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ORIGINAL PAPER

Quantum Dots (QDs) Based Fluorescent Sensor for the Selective Determination of Nimesulide

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Abstract Fluorescent PET (Photoinduced Electron Transfer) has been of particular growth in recent times. A novel PET based fluorescent sensor using unmodified CdSe quantum dots (QDs) has been developed for the trace determination of Nimesulide (NIM). The sensor is based on the selective fluorescence quenching of quantum dots by NIM in presence of other NSAIDs and is found that intensity of quenching is linearly related to NIM concentration in the range 8.2×10^{-7} – 4.01×10^{-5}M. The mechanism of interaction is discussed. Finally, the potential application of the proposed method for the trace determination of NIM in pharmaceutical formulation is demonstrated.

Keywords Photoinduced Electron Transfer · CdSe quantum dots (QDs) · Fluorescent sensor · Nimesulide

Introduction

The unique properties of colloidal luminescent semiconductor nanoparticles or quantum dots (QDs), attributed to quantum confinement effects have elicited intensive research for sensing, labeling and imaging applications. QDs have high quantum yields, broad absorption spectra, narrow size tunable emissions and are resistant to photo bleaching as well as to chemical degradation [1]. The QD surfaces are usually capped by long chain organic moieties such as trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO) [2, 3], as part of their stable synthesis.

QDs with variable surface capping ligands have been extensively used as fluorescent species for cell labeling, tumor imaging and clinical diagnosis [2, 4]. They have been applied for quantitative determination of biological macromolecules [5, 6] and drugs [7, 8] based on their fluorescence quenching, which may be due to the changes of the surface states of QDs.

Non-steroidal anti inflammatory drugs (NSAIDs) are of great interest in medicine as they are widely used for mild to moderate pain relief as well as in the treatment of osteoarthritis and rheumatoid arthritis. Their action is attributed to the inhibition of cyclooxygenase enzyme, which in turn prevents the biosynthesis of certain prostaglandins [9].

As a continuation to our work on development of voltammetric sensors for pharmaceuticals [10–12], herein we present a simple and selective method for determination of Nimesulide (NIM) (Fig. 1) using unmodified CdSe fluorescent probe. NIM, (4-Nitro-2-phenoxy-methanesulfonanilide) is a non-steroidal antiinflammatory drug (NSAID). It has potent analgesic, anti-inflammatory and antipyretic activities on oral and rectal administration. Even though this drug has been projected as a useful alternative to other NSAIDs, serious hepatic [13, 14], renal [15, 16] and other adverse effects [17–19] followed by the administration of NIM has been reported. Several analytical methods such as HPLC [20–22], spectrophotometry [23, 24], electrochemical methods [25, 26], ion association methods [27], etc. have been reported for the quantitative determination of NIM. However, the above methods are expensive and time-consuming. A novel method for the selective determination of NIM, among other NSAIDs such as mefenamic acid (MEF), rofecoxib (ROF), ibuprofen (IBU) and diclofenac sodium (DIC) was developed based on the fluorescence quenching of CdSe QDs in organic media. The quenching mechanism was explained based on Photo Induced Electron Transfer [28] (PET). In PET the possibility of electron transfer can be predicted from the reduction potential of CdSe QDs and drugs that were obtained using cyclic voltammetric studies.

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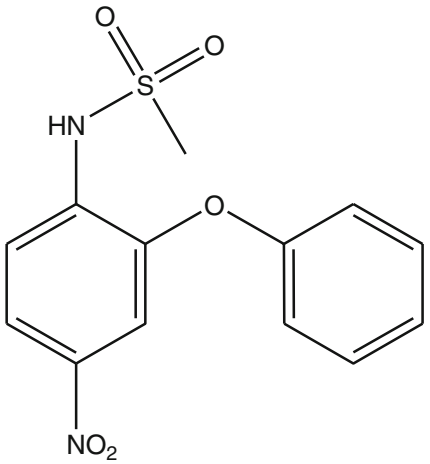


Fig. 1 Structure of NIM

Experimental

Materials and Methods

Nimesulide, mefenamic acid, ibuprofen, rofecoxib, diclofenac sodium, sulfamethoxazole and tinidazole were obtained as gift samples. All solvents and reagents were of analytical grade and used without further purification. The water used in all experiments had a resistivity higher than 18 MΩcm^{-1}.

A standard stock solution (1×10^{-4}M) was prepared by dissolving NIM in acetone. Lower concentration of NIM solution was prepared by serial dilution of the stock solution (1×10^{-4}M).

