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Quantum dots-based ratiometric fluorescence probe for mercuric ions in biological fluids

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ABSTRACT

Fluorescence analysis by means of a single fluorescence signal output usually leads to the signal fluctuation caused by various external factors. Ratiometric fluorescence probes that can significantly eliminate the external effects by self-calibration of two different emission bands are preferable for the detection of real samples. In this work, we designed a dual-emission quantum dots (QDs) nanocomposite as a ratiometric probe for the visual detection of Hg^{2+}. The dual-emission QDs nanocomposite consists of two differently sized CdTe/CdS QDs. The red-emitting larger sized CdTe/CdS QDs embeded in silica nanoparticles are insensitive to Hg^{2+}, while the green-emitting smaller sized ones are covalently conjugated onto the silica nanoparticles surface and sensitive to Hg^{2+}. The addition of Hg^{2+} can only quench green fluorescence in the dual-emission QDs nanocomposites, which triggers the change of fluorescence intensity ratio of two different emission wavelengths and hence induces the evolution of fluorescence color of the probe solution with variation of Hg^{2+} concentration. Based on this feature, the dual-emission QDs nanocomposites can be used to develop a ratiometric fluorescence probe for the visual detection of Hg^{2+}. Under the optimized conditions, the ratiometric fluorescence QDs probe shows a linear relationship between fluorescence intensity ratio and Hg^{2+} concentration in the range of 5–300 nM. The detection limit of this probe was found to be 3.1 nM. This ratiometric assay also exhibits a high selectivity and it has been successfully used in the determination of Hg^{2+} content in fetal bovine serum and human urine.

1. Introduction

Mercury ion (Hg^{2+}) is a global pollutant issue due to its extensive distribution and serious deleterious effects on human health and environment [1,2]. Therefore, the development of a facile detection method for Hg^{2+} in aqueous media is highly desired. Although many traditional instrumental analytical methods (such as cold-vapor atomic fluorescence spectrometry (CV-AFS), cold-vapor atomic absorption spectrometry (CV-AAS), inductively coupled plasma-mass (ICP-MS) and X-ray absorption spectroscopy *etc.*) can provide a low detection limit and wide detection range, they are always time-consuming, labor-intensive, laboratory-based and usually require the certain expensive sophisticated analytical instruments, which significantly limits their widespread and fast detection applications [3,4]. Thus, there is a great demand for the development of rapid, sensitive, and selective sensing assays for Hg^{2+}. Fluorescence method maybe a preferable choice due to its low cost, simplicity, and sensitivity features. Till now, a large number of organic fluorophore-based fluorescent sensors for Hg^{2+} have been developed [5–10]. However, the relatively tedious synthesis and purification, low fluorescence quantum yields, low water solubility, and poor photostability of organic fluorophores are always the problems to be overcome [11].

As an alternative emitter, semiconductor quantum dots (QDs) have attracted extensive interest and exhibit a promising prospect in various fields due to their unique photophysical properties, such as high photoluminescence quantum yields (PL QYs), substantial photostability, size-dependent PL, high extinction coefficients, and broad excitation window *etc.* [12]. The use of QDs as an optical reporter in analytical fields has been extensively exploited [13–17]. However, most of QDs-based sensors utilize single optical signal output (*i.e.* the fluorescence intensity change of single emission band) for the detection of certain analytes. This single signal mode easily leads to the fluctuation of experimental results due to the non-constant external environment or instrumental conditions. In contrast, sensors on the basis of ratiometric fluorescence can significantly eliminate or reduce the most ambiguities by the self-calibration of two different emission bands [18]. Although some external factors, such as excitation source fluctuations and

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probe concentration may simultaneously affect the fluorescence intensity of the two different emission wavelengths, the fluorescence intensity ratio remains relatively constant. This unique feature evades the possible interferences from the various factors instead of analyte itself and enables ratiometric fluorescence probes to be a promising analysis method.

