Journal of Luminescence 160 (2015) 299–304

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Label-free room-temperature phosphorescence turn-on detection of tiopronin based on Cu^{2+}-modulated homocysteine-capped manganese doped zinc sulfide quantum dots

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ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form
16 December 2014
Accepted 18 December 2014
Available online 29 December 2014

Keywords:
Hcy-capped Mn-doped ZnS quantum dots (QDs)
Room-temperature phosphorescence sensor
Tiopronin

ABSTRACT

A label-free turn-on room-temperature phosphorescence (RTP) sensor for tiopronin based on Cu^{2+}-modulated homocysteine (Hcy)-capped Mn-doped ZnS quantum dots (QDs) was described in this paper. The RTP of Hcy-capped Mn-doped ZnS QDs can be effectively quenched by Cu^{2+} due to the binding of Cu^{2+} to the Hcy on the surface of the QDs and the electron transfer generated from the photoexcited QDs to Cu^{2+}. The high affinity of tiopronin to Cu^{2+} enables the dissociation of the ion from the surface of the QDs, thereby forming a stable complex with tiopronin in the solution, and recovering the RTP of the QDs. The Cu^{2+}-induced RTP quenching and subsequent tiopronin-induced RTP recovery for MPA-capped ZnS QDs provide a solid basis for the present RTP sensor based on QDs for the detection of tiopronin. The detection limit for tiopronin is 0.18 ng mL^{-1}, the relative standard deviations is 1.9%, and the recovery of urine and serum samples with tiopronin addition range from 96% to 106% under optimal conditions. The proposed method was successfully applied to biological fluids and obtained satisfactory results.

1. Introduction

Tiopronin (N-(2-mercaptopropionyl)-glycine) is a synthetic sulphydryl compound that acts as a potent free radical scavenger [1]. It is a weak acidy compound for the treatment of cystinuria, rheumatoid arthritis, hepatic disorders as well as an antidote to heavy metal poisoning [2]. Tiopronin could be rapidly absorbed from the gastrointestinal tract and oxidized to various disulfide forms, it may cause some unwanted effects such as muscle pain, yellow skin or eyes, sore throat and fever, change in taste or smell, etc. [3]. Because of its relatively high frequency of side effects and dose-related, the sensitive determination of tiopronin in biological matrices and pharmaceutical preparation is highly desirable.

A number of analytical methods have been developed for the determinations of tiopronin in various kinds of samples, such as capillary electrophoresis [4], HPLC with UV detection [5], mass spectrometry [6], fluorescence detection [7–9] and electrochemical detection [10]. These techniques require expensive apparatus and reagents as well as skilled operators, they are also time-consuming. Tedious pre- and post-column derivatization is often required for liquid chromatography. Studies have reported a method for the determination of tiopronin based on the fluorescence quenching of ZnS quantum dots (QDs) caused by pH changes when adding tiopronin in aqueous medium [11]. However, fluorescence of ZnS QDs has a short average life and thus can be disturbed by background fluorescence and scattered light.

Sensor design based on the unique properties of various nanomaterials in conjugation with natural or artificial molecular recognition units has gained considerable attention in the past decade [12,13]. Photoluminescent semiconductor QDs with particle sizes between 1 and 20 nm exhibiting higher quantum yield and surface functionality [14–16]. In comparision with bulk materials or traditional organic dyes, QDs exhibit excellent optical properties, such as high quantum yield, tunable size-dependent emission, resistance to photobleaching, and narrow emission peaks, which have gained significant interest in fundamental research and technical applications [17]. The modification of QDs with biomolecules [2] and metal ions [18,19] has emerged as an important field in sensor/biosensor applications [20–22], such as ions [23,24], biomacromolecules [25–29], and small molecules [30–33]. Compared to the fluorescence, the phosphorescence of doped-QDs demonstrates longer average life, allowing an appropriate delay time to prevent fluorescence emission and light scattering [34].

