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Bioconjugation of CdTe quantum dot for the detection of 2,4-dichlorophenoxyacetic acid by competitive fluoroimmunoassay based biosensor

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ABSTRACT

Quantum dots (QD) are semiconductor fluorescent nanoparticles, which can be made use of for environmental monitoring with high sensitivity. In view of the alarming levels of pesticides and herbicides being used in agriculture practices, there is a need for their rapid, sensitive and specific detection in food and environmental samples, as pesticides and herbicides are harmful to living beings even at trace levels. Present study was carried out to develop a reliable and rapid method for analysis and detection of 2,4-D (herbicide) using cadmium telluride quantum dot nanoparticle (CdTe QD). Fluoroimmunoassay based on the fluorescent property of quantum dot was used along with immunoassay to detect 2,4-D. CdTe capped with mercaptopropionic acid, was conjugated using *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) and a coupling reagent like *N*-hydroxysuccinimide (NHS) to alkaline phosphatase (ALP) which was in turn conjugated to 2,4-D molecule. Anti 2,4-D-IgG antibodies were immobilized in an immunoreactor column using Sepharose CL-4B as an inert matrix. The detection of 2,4-D was carried out by fluoroimmunoassay-based biosensor using competitive binding between conjugated 2,4-D–ALP–CdTe and free 2,4-D with immobilized anti 2,4-D antibodies in an immunoreactor column. It was possible to detect 2,4-D upto 250 pg mL^{-1}. Present study also emphasizes on the resonance energy transfer between ALP and CdTe QD as a result of bioconjugation, which can be used for future biosensor development based on quantum dot-biomolecular interactions.

1. Introduction

Food and environmental safety is a serious concern for modern society. Toxicants and pollutants are harmful even at trace level. Therefore, there is a need for novel strategies and techniques, which are fast, reliable and highly sensitive for their detection. Conventional analytical techniques such as HPLC, LCMS, and GC have several limitations (Lancas et al., 1999), which can be overcome by the application of a modern analytical tool such as Biosensor. Biosensor devices are emerging as one of the foremost relevant tools in food and environmental safety due to their rapidity, specificity, ease for mass fabrication and field applicability.

Recent developments in smart Nanomaterials have paved the way for nanobiosensor development (Bruchez et al., 1998). Nanotechnology is at the core of advances in the biosensor field through the use of novel materials such as quantum dot semiconductor nanoparticle. Quantum dots consist of II–VI, III–V and IV–VI group elements of the periodic table. These are unique with respect to their broad excitation and narrow emission spectra, and water solubility, which make them useful for biological labelling (Hall et al., 1999; Ramadurai et al., 2006). Moreover, they are superior to any conventional organic fluorescent dyes for biological labelling in terms of photostability, phtobleaching threshold, quantum yield and controlled absorption and emission spectra with respect to their size (Bruchez et al., 1998; Chan and Nie, 1998). The shift in the band gap energy as a function of quantum confinement and size can be made useful to study optical properties of biomolecules by conjugation. The effects of quantum confinement can be observed by measuring the absorption spectrum and in turn the band structure and band gap of the quantum dot. This will provide an insight into the overall structural changes and optical properties of quantum dots as well as biomolecules by conjugation. It was reported that quantum dots such as CdTe and CdSe were able to participate in resonance energy transfer processes that resemble forster resonance energy transfer (FRET) (Mamedova et al., 2001; Willard et al., 2001; Ma et al., 2005). The combina-

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tion of small size, high photostability and size tuneable emission properties makes quantum dots highly attractive probes for biological analysis (Grieve et al., 2000; Aoyagi and Kudo, 2005). They obtain their specificity from biological binding reactions, which are derived from a range of interactions that include antigen/antibody, enzyme/substrate/cofactor, receptor/ligand, chemical interactions and nucleic hybridisation in combination with a range of transducers (Thakur and Karanth, 2003). The fluorescence can be scanned even at very low concentration, which is helpful for measuring the analyte by conjugating quantum dots with the suitable tracer molecule (Huang et al., 2005; Wang et al., 2007). They are emerging as powerful tools for biosensors, bioanalytical methods for sensitive detection in health, food, water and environmental safety.