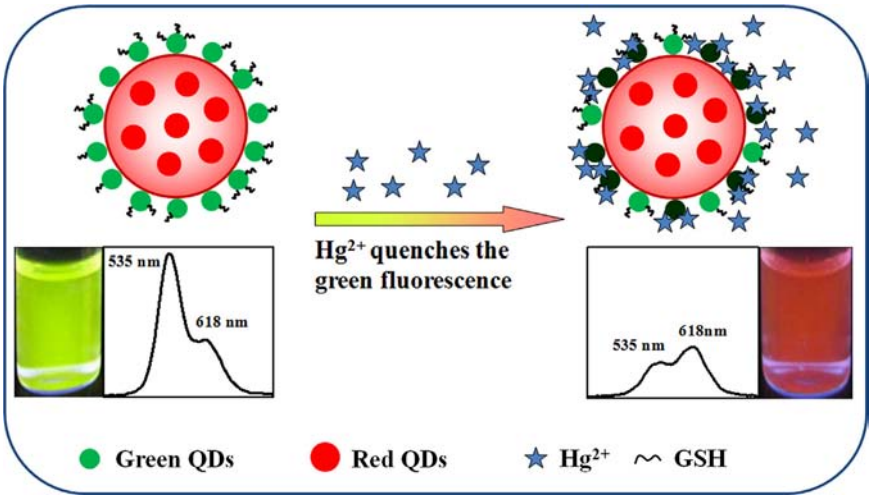
Indeed, QDs-based ratiometric fluorescence probes have been extensively exploited to probe metal ions [19–21], intracellular pH [22,23], nucleic acid hybridization [24], physiological temperature [25], and other small molecules [26–29] in the past few years. However, the detection of Hg^{2+} by means of QDs-based ratiometric fluorescence sensors are rarely studied [30–35]. The probes consisted of a fluorescent QDs and a rhodamine-based Hg^{2+}-sensitive organic dye for the visual detection of Hg^{2+} have been developed [30–32]. They worked in ratiometric fluorescence mode *via* Förster resonance energy transfer (FRET) [30,31] or the change of fluorescence intensity [32]. Recently, a QDs-based dual-emission ratiometric nanoprobe for Hg^{2+} has been reported, in which a fluorescent sphere embedded with CdS QDs acting as internal standard was modified by the outer Hg^{2+}-sensitive CdTe QDs shell [33]. More recently, a single emission source QDs nanoprobe for the visual Hg^{2+} detection by the surface chelating reaction between QDs surface ligands and Hg^{2+} has been developed [34]. Besides, a dual-emission nanohybrid for the visual Hg^{2+} detection has been recently developed by simple mixing blue-emitting carbon dots with red-emitting CdSe/ZnS QDs. The visual detection for Hg^{2+} was realized through the selective fluorescence quenching of red QDs based on the strong chelating ability of surface ligands of QDs to Hg^{2+} [35]. For these QDs-based ratiometric fluorescence probes for Hg^{2+}, the multi-step synthesis and purification of Hg^{2+}-sensitive organic dye [30–32] as well as the relatively hard discrimination from the limited transition fluorescence colors by the naked eye [34] are always the barriers for the visual detection. Moreover, the lack of detection performance for Hg^{2+} in real samples, especially in biological fluids [32,33,35] is really difficult to reasonably evaluate the practicability of the ratiometric fluorescence QDs probes.

In order to further exploit luminescent QDs as the optical signal reporters in ratiometric fluorescence probes, herein we report a QDs-based dual-emission ratiometric fluorescence probe for the visual detection of Hg^{2+} in biological fluids (Scheme 1). The probe consists of two differently sized CdTe/CdS QDs with red and green emission colors, respectively. The red-emitting QDs embedded in silica nanoparticles are insensitive to Hg^{2+} due to the passivation by outer silica shell and the green-emitting smaller QDs attached onto the silica nanoparticles surface is significantly sensitive to Hg^{2+}. The presence of Hg^{2+} can only quench the PL of green QDs, which brings forward a ratiometric fluorescence response with variation of Hg^{2+} concentration. The variation of fluorescence intensity ratio at two emission positions produces an evident evolution of the fluorescence color, which readily facilitates the visual detection of Hg^{2+}. The obtained ratiometric fluorescence probe has good photostability, excellent sensitivity as well as selectivity and has been successfully applied in fetal bovine serum and human urine for the detection of Hg^{2+}.

2. Material and methods

2.1. General

Tellurium powder (200 mesh, 99.8%), cadmium chloride hemi (pentahydrate) ($CdCl_2 \cdot 2.5H_2O$, 99.995%), sodium borohydride ($NaBH_4$, 96%), thiourea (99%), tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTS, 99%), 3-mercaptopropionic acid (MPA, 99%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98.5%), 3-mercaptopropyltrimethoxysilane (MPS, 97%), N-hydroxysuccinimide (NHS, 98%), 2-(N-morpholino) ethanesulfonic acid (MES, 99%), and glutathione (GSH, reduced form, 99+%) were purchased from Sigma-Aldrich. Ammonium hydroxide (25%), $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, and other metal salts ($HgCl_2$, $Mg(Ac)_2$, $Zn(Ac)_2$, $Mn(Ac)_2$, $Cd(Ac)_2$, $BaCl_2$, $NiCl_2$, $CoCl_2$, NaCl, KCl, $Al_2(SO_4)_3$, $Pb(Ac)_2$, $CaCl_2$, $FeCl_3$, $AgNO_3$, $Cu(Ac)_2$) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The certified reference material (GSB 04-1729-2009, Hg^{2+} concentration 1.000 g/L) was provided by National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials (NCATN) and used after dilution. Fetal bovine serum (FBS) was obtained from Gibco. All chemicals were analytical grade and were used as received without further purification. For all operations performed in aqueous media, Milli-Q water was used throughout unless otherwise stated. UV–vis and PL spectra were obtained on a Shimadzu UV-2450 spectrometer and a Cary Eclipse (Varian) fluorescence spectrophotometer, respectively. Transmission electron microscopy (TEM) images were taken on a JEOL JEM-1400 at an acceleration voltage of 100 kV. TEM samples were prepared by depositing a drop of dilute suspension