Absorption spectra were recorded with a Spectro UV-Visible double beam UVD-3500 instrument, using quartz cuvettes of 1.0 cm optical path length. Fluorescent measurements were performed on a JAZ-EL-200-X spectrometer. Electrochemical measurements were performed with an Electrochemical analyzer (BAS Epsilon Bioanalytical system, USA) under N_2, using a glassy carbon working electrode, a platinum counter electrode, and an Ag/AgCl reference electrode. A 1×10^{-3}M drug solution in phosphate buffer solution was used for voltammetric studies of drugs. CdSe nanocrystals were dispersed in chloroform within nitrogen atmosphere. Cleaned glassy carbon electrode was dip-coated with this nanocrystal dispersion and dried for 2 h at 120 °C. This process led to adsorption of nanocrystals on the electrode surface. Subsequently, the nanocrystal-coated electrodes were transferred to the electrochemical cell. Tetra butyl ammonium hexafluorophosphate ($TBAPF_6$) was used as the supporting electrolyte for the voltammetric studies of quantum dots.

Transmission electron microscopy was performed with a Hitachi H600, operating at an accelerating voltage of 80 kV. Purified QDs in dry form was redispersed in chloroform and deposited on the surface of the copper grid with a sprayer.

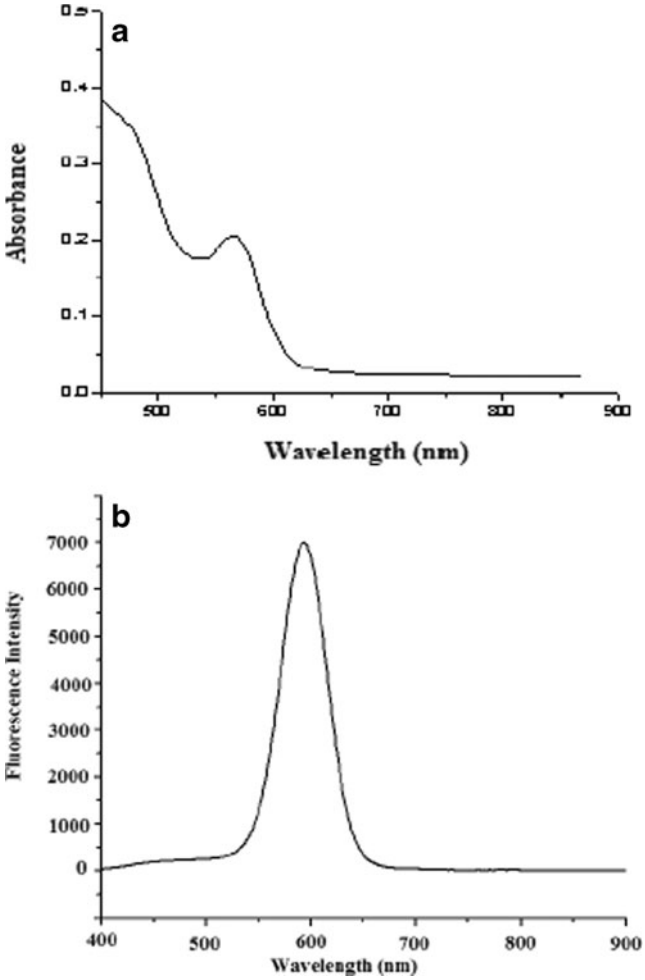


Fig. 2 **a** Absorption spectra of CdSe QDs. **b** Emission spectra of CdSe QDs

Synthesis of CdSe Quantum Dots

CdSe QDs were synthesized as reported earlier [29]. A typical synthesis is as follows. CdO, 0.0127 g (0.1 mmol),

