CdS nanocrystals as fluorescent probe for detection of dolasetron mesylate in aqueous solution: Application to biomedical analysis

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ABSTRACT

A simple and straightforward method for the determination of dolasetron mesylate (DM) in aqueous solution was developed based on the fluorescence quenching of 3-Mercaptopropionic acid (MPA) capped CdS quantum dots (QDs). The structure, morphology, and optical properties of synthesized QDs were characterized by using UV-Vis absorption spectroscopy, fluorescence spectroscopy, transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements. Under the optimum conditions, the MPA-CdS QDs fluorescence probe offered good sensitivity and selectivity for detecting DM. The probe provided a highly specific selectivity and a linear detection of DM in the range of 2–40 µg/mL with detection limit (LOD) 1.512 µg/mL. The common excipients did not interfere in the proposed method. The fluorescence quenching mechanism of CdS QDs is also discussed. The developed sensor was applied to the quantification of DM in urine and human serum sample with satisfactory results.

1. Introduction

Dolasetron mesylate (DM, Fig. 1) is an antiemetic and antinauseant agent. It is a highly specific and selective serotonin subtype 3 (5-HT3) receptor antagonist both in vitro and in vivo.

DM is currently being used for the management of nausea and vomiting associated with cancer chemotherapy, radiotherapy and surgical procedures [1,2]. Its main effect is to reduce the activity of the vagus nerve that activates the vomiting center in the medulla oblongata. Dolasetron breaks down slowly, staying in the body for a long time. One dose usually lasts 4–9 h and is usually administered once or twice daily. This drug is metabolized in the liver and removed from the body by the kidneys. It is also sometimes used as an antiemetic (anti-vomiting medication) in veterinary medicine for dogs and cats. Dolasetron is a well-tolerated drug with few side effects. Headache, dizziness, and constipation are the most commonly reported side effects associated with its use. However, the FDA has recently issued a drug communication, stating that the injection form of DM is currently being used for the management of nausea and vomiting associated with cancer chemotherapy, radiotherapy and surgical procedures. In addition, these methods have some limitations like use of expensive instruments, long operation time and reagents required not easily available in many laboratories. Therefore, it is necessary to develop a fast, simple, sensitive, selective and straightforward method for analysis of DM in human urine and blood serum.

Nowadays, fluorescence-based sensors have attracted much attention due to its simple operation, low cost, selectivity, and high sensitivity and thus can be used for real sample analysis. Mostly conjugated polymers, organic fluorescent molecules, fluorescent dyes, semiconducting nanoparticles and fluorescent proteins are used as fluorescence sensors. Among them semiconducting nanocrystals or quantum dots (QDs) have attracted much attention of researchers towards development of sensors. QDs possess outstanding optical properties, such as broad absorption spectra, narrow and symmetric size-tunable emission, high quantum yields, and high photobleaching stability, which make them advantageous over traditional fluorophores for bioimaging and biosensing applications [9,10]. In 1998, Bruchez and co-workers [11] and Chan and Nie [12] were the first to recognize...
the applications of QDs for biology. So far, many fluorescence-based methods have been developed by using semiconducting QDs for analysis of ions [13–16], imaging [17–21], biosensing [22–25], and explosives [32].

Chen et al. [25] developed a chemiluminescence probe based on hydrogen peroxide—sodium hydrogen carbonate-CdSe/CdS QDs system for the determination of l-ascorbic acid in human serum. The chemiluminescence intensity and the concentration of l-ascorbic acid have a good linear relationship in the ranges of 1.0×10^{-7}–1.0×10^{-4} mol/L and detection limit (LOD) for l-ascorbic acid is 6.7×10^{-9} mol/L. Hou et al. [23] reported a fluorescence sensor for the determination of spironolactone (SPFX) based on fluorescence quenching of CdSe/CdS QDs at 556 nm wavelengths. The fluorescence quenching intensity of QDs is linearly proportional to the concentration of SPFX ranging from 0.5 µg/mL to 40 µg/mL, with LOD of 0.1391 µg/mL. Further this method was applied for the determination of SPFX in pharmaceutical tablets and milk samples. Liang et al. [27] developed a fluorescence probe for spironolactone detection based on the fluorescence quenching of CdSe QDs with good linearity between 2.5 µg/mL and 700 µg/mL and LOD was 0.2 µg/mL. Fluorescence quenching probe for detection of dopamine has been reported by using functionalized CuInS2 QDs [33]. The turn-on fluorescent biosensor for herring sperm DNA (hsDNA) detection was designed by Shen et al. [22] based on hsDNA-induced fluorescence enhancement of Sm^{3+} modulated glutathione (GSH)-capped CdTe QDs. Also, there is development of turn-on fluorescence sensor for ATP detection based on cysteamine capped CdS QDs in aqueous solution with very LOD (17 µM), which is applied to determine ATP in spiked urine samples [34].

