Original Research Article

Cadmium selenium quantum dot based nanosensor with femtometer level sensitivity for the detection of the pesticide endosulfan

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ABSTRACT

Endosulfan (6,7,8,9,10-Hexachloro-1,5,5a,6,9-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) is an off-patent insecticide used in agricultural farms. Its usage as a pesticide has become highly controversial during the last decades. This is due to its reported hazardous nature to the health and side effects like growth retardation, hydrocephalus, and undesired changes in the male and female hormones leading to complications in sexual maturity. Endosulfan is the main culprit among all pesticide poisoning incidents around the world. Though the usage of this dreaded pesticide is banned by most countries, the high stability of this molecule to withstand degradation for a long period poses a threat to mankind even today. So, it has become highly essential to detect the presence of this poisonous pesticide in the drinking water and milk around these places. It is also advisable to check the presence of this toxic material in the blood of the population living in and around these places so that an early and appropriate management strategy can be adopted. With this aim, we have developed a sensor for endosulfan that displayed high selectivity and sensitivity among all other common analytes in water and biological samples, with a wide linear concentration range (2 fM to 2 mM), a low detection limit (2 fM), and rapid response. A citrate functionalized cadmium selenium quantum dot was used for this purpose, which showed a concentration-dependent fluorescence enhancement, enabling easy and sensitive sensing. This sensor was utilized to detect endosulfan in different sources of water, human blood serum and milk samples with good recoveries. It is also noted that the quantum dot forms a stable complex with endosulfan and is easy to separate it from the contaminated source, paving a solution for purifying the contaminated water. More detailed tests and validation of the sensor is needed to confirm these observations.

Keywords: endosulfan; quantum dot; sensor; femtomolar sensitivity; water pollution; pesticide

1. Introduction

Pesticides are chemical substances used to destroy pests that attack crops in the agricultural field. Endosulfan is an off-patent organochlorine insecticide. It belongs to a class of persistent organic pesticides (POP), which are a set of toxic chemicals that persist in the environment for years before breaking down into harmless molecules[1]. These chemicals circulate globally; those released in one part of the world can get deposited far away from their original source through a process of deposition and evaporation. Since it is sprayed onto the crops, the whole food chain gets affected by the repeated circulation of the pesticide.

Endosulfan exposure is linked to a different medical conditions, such as physical deformities, neurotoxicity, poisoning, etc., A large
number of neighboring populations have been victimized by the use of this pesticide with a broad range of diseases from growth retardation to hydrocephalus which has created horror in such areas\textsuperscript{[2]}. It is reported that it can imitate or enhance the effect of female hormones\textsuperscript{[3]}. Delayed sexual maturity in boys was reported in many places as an after-effect of the influence of this pesticide\textsuperscript{[4]}. POPs are lipophilic, and hence they accumulate in fatty tissues. Concentrations of these chemicals in fatty tissues can be magnified to about 70,000 times higher than the background levels. It is a widely banned pesticide due to its hazardous effects on human endocrine and genetic systems. These pesticides get accumulated in the environment and thereby affects food chains. Moreover, its presence in water can bioconcentrate in aquatic organisms.

It is known that endosulfan tends to persist in soil without degradation for a longer duration, and transport to long distances, accumulates in living organisms creating harmful effects to them. Hence the detection of the remaining residue and its removal plays an important role in the near future. Even after the banning, the presence of residual pesticides in the soil and water sources in very low concentration cannot be ruled out and hence demands the need of a very sensitive sensor. The commonly employed techniques for the detection of endosulfan include liquid chromatography mass spectrometry (LC-MS)\textsuperscript{[5]}, gas chromatography mass spectrometry (GC-MS)\textsuperscript{[6]}, high performance liquid chromatography (HPLC)\textsuperscript{[7]}, gold immunochromatographic strip and indirect competitive ELISA\textsuperscript{[8]}, multiwalled carbon nanotube based sensor\textsuperscript{[9]} etc. Currently available detection techniques are limited by several factors including low sensitivity. It is reported that 0.21 to 54 ng/L of endosulfan is present in the drinking water near the endosulfan sprayed farms\textsuperscript{[10]}.