A room-temperature phosphorescence (RTP) turn-on sensor for selective detecting tiopronin based on Cu^{2+} quenched RTP of Hcy-capped Mn-doped ZnS QDs was described in this paper. The RTP intensity of Hcy-capped Mn-doped ZnS QDs is firstly quenched by Cu^{2+}, and then rapidly recovered by tiopronin. The developed QDs-based RTP sensor acts in a turn-on mode and offers high

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http://dx.doi.org/10.1016/j.jlumin.2014.12.043
0022-2313/

sensitivity for tiopronin. Under the optimal conditions, the detection limit of tiopronin can be down to 0.18 ng mL^{-1}. Moreover, the proposed method has some advantages such as simplicity, high sensitivity, selectivity and wide linear range. Importantly, it has been successfully applied to determine tiopronin in biological fluids.

2. Materials and methods

2.1. Materials and chemicals

All chemicals used were of analytical reagent grade. Homocysteine (Hcy), $ZnSO_4 \cdot 7H_2O$, $Na_2S \cdot 9H_2O$, $CuSO_4 \cdot 5H_2O$ and $MnCl_2$ (Tianjing Kermel Chemical Reagent Co., China) were used to prepare Hcy-capped Mn-doped ZnS QDs. Ultrapure water (18.2 MΩ cm) was obtained using a Water Pro water purification system (Labconco Corporation, Kansas City, MO). Tiopronin was provided by (J&K Scientific, Beijing, China).

2.2. Instrumentation

A Cary Eclipse phosphorescence spectrophotometer with excitation wavelength at 295 nm (Varian, American) and equipped with a plotter unit and a quartz cell (1.0 cm × 1.0 cm) was used in this study. The slit widths of excitation and emission were 10 and 20 nm, respectively, for phosphorescence mode with an excitation wavelength of 295 nm and emission wavelength of 590 nm. UV–visible absorption spectra were acquired using a UV-4100 spectrophotometer (Shimadzu, Japan). The QDs were characterized with a JEM-2100 (JEOL, Japan) transmission electron microscope (TEM), and a D8 Advance (Bruker, Germany) X-ray diffractometer (Cu Kα). The samples for TEM were obtained by drying the sample droplets from water dispersion onto a 100-mesh Cu grid coated with a lacey carbon film. The grid was then dried prior to imaging. The resonance light scattering spectra (RLS) were recorded using the same spectrofluorometer by simultaneously scanning the excitation and emission monochromators ($\Delta\lambda = 0$) from 200 nm to 700 nm.

2.3. Synthesis of aqueous Hcy-capped Mn-doped ZnS quantum dots

Hcy-capped Mn-doped ZnS QDs were prepared in our laboratory via previously described procedures [35,36] with minor modifications. The whole process was carried out at deoxygenated condition in an argon atmosphere. Briefly, 50 mL of 0.02 M Hcy, 5 mL of 0.1 M $ZnSO_4$, and 2 mL of 0.01 M $MnCl_2$ were sequentially added into a 100 mL three-necked flask, and pH was adjusted to 11 with 1 M NaOH. After stirring at room temperature for 30 min, 5 mL of 0.1 M Na_2S was rapidly injected into the solution with deoxygenation to allow the nucleation of the nanoparticles. The mixture was stirred for another 20 min, and then the solution was aged at 50 °C under air for 2 h to form Hcy-capped Mn-doped ZnS QDs. The QDs were precipitated with the same volume of ethanol, centrifuged at 4000 rpm for 10 min, washed with ethanol three times to remove unreacted Hcy and dried in a vacuum. The prepared QD powder is highly soluble in water.

2.4. Analytical procedures

To determine the quenching effect of Cu^{2+} on the RTP of Hcy-capped Mn-doped ZnS QDs, we sequentially added 500 μL of 0.01 M PBS buffer solution (pH 9.5), 250 μL of 1 μg mL^{-1} Hcy-capped Mn-doped ZnS QDs, and 200 μL of 1 μg mL^{-1} Cu^{2+} to a 10-mL calibrated test tube. The mixture was diluted to volume with ultrapure water. RTP intensity was recorded every minute for a total 15 min to observe the time course of RTP enhancing.

