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CdSe quantum dots as luminescent probes for spironolactone determination

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Abstract

Based on the quenching of the fluorescence of CdSe quantum dots (QDs) by spironolactone, a simple, rapid and specific method for spironolactone determination was proposed. In the optimum conditions, spironolactone concentration versus quantum dot fluorescence gave a linear response with an excellent 0.997 correlation coefficient, between 2.5 and 700 mg/mL (6.0–1680 μmol/L) and the limit of detection (S/N = 3) was 0.2 μg/mL (0.48 μmol/L). The contents of spironolactone in pharmaceutical tablets were determined by the proposed method and the results agreed with the claimed values. The possible mechanism for the reaction was also discussed.

Keywords: Quantum dots; Spironolactone; Determination

1. Introduction

Luminescent semiconductor quantum dots (QDs), also called nanocrystals (NCs), have gained increasing attention in the past decade [1–3]. In comparison with organic dyes and fluorescent proteins, QDs have high quantum yield of fluorescence, broad excitation spectrum and narrow/symmetric emission spectrum [4–6]. In addition, QDs exhibit high photobleaching threshold and excellent photostability. In 1998, Alivisatos and co-workers [7] and Chan and Nie [8] demonstrated the first applications of QDs for biology. Since those foundational papers, the use of QDs has been demonstrated in biology and medicine as fluorescent probes and more recently in analytical chemistry. Some inorganic cations, such as Cu(II), Hg(II) and Ag(I) had been detected by utilizing luminescent QDs based on changes in the luminescence intensity of the QDs [9–14]. Jin et al. proved luminescent CdSe QDs could be used for the selective determination of free cyanide in both aqueous solution and organic media [15,16]. Ji et al. [17] and Constantine et al. [18,19] demonstrated QDs could be a promising biosensor for the detection of paraoxon. Investigations of the effects of the adsorption of different gaseous analytes on the emission properties of colloidal NCs in polymer thin films have attracted some interest recently. Peng and co-workers reported the photostimulated responses of the photoactivated CdSe NCs to different gases vary dramatically [20]. They believe it is possible to develop sensitive and simple sensors using semiconductor NCs. The same group also reported environmental effects on photoluminescence of highly luminescent CdSe and CdSe/ZnS core/shell NCs in polymer thin films. It is thought that the oxygen and water molecules act as a surface adsorbant or oxidant which passivates the remaining surface states, hence resulting in a reversible, large blue shifted enhancement [21]. These studies reveal that some changes of the surface charges or components of QDs would change their photophysical properties.

Spironolactone (Scheme 1) is a type of medication called a "potassium sparing" diuretic, which is used to remove a surplus of fluid from the body's bloodstream or tissues. It also acts as an aldosterone inhibitor (prevents salt retention), and is used to treat advanced heart failure when symptoms persist after other drug therapies are maximized [22]. Spironolactone is banned in sports by the Medical Commission of the International Olympic Committee (IOC), since it may be abused by competitors to reduce weight quickly, to dilute urine or change pH in order to prevent detection of other drugs and to control the retention of water produced by anabolic steroids [23]. Some methods based on various analytical techniques have been reported for the determination of the spironolactone, such as spectrophotometric, chromatographic and spectrofluorimetric methods [24–26]. However, the above methods are expensive, time-consuming or requiring complex mathematic dealing.

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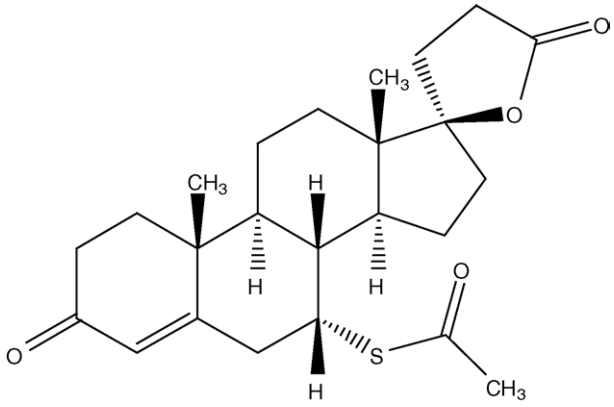

Scheme 1. Structure of spironolactone.