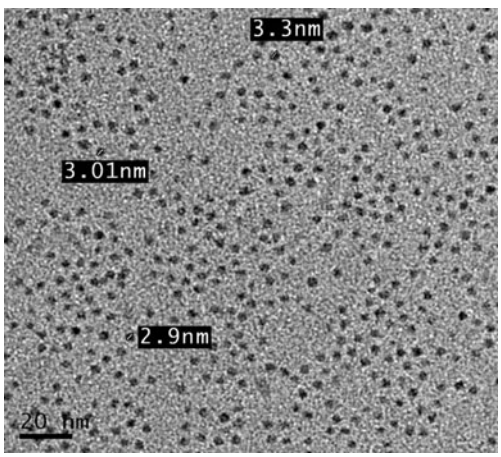


Fig. 3 TEM image of CdSe QDs

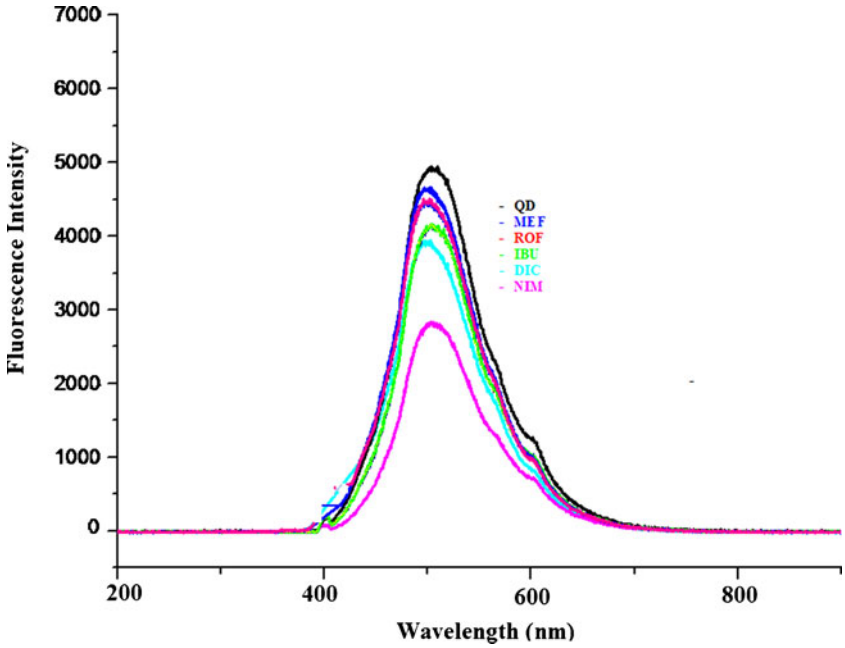
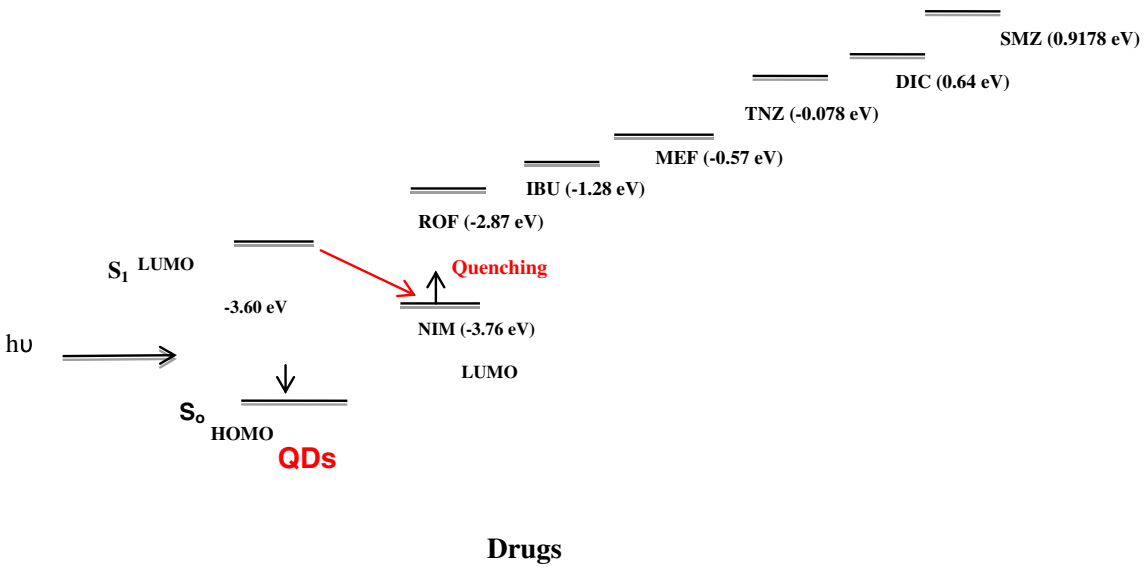