Scheme 1. Schematic illustration of the working principle of the dual-emission QDs probe for the visual detection of Hg^{2+}. Red QDs are stabilized within the silica nanoparticle and insensitive to Hg^{2+}. Green QDs conjugated covalently to the silica nanoparticle surface are sensitive to Hg^{2+}. The bottom panel shows the PL spectra of the ratiometric fluorescence QDs probe in the presence and absence of Hg^{2+} together with the corresponding fluorescence images under UV light.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of nanoparticles on a copper grid coated with carbon film. The fluorescence lifetime study was performed on an Edinburgh Instruments LifeSpec-ps spectrometer equipped with a Hamamatsu C8898 picosecond light pulser. The excitation light (440 nm) was obtained from a 441 nm laser with frequency of 1.0 MHz.

2.2. Synthesis of the dual-emission ratiometric fluorescence QDs probe

The dual-emission ratiometric fluorescence QDs probe used in this work was obtained through four steps as shown in Scheme 2. The experimental details were described as following and the reaction equations involved in each step were illustrated in Fig. S1.

2.2.1. The synthesis of MPA-capped green and red CdTe/CdS QDs

Water soluble MPA-capped CdTe/CdS QDs were synthesized according to the literature method [36]. Typically, 0.183 g (0.8 mmol) of $CdCl_2 \cdot 2.5H_2O$ and 1.6 mmol (135 μL) MPA was mixed in 40 mL deionized water, and the pH of the mixed solution was adjusted to 10.0 by dropwise addition of 2.0 M NaOH solution under stirring. The mixture placed in a three-necked flask was heated with a heating jacket under N_2 atmosphere. When the temperature reached 100 °C, 8 mL of the pre-prepared NaHTe solution was injected. The mole ratio of Cd:MPA:Te in the reaction solution maintained 1:2:0.2. Nitrogen was steadily pumped in and the temperature was maintained to complete this reaction. Aliquots of sample were taken at different time intervals and their PL spectra were recorded until the PL maximum reached 520 nm. Cooled down the CdTe core suspension to room temperature by removing the heating source. Then, thiourea (0.152 g, 2 mmol) dissolved in ultrapure water was added into the cooled CdTe core solution and the pH of the mixture was adjusted to 10.0 again. The producing mixture was heated to 100 °C under N_2 atmosphere. Timing started when the temperature was up to 100 °C. Aliquots of the sample were taken at different time intervals and their UV–vis and PL spectra were record to monitor the reaction. The red and green emitting CdTe/CdS core/shell QDs used in this work were obtained under different periods of growth, respectively.

2.2.2. Amino groups functionalized silica nanoparticles doped with red CdTe/CdS QDs

For the synthesis of the dual-emission QDs probe, the silica nanoparticles doped with red QDs was firstly obtained according to Stöber method with a slight modification [37]. Typically, 15 mL of EtOH, 5 mL of red QDs (*ca.* 1.5×10^{-5} M) [38], and 20 μL of MPS were mixed and the resulting mixture was stirred for 6 h, after which a mixture containing 0.8 mL of TEOS and 0.8 mL of $NH_3 \cdot H_2O$ was added and the solution was continuously stirred for another 12 h. To obtain a silica surface with amino groups, 70 μL of APTS was added to the above solution and the mixture was stirred for 12 h. The resulting silica nanoparticles doped with red QDs were centrifuged and washed with ethanol for three times and redispersed in ultrapure water.

2.2.3. The synthesis of dual-emission ratiometric fluorescence QDs probe

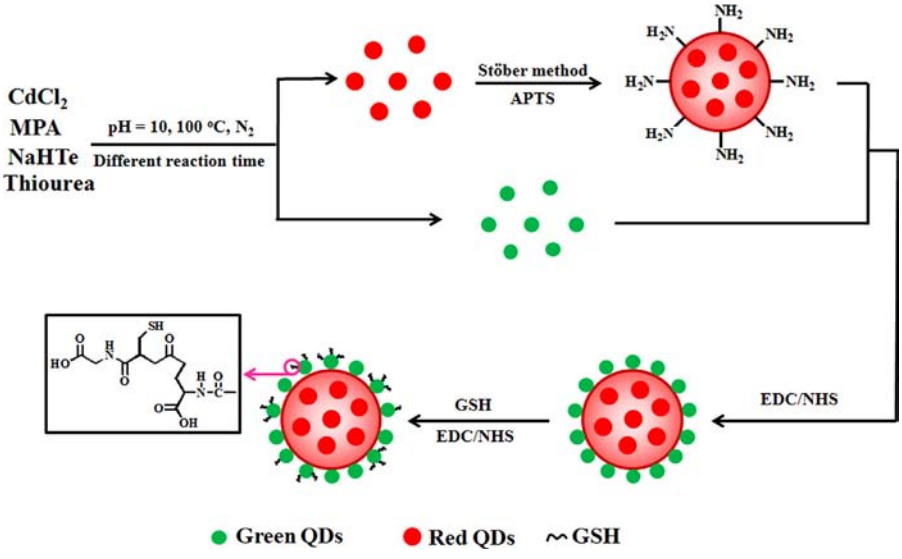
The attachment of green QDs to silica nanoparticles surface was achieved by EDC/NHS coupling method. Typically, the above silica nanoparticles (1 mL, *ca.* 27.6 mg mL^{-1}) was treated with 2 mL of green QDs (*ca.* 3.8×10^{-5} M) in 20 mL of MES buffer (pH 5.6, 0.1 mM) in the presence of EDC/NHS (5 mL, 1 mg mL^{-1}).