In modern agricultural practices, pesticides are commonly used on a large scale in the control of weeds, insects and rodents (Mazzullo et al., 1997) and have been detected in number of foods and drinking water samples due to their accumulation in the food chain (Gruzdyev et al., 1983; Belfroid et al., 1998). 2,4-D is an herbicide, generally used in the fields of barley, wheat, corn and sorghum to control broad leaf weeds (U.S. Dept., 1993). It is an active ingredient of more than 1000 pesticide preparations. It has also appeared in drinking water due to its vast usage in agriculture, which is not detectable as per recent surveys (U.S. Envr., 1987; American Conf. Govt. Indust. Hygi., 1999).

These pesticides can cause human health problems even at trace level and therefore, their sensitive detection and quantification is very important. Conventional analytical techniques like HPLC and GC/MS can detect pesticides upto μg level (Trau et al., 1997; Chouhan et al., 2006). It was reported that an amperometric flow injection immunoanalysis (FIIA) system could detect 2,4-D in the range of 0.2–70 $\mu g\,L^{-1}$ (Trau et al., 1997). We have attempted for the first time, the detection 2,4-D, a commonly used herbicide, based on a simple strategy of integrating the fluorescence property of quantum dot and immunological techniques for the quantification of residual analyte.

2. Experimental

2.1. Materials

Antibodies (IgG) against 2,4-dichlorophenoxyacetic acid (2,4-D) were procured from Institute of Microbial Technology (IMTECH), Chandigarh. CdTe quantum dot capped with mercaptopropionic acid (CdTe-QD) was obtained from Laboratory of Photonics and Interfaces, ISIC, EPFL, Switzerland. 2,4-D, Alkaline Phosphatase (ALP, Source: Calf intestine), *N*-hydroxisuccinimide (NHS), 1 ethyl 3(3-dimethyl amino propyl) carbodiimides hydrochloride (EDC) and sodium cyanoborohydride were obtained form Sigma Chemicals, St. Louis, USA. Sepharose gel CL-4B was obtained from Amersham Biosciences AB, Sweden and Dialysis membranes of 6–8 kDa cut off range from Spectra/Por, USA. All reagents used during this study were of analytical grade and obtained from standard sources.

2.2. Apparatus

The instruments used were Spectrofluorimeter (RF-5301 PC, Shimadzu), UV–vis spectrophotometer (UV-1601, Shimadzu), VERSA max tunable microplate reader, (Molecular devises, USA), and Nunc-Immuno plate, (F96 CERT-Maxisorp, Nunc A/C, Denmark), and water bath (Julabo SW 20-C), Alitea-XV peristaltic pump (Sweden).

2.3. Preparation of 2,4-D conjugate with alkaline phosphatase

1 mg of 2,4-D was dissolved in 100 μL of dimethyl formimide (DMF) and 400 μL of PBS (phosphate buffer saline, pH 7.4, 50 mM), and activated for 20 minutes at room temperature using 0.05 M *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) as an activator, and 5 mM *N*-hydroxysuccinimide (NHS) as a coupling reagent. To this reaction mixture 1 mg of alkaline phosphatase (ALP) dissolved in 500 μL of PBS was added and incubated for 2 h at room temperature (Hermanson et al., 1992; Svitel et al., 2001). This mixture was further incubated for 16 h at 8 °C, to de-activate unreacted EDC (Scheme 1S, see Supplementary material). The sample was later dialyzed using Spectra/Por dialysis bag against a molecular weight cut off limit of 6–8 kDa for 24 h with 4 changes of buffer (PBS) to remove unreacted 2,4-D, EDC, and NHS. 2,4,6-Tri-nitrobenzenesulfonicacid (TNBS) assay was performed to determine the percentage conjugation and the number of ε-amino groups remaining free in ALP after conjugation with 2,4-D. Percentage conjugation was found to be 64.70% (Sashidhar et al., 1994; Sreenath and Venkatesh, 2007). The resulting 2,4-D-ALP conjugate was further conjugated with CdTe QD as described below.