To our knowledge, there is no report of drug determination based on changes in the luminescence intensity of the QDs in organic media until now. In the present work, a new method for the determination of spironolactone was developed based on the quenching of the fluorescence of CdSe QDs by spironolactone in organic media.

2. Experimental

2.1. Apparatus

The absorption spectra were acquired on a TU-1900 UV–vis spectrometer (Beijing, China). All fluorescence measurements were made with Perkin-Elmer model LS-55 luminescence spectrometer equipped with a 20 kW xenon discharge lamp as a light source. The transmission electron microscopy (TEM) images of the QDs were acquired on a JEOL-JEM-2010 transmission electron microscope (Japan). The colloidal solution of the QDs was dropped onto copper grids covered with a thin film of amorphous carbon and the excess solution immediately wicked away.

2.2. Reagents

Hexane, methanol and CdO were purchased from Shanghai Reagent Factory and used as received without further purification. Selenium (Se), tributylphosphine (TBP) and tri-*n*-octylphosphine oxide (TOPO) were purchased from Aldrich (Milwaukee, WI). Spironolactone was purchased from ICN Biomedicals Inc. The spironolactone tablets were the products of Hangzhou Minsheng Pharmaceutical Group Co. Ltd. All other chemicals used were of analytical-reagent grade. Doubly deionized water was used throughout the experiment.

2.3. Procedure

Nearly monodisperse CdSe QDs were synthesized by our group according to the scheme reported by Qu and Peng with minor modifications [27]. Briefly, 0.0254 g CdO and 0.2280 g stearic acid were heated to 150 °C under Ar flow. After CdO was completely dissolved, the mixture was allowed to cool to room temperature, 3.88 g trioctylphosphine oxide and 3.88 g hexadecylamine were added, and the mixture was heated to 310 °C under Ar flow. At this temperature, the 0.158 g Se solution in 0.476 g tributylphosphine and 3.362 g dioctylamine was swiftly injected into the reaction flask. The reaction was stopped 3 min after the injection and heat was immediately removed. After purification by precipitation, centrifugation and decantation, the vacuum-dried CdSe QDs (more than 50 mg) were redispersed in hexane and kept in the dark for future use. QDs solution concentrations were estimated from the absorption spectra using the molar absorptivity at the first absorption maximum for QDs of this size reported by Schmelz et al. ($\sim 1.8 \times 10^5\ M^{-1}\ cm^{-1}$) [28].

2.5 mg/mL spironolactone was prepared by dissolving spironolactone in chloroform, and diluted to appropriate concentration by hexane, then transferred into a calibrated 5 mL test tube. After addition of CdSe QDs solution, hexane was added to the mark. The fluorescent intensity was measured with the following settings of the spectrofluorometer (excitation wavelength (λ_{ex}), 388 nm; excitation slit (EX), 10.0 nm; emission slit (EM), 5.0 nm).

2.4. Sample treatment

For the pharmaceutical analysis, 10 tablets were weighed and powdered in a mortar. The average weight of a tablet was calculated. The powder was dissolved in chloroform/hexane (1:9 (v/v)) solution, and insoluble excipients were removed from solution with centrifugation at 12,000 rpm for 5 min.

3. Results and discussion

3.1. Characterization of CdSe QDs

Fig. 1 shows the absorption (a) and room temperature fluorescence (b) spectra of CdSe QDs. It can be seen that the line width of the fluorescence spectrum is narrow (with the full width at half-maximum about 30 nm), showing that as-prepared CdSe QDs are nearly monodisperse and homogeneous. The TEM image (Fig. 2a) of CdSe QDs indicates much less agglomerated particles with average size of $\sim 3.8 \pm 0.2$ nm. The TEM image (Fig. 2b) CdSe QDs in spironolactone solution shows also very

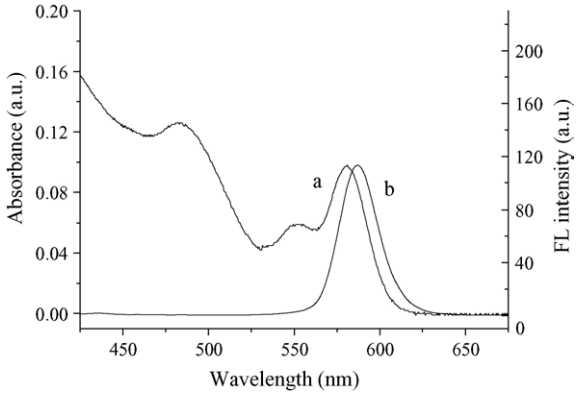

Fig. 1. Absorption (a) and room temperature fluorescence (b) spectra of CdSe QDs.