2.2.4. The post-modification of dual-emission ratiometric fluorescence QDs probe by GSH

To improve the photostability of the producing dual-emission QDs probe, the further modification was performed by covalently conjugating GSH onto the outer green fluorescent QDs surface *via* EDC/NHS method. The obtained dual-emission QDs probe was centrifuged and washed with ultrapure water and redispersed in 3 mL of ultrapure water (*ca.* 4.7 mg mL^{-1}) for further use.

2.3. Detection of Hg^{2+} ions in aqueous solution

All PL spectra measurements were performed under the same conditions: the slit for both excitation and emission were set at 10 nm, and the excitation wavelength was set at 350 nm. The emission was scanned from 400 to 750 nm. All the detection experiments were carried out at room temperature. A typical procedure for the detection of Hg^{2+} is described as follows: 20 μL of dual-emission ratiometric QDs probe was added to a 2.0 mL of phosphate buffer solution (PBS, 10 mM, pH 8.0) in



Scheme 2. Schematic illustration of the formation of dual-emission ratiometric fluorescence QDs probe for the visual detection of Hg^{2+}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

a 10×10 mm quartz cell under stirring to give a QDs probe solution (*ca.* 47 μg mL^{-1}). The fluorescence spectrum was recorded subsequently and the corresponding fluorescence intensity ratio I_{535}/I_{618} was calculated. Then, 2 μL of Hg^{2+} stock solution with certain concentration was added to the above QDs probe solution and the corresponding PL spectra were recorded at different time intervals. It usually took about 10 min for the stabilization of the PL profile and the relative fluorescence ratios were also calculated. By plotting a series of I_{535}/I_{618} values *vs.* the varying Hg^{2+} concentration, a linear working plot was established.

2.4. Detection of Hg^{2+} ions in biological fluids

To evaluate the applicability of the dual-emission ratiometric QDs probe, the determination of Hg^{2+} in commercial FBS and human urine samples was performed. The human urine samples were collected from two healthy volunteers and used after 100-time dilution. The commercial FBS (used after 50 or 100-times dilution) and original human urine was firstly diluted by PBS (10 mM, pH 8.0) and the resulting diluted solution was used as the probe and analyte carriers. Then 20 μL of ratiometric QDs probe suspension was introduced into the above diluted solution spiked with the known amounts of Hg^{2+} at different concentrations under stirring. After 10 min, the fluorescence spectra were recorded and the fluorescence intensity ratios I_{535}/I_{618} of QDs suspension were calculated. The Hg^{2+} concentrations in real samples were determined by comparing the corresponding I_{535}/I_{618} with the linear working plot (obtained in Milli-Q water).

3. Results and discussion

3.1. Synthesis and characterization of the dual-emission QDs probe

The ratiometric fluorescence QDs probe was readily obtained through two-step reactions (see Experimental 2.2). The ratiometric QDs probe showing a combined yellow green fluorescence color exhibited a dual-emission PL spectrum (535 and 618 nm) in aqueous media (Fig. S2) and the emission positions did not change even the excitation wavelength was adjusted. By modification with GSH the resulting dual-emission QDs nanocomposite showed a relatively high stability (Fig. S3), which may stem form the hindrance effect of the resulting GSH shell. In addition, no significant change of the relative fluorescence ratio (I_{535}/I_{618}) ($< 5\%$) was observed upon exposure to the normal laboratory light for 3 h in PBS (10 mM, pH 8.0) demonstrating a long-term photostability of the dual-emission QDs probe. The prepared ratiometric fluorescence QDs probe was also characterized by TEM technique and the result showed that the dual-emission QDs nanocomposites became nearly monodispersed in aqueous media with an average size of about 24 nm based on the statistic analysis of more than 100 particles (Fig. S4).