Nanoparticles being extremely smaller in size have high reactivity and size tuneable properties which makes them suitable for many applications\textsuperscript{[11–25]}. Quantum dots (Qd) have got wide attention in this field due to its favourable optical property and low cost\textsuperscript{[26–28]}. Cadmium sulphide (cds) quantum dots were previously shown to be a sensor for endosulfan using fluorescence immunoassay technique\textsuperscript{[29]}. A spectrophotometric mode of detection of endosulfan using metal nanoparticles has been previously reported by Sreekumaran Nair et al.\textsuperscript{[30]}. Electrochemical, enzymatic, and non-enzymatic-based endosulfan detection was demonstrated using different nanoparticles\textsuperscript{[31–33]}.

With this background, we aim to develop a highly selective and sensitive nanosensor using citrate stabilized cadmium selenium quantum dots for the detection of endosulfan, augmented by the new science of nanotechnology. The sensor, with femtomolar level sensitivity, has the potential to detect endosulfan in different environments.

2. Materials and method

All the reagents were used as supplied without further purification unless stated. Cadmium acetate, trisodium citrate, selenium, sodium sulphite of analytical grade was purchased from Merck India.

2.1. Instrumentation

UV–Visible (UV-Vis) absorbance spectra were recorded using a double beam spectrophotometer (Shimadzu, Japan) from 300 nm to 800 nm. Photoluminescence study was performed using a Cary Bio 500 instrument using 1 cm path length cuvette. A Nicolet 5700, Madison, USA recorded the Fourier transform infrared spectroscopy—attenuated total reflectance (FTIR-ATR mode). JEOL 3010; 300 KV was used to obtain high resolution transmission electron microscope (HRTEM) micrographs of the material. For HRTEM analysis samples were drop casted into a formvar coated copper grid. Samples were dried before the analysis. Dynamic light scattering (DLS) was performed with Nano ZS (Malvern, UK) for zeta potential measurement by dispersing the sample in water.
2.2. Synthesis of CdSe quantum dots

Cadmium selenium quantum dots were prepared using trisodium citrate as the stabilizing agent. Synthesis is done in a two-step process. Initially, sodium selino sulphite was synthesized. Briefly, 40 mM sodium sulphite solution was mixed with selenium metal powder and stirred at 75 °C till the solution became colourless. For the synthesis of quantum dots, 30 mM cadmium acetate was dissolved in 5 mL deionized (DI) water. 60 mM trisodium citrate was added to the cadmium solution in 15 mL dimethylformamide (DMF). When the solution became turbid, 55 mL water was added and stirred. To the above system $8 \times 10^{-4}$ moles of freshly prepared selenium solution was added and stirred till the solution became colourless to reddish colour.

The prepared quantum dots were washed with isopropanol and centrifuged twice to purify the same.

2.3. Sensing of endosulfan in DI water, pipe water, well water and pond water

About 1mg of the developed quantum dots were dispersed in 1 mL of DI water to be used as the nanosensor. Different concentrations of endosulfan containing water were added to the quantum dot solution.

2.4. Sensing of endosulfan in serum and milk

Human blood serum collected from the blood bank (Ethics committee approval number SCT/IEC/594/APRIL-2014) was separated and incubated with different concentrations of endosulfan. Concentration dependent fluorescence was recorded upon addition of endosulfan incubated serum with Qd. Similarly, endosulfan in milk was also detected.

3. Results and discussion

3.1. Preparation of citrate capped cadmium selenium quantum dot

Quantum dots have got wide attention in the field of imaging, diagnosis, sensing etc. due to its size tuneable optical property, low cost, high stability and ease of synthesize. Cadmium selenium quantum dots were synthesized by pure wet chemical method. Stability of the QDs can be enhanced via surface functionalization or interaction with other molecules. Substances such as trioctyl phosphine (TOP)) and tri-n-octylphosphine oxide (TOPO) are commonly used capping agents, which forms water insoluble QDs due to the hydrophobic nature of these organic solvents. Here trisodium citrate is used as the capping agent.