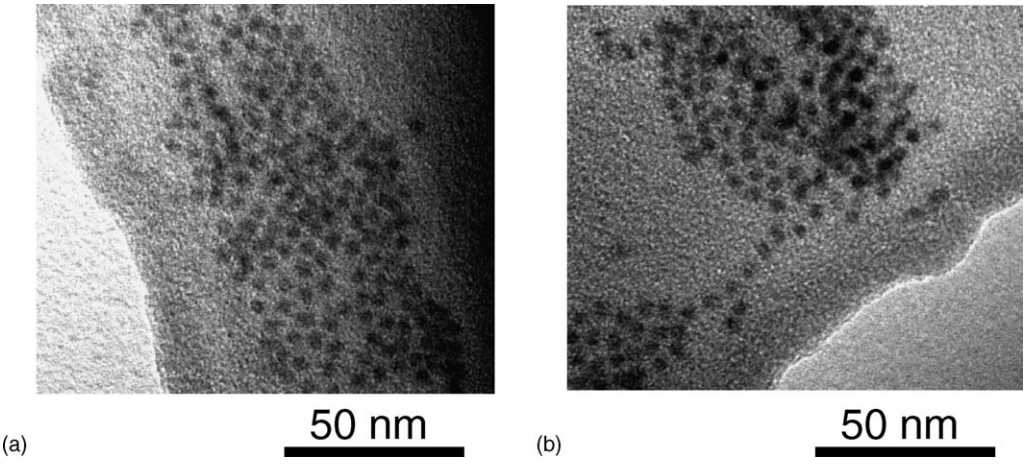

Fig. 2. (a) TEM image of CdSe QDs and (b) TEM image of CdSe QDs after adding spironolactone.

clearly that these QDs are monodisperse. Thus, we believe the quench of QDs fluorescence is not due to the agglutination or becoming smaller of QDs induced by spironolactone.

3.2. Effect of reaction time

Initial experiments demonstrated that the spironolactone quenching of the QDs was finished within 5 min and the fluorescence signals are stable for more than 30 min. We recorded the fluorescence intensity after the system had reacted for 10 min.

3.3. Calibration and sensitivity

The emission spectra of CdSe QDs and its fluorescence titration with spironolactone were recorded in hexane, the results of which are shown in Fig. 3. The observed fluorescence band centered 587 nm (excitation 388 nm) is commonly attributed to the recombination of the charge carriers within surface states [29]. When spironolactone was added to the CdSe QDs, a significant decrease of QDs fluorescence emission was observed.

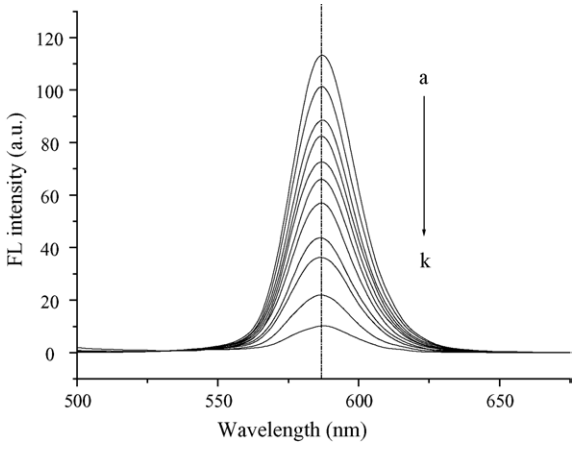

Fig. 3. Fluorescence response of CdSe QDs to addition of spironolactone in hexane. Aliquots of 2.5, 0.5 and 0.1 mg/mL spironolactone were added to yield final spironolactone concentrations of (a) 0, (b) 2.5, (c) 7.5, (d) 12.5, (e) 20, (f) 30, (g) 50, (h) 100, (i) 150, (j) 300 and (k) 700 μg/mL. λ_{ex} = 388 nm. The concentrations of CdSe QDs are 1.5×10^{-7} mol/L.