2.4. Conjugation of CdTe QD with 2,4-D-ALP moiety

MPA coated CdTe QD were initially activated using an activator EDC and NHS (Hermanson et al., 1992; Svitel et al., 2001) and further reacted with 2,4-D-ALP (prepared as above) to establish an amide linkage between –COOH group of MPA coated CdTe QD and free side chain $–NH_2$ group of ALP to form a bioconjugate. After appropriate incubation as mentioned above, the sample was purified by dialysis against a molecular weight cut off limit of 6–8 kDa for 24 h with 4 changes of buffer (PBS) to remove any unreacted excess CdTe QD left in the sample (Scheme 2S, see Supplementary material). The conjugation was confirmed by gel electrophoresis.

2.5. Gel electrophoresis

Both native and SDS-PAGE were run on 10% separating gel and 5% stacking gel (Laemmli, 1970). Luminescent image of native PAGE was taken under UV chamber at 366 nm before staining the gel with silver stain, to confirm the bioconjugation and to monitor the effects of CdTe QD on the mobility of 2,4-D–ALP–CdTe under charge.

2.6. Quantitative detection of 2,4-D by indirect ELISA

Indirect competitive ELISA was performed to determine the detection limit of 2,4-D. ELISA plate (96-well polystyrene plate from Nunc) was coated with 100 μL of 2,4-D-ALP conjugate (1:250 dilutions from above prepared stock) in carbonate buffer (pH 9.6) overnight at 4 °C. The plate was washed thoroughly with 0.05% PBS–Tween (PBST) followed by blocking with 1% BSA in PBS. After washing, 100 μL of anti-2,4-D antibody (IgG, 1 μg per well concentration) along with free 2-4-D at the range of 1000 $ng\,mL^{-1}$ to 50 $pg\,mL^{-1}$ was added in the wells and incubated for 2 h at 37 °C. The plate was washed as described above and further incubated with 100 μL of anti-rabbit IgG antibody (1:10,000 concentration) conjugated to HRP at 37 °C. The plate was washed again followed by addition of 100 μL of HRP substrate solution to each well (0.03% TMB, 0.014% urea H_2O_2 in 0.1 M citrate buffer, pH 4.9). The reaction was stopped by adding 1N HCL. Absorbance was recorded at 450 nm in a tunable microplate reader after 30 min of incubation at room temperature (Kolosova et al., 2004).

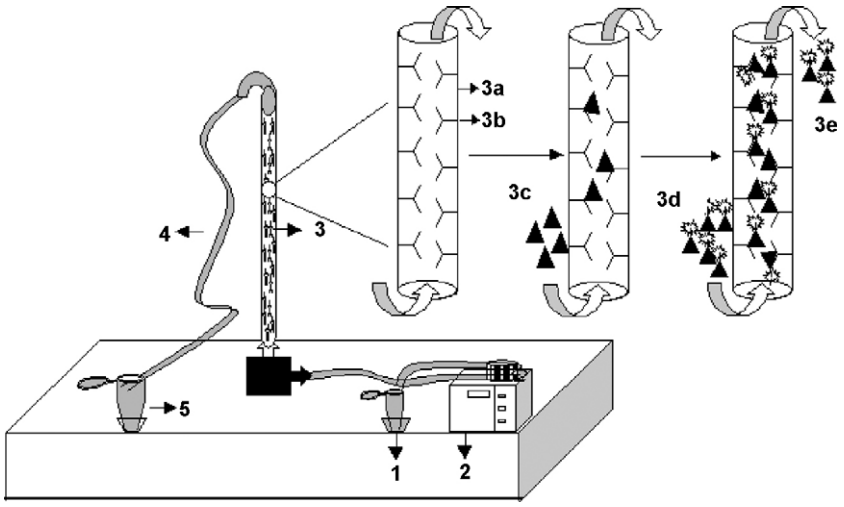

Fig. 1. Immunoreactor column showing competitive binding. (1) Sample Inlet. (2) Peristaltic pump. (3) Immunoreactor column. (3a) Glass capillary column. (3b) Immobilized anti-2,4-D antibodies. (3c) 2,4-D. (3d) 2,4-D–ALP–CdTe conjugate. (3e) Unbound 2,4-D–ALP–CdTe conjugate. (4) Polystyrene tubing. (5) Sample collection point.