The possible sensing mechanism was based on the charge—charge interaction concurrent with the hydrogen bonding between ATP and protonated amine group on the Cys-CdS QDs surface. Recently, we have presented a novel turn-on fluorescence sensor for determination of μ-penicillamine (μ-PA) in pharmaceutical formulation by CdS QDs [29]. The key sensing mechanism of fluorescence enhancement of QDs was attributed to the passivation of the trap states of MPA-CdS QDs. After stirring for 12 h, the key sensing mechanism of fluorescence enhancement of QDs was attributed to the passivation of the trap states of MPA-CdS QDs. After stirring for 12 h, the mixture was diluted to 100 mL with double distilled water. The concentration of prepared CdS QDs was 0.02 mol/L, which was determined as per the concentration of S^{2-} added.

2. Experimental

2.1. Chemicals

All chemicals which were used for synthesis of QDs are of analytical reagent grade and used as received without further purification. Cadmium chloride (CdCl2·H2O) and sodium sulfide (Na2S) were procured from s d Fine-CHEM Ltd. (Mumbai, India), 3-Mercaptopropionic acid (MPA), di-potassium hydrogen orthophosphate (K2HPO4) and potassium dihydrogen orthophosphate dihydrate (KH2PO4·0·H2O) for phosphate buffer preparation were purchased from Spectrochem Chemicals (Mumbai, India). The stock solution of DM was prepared in doubly distilled water which was purchased from Sigma-Aldrich (Mumbai, India). Solutions of all coexisting substances were prepared in the double distilled water and stored at room temperature.

2.2. Instruments

Fluorescence measurement of solutions was made with PC based Spectrofluorophotometer (JASCO Model FP-8300, Japan) equipped with Xenon lamp source and 1.0 cm quartz cell. Both excitation and emission slits were fixed at 5 nm with medium sensitivity. The absorption spectrum was recorded on ultraviolet-visible near infrared (UV-Vis-NIR) spectrophotometer (Specord 210, analytic jena) with 1.0 cm quartz cell. Particle size of CdS QDs was measured by using transmission electron microscopy (TEM) (TEM, FEI Tecnai 300) and dynamic light scattering (DLS) (Malvern Instruments Ltd., UK). The fluorescence lifetime of CdS QDs was measured on time resolved fluorescence spectrometer (Horiba’s Jobin-Yv on-IBH). High-speed centrifuge model C-24 BL (REMI Instrument Ltd, Mumbai, India) was used for centrifugation operation.

2.3. Synthesis of functionalized CdS QDs (MPA-CdS QDs)

Water soluble functionalized CdS QDs were synthesized by the method described in our previous report [29]. In brief, the 20 mL of 0.1 mol/L CdCl2·H2O solution was dropped into the 5.0 mL of 0.1 mol/L MPA solution slowly with constant stirring at room temperature. Then pH was adjusted at 6.0–7.0 by the dropwise addition of 0.1 mol/L NaOH solution followed by 10 mL of 0.1 mol/L Na2S. Under vigorous stirring, a clear yellowish suspension was obtained, which clearly indicated that formation of MPA-CdS QDs. After stirring for 12 h, the mixture was diluted to 100 mL with double distilled water. The concentration of prepared CdS QDs was 0.02 mol/L, which was determined as per the concentration of S^{2-} added.

2.4. Fluorescence measurement

Initially, 100 µg/mL of standard DM solution was prepared by dissolving 5 mg pure DM in double distilled water in a 50 mL volumetric flask. For the determination of DM by CdS QDs, we adopted the following procedure: to a 10 mL volumetric flask, 2.0 mL CdS QDs solution having concentration of 5×10^{-5} mol/L was added and then known volume of standard DM solution was added into it. Finally, the mixture was diluted up to the mark with double distilled water and mixed thoroughly. After 5.0 min, diluted solution was transferred into the quartz cell and the fluorescence intensity was measured at λex=370 nm with a slit width of 5.0 nm for excitation and emission at medium intensity. Similar procedure was used for determination of DM in human urine and blood serum by standard addition method.
2.5. Selectivity

The selectivity of the proposed method for DM was studied by performing the following procedure. Stock solutions of various substances and some common ions were prepared by dissolving pure substances and salts in deionized water, respectively. To a standard volumetric flask (10 mL), 2.0 mL of CdS QDs solution, DM and known volume of standard solutions of interfering substances (double concentration) were added. Then the solutions were diluted with water to 10 mL and mixed thoroughly. After 5.0 min, the fluorescence was measured.