Fig. 4 Effect of various NSAIDs on fluorescence intensity of CdSe QDs

and 0.1140 g (0.4 mmol) of stearic acid were loaded into a 25 mL three-neck flask and heated to 150 °C under N_2 flow. After complete dissolution of CdO, the mixture was allowed to cool to room temperature. 1.94 g of TOPO and hexadecylamine were added to the flask, and the mixture was heated to 320 °C under N_2 flow to form an optically clear solution. At this temperature, the Se solution containing 0.079 g (1 mmol) of Se dissolved in 0.238 g (1.18 mmol) of TOP and 1.681 g of dioctylamine was swiftly injected into the reaction flask. After the injection, the temperature was set at 290 °C for the growth of the nanocrystals. 15 mL of chloroform was added to the reaction mixture at 30–50 °C. The nanocrystal solution was separated from the insoluble white or reddish solid floating on the top of the chloroform solution by centrifugation and decantation. The nanocrystals were precipitated by adding methanol into the chloroform solution and isolated by centrifugation and decantation. The purified quantum dots were redispersed in chloroform and were left for evaporation in nitrogen atmosphere for 48 h.

Fluorescence Quenching by NIM

For investigating the fluorescence quenching of QDs by NIM, different concentration of NIM was mixed with 500 μL of QDs and diluted to 2 ml with chloroform and fluorescence intensity was measured. All the samples were excited at a wavelength of 365 nm, and the



Scheme 1 Mechanism for selective fluorescence quenching of CdSe QDs by NIM in presence of other drugs

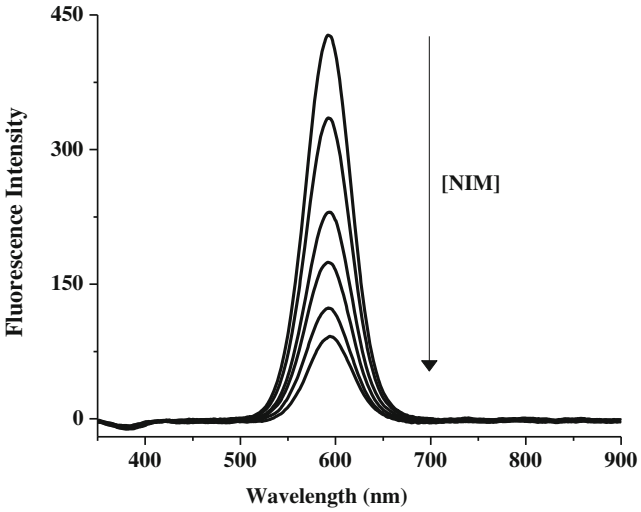


Fig. 5 Effect of NIM concentration on the fluorescence intensity of CdSe QDs

fluorescence emission was scanned from 400 to 900 nm at room temperature.

Results and Discussions

Characterization of CdSe QDs

Figure 2a, shows the absorption spectrum of CdSe QDs with a shoulder centered at (564 nm). Figure 2b, shows the emission spectrum of CdSe QDs with maximum emission at (589 nm). The particle size of CdSe QDs is determined from the absorption maximum by using the empirical formula $D = (1.6122 \times 10^{-9})\lambda^4 - (2.6575 \times 10^{-6})\lambda^3 + (1.6242 \times 10^{-3})\lambda^2 - (0.4277)\lambda + (41.57)$ and was found to be 3.3 nm. The particle size was confirmed from the TEM image of CdSe QDs (Fig. 3).

Sensor for Nimesulide and Quenching Effect of NIM on Fluorescence Intensity of CdSe QDs

To better understand the mechanism of fluorescent sensor, the fluorescence turn-on selectivity to various NSAIDs of different class was compared. From Fig. 4 it was found that only NIM was able to turn-off the fluorescence intensity of CdSe QDs. In order to study the effect of functional groups in NIM on fluorescence quenching of CdSe QDs, the effect of drugs such as sulfamethoxazole (which have a sulfonyl group as in NIM) and tinidazole (contains a nitro group) on fluorescence intensity was also studied. Even these drugs do not affect the fluorescence intensity.

Quenching mechanisms include inner filter effects, non-radiative recombination pathways, electron transfer processes and ion binding interactions [30, 31]. In the case of quantum dots, different electron transfer mechanisms have been considered, with and without photoinduction of either QD or receptor. For the NIM receptor to be an effective quencher of QD emission, the suggestion is that it needs to be able to interact directly with one of the QD charge carriers (i.e., valence band holes or conduction band electrons), there by disrupting the radiative recombination process.