2.7. Immobilization of 2,4-D antibodies (IgG) and detection of 2,4-D using immunoreactor column

2.7.1. Activation of Sepharose beads

2 mL of Sepharose-CL 4B was activated by reductive amination (Borch et al., 1971) using 85.6 mg of sodium *meta*-periodate in 2 mL of PBS (50 mM, pH 7.4). 0.2 M of 1,6-diaminohexane was dissolved in 1 mL of PBS and pH was adjusted to 7.4 using concentrated HCl while maintaining the solution at 4 °C and added to periodate activated Sepharose along with 12 mg of sodium cyanoborohydride as a Schiff base reductant followed by incubation for 2 h at room temperature and overnight at 4 °C (O'Carra and Barry, 1972).

2.7.2. Immobilization of anti-2,4-D antibody

The concentration of antibody was fixed to 20 μg per 150 μL of Sepharose, as the maximum loading capacity of immunoreactor column was 150 μL. 266.66 μg (20 μg per 150 μL of Sepharose) of anti-2,4-D antibody (IgG) was added to activated Sepharose along with 2 mM of NHS (Grabarek and Gergely, 1990) and incubated for 2 h at room temperature with intermittent shaking and overnight at 4 °C. The immobilized Sepharose was washed thoroughly with PBS.

2.7.3. Detection of 2,4-D by fluoroimmunoassay

Antibody immobilized Sepharose was packed in a glass capillary column of 150 μL capacity. The packed column was washed and equilibrated with phosphate buffer (50 mM, pH 7.4). Different concentrations of 2,4-D–ALP–CdTe conjugate was passed through the packed column (Fig. 1) and the unbound 2,4-D–ALP–CdTe conjugate was collected in flow through fraction as non-retained effluent. The collected fractions were observed for fluorescence intensity in a fluorimeter and exact concentration required was optimised based on lowest fluorescence intensity shown by the fraction that has shown maximum binding in the column. Different concentrations of 2,4-D in the range of 1000 ng mL^{-1} to 100 pg mL^{-1} was passed through the column followed by 10 μL of the 2,4-D–ALP–CdTe conjugate, which was found optimum, at a flow rate of 50 μL per minute using a peristaltic pump to facilitate optimal binding to the immobilized IgG in the immunoreactor column. The unbound 2,4-D–ALP–CdTe conjugate that was in excess was collected in flow through fraction as non-retained effluent (Fig. 1, 5). Collected fractions were

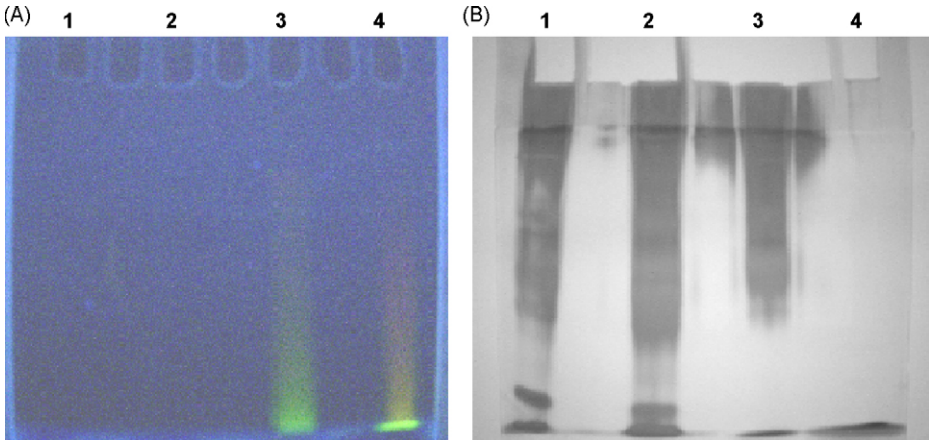

Fig. 2. Native PAGE of CdTe and 2,4-D–ALP–CdTe conjugate. (A) Luminescent image of native PAGE (excited at 366 nm). (B) Silver stained image of native PAGE. Lane (1) Standard ALP, lane (2) 2,4-D-ALP, lane (3) 2,4-D–ALP–CdTe and lane (4) standard CdTe.

analyzed in a spectrofluorimeter for fluorescence, exciting the reaction sample at 350 nm and the emission spectra was monitored and recorded.