It was found that spironolactone quenches the fluorescence of QDs in a concentration dependence that is best described by a Stern–Volmer type equation:

$$\frac{I_{max}}{I} = 1 + K_{SV}[S].$$

I and I_{max} are the fluorescent intensities of the QDs at a given spironolactone concentration and in a spironolactone free solution, respectively. [S] is the spironolactone concentration and K_{SV} is found to be 6.0×10^3 M^{-1}. The calibration plot (Fig. 4) of I_{max}/I with concentration of spironolactone is linear in the range 2.5–700 μg/mL (6.0–1680 μmol/L). The correlation coefficient is 0.997. The limit of detection (S/N = 3) is 0.2 μg/mL (0.48 μmol/L). The relative standard deviation for five determinations of 10.0 μg/mL spironolactone was 3.7%.

Even though the same experimental procedure is employed in the procedure of the QD synthesis, the many experimental variables will mean that each batch of QDs will have different fluorescence emission/absorption profiles. Thus, for each batch of QDs, a calibration curve (fluorescence versus spironolactone concentration) must be constructed.

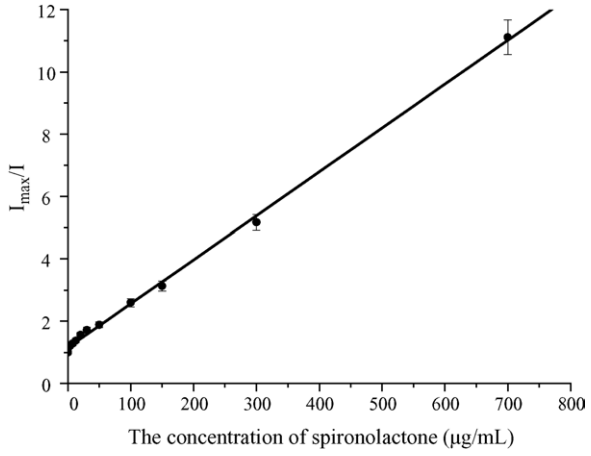

Fig. 4. Stern–Volmer plot of spironolactone concentration dependence of the FL intensity of QDs with a 0.997 correlation coefficient.

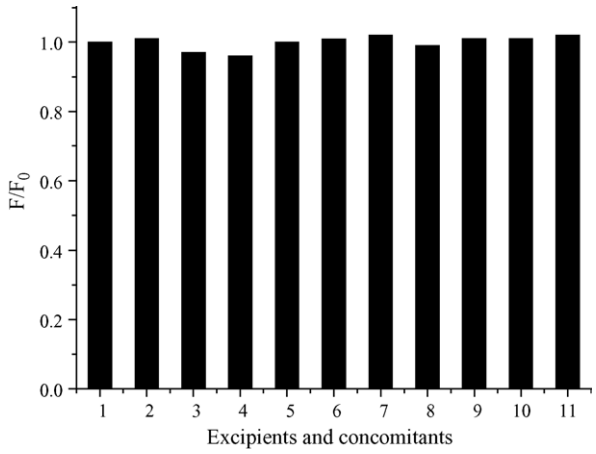

Fig. 5. Effect of several excipients and concomitants on the fluorescence of CdSe QDs. (1) contr., (2) EDTA, (3) cellulose acetate, (4) $CaSO_4$, (5) SDS, (6) glucose, (7) sucrose, (8) starch, (9) mannitol, (10) sorbitol and (11) *o*-phthalic acid. The excipients and concomitants are prepared in chloroform/hexane (1:9 (v/v)) saturated solution. The concentrations of CdSe QDs are 1.5×10^{-7} mol/L.