3.2. PL response of the dual-emission ratiometric QDs probe to Hg^{2+}

The dual-emission QDs nanocomposites showed a high sensitivity to Hg^{2+} in aqueous media. Upon excitation at 350 nm, the ratiometric QDs probe exhibited two emission peaks centered at 535 and 618 nm, which corresponded to the PL of green QDs on the silica nanoparticles surface and red QDs within the silica nanoparticles, respectively. When introducing Hg^{2+} into the QDs suspension, the green fluorescence from smaller sized QDs was gradually quenched with the increase of Hg^{2+} concentration (from 0 to 1000 nM), while the larger QDs still emitted red fluorescence with a relatively constant intensity (Fig. 1a). Accordingly, the change of the fluorescence intensity ratio at two emission wavelengths triggered a continuous evolution of fluorescence color of the probe solution from yellow green to pale orange and finally to pink color under the irradiation of UV light (Fig. 1a, bottom panel). In addition, the whole fluorescence color change of the dual-emission QDs nanocomposite can be completed within 10 min (Fig. S5). The relatively long incubation time reflects the slow reaction kinetics between QDs nanocomposite and Hg^{2+}. It should be noted that even a slight change in PL intensity ratio can result in a visual color difference from the original background by the naked eye, which enables the dual-emission QDs nanocomposite as the ratiometric fluorescence probe for the visual detection of Hg^{2+}. The advantage of the dual-emission ratiometric fluorescence QDs probe can be demonstrated by comparing the fluorescence color change between our ratiometric fluorescence QDs probe and the single green-emitting QDs by Hg^{2+}, respectively. As shown in Fig. 1b, the identical quenching extent of green QDs by Hg^{2+} does result in the most evident difference in fluorescence color. The resulting fluorescence images of single green QDs suspensions under a UV lamp are yet hard to distinguish each other by the naked eye. The comparison clearly suggests that the dual-emission ratiometric QDs

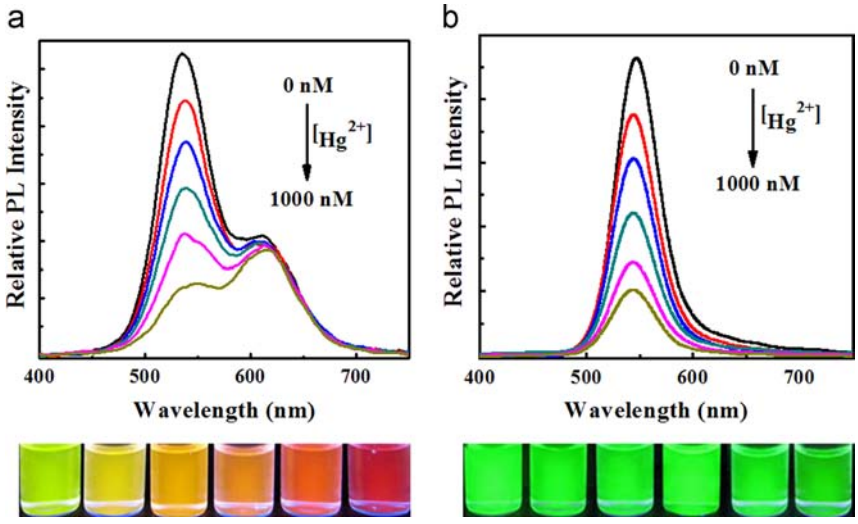


Fig. 1. The comparison of PL spectra (λ_{ex}=350 nm) of (a) ratiometric fluorescence QDs probe and (b) the single green QDs upon exposure to the varying Hg^{2+} concentrations (0, 10, 100, 200, 400, 1000 nM). Bottom panel shows the corresponding fluorescence images in (a) and (b). All photos were taken under a UV lamp.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

probe may be more suitable for the visual detection of Hg^{2+} than the traditional single fluorescence quenching method [39–41].

3.3. Effect of experimental variables on optical response of the ratiometric fluorescence QDs probe toward Hg^{2+}

As mentioned above, the dual-emission ratiometric QDs nanocomposites can be utilized to develop a ratiometric fluorescence probe for Hg^{2+}. So it is necessary to optimize the experimental variables for Hg^{2+} detection by the use of the designed ratiometric fluorescence QDs probe. In order to make the probing system work well under specific conditions, we investigated the effects of two experimental parameters, pH value of QDs suspension and the mole ratio between green QDs and red QDs in ratiometric fluorescence QDs nanocomposites, on the detection performance of the probe.

3.3.1. Effect of pH value of dual-emission ratiometric QDs probe suspension on its PL response toward Hg^{2+}

As for metal ions probes, the pH value of solution is an important factor to affect the detection sensitivity of the probe due to many metal ions such as Cu^{2+}, Zn^{2+}, Hg^{2+}, and Cd^{2+} *etc.* can be converted into the corresponding metal hydroxide or oxide under certain pH. In this work, the dual-emission ratiometric QDs probe showed the best stability at pH 8.0 (PBS, 10 mM) as shown by the ratio of I_{535}/I_{618}, which reflects to some extent the stability of the ratiometric fluorescence QDs probe. Acidic and high basic conditions would significantly influence the I_{535}/I_{618} of the ratiometric fluorescence QDs probe (Fig. 2), which could be derived from high ionic strength or the interparticle crosslink of QDs nanocomposites that induced the aggregation of QDs nanocomposite. Considering the fact that the normal physiological environment is less alkalescent and higher pH prefers to convert Hg^{2+} to the corresponding $Hg(OH)_2$, the pH 8.0 was used as the standard value in subsequent detection.