The developed quantum dot has a characteristic broad absorption peak (Figure 1a) at 552 nm. The bulk CdSe particles have absorbance maximum at 698 nm which is absent in the prepared quantum dots and the blue shift in the absorbance peak indicate quantum confinement effect confirming the formation of CdSe quantum dots\[16,34,35\]. The band gap of the CdSe quantum dot capped with trisodium citrate was estimated as 2.07 eV, which determines the unique properties of the developed nanomaterial. It has a maximum excitation at 448 nm with a shoulder band at 482 nm (Figure A1).

Excitation at 448 nm resulted in the maximum fluorescence emission at 570 nm (Figure 1b). Quantum confinement and interaction of ligand with CdSe contributes to the green emission of the quantum dots. Qds showed a greenish yellow emission under UV irradiation (Figure 1b inset). Transmission electron micrographs of CdSe quantum dots showed dispersed spherical particles (Figure 2a). The particle size distribution (Figure 2b) of quantum dot showed an average size of 3.5 nm from the TEM micrographs (Figure 2b).
Figure 1. (a) UV-Vis absorption and (b) emission spectra of developed CdSe quantum dots. Inset of (b) shows the photograph of quantum dots under UV light irradiation.

Figure 2. (a) Transmission electron micrographs of CdSe quantum; (b) corresponding particle size distribution of Qd.

3.2. Endosulfan detection

1 mg of citrate capped quantum dots dissolved in 1 mL of DI water formed the nanosensor. Different concentrations of endosulfan (2 fM to 10 mM) were added to this colloidal nanosensor, i.e., citrate stabilized quantum dots. The tested water was confirmed for the absence of endosulfan spectrometrically before the sensing efficacy was attempted.

To check the concentrations of endosulfan in real water sources (well water, pond water, pipe water), the fluorescence spectrum was recorded after addition of different concentrations of analyte. Sensing experiments were repeated thrice in different media. As a sensing indicator, a concentration dependent fluorescence enhancement of the 570 nm emission peak was observed on addition of endosulfan to Qd sensor (Figure 3a,c and Figure A2). In all the cases, fluorescence intensity increased with the concentration of endosulfan (Figure 3a,c and Figure A2). The linear dependency of endosulfan detection was checked in the range from millimolar to femtomolar levels. DI water showed regression co-efficient value of 0.98 and well water showed 0.99 (Figure 3b,d) indicating the high efficacy of developed sensor in the detection of endosulfan from these sources. In the case of pipe water and pond water, the regression co-efficient was slightly less which may be due to the presence of other molecules of water purifying agents and detergents respectively, which we haven’t included in the selectivity tests (Figure A3). The increase in fluorescence intensity is attributed to the binding of endosulfan to the quantum dot and subsequent enhancement in the light absorption by the quantum dot-endosulfan complex as reflected in the absorption spectra of the complex with different concentrations of endosulfan (Figure A4). Scheme 1 represents the binding of endosulfan to CdSe quantum dots.
Figure 3. Endosulfan detection using the quantum dots in (a) DI water, (b) corresponding linear relation from millimolar to femtomolar range, (c) well water and (d) corresponding linear relation from millimolar to femtomolar range.

To understand more about the interaction of endosulfan with Qd and to explain the observed mechanism detailed analysis of the complex was carried out using XRD, FT-IR and zeta potential studies. A concentration dependent increase in the surface charge was observed in the zeta potential (Figure 4a) of the sensor with different concentrations of endosulfan. This can be attributed to the presence of endosulfan, one of the most stable pesticides over Qd. Due to the presence of endosulfan over the quantum dot surface, the stability of the system increases as is clear from the significant increase in the zeta potential with the increase in concentration.

Figure 4. (a) Zeta potential evaluation of Qd and Qd with different concentrations of endosulfan (n = 3). (b) XRD pattern of Qd and endosulfan-treated Qd along with JCPDS data.

