necessary materials including chromatophores purified from bacteria *Rhodospirillum rubrum*, recombinant F$_0$F$_1$-ATPase β-subunit and anti-F$_0$F$_1$-β-subunit antibodies, and anti-H5N1 antibodies. Details of the fabrication of biosensor for detection of H5N1 avian influenza virus were described elsewhere [47]. The working principle of the QDs-ATPase-based biosensor is based on the change of the PL intensity from QDs in the presence of virus, which makes changing the ATP (adenosine tri-phosphate)-ADP (adenosine diphosphate) transformation to release energy for metabolic processes and therefore making the proton (H$^+$) flux change. In the biosensors fabricated, we clearly determined the core part (consists of CdTe/CdS QDs bound on the surface of chromatophores with the F$_0$F$_1$-ATPase antibody of β-subunit) which is similar for most antigens, while the peripheral part (consists of the antibody of H5N1 avian influenza virus and H5N1 avian influenza virus) could be varied to target antigens which we want to detect. The antibody of β-subunit (in the core part) and the antibody of H5N1 avian influenza virus (in the peripheral part) were biotinylated and joined each other by the streptavidin bridge. This sensor design is very flexible for specific detection of different kinds of antigens by using the same core part, just by changing only the antibody–antigen targeting in the peripheral part.

### 2.3. Characterizations

#### 2.3.1. Morphology and structural characterizations.

Morphology of the QDs was characterized by using a TEM (Tecnai 20ST, or JEOL 4000EX). The powder XRD patterns were taken by using a Siemens D5000 powder x-ray diffract meter equipped with a graphite monochromatized high-intensity Cu $\text{K}\alpha$ radiation ($\lambda = 1.54178$ Å).

#### 2.3.2. Optical characterizations.

The absorption and PL spectra were taken by using a Varian Cary 5000 UV-Vis-NIR spectrophotometer and an iHR550 Horiba spectrometer equipped with a thermoelectrically cooled Si-CCD camera (Synapse), respectively. In PL measurement, a 377 nm LED or a 405 nm diode laser were used as the excitation sources. The size of QDs so that can be controlled by the growth temperature and/or duration time. One should compromise between these two technological factors, but we note that the short growth time is usually giving rise to the better monodisperse size distribution. For most biolabeling, the high LQY is more important than the exact peak wavelength.

![Figure 2. Photos of colloidal CdTe QDs in different sizes (three left) and CdSe QDs (last right) showing strong luminescence under room light.](image-url)

### 3. Results and discussion

#### 3.1. CdTe and CdSe semiconductor QDs

For rather a long time working on the synthesis of various nanocrystals using chemical route we have successfully produced the high quality CdTe, CdSe, InP and CuInS$_2$ QDs [7, 10, 27, 65–67]. By fully controllable technology these QDs have been produced with different size in the range of 2.5–5 nm and various structures including the CS and CSS. Our high quality synthesized QDs have been used for basic researches and applications [10, 21, 27, 37, 47, 68]. In fabricating fluorescence sensors, we have mostly used CdTe/CdS and CdSe/ZnSe/ZnS QDs which have nice excitonic absorption and could strongly emit luminescence at a designed spectral range. The PL spectra from CdTe/CdS QDs normally show full-width of half-maximum (FWHM) of 40–50 nm while those from CdSe/ZnS or CdSe/ZnSe/ZnS QDs show narrower FWHM of 20–35 nm.