3.3.2. Effect of the mole ratio of green QDs to red QDs in dual-emission ratiometric QDs probe on its PL response toward Hg^{2+}

The advantage of ratiometric fluorescence probe is to utilize the systematic fluorescence ratio change of the probe solution to reflect the change of analyte concentration, which significantly improves the detection accuracy and sensitivity. Thus, it is anticipated that the original fluorescence intensity of two emissions will greatly affect the overall detection sensitivity of ratiometric probes. In our case, it refers to the PL intensity of green QDs and red QDs, respectively. Assuming two differently sized QDs obtained from the same synthetic method have the same level for PL QYs, the original PL intensity of green and red QDs are basically proportional to the amounts of the corresponding sized QDs in the resulting ratiometric fluorescence probe. As shown in Fig. S6, the dual-emission ratiometric QDs probe with different mole ratios between green and red QDs exhibited the similar PL spectra change. With the increase of Hg^{2+} concentration, the PL intensity of green QDs was gradually decreased while the red ones remained constant. However, the fluorescence color change of the corresponding probe systems showed the very different trends (Fig. 3). The dual-emission ratiometric QDs probe with the larger mole ratios of green to red QDs (8.7:1 and 5.5:1) exhibited a color transition from green to pale green color and those with the smaller mole ratios (0.6:1 and 1.4:1) possessed a color transition from pale yellow to pink color. No richer intermediate colors that can be distinguished by the naked eye are observed in these cases. On the contrary, the ratiometric fluorescence QDs probe with mole ratio of 2.5:1 demonstrated the richest combination fluorescence color transition from yellow green to orange, and finally to pink, which facilitated the visual detection of Hg^{2+}. Therefore, the dual-emission ratiometric QDs probe with mole ratio of green to red QDs (2.5:1) was used as ratiometric fluorescence QDs probe in subsequent Hg^{2+} detection.

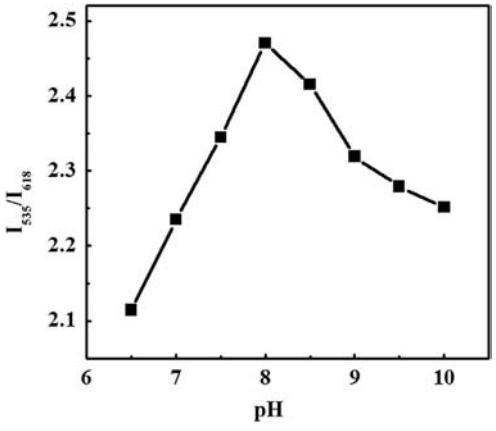


Fig. 2. The fluorescence intensity ratios I_{535}/I_{618} of the dual-emission ratiometric QDs probe under different pH values. (λ_{ex}=350 nm).

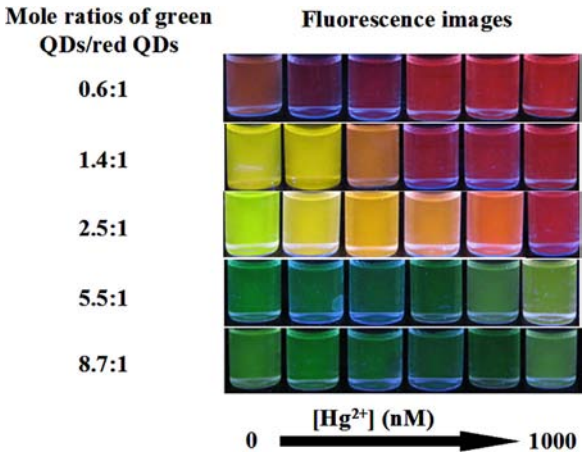


Fig. 3. The photographic images of the dual-emission ratiometric QDs probe with different mole ratio of green to red QDs in the presence of the varying Hg^{2+} concentration (from left to right: 0, 10, 100, 200, 400, 1000 nM). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

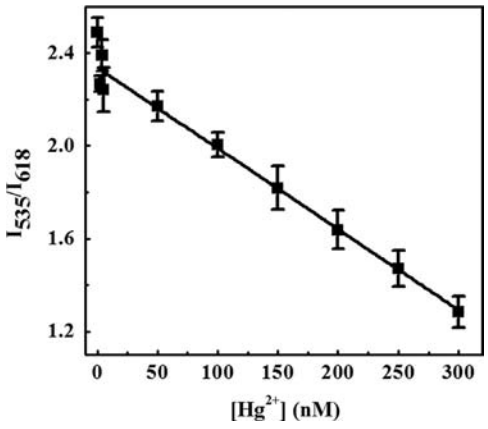


Fig. 4. Linear plot of relative PL response (I_{535}/I_{618}) of the ratiometric fluorescence QDs probe toward Hg^{2+} *vs.* Hg^{2+} concentration. The error bars represent the standard deviation of three measurements.