To evaluate the effect of tiopronin on the RTP restoration of the Cu^{2+}-modulated Hcy-capped Mn-doped ZnS QDs, we sequentially added 500 μL of 0.01 M PBS buffer solution (pH 9.5), 250 μL of 1 μg mL^{-1} Hcy-capped Mn-doped ZnS QDs, 200 μL of 1 μg mL^{-1} Cu^{2+} to a 10-mL calibrated test tube. The mixture was diluted to about 3 quarters of the total volume of the test tube with ultrapure water, and left for 15 min. Then, tiopronin standard solution was added and the mixture was further diluted to volume with ultrapure water. The RTP intensity was recorded every minute for a total time of 15 min to observe the time course of RTP restoration.

To determine Cu^{2+} in the solution of Cu^{2+}-modulated Hcy-capped Mn-doped ZnS QDs in the absence and in the presence of tiopronin, 10 mL of the Cu^{2+}-modulated Hcy-capped Mn-doped ZnS QDs solution was dialyzed against 100 mL Tris–HCl buffer (0.01 M, pH 9.5) for 6 h under continuous gentle shaking. The dialysis bag has molecular weight cut-off of 12,000–14,000. Then, the dialysate was directly analyzed by ICPMS, and calibrated against aqueous standards prepared in Tris–HCl buffer (0.01 M, pH 9.5).

2.5. Real samples

Tiopronin tablets (containing 20 mg) were dissolved in doubly distilled water and then the solution was filtered to obtain a solution of 100 μg mL^{-1} before analysis; The urine and serum samples were collected from healthy volunteers. An appropriate (1000-fold) dilution of all the samples was made before analysis to reduce the effect of matrix. No further complex pretreatment procedures were needed in the sample preparation.

3. Results and discussion

3.1. Characterization of the Hcy-capped Mn-doped ZnS QDs

Size was characterized by measuring the diameter of the QDs via TEM, as shown in Fig. S1A. The image reveals Mn-doped ZnS QDs was nearly monodispersed and exhibited an average diameter of 2.5 nm. The XRD spectra were scanned over the 2θ range of 10–80°, as shown in Fig. S2, the XRD pattern of Hcy-capped Mn-doped ZnS QDs presented a cubic structure, the diffractive peaks of the QDs at 28°, 46° and 57° indicated well-crystallized QDs were obtained by this method. The as-prepared Hcy-capped Mn-doped ZnS QDs demonstrated the maximum excitation peak at 295 nm and a narrow emission band centered at 590 nm (Fig. 1). The energy transfer from the band gap of ZnS to Mn^{2+} dopant and the subsequent transition

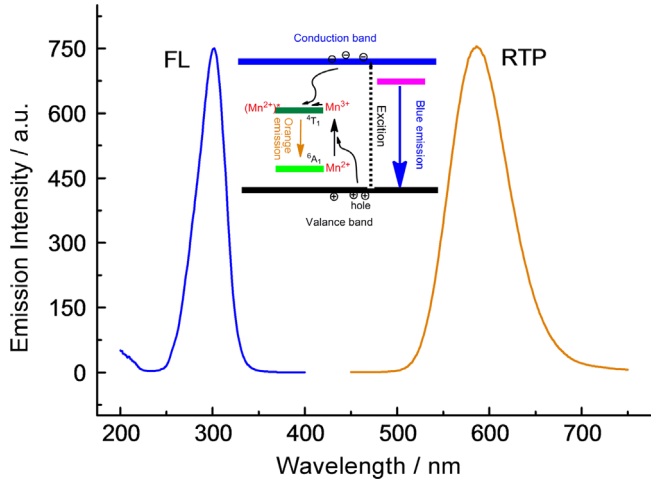

Fig. 1. The excitation and RTP emission spectra of Hcy-capped Mn-doped ZnS QDs (10 ng mL^{-1}). Solutions were prepared in PBS buffer (0.02 M, pH 9.5).