3. Results and discussion

3.1. Conjugation of CdTe QD with 2,4-D-ALP

The carboxylic group of 2,4-D got activated by EDC reaction and conjugated with NH_2 group (ε-amino groups) of ALP (Scheme 1S, see Supplementary material). As the percentage conjugation was determined to be 64.70% by TNBS assay, the ε-amino groups remaining free in ALP after conjugation with 2,4-D were targeted for conjugation with CdTe QD. Further 2,4-D–ALP–CdTe conjugate (Scheme 2S, see Supplementary material) was obtained by reacting 2,4-D-ALP with MPA coated CdTe QD. This bioconjugate was confirmed by gel electrophoresis.

3.2. Gel electrophoresis

Both native (Fig. 2A and B) and SDS-PAGE (Fig. 3) confirmed 2,4-D-ALP and 2,4-D–ALP–CdTe conjugate. Both 2,4-D–ALP–CdTe conjugate (lane 3 of Fig. 2A) and standard CdTe QD (lane 4 of Fig. 2A) have displayed strong luminescence under luminescent image of native PAGE. The luminescence of 2,4-D–ALP–CdTe conjugate (lane 3 of Fig. 2A) was possible only if the CdTe QD was covalently conjugated to 2,4-D-ALP. As the conjugated sample was properly dialyzed to remove any excess free CdTe QD, the luminescence of 2,4-D–ALP–CdTe conjugate under UV chamber at 366 nm was only because of the covalent conjugation of CdTe QD. Moreover, the absence of detectable luminescence signals in lanes 1 and 2 of Fig. 2A for standard ALP and 2,4-D-ALP, respectively, justifies this fact. When the native gel was silver stained to determine the mobility of standard ALP and 2,4-D-ALP along with 2,4-D–ALP–CdTe and standard CdTe QD, It was observed that both 2,4-D–ALP–CdTe (lane 3 of Fig. 2B) and standard CdTe (lane 4 of Fig. 2B) bands have become more mobile than standard ALP (lane 1 of Fig. 2B) and 2,4-D-ALP (lane 2 of Fig. 2B) bands. It was obvious that standard CdTe QD having low molecular weight will migrate along with the electrophoretic front. But the higher mobility of 2,4-D–ALP–CdTe conjugate (lane 3 of Fig. 2B) compared to standard ALP (lane 1 of Fig. 2B) and 2,4-D-ALP (lane 2 of Fig. 2B) was because of the conjugation of CdTe QD. The high negative charge and compactness of CdTe QD has apparently overcome the increased molecular weight of 2,4-D–ALP–CdTe conjugate (lane 3 of Fig. 2B) and made the conjugate to become more mobile under electric charge compared to that of standard ALP (lane 1 of Fig. 2B) and 2,4-D-ALP (lane 2 of Fig. 2B). This makes it evident that the CdTe QD has got conjugated to 2,4-D-ALP. Wang et al. (2002) have made similar observation regarding the conjugation of CdTe QD with protein BSA (bovine serum albumin).

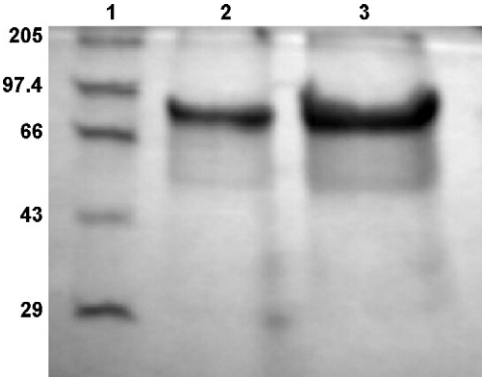

Fig. 3. SDS-PAGE of standard ALP and 2,4-D–ALP–CdTe conjugate stained by Coomassie Brilliant Blue R-250 stain. Lane (1) Standard molecular markers, lane (2) standard ALP and lane (3) 2,4-D–ALP–CdTe.