In general view, the as-synthesized QDs are covered by ligand molecules. Depending on the polarization of these ligands the complex QDs/ligands could be dispersive in water or not. For CdTe QDs normally covered by MPA or MSA with the COOH end and the SH end out. Both these ends make the complex of CdTe QDs/MPA (or MSA) easily dispersive in water. For CdSe QDs, many authors have performed the syntheses in high boiling temperature organic solvents like TOPO, TOP and/or amines [51–53, 61–64] or a non-coordinating solvent such as ODE or diesel [55, 69]. As-prepared CdSe NCs generally show weak luminescence because of the existence of non-radiative channels originating from surface states. After appropriate passivation by shelling with the CdS, ZnSe and/or ZnS monolayers [2, 10, 24, 27, 63, 64] these core/shell QDs become highly luminescent. However, after doing ligands exchange to get water-soluble CdSe QDs, the luminescence decreases significantly. Figure 2 shows the photos of CdSe and CdTe QDs synthesized for the fluorescence biolabeling purpose. These QDs emit strong luminescence under room light, meaning that their LQY must be at least 50%. Because of the quantum confinement, the peak emission is dependent on the size of QDs so that can be controlled by the growth temperature and/or duration time. One should compromise between these two technological factors, but we note that the short growth time is usually giving rise to the better monodisperse size distribution. For most biolabeling, the high LQY is more important than the exact peak wavelength.
Figure 3. TEM images of colloidal CdTe (left) and CdSe QDs (right). High-resolution TEM image shows clearly the atomic arrangement of CdTe nanocrystals.

(in the visible range); however, for certain application it needs the overlapping between the emission of QDs and the absorption of transducer for getting high efficiency detection. Below, we present the change in the 520 nm PL band due to the energy transfer to DZ in such a case the peak emission of QDs synthesized must be controlled exactly.

Figure 3 demonstrates the morphology of some QD examples showing that the QDs have spherical shape. With shelling, the size of QDs become bigger. In many cases from the high resolution TEM images one can see clearly the lattice facets of nanocrystals. The XRD patterns confirm the good crystalline structures with broad peaks due to the nanometer size of CdTe and CdSe QDs (figure 4).

The optical properties of CdTe and CdSe QDs have been extensively studied and published [1–64, 68, 69]. We have contributed to the optical studies of polarized CdSe, CdSe/ZnSe, and CdSe/CdS/ZnS QDs dispersed in various polar solvents to show the quantum Stark effect on the nanometer scale [27]. This effect plays an important role in the change of the PL intensity and peak position as well. This is essentially due to the effective electric field induced by the polarization of QDs, ligand molecules, polarity of solvent and surface states (figure 5). In other words, any change in the electrical situation local at the surface of QDs could influence their PL intensity. This behavior is beneficial to the design of fluorescence biosensors. Note that the quantum Stark effect makes such PL change in the core QDs by the electric field so that it can happen not only for the barely core QDs but also for the core/shell or core/shell/shell ones. Usually the core only QDs have low LQY and are not very resistant to environment. Shelling QDs with large bandgap semiconductors (e.g. CdS or ZnS) makes the core/shell or core/shell/shell CdTe and CdSe QDs emission with much higher LQY and more stable. This is due to passivation of dangling bonds on the surface of QDs and isolation of QDs with environment. It is interesting to reveal that dangling bonds on the surface of QDs can be passivated by shelling with large bandgap semiconductors or simply with H+ and OH− ions generated from dissociation of water molecules by UV light irradiation [68]. With such a manner, using a small 377 nm LED to passivate the water-soluble CdSe QDs, the PL intensity from the passivated CdSe QDs could increase up to almost two orders of magnitude and the peak emission shifted to the higher energy (figure 6).

As a preparation step for realization of fluorescence sensors using highly luminescent QDs, we need first to study the detailed pH-dependent PL of CdTe/CdS and CdSe/ZnSe/ZnS QDs. The colloidal CdTe/CdS and CdSe/ZnSe/ZnS QDs used in our study have been capped with MPA ligands or MSA ligands (due to the direct aqueous phase synthesis or from the ligand exchange), in which the thiol (SH−) and carboxylic (COOH+) groups exist on the surface; they have therefore negatively charged surface and are very sensitive to pH of the surrounding medium. In the AChE enzyme-based sensors for the pesticide residual detection, the presence of pesticide changes the pH of the environment surrounding the QDs, since the hydrolysis of ACh produces thiolcholine (Tch) and acetic acid. In the ATPase-based biosensor the proton (H+) flux change during the reaction with the H5N1 avian influenza virus makes the pH change. It is worth noting that depending on the preparation technology the QDs have a different tendency in changing their PL intensity, namely positive or negative with increasing pH [16]. Our CdTe/CdS QDs synthesized with MPA or MSA ligands exhibit the PL intensity change with pH (figure 7) in good consistency with those used in Deng’s group [16], i.e. we observed an increase in the PL intensity with decreasing pH value (equivalently the increase of proton flux).