2.6. Real sample analysis

A urine sample from a healthy volunteer was collected and filtered through a Whatman No. 42 filter paper. Then the filtered urine sample was diluted to 100 times with double distilled water. Different concentrations of standard DM solution were added to the diluted urine samples to prepare spiked samples.

Blood sample was collected from the healthy volunteer, centrifuged at 4500 rpm for 10 min and kept for settling for 3 h. From centrifuged sample, serum was isolated and 1 mL of serum was further diluted to 10 mL. Different concentrations of standard DM solution were added to the diluted serum samples to prepare spiked samples.

3. Results and discussion

3.1. Characterization of functionalized CdS QDs

The optical properties of synthesized MPA-CdS QDs were characterized and the results are shown in Fig. 2. The synthesized QDs showed a narrow and symmetrical emission spectrum, suggesting that the prepared CdS QDs were nearly homogenous and monodisperse [35]. The particle size of QDs was measured by HR-TEM and DLS and the results are shown in Fig. 3. From the DLS study, the average particle size was found to be 3.86 nm. Moreover, the particle size of the prepared CdS QDs was calculated from absorption maximum of the UV-Vis spectrum according to the literature [36] and found around 2.50 nm.

3.2. Fluorescence quenching study

In the present work, the interaction between MPA-CdS QDs and DM was systematically studied based on the fluorescence change of MPA-CdS QDs induced by DM. It could be seen from Fig. 4A that there was quenching of the fluorescence intensity of CdS QDs with increasing concentration of DM. Furthermore, Fig. 4B shows there was a good linear relationship between \( F_0/F \) (F and \( F_0 \) are fluorescence intensity of QDs with and without DM) and the DM concentration ranging from 2 µg/mL to 40 µg/mL. The corresponding linear correlation coefficient \( (R^2) \) was 0.9989 and the LOD was 1.512 µg/mL. LOD was calculated by equation, 3σ/k, where σ is the standard deviation of the y-intercept of regression line and k is the slope of the calibration graph [37].

3.3. Possible sensing mechanism

The QDs have been regarded as good fluorophores for different analytes because of their unique optical properties and surface modification. The fluorescence of QDs results from radiative recombination of photo-induced electron and hole located at the conduction band and valence band, respectively. Therefore, QDs have been used as a good sensor based on fluorescence enhancement and fluorescence quenching by analytes. Based on fluorescence enhancement [23,29,34,38] and fluorescence quenching [13,24,27,28,33,39,40], many methods have been developed for detection of different analytes.

There are two kinds of fluorescence quenching: dynamic quenching and static quenching. Quenching mechanisms include inner filter effects, nonradiative recombination pathways, electron transfer processes, and ion binding interaction. Liu et al. [33] have developed functionalized CuInS2 QDs as a near-infrared fluorescence probe for the determination of dopamine based on fluorescence quenching of QDs due to the strong interaction of vicinal dial groups on the surface of CuInS2 QDs with dopamine molecules by covalent bonding to form five membered cyclic boronate ester, which leads to an increase in thickness of the surface capping layer of QDs. Koneswaran and Narayanaswamy [28] described the quenching of L-cysteine capped CdS/ZnS QDs by vitamin B6 based on nonradiative recombination of excited electrons and holes which is attributed to the effective electron transfer from QDs to vitamin B6. Very recently, there has been one report on determination of 2,4,6-trinitrotoluene (nitro-explosive) based on fluorescence quenching using CdTe(S)@PAM QDs [32]. The fluorescence quenching of QDs was due to resonance energy transfer, which was confirmed by the fluorescence life time measurement and electron transfer from QDs core to 2,4,6-trinitrotoluene.