Contrary to native PAGE, in SDS-PAGE (Fig. 3) the mobility of the standard ALP (lane 2 of Fig. 3) and its conjugate 2,4-D–ALP–CdTe (lane 3 of Fig. 3) were due to the mass/charge ratio of the denatured ALP carrying sodium dodecyl sulfate (SDS). SDS imparts negative charge to the denatured ALP and thus the difference in mobility will be based on the mass difference between ALP and 2,4-D–ALP–CdTe conjugate, as charge remains equal between ALP and 2,4-D–ALP–CdTe conjugate. But in SDS-PAGE (Fig. 3), it was observed that the mobility of 2,4-D–ALP–CdTe conjugate (lane 3 of Fig. 3) was almost coinciding with standard ALP (lane 2 of Fig. 3). Wang et al. (2002) has made similar observation with conjugated CdTe with BSA in SDS-PAGE. This was due to the negatively charged CdTe QD that has affected the overall charge density of the conjugate. This property of nanoparticles after conjugation has been reported by Schroedter et al. (2002). The increase of the overall charge density of the conjugate has compensated the increase of mass due to conjugation (Mamedova et al., 2001; Wang et al., 2002). Therefore, band positions of nanoparticle labeled and unlabelled proteins virtually coincide. ALP (calf intestinal) was a homodimer of 69 kDa (Fosset et al., 1974; Yan et al., 2003), and thus it has shown single band instead of giving different bands for each subunits in SDS-PAGE.

3.3. Indirect competitive ELISA

Indirect competitive ELISA was performed to detect the sensitivity and the binding efficiency of anti-2,4-D antibodies with 2,4-D/2,4-D-ALP. With optimized conjugate and antibody concentration, the detection sensitivity for 2,4-D was able to reach up to 1000 pg mL^{-1}. Below this range, the graph (Fig. 1S, see Supplementary material) was almost flat and there was not much difference in the absorption. Hence the detection limit was found to be 1000 pg mL^{-1} by indirect competitive ELISA.

3.4. Studies on absorption and emission spectra of the conjugate

The absorption spectrum of quantum dot offers a simple way to investigate their band structure and band gap energy. This in turn provides a way to determine the resonance energy transfer coupled with biomolecular conjugation. It was reported that quantum dots were able to participate in resonance dipole–dipole interactions when they got conjugated with biomolecules forming donor–acceptor pair (Mamedova et al., 2001; Willard et al., 2001; Ma et al., 2005). The absorption spectra of the bare CdTe QD and the 2,4-D–ALP–CdTe conjugate were recorded (Fig. 4). It was observed that the absorption spectra of 2,4-D–ALP–CdTe conjugate differs from that of standard ALP and CdTe QD alone. The absorption peak observed at 320 nm for 2,4-D–ALP–CdTe conjugate was due to conjugation, which was absent in standard ALP and standard CdTe QD (Fig. 4). The conjugation has changed the overall structure and increased the mass of 2,4-D–ALP–CdTe conjugate. This has resulted in a strong overlap of emission and absorption spectra of ALP and CdTe QD, respectively. The spatial closeness of ALP and CdTe QD in the tightly bound covalent attachment has allowed sufficient resonance energy transfer between ALP and CdTe QD resembling FRET. This was possible as ALP and CdTe QD behaved as donor–acceptor pair after conjugation allowing CdTe QD to absorb energy emitted by ALP at 310–320 nm range (Mamedova et al., 2001). This increase in the absorbance of 2,4-D–ALP–CdTe conjugate has further confirmed the conjugation of ALP with CdTe QD.

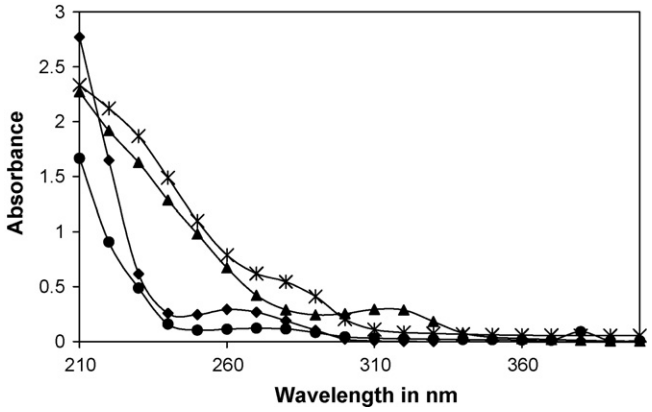

Fig. 4. UV–vis absorption spectra of ALP (●), 2,4-D-ALP (♦), 2,4-D–ALP–CdTe (▲) and CdTe (*).