3.2. Testing applications

Examples for the applications of CdTe and CdSe QDs in agricultural production have been realized. Concretely, in the following we demonstrate the applications of QDs in fabricating three kinds of biosensors for the detection of: (i) residual pesticides, (ii) clenbuterol and (iii) the H5N1 avian influenza virus.

3.2.1. Detection of residual pesticides. OP-based pesticides (e.g. paraxoxon and parathion) are most widely used in agricultural production. The toxicity of these compounds comes from their irreversible binding to AChE, an enzyme that is essential for hydrolysis of the neurotransmitter acetylcholine (ACh)—each molecule of AChE degrades about 25 000 molecules of ACh per second. Therefore, the inhibition of AChE by pesticide leads to in vivo accumulation of ACh, which results in wrong actions of the neurotransmitters that produce respiratory disorders, paralysis or even death. Here we present the testing results on the fluorescence biosensors fabricated using QDs to indicate the residual pesticide amount. In the sensors, ATCh was used to monitor the presence of the AChE enzyme because ATCh is a powerful hydrolyte with the presence of AChE enzyme to change the pH, consequently changing the PL intensity of QDs. In order to exclude the interference from each ATCh or AChE or PM on the operation of the biosensor, the PL spectra of QDs (CdTe/CdS CS and CdSe/ZnSe/ZnS CSS QDs in the present study) were taken separately, adding one of the mentioned ingredients. The results showed that almost no change was observed in the PL spectra of both CdTe/CdS CS and CdSe/ZnSe/ZnS CSS QDs. In other words, there is no separate influence of ATCh or AChE or PM on the PL of
QDs. Also, we observed no change in the PL spectra of QDs in the mixed solution with ATCh or AChE and then added with PM. This is because the pH of the media surrounding the QDs was not changed. As presented in section 2.2, fluorescence biosensor is composed of the three constituents: QDs, AChE and ATCh. Therefore, the PL spectra of the biosensors must be measured before adding PM pesticide for the calibration purpose. In the biosensor, the PL intensity from QDs was much decreased because AChE catalyzed the hydrolysis of ATCh to produce ACh with more SH⁻ group to increase the local pH. This value of the PL intensity is depending on the ratio of QDs and AChE and ATCh, is minimum corresponding to a certain amount of QDs. The maximum PL intensity is of course as high as that of the QDs only without hydrolysis process. The difference between the minimum and maximum PL intensity is considered to be the dynamic range of the biosensor. PM pesticide is detectable because it inhibits the AChE enzymatic activity and consequently decreases the hydrolysis of ATCh, making a decrease of pH that then releases the PL intensity from QDs. Figure 8 shows the PL spectra taken from a fluorescence biosensor fabricated with CdSe/ZnSe/ZnS CSS QDs. By adding both AChE (1 unit) and ATCh (in the QDs:AChE:ATCh ratio of 1:1.5:1) the PL intensity greatly decreased to get the minimum value. PM was then added to inhibit the AChE enzymatic activity, consequently making a respective increase in the PL intensity. The sensitivity of 0.05 ppm PM has been achieved that corresponds to the limit detection of the EU standard 2010 (the EU Reg. No. 600/2010 of July 8, 2010) and much lower than the residual amount around ppm that is allowed to be residual in foods. The detection dynamic range of this biosensor is between 0.05 ppm and 0.5 ppm. The results on the activity of fluorescence biosensors fabricated from CdTe/CdS CS QDs are quite similar to those obtained from CdSe/ZnSe/ZnS CSS QDs [37]. Therefore, we used CdTe/CdS CS QDs (because of their simpler synthesis) for the experiments to check the dynamic range of biosensors. With a certain concentration of CdTe/CdS QDs, the dynamic range for pesticide detection can be expandable by increasing the AChE content. Figure 9 shows the results extracted from the two biosensors fabricated with the two different AChE amounts of 1 and 3 units, respectively.