The UV-Visible absorption spectra of NIM shows no absorption band in the range 400–700 nm, illustrating that the quenching effect is not due to an inner filter resulting from the absorption of the emission wavelength by NIM. There was no obvious change in the absorption spectra of CdSe QDs before and after addition of NIM. Absence of blue-shift or red-shift in the fluorescence emission spectra with increasing concentration of NIM indicates that the QDs do not aggregates in the presence of NIM. Instead the emission intensity decreases significantly in agreement with the expected photoinduced electron transfer from the nanoparticles to the quencher [32]. The energy change for PET is obtained using Rehm-Weller equation [33] (Eq. 1) considering the reduction potential of CdSe quantum dots (−0.38 V) and NIM (−0.64 V).

$$\Delta G_{PET} = E_{D+/D} - E_{A/A-} - E_{00} \quad (1)$$

$E_{D+/D}$ and $E_{A+/A-}$ are the redox potentials of the electron donors and acceptors respectively. The redox potentials were measured using cyclic voltammetry. The excitation energy of CdSe quantum dots (E_{00}) is estimated by using the emission wavelength (λem), where $E_{00} = h\nu = 12398.1/\lambda em$ (Å). With use of the redox potentials and excitation energy, the ΔG_{PET} values were calculated. ΔG values for electron transfer for all the drugs except NIM (ΔG=−2.08 eV) is positive. Therefore, photoinduced electron transfer may take place between the CdSe quantum dots and NIM. This demonstrates the selective quenching of fluorescence intensity of CdSe quantum dots by NIM.

Selective quenching of fluorescence intensity of CdSe QDs by NIM can be explained on the basis of band gap

Table 1 Determination of NIM in pharmaceutical sample

Sample	Declared amount (mg/tablet)	Method adopted	Found[a] (mg/tablet ± R.S.D)
Nise (Dr. Reddy's, India)	100.0	Proposed method	100.8±0.19
		Standard method	101.5±0.11

[a] Average of six determination ± relative standard deviation

energy. The conduction band (LUMO) and valence band (HOMO) edges were calculated using the equations:

$$E_{HOMO} = -(E_{ox} + 4.71)eV \quad (2)$$

$$E_{LUMO} = -(E_{red} + 4.71)eV \quad (3)$$

where 4.71 eV is the difference between vacuum level potential of the normal hydrogen electrode and the potential of the Ag/AgCl electrode [34, 35], E_{ox} and E_{red} are the onset potentials of the oxidation and reduction process. For quenching to occur the energy of Lowest Unoccupied Molecular Orbital (LUMO) of quencher should lie between the band gap energy of fluorophore. Scheme 1 demonstrates that the LUMO of NIM (−3.76 eV) lies within the band gap of CdSe QDs, where as the LUMO for other drugs under consideration lies above the band gap energy. These results also support the selective fluorescence quenching of CdSe QDs by NIM.

The experimental results on effect of reaction time and mixing sequence showed that reactions were complete within 5 min and the relative fluorescence intensity remained stable for at least 1.5 h. Therefore, the fluorescence spectrum was recorded after 5 min.

Under optimum conditions, the fluorescence intensity for different NIM concentrations is as shown in Fig. 5. There was a good linear relationship between fluorescence intensity and NIM concentration in the range $8.2\times10^{-7} - 4.01\times10^{-5}$M. The limit of detection (S/N=5) for NIM was 1.1×10^{-8}M. These results illustrate that this method can be successfully applied for the selective ultra trace determination of NIM.

Interference Study

The effect of coexisting substances, which pharmaceuticals often contains, on the fluorescence intensity of CdSe QDs was also carried out to evaluate the selectivity of the proposed method. It was found that the major interfering species such as ascorbic acid, citric acid, urea, glucose, lactose, Na^+, K^+, SO_4^{2-} and Cl^- did not cause any observable interference when present in 100 molar excess compared with concentration of NIM [1×10^{-6}M].

Application Studies

Application to Pharmaceutical Preparations

The proposed method has been effectively applied to the determination of NIM in commercially available pharmaceutical formulation. The sample was powdered well and dissolved in acetone. The solution was filtered through a Whatman No. 41 filter paper and the filtrate was used for further studies. The analytical results of the proposed method were compared with standard method for the determination of NIM [36]. According to standard method an adequate amount of the tablet powder containing NIM was accurately weighed and dissolved in 30 mL of acetone. Then 20 mL of double distilled water was added to it and titrated against 0.1 M NaOH solution. The end point was determined potentiometrically. The obtained results, summarized in Table 1, are found to be in good agreement with the standard method indicating the utility of the proposed CdSe based fluorescent sensor.

Conclusion

We have demonstrated the use of unmodified CdSe QDs for the selective determination of the drug nimesulide in presence of other non steroidal anti inflammatory drugs. The good linear range and lower limit of detection proves the utility of the proposed nanosensor for the trace analysis of NIM.

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