FT-IR spectroscopy and XRD evaluation further confirmed the binding of ES with Qd. In FT-IR spectrum (Figure A5) of endosulfan treated quantum dot showed all the characteristics peaks of both Qd and endosulfan with a slight shift, especially in the peaks of endosulfan. The characteristic -OH vibration was observed at 3353 cm$^{-1}$ in CdSe Qd. The same peak was observed after ES complexation indicating the presence of hydroxyl group in the combined system. COO- peak at 1598 cm$^{-1}$ was observed for both Qd sensor as well as for the combined system. The characteristic peaks of endosulfan seen at 1604 cm$^{-1}$, 1270 cm$^{-1}$, 1070 cm$^{-1}$, C = C, S = O, C-Cl got shifted to 1597 cm$^{-1}$, 1256 cm$^{-1}$, 1067 cm$^{-1}$ after binding to the Qd. The shift in the vibrational modes to the lower wavenumber region indicates the formation of strong hydrogen bonds during the complex formation. In the case of S = O, a shift in wavelength of more than 10 cm$^{-1}$ was observed, which indicates that
an electronic interaction followed by bond change occurred in ES on adding to Qd. From this observation it can be confirmed that ES binds to Qd through S = O bond. The peaks corresponding to citrate were also present in the combined system which indicate that the ES binding occurs through only one plane without disturbing the other planes of Qds.

![Scheme 1. Schematic representation of detection of endosulfan using CdSe Qd.](image)

Again, XRD analysis confirmed the crystal structure of Qd and Qd-ES system. XRD (Figure 4b) of Qd shows cubic crystal structure with (111), (220), (311) and (222) planes at 2 theta value of 25.6°, 43.007°, 49° and 52.66°. Upon addition of ES, the crystal structure of Qd changed from cubic to hexagonal. It is due to the binding of ES molecule through –S = O bonding (as reflected by the change in wavelength in FTIR spectrum Figure A5) to metal surface of Qd and due the involvement of hydrogen bond during the complexation. Due to the bulky nature of ES molecule, the bonding takes place diagonally which leads to change in crystal structure from cubic to hexagonal. After ES binding, crystal planes of the system are (100), (002), (101), (102), (110), (103) and (201) at 2 theta values of 23.9°, 25.2°, 26.87°, 34.4°, 41.1°, 46.22° and 52.0° corresponding to more stable hexagonal structure of CdSe quantum dots (Figure 4b) supporting the finding of zeta potential study. All other un-assigned peaks in XRD correspond to planes of stabilizing agent of Qd and ES (Figure 4b). All the above observations are in favor of the attachment of endosulfan onto the surface of Qd enabling electronic transition and enhanced fluorescence with increase in concentrations of ES.

The presence of endosulfan could be detected from the fluorescence enhancement with a lowest limit of detection 2 fM and the highest detection limit as 2 mM. Increasing the concentration of endosulfan beyond 2 mM showed saturation in the fluorescence signal, without appreciable increase.

Selectivity (Figure 5) of the quantum dot sensor towards other common analytes present in water was tested by adding 2 mM of the analytes to the sensor solution. There was no change in the fluorescence intensity with any of the commonly occurring analytes like cadmium (Cd), lead (Pb), copper (Cu), cobalt (Co), zinc (Zn), mercury (Hg), manganese (Mn) and magnesium (Mg). However, on addition of endosulfan to these analytes containing solution, an increase in the fluorescence was observed indicating the high selective detection of the pesticide in the presence of other analytes.
3.3. Detection of endosulfan in blood and milk

Detection of the presence of endosulfan in blood is equally or more important than the detection in the environment because overexposure of the chemical can lead to its entry into the blood stream through pesticide-contaminated food or water. So, we checked the efficacy of the Qd-based sensor in detecting endosulfan in blood (Figure 6c,d). It also showed a concentration-dependent fluorescence enhancement, similar to that of water.

Another route of entry of endosulfan is through cattle. If the cattle happen to feed on the endosulfan-treated fields, there is a chance of contamination of the cattle milk, which in turn can contaminate humans. So, the developed sensor was also checked for the detection of endosulfan from contaminated milk (Figure 6a,b). In the case of milk, a slight broadening of the emission peak was observed on the addition of Qd based nanosensor. This can be due to the interaction of milk protein with Qd. But here also the detection mechanism works perfectly showing a concentration-dependent fluorescence enhancement on the addition of endosulfan-containing milk while retaining the broad emission pattern.
3.4. Comparison of the sensor in different environment

Efficacy of the developed sensor in the different environment was checked in comparison with the fluorescence intensity of quantum dot. The comparison is shown in Figure A6a,b. Presence of endosulfan in well water, pond water and DI water shows almost uniform fluorescence response upon addition of quantum dot sensor. However, in the case of pipe water, the fluorescence enhancement is slightly less, especially at higher concentration. This may be either due to the slight aggregation of quantum dots or the hindrance of some other molecules present in pipe water. But below 20 nM, the response of endosulfan on quantum dot is almost uniform.