The PL intensity of CdTe or CdSe QDs in fact could be varied also due to energy transfer. Based on the FRET
process, we fabricated another kind of on-off fluorescence biosensor in which the DZ–Cd\(_{2}^{+}\) ions complex on the surface of CdTe/CdS QDs works as the PL quencher by the FRET. This is a sensitive indicator to the presence of OP-based pesticide because the hydrolysis of organophosphorothioate molecules releases diethyl phosphorothioate (DEP) which easily forms the strong DEP–Cd\(_{2}^{+}\) ions complex to replace the DZ–Cd\(_{2}^{+}\) ions complex on the surface of CdTe or CdSe QDs, consequently to release the PL from CdTe QDs from the FRET. Thus, the DZ–Cd\(_{2}^{+}\) ions complex ligands once they are released from their energy acceptor role can turn-on luminescence of CdTe QDs. The PL intensity change gives rise to the detection limit of 0.1 nM for chlorpyrifos, a kind of organophosphorothioate. Our study showed that the stability of such turn-on luminescence biosensor was not so good as the DZ ligands could significantly release themselves from QDs after hours to clearly allow the PL of QDs to recover without the presence of pesticides.

3.2.2. Detection of residual clenbuterol. The CdTe/CdS QDs conjugated with 2-amino-8-naphthol-6-sulfonic acid were used as the clenbuterol recognizable probe. Our TEM study showed the super-molecule QDs were coated by clenbuterol recognizable naphthol. These super-molecule QDs could react with diazotized clenbuterol via coupling of the naphthol group (on the surface of QDs) and the diazo group (of the diazotized clenbuterol) (see figure 1). This reaction could happen only with the diazo group and forms the specific orange color of diazo compound. In the FRET-based sensor, the energy transfer happened between QDs (donors) and the remaining part, including diazotized clenbuterol and modified 2-amino-8-naphthol-6-sulfonic acid that act as acceptors, gives rise to the change in the PL intensity of QDs. Thus, by measuring the PL intensity of QDs in the nanosensor as a function of diazotized clenbuterol we could calibrate the PL intensity change with the clenbuterol content. Figure 10 shows that there is a linear correlation between the clenbuterol content and the PL intensity taken.
3.2.3. QDs-chromatophore for the detection of H5N1 avian influenza virus. We have successfully prepared all the constituents, checked their functions and fabricated the fluorescence biosensor for detection of H5N1 avian influenza virus. Figure 11 shows the TEM image of chromatophores purified from bacteria *Rhodospirillum rubrum*. The average size of these chromatophores is 70 nm. Figure 12 shows the PL image of chromatophores conjugation with CdTe/CdS QDs. One can clearly see (under epifluorescence microscope Olympus IX71) the luminescence of CdTe/CdS QDs bound on chromatophores. By electrostatic force, the negatively charged MPA-capped CdTe/CdS QD could easily bind to the positively charged chromatophores and many CdTe/CdS QDs could bind on the surface of one chromatophore. The resultant QDs–chromatophore conjugates were stable as a colloidal solution. In fact, chromatophore and H5N1 avian influenza virus are protein in nature; consequently they can emit strong luminescence under UV light excitation. Therefore, we measured the PL spectrum of not only the CdTe/CdS QDs but also all the chromatophores and H5N1 avian influenza virus (figure 13). This is the important procedure for analyzing the final PL signal obtained from the whole biosensor. The overall PL spectrum (curve (d) of figure 13) shows clearly the superposition of the PL spectrum from CdTe/CdS QDs (peaking at 527 nm), from H5N1 avian influenza virus (with the peaks at 523, 618 and 681 nm), chromatophores (with the peaks at 597, 636 and 705 nm). In the present study, the CdTe/CdS QDs used emit PL at the same peak wavelength of H5N1 avian influenza virus but we can distinguish the right PL signal for specific detection of H5N1 avian influenza virus upon the proper spectrum of each component in the whole biosensor.