To explore the mechanism of fluorescence quenching of MPA capped CdS QDs by the successive addition of DM, we used different analytical techniques such as fluorescence spectroscopy, UV–Vis absorption spectroscopy and fluorescence life time measurement. Fluorescence quenching spectra of CdS QDs before and after successive addition of DM show that there was no significant shift in emission wavelength (Fig. 4A), which confers that there is no change on the surface of CdS QDs and fluorescence quenching may be due to either electron transfer from QDs to DM [28] or resonance energy transfer [32]. Moreover, there is no significant change in absorption spectra of QDs before and after addition of DM (Fig. 5), which confirms that there is no aggregation of CdS QDs after addition of DM [28]. Here, DM with indole structure could serve as an efficient electron acceptor due to electron withdrawing group in its vicinity. Hence, the fluorescence quenching of QDs may be due to nonradiative recombination of excited electrons and holes [28]. Further, CdS QDs are electron donor, and electron transfer from the QDs cores to the DM may be another cause of fluorescence quenching [32]. The type of fluorescence quenching of the present system was also investigated by the fluorescence life time measurements. It can be seen from Fig. 6 that there is no change in the fluorescence life time of QDs (around 5.48 ns) in absence and presence of different concentrations of DM. This result suggests that the mechanism of the quenching is static quenching rather than dynamic quenching. Moreover, it is also supported using Stern–Volmer equation, 

\[ F_0/F = 1 + K_{SV}[Q] \]

where F and \( F_0 \) are the fluorescence intensities of the QDs with and...
without DM, \([Q]\) is the concentration of quencher (DM) and \(K_{sv}\) is the Stern–Volmer quenching rate constant. A very good linear relationship was observed in the concentration range of 2 µg/mL to 40 µg/mL of DM. The Stern–Volmer quenching constant was found to be \(3.84 \times 10^3 \text{ dm}^3/\text{mol}\), and linear correlation coefficient (\(R^2\)) was 0.9989. For dynamic type quenching, the quenching constant is always less than 200 dm³/mol while for static type of quenching it is greater than 200 dm³/mol; therefore, the present quenching process is static type (\(K_{sv} > 200 \text{ dm}^3/\text{mol}\)) [24,41]. In static quenching, formation of ground state complex takes place between fluorophores and quencher. Conversely, there is a possibility of ground state complex formation between the QDs and DM due to electrostatic interaction between them and it may be the one more cause of fluorescence quenching. The schematic illustration of the plausible quenching mechanism is shown in Scheme 1.

3.4. Selectivity of the sensor

To evaluate the selectivity of the CdS QDs as a fluorescent quenching probe for DM, the fluorescence response of the QDs was investigated towards other biomolecules and some ions, having con-
centrations of 40 µg/mL and 80 µg/mL, respectively. As shown in Figs. 7 and 8, only DM resulted in significant fluorescence quenching of the CdS QDs. It was found that the common interfering substances like sucrose, glucose, maltose, dextrose, starch, PEG, SDS, \(K^+\), \(Na^+\) and urea did not affect the fluorescence intensity even at high concentration. However, there were some effects of coexisting substances on the fluorescence of CdS QDs, but it was not much significant in our proposed method. The results revealed that the proposed method can be applied to the detection of DM in human urine and blood serum samples.

3.5. Comparison with other methods

There are only few methods developed for the determination of DM based on different analytical techniques. Most of them require sophisticated and expensive instruments, costly chemicals, and operation seems to be complex prior to analysis. For comparison, analytical performances of different methods for the determination of DM are summarized in Table 1. The proposed method based on fluorescence quenching of QDs is superior because of simple experimental procedure, easy synthesis of QDs, short analysis time and no need for costly solvents. Therefore, the proposed method is the best alternative to determine the amount of DM in real samples.

3.6. Analytical applications of the proposed sensor

To confirm the feasibility of the proposed fluorescence sensor, recovery experiments were performed to determine the concentration of DM in real human urine and blood serum samples of a healthy volunteer using standard addition method and the results are shown in Table 2. The relative standard deviation (RSD) was lower than 1%, and the average recoveries of DM in the real sample were 99%–101%. The excipients in real samples did not interfere with the determination. These results confirm that the proposed sensor is reliable for the practical use.
A fluorescence sensor was developed for DM sensing in aqueous solution. The key sensing mechanism of fluorescence quenching was based on the nonradiative recombination of electron and hole. The quenching of fluorescence intensity of QDs with an increasing concentration of DM was in the linear range of 2–40 µg/mL with LOD being 1.512 µg/mL, indicating that this sensor can be used to detect DM in real sample. The proposed method was successively applied to the detection of DM in human urine and blood serum sample with satisfactory results.

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