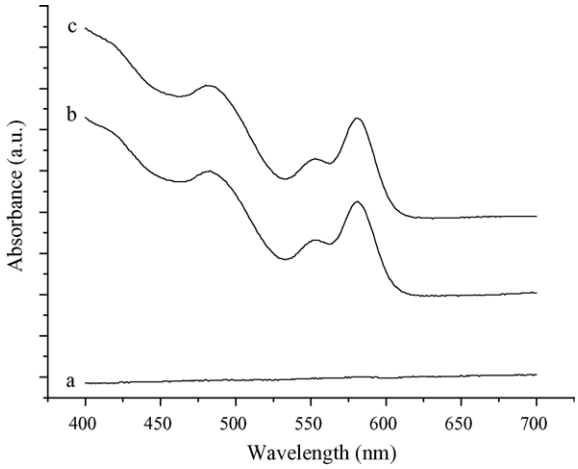

Fig. 6. UV–vis absorption spectra of (a) 500 μg/mL spironolactone, (b) 5.0×10^{-7} mol/L CdSe QDs and (c) 5.0×10^{-7} mol/L CdSe QDs + 500 μg/mL spironolactone.

3.4. Effect of foreign substance

Many compounds have the potential to quench QD fluorescence emissions [15,17,20]. The tablets often contained the following excipients: EDTA, cellulose acetate, $CaSO_4$, sodium dodecyl sulphate (SDS), glucose, sucrose, starch, mannitol, sorbitol and *o*-phthalic acid [30]. Accordingly, we set out to establish that these excipients did not quench QD fluorescence emissions which in turn would give rise to erroneous spironolactone levels being determined. Because most of the excipients and concomitants such as EDTA, cellulose acetate, etc., are hardly dissolved in chloroform/hexane, their saturated solutions in chloroform/hexane (1:9 (v/v)) are selected to study the effects. As shown in Fig. 5, EDTA, cellulose acetate, $CaSO_4$, sodium dodecyl sulphate (SDS), glucose, sucrose, starch, mannitol, sorbitol and *o*-phthalic acid in chloroform/hexane (1:9 (v/v)) saturated solution have hardly any effect on fluorescence of CdSe QDs.

3.5. Application

The present method was applied to determine spironolactone in spironolactone tablets. Results are given in Table 1. As shown in Table 1, the R.S.D. was 2.4%, and the results obtained by the present method agreed with the labeled values for spironolactone tablets.

Table 1
Determination of spironolactone in tablets by the proposed method

Sample	Spironolactone tablets
Labeled values (mg/tablet)	20
Found (mg/tablet)	20.04
R.S.D.[a] (%)	2.4
E_r	0.2

[a] The R.S.D. value is mean of five determinations.

3.6. The mechanism of reaction

Fig. 6 shows UV–vis absorption spectra of (a) spironolactone, (b) CdSe QDs and (c) CdSe QDs + spironolactone. No absorption band was observed in the 400–700 nm wavelength range for spironolactone (Fig. 6a), so the quenching effect of spironolactone on the fluorescence of CdSe QDs is not due to an inner filter resulting from the absorption of the emission wavelength by spironolactone. No obvious change was observed for the CdSe QDs absorption spectra before and after adding spironolactone (Fig. 6b and c), and a blue-shift or red-shift of the fluorescence emission spectra was also not seen when the concentration of spironolactone is changed from 2.5 to 700 μg/mL (Fig. 3), which also means CdSe QDs do not aggregate or become smaller after adding spironolactone [4,31]. The result is in agreement with that from TEM images.

The surface-bound organic molecules play an important role in determining fluorescent properties of QDs [21]. Peng and coworkers reported highly emitting CdSe QDs showed no emission after surface-bound amine ligands were replaced by hydrophilic thiols [32]. The quenching of the fluorescence of CdSe QDs may be due to the changes of the surface-bound organic molecules of QDs induced by spironolactone [29].

4. Conclusion

A novel method for spironolactone analysis has been developed based on the quenching of the fluorescence of CdSe QDs by spironolactone. In the optimum conditions, calibration graph was linear in the range 2.5–700 μg/mL (6.0–1680 μmol/L). The correlation coefficient is 0.997. The limit of detection (S/N = 3) is 0.2 μg/mL (0.48 μmol/L). The relative standard deviation for five determinations of 10.0 μg/mL spironolactone was 3.7%. The present method was applied to determine spironolactone in spironolactone tablets and the results agreed with the claimed values. The possible quenching mechanism is due to the changes of the surface-bound organic molecules of QDs induced by spironolactone.

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