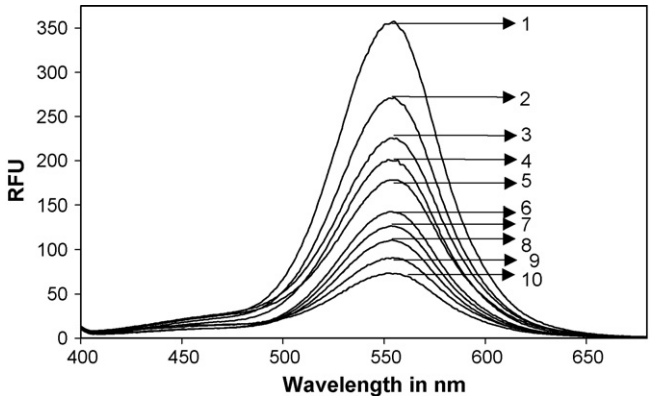

Fig. 5. Relative fluorescence intensity of 2,4-D–ALP–CdTe conjugate with respect to different concentration of 2,4-D passed through the column. (1) CdTe control, (2) 2,4-D–ALP–CdTe control, (3) 1000 ppb, (4) 500 ppb, (5) 250 ppb, (6) 1 ppb, (7) 0.75 ppb, (8) 0.5 ppb, (9) 0.25 ppb and (10) 0 ppb.

The comparative emission spectra of the CdTe QD and the 2,4-D–ALP–CdTe bioconjugate were recorded from 400 to 700 nm at a fixed excitation wavelength of 350 nm (Fig. 2S, see Supplementary material). A blue shift of 4 nm was recorded for the 2,4-D–ALP–CdTe bioconjugate (549 nm) when compared to that of CdTe QD alone (553 nm). Similar observations were made by Wan et al. (2004), who suggested that the blue shift was due to the binding of the protein to CdTe QD, which was resulted in the alteration of polymer coating around the QD surface, leading to a reduction in the surface charges of the CdTe QD and the directional polarizabilty of molecules surrounding the QD. The stoke shift of CdTe QD was reduced resulting in blue shift of emission peak (Fig. 2S, see Supplementary material).

3.5. Immobilization of anti-2,4-D antibody

Fluoroimmunoassay was performed in an immunoreactor column which was packed by immobilized anti-2,4-D antibodies. Sepharose-CL 4B was used as an inert matrix for the immobilization of anti-2,4-D antibodies. The functional group of Sepharose-CL 4B was modified with 1,6-diaminohexane to assign primary amino group as a functional group, which was confirmed by TNBS test. The purpose of modification of the functional group with a spacer was to facilitate the immobilization of anti-2,4-D antibodies through their Fc portion. This was found advantageous to retain the antigen binding Fab site of antibody (IgG) molecule free for reactivity, facing away from the matrix (Ey et al., 1978; Schneider et al., 1982; Lindmark et al., 1983). The retention of effective antigen binding activity of an antibody was of great concerned area, as antibodies can lose their effective antigen binding activity, if their Fab portions are blocked during immobilization process, which was positioned close to the N-terminals of antibody molecule (Ey et al., 1978; Schneider et al., 1982; Lindmark et al., 1983).