3.5. Removal of endosulfan from water

As the attachment of endosulfan to the Quantum dots during its detection is strong and stable as indicated by the enhanced fluorescence and changes in zeta potential and XRD analysis, endosulfan along with Qd can be removed from the contaminated system without its dissociation. Easy methods like centrifugation would suffice for this purpose. This was tested in water treated with different concentrations of endosulfan by centrifugation at 15,000 rpm for 15 min. Subsequently the colourless supernatant and the pellet were independently treated with freshly prepared quantum dot to test the presence of endosulfan. The supernatant of water treated with endosulfan with concentrations up to 2 mM didn’t show any considerable change in the fluorescence of the quantum dot indicating that the developed system can remove the pesticide up to this concentration. When higher concentration of the order of 10 mM endosulfan was added, the supernatant showed a fluorescence enhancement corresponding to 20 fM (Figure A7).

4. Conclusion

In the present study, we have developed a highly selective sensor for the detection of endosulfan based on the changes in the emission of the sensor in the visible range. The sensing mechanism has been successfully demonstrated in different endosulfan contaminated environments like pond water, well water, pipe water, blood and milk. The sensor works successfully to detect the presence of this harmful pesticide at a limit as low as 2 fm. The higher limit was 2 mM. The presence of other heavy metal ions present in water has not affected the sensing, proving its high selectivity. It is also shown that the pesticide can be removed from the contaminated system at concentrations of the order of 2 mM. Validation of the current system with other existing methods is in progress.

Author contributions

Conceptualization, LVN and RSJ; methodology, LVN and RVN; validation, LVN, RVN and RSJ; formal analysis, LVN, RVN and RSJ; investigation, LVN, RVN and RSJ; resources, RSJ; writing—original draft preparation, LVN and RVN; writing—review and editing, RSJ; supervision, RSJ; project administration, RSJ; funding acquisition, RSJ.

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Conflict of interest

The authors declare that they have no conflict of interest.
References


Appendix

Supplementary materials that are excluded in the main body of the paper are included in the appendix. These includes additional supporting data, like excitation spectrum of CdSe quantum dots which shows a maximum excitation at 448 nm with a shoulder band at 482 nm (A1), the fluorescence variation of the CdSe quantum dot sensor in different water sources (A2), the linear dependency of endosulfan detection in pond water and pipe water represented as regression co-efficient value (A3) UV-Visible absorbance spectra of Qd and Qd with different concentrations of endosulfan (A4), FT IR spectra of the sensor, endosulfan and endosulfan treated sensor (A5), Comparison of CdSe quantum dot in different environments containing different concentrations of endosulfan (A6) and the removal of endosulfan as tested on the supernatant of DI water treated with 10 mM of endosulfan (A7) showing only insignificant enhancement in the fluorescence even at higher concentration.

Figure A1. Excitation spectra of CdSe quantum dot.

Figure A2. Endosulfan detection using the quantum dots in different water sources. Changes in the fluorescence of (a) DI water (b) well water, (c) pond water and (d) pipe water.
Figure A3. Endosulfan detection using the quantum dots in (a) Pond water, (b) corresponding linear relation from millimolar to femtomolar range, (c) pipe water and (d) corresponding linear relation from millimolar to femtomolar range.

Figure A4. UV-visible absorbance spectra of Qd and Qd with different concentrations of endosulfan.
Figure A5. FT IR spectra of Qd, endosulfan and endosulfan treated Qd.

Figure A6. (a) Comparison of CdSe quantum dot in different environments containing different concentrations of endosulfan ranging from 2 fM to 20 µM, (b) shows the lower concentration (1 fM–20 pM) portions of (a).

Figure A7. Fluorescence spectra of supernatant of Qd along with 10 mM endosulfan after adding freshly prepared Qd.