3.4. Detection of Hg^{2+} based on the dual-emission QDs composite

The specific detection performance of the dual-emission ratiometric QDs probe for Hg^{2+} was subsequently evaluated in aqueous media. Under the optimized conditions, I_{535}/I_{618} showed a linear relationship with the Hg^{2+} concentration from 5 to 300 nM with the correlation coefficient $R^2=0.988$ as shown in Fig. 4. According to the IUPAC 3σ criterion, as the concentration of analyte that produces an analytical signal equaling to three times the standard deviation of the background fluorescence signal, the limit of detection (LOD) was calculated to be 3.1 nM. The detection limit of our ratiometric fluorescence QDs probe for Hg^{2+} is comparable with that of the QDs-based dual-emission nanoprobe (5.6 nM) [33] and much lower than those by other ratiometric fluorescence probes [30,31,33–35,42]. To provide a detailed comparison we compiled Table 1 for the specific comparison of this work and selected fluorimetric methods in the linear range and detection limit. Furthermore, the relative standard deviation for three independent measurements with Hg^{2+} (200 nM) is 5.0% implying that the optical response of the ratiometric fluorescence probe toward Hg^{2+} is relatively reproducible. The high sensitivity of this probe for Hg^{2+} can be attributed to the native feature of ratiometric fluorescence probes, *i.e.* a slight change in the ratio of two emission intensity can result in a visual fluorescence color change. Moreover, the fluorescence intensity ratio is independent of the various external variables. In addition, high PL QYs of CdTe/CdS core/shell QDs also contributed partly to the sensitivity. To demonstrate the utility of our probe for the visual detection of Hg^{2+}, the certified reference material GSB 04-1729-2009 was selected to analyze by our probe and the found value (10.5 μg/L) was very close to the real value (10.0 μg/L). The relative standard deviation (RSD) for five repeated measurements was 2.6% showing the reliability of our ratiometric fluorescence probe. Generally, the high sensitivity together with the easy operation enables this ratiometric fluorescence QDs probe suitable for the visual detection of Hg^{2+} in aqueous media.

3.5. Selectivity

In order to evaluate the selectivity to Hg^{2+}, the PL response of this dual-emission ratiometric QDs probe in the presence of other common ions were also investigated. As shown in Fig. 5, the presence of metal ions such as Mg^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Ba^{2+}, Ni^{2+}, Na^{+}, K^{+}, Al^{3+}, Pb^{2+}, Ca^{2+}, Co^{2+}, and Fe^{3+} even with concentrations 100 times higher than Hg^{2+} concentration can not give the most distinctive fluorescence color difference as Hg^{2+} did, which suggested an excellent selectivity of this ratiometric fluorescence QDs probe over these metal ions. Treated the ratiometric fluorescence QDs probe with Cu^{2+} or Ag^{+} at the same concentration as Hg^{2+} (1 μM), a slightly positive optical response was observed (Fig. 5). It should be noted that the PL quenching of QDs by certain transition metal ions like Cu^{2+}, Ag^{+}, and Hg^{2+} *etc.* is a common phenomenon [43,44]. The present result shows the ratiometric fluorescence QDs probe has the higher selectivity for Hg^{2+} than Cu^{2+} and Ag^{+}. The practical interference from these two metal ions, especially for Cu^{2+}, can be significantly eliminated by diluting the original samples. In addition, the existence of Zn^{2+} or Cd^{2+} produced a verse effect with a larger fluorescence intensity ratio value, which could stem from the re-passivation of green emitting QDs by the instantaneously formed microcrystals [45]. In a word, the dual-emission ratiometric QDs probe exhibits a high selectivity to Hg^{2+}.

In order to verify the excellent probing performance of our ratiometric fluorescence QDs probe toward Hg^{2+} in practical applications, the selective optical response with the co-presence of other mental ions was also evaluated and the results were illustrated in Fig. 6. Experimental results indicated that the ratiometric fluorescence QDs probe suspension with the co-presence of common ions including Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+},

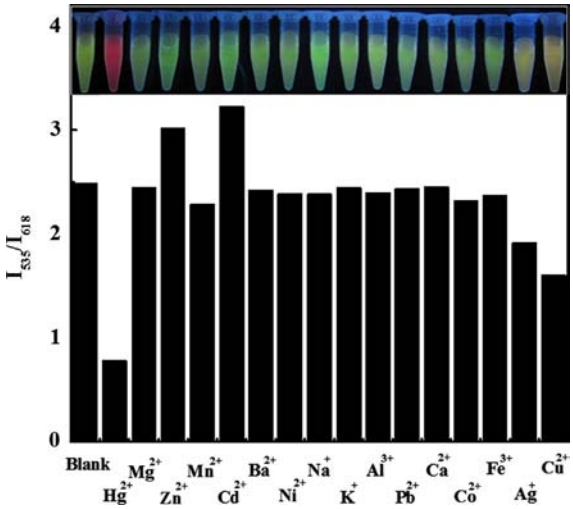


Fig. 5. The I_{535}/I_{618} ratios of the dual-emission ratiometric QDs probe (47 μg mL^{-1}) containing various metal ions (Hg^{2+}, Ag^{+}, and Cu^{2+} at 1.0 μM; Fe^{3+} and Co^{2+} at 10.0 μM; others at 100.0 μM) in PBS (10 mM, pH 8.0) (λ_{ex}=350 nm). The inset shows the corresponding photographic images of QDs probe suspensions under a UV lamp.