3.6. Detection of 2,4-D using immunoreactor column with 2,4-D–ALP–CdTe conjugate

The immunoassay development was based on the optimization of several parameters to achieve high sensitivity of detection using quantum dot based analytical system. It has been reported that the specificity of immunoassay generally depends on the immunogenic nature of the hapten and the competitiveness of the hapten determines the sensitivity of the immunoassay (Kolosova et al., 2004). Therefore the hapten was designed without altering the immunogenic nature of 2,4-D conjugating ALP to the carboxyl group of 2,4-D. Quantification of 2,4-D by fluoroimmunoassay was based on the competitive binding among 2,4-D and known concentration of 2,4-D–ALP–CdTe conjugate. As the concentration of immobilized antibody was fixed (20 μg per 150 μL of Sepharose), the optimized 2,4-D–ALP–CdTe conjugate passed (10 μL) has shown maximum binding in the absence of any competitors (in this case 2,4-D). The 2,4-D–ALP–CdTe conjugate was in little excess concentration for the immobilized antibody and eluted out of the column as a non-retained effluent (peak 9 of Fig. 5) with 73.449 units of relative fluorescence intensity. 2,4-D–ALP–CdTe conjugate was passed in little excess concentration to avoid any non-specific signals due to noise. This was considered as control for zero concentration of 2,4-D passed (peak 9 of Fig. 5) or in the absence of 2,4-D where there was no competitors for 2,4-D–ALP–CdTe conjugate to form immunocomplex with immobilized anti-2,4-D antibody. During competitive binding in presence of 2,4-D, the binding of 2,4-D–ALP–CdTe conjugate depended on the antibodies freely available after the binding of 2,4-D. This has caused the unbound 2,4-D–ALP–CdTe conjugate to elute out of the column as a non-retained effluent (Fig. 1), which was directly proportional to the bound 2,4-D in the column. Therefore, quantification of 2,4-D was made possible by detecting the relative fluorescent intensity of unbound 2,4-D–ALP–CdTe conjugate, which was eluted out. It was observed that the fluorescence intensity of 2,4-D–ALP–CdTe (peak 9 of Fig. 5) conjugate was reduced considerably as most of the 2,4-D–ALP–CdTe conjugate was bound to immobilized anti-2,4-D antibody compared to standard CdTe of same concentration (peak 1 of Fig. 5). The fluorescence intensity of 2,4-D–ALP–CdTe conjugate was found to increase in accordance with free 2,4-D passed in the range of 250 pg mL^{-1} to 1000 ng mL^{-1}, respectively, as shown in Fig. 5. It was also observed that, below 250 pg mL^{-1} of 2,4-D, the fluorescence intensity of 2,4-D–ALP–CdTe conjugate passed was following very close to the base line of the conjugate fluorescence, and therefore the lower limit of detection can be considered to be 250 pg mL^{-1}.

Hence it was possible to detect 2,4-D upto 250 pg mL^{-1} through fluoroimmunoassay using immunoreactor column. The sensitivity of fluoroimmunoassay was found higher as compared with indirect competitive ELISA. A standard graph was plotted for 2,4-D in the concentration range 250–1000 pg mL^{-1} against relative fluorescence intensity (RFU) with a convincing regression value of $R^2 = 0.9983$ (Fig. 6).

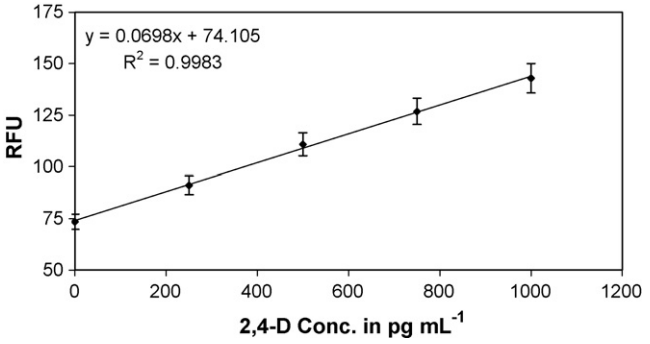

Fig. 6. Standard graph obtained by fluoroimmunoassay for the detection of 2,4-D.

4. Conclusions

The present study was conducted for the detection of 2,4-D using CdTe QD. Fluoroimmunoassay based biosensors using quantum dots can provide better sensitivity than any other conventional methods. The absorption spectra of 2,4-D–ALP–CdTe conjugate has shown the possible resonance energy transfer between quantum dot and a biomolecule. This phenomenon can be utilized for more efficient biosensor development based on the interactions between biomolecules and quantum dot after conjugation. Quantum dots can be used for future biosensor developments for rapid and sensitive detection of food and environmental monitoring.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.08.042.

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