After adding ADP to promote the proton pumping from chromatophores during the synthesis of ATP, the PL spectra were recorded using 405 nm laser light excitation. Figure 14 shows the relationship between the PL intensity (taken at the peak wavelength around 525 nm) of H5N1 avian influenza viruses and of the biosensor composed of CdTe/CdS QDs and chromatophores and the virus contents. The increase of the PL intensity proper to H5N1 avian influenza virus directly resulted from the increase of the virus amount (curve (a) of figure 14). Compared to that, the increase of the PL intensity from CdTe/CdS QDs-based biosensor was caused by lowering pH due to the proton (H$^+$) flux which pumped out of the chromatophores during the synthesis of ATP catalyzed by the F$_o$F$_1$–ATPase enzymes. The proton flux was increased.
with increasing H5N1 avian influenza virus loaded into the biosensor because the antibody-antigen reaction enhances the activity of the F$_6$F$_1$–ATPase enzymes. As the H5N1 avian influenza virus increased, the overall PL intensity was increased by both the PL from H5N1 avian influenza virus itself and from CdTe/CdS QDs at lower pH values. This adding effect was clearly observed and demonstrated in curve (b) of figure 14. Note that in the literature [10], the integral PL signal has been presented instead of the proper spectrum from H5N1 avian influenza viruses and that from the CdTe/CdS QDs-based biosensor. However, at this stage we have not yet exactly determined the virus concentration (number of viruses per volume unit), but we have obtained the linear dependence between the PL recorded from biosensor and the amount of H5N1 avian influenza virus in the range of 3–50 ng µL$^{-1}$ with a detection limit of 3 ng µL$^{-1}$.

4. Conclusion

In conclusion, we have synthesized high-quality CdTe/CdS CS QDs in aqueous phase, and CdSe/ZnS CS and CdSe/ZnSe/ZnS CSS QDs in high-boiling organic solvents. The optimal technological parameters were determined for synthesizing high-quality QDs which strongly emit in the spectral region between ~500 and 700 nm as designed. The synthesized QDs generally possess a luminescence quantum yield of 30–85%. The morphology, structural phase and optical properties of QDs were characterized by using appropriate techniques such as TEM, XRD and absorption, PL, including the PL as a function of temperature or pH or dependent on the resonant energy transfer. Studying the high quality of QDs enables us to interpret the optical transitions and quantum confined Stark effect at the nanometer scale, and the passivation of dangling bonds on the QDs’ surface by the H$^+$ and/or OH$^-$ ions. For testing applications, three kinds of fluorescence biosensors using CdTe/CdS and CdSe/ZnS CS, CdSe/ZnSe/ZnS CSS QDs were fabricated to trace residual pesticide in agricultural products, residual clenbuterol in meat and for detection of H5N1 avian influenza virus in breeding farms. PM pesticide at a content as low as 0.05 ppm has been specifically detected by using the biosensor made from CdTe/CdS or CdSe/ZnSe/ZnS QDs and the AChE enzymes. CLENBUTEROL at a content as low as 10 µg ml$^{-1}$ could be detected by using FRET-based nanosensors in which the PL intensity from the CdTe/CdS QDs conjugated with 2-amino-8-naphthol-6-sulfonic acid was measured. H5N1 avian influenza virus of 3 ng µL$^{-1}$ was detected with a fluorescence biosensor based on the antibody-antigen recognition. This biosensor using CdTe/CdS QDs bound on the surface of chromatophores purified from bacteria Rhodospirillum rubrum could be extendable to application for other kinds of antigens. This could be a good issue for promoting complementary collaboration between researchers working in materials science and biological technology to realize various kinds of sophisticated biosensors.

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