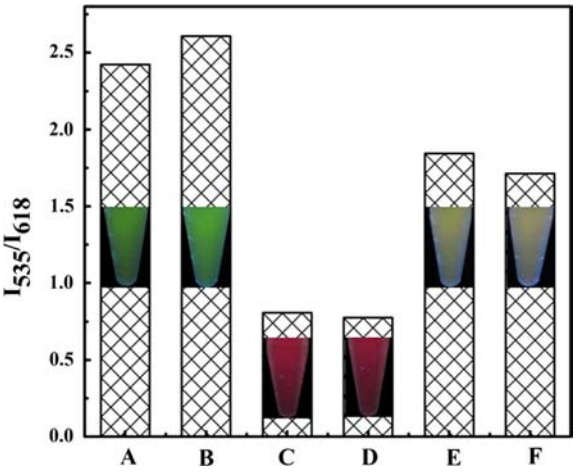


Fig. 6. The ratios I_{535}/I_{618} of ratiometric fluorescence QDs probe suspension (47 μg mL^{-1}) in PBS (10 mM, pH 8.0) (A), A solution with the co-presence of Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Fe^{3+}, and Pb^{2+} (each at 10.0 μM) (B), B solution with Hg^{2+} (1.0 μM) (C), A solution with Hg^{2+} (1.0 μM) (D), and B solution with Ag^{+} (1.0 μM) (E) or Cu^{2+} (1.0 μM) (F) (λ_{ex}=350 nm). The insets show the corresponding photographs of various solutions from A to F.

Table 1
Comparison of the main characteristics of the selected fluorimetric methods for the determination of Hg^{2+}.

Probes	Linear range ($\times 10^{-7}$ M)	LOD (nM)	References
DOB-CdTe QDs	0.08–30	4.2	[16]
Bi-color CdTe QDs multilayer films	0.1–10	4.5	[17]
RhB-CdSe/ZnS QDs	–	395	[30]
RhB-CdTe QDs/SiO_2	0–100	260	[31]
RhB6G-CdTe QDs/SiO_2	0.4–8	2.59	[32]
CdTe/CdS-chitosan	0–18	5.6	[33]
HDTC-CdSe/ZnS QDs	0.1–1.4	5	[34]
C-dots/GDTC-CdSe/ZnS QDs	2–10	100	[35]
This work	0.05–3	3.1	–

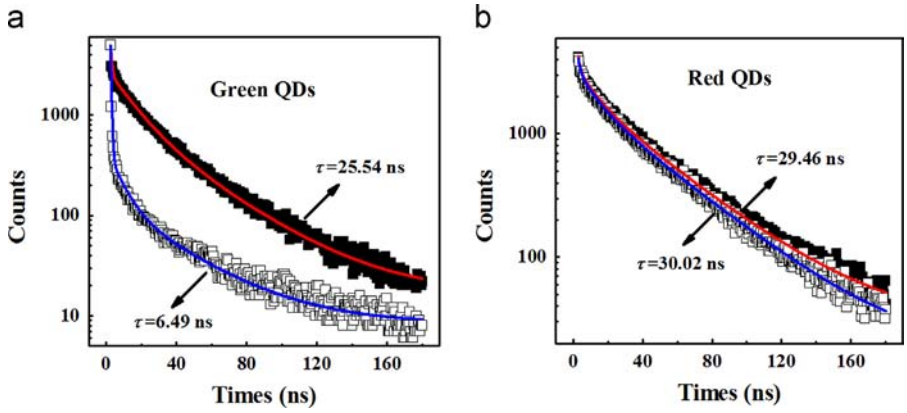


Fig. 7. Normalized PL decay curves of green QDs (a) and red QDs (b) in the dual-emission ratiometric QDs probe in the absence (closed squares) and presence of Hg^{2+} (1 μM, open squares). The red and blue lines represent the corresponding fitting curves. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Cd^{2+}, Mn^{2+}, Fe^{3+}, and Pb^{2+} except for Hg^{2+} did not induce the evident change in PL spectra and the solution fluorescence color was basically as same as the blank suspension (without any metal ions) (bar A and B in Fig. 6). We also performed the control test by the addition of Hg^{2+} (1.0 μM) into the mixed ions (each at 10.0 μM) (bar C in Fig. 6). It was found that the PL spectrum (or fluorescence color) of QDs probe suspensions with Hg^{2+} gave the similar response in comparison with the system containing Hg^{2+} alone (bar D in Fig. 6). The control studies by mixing Cu^{2+} (1.0 μM) or Ag^{+} (1.0 μM) with the above metal ions mixture except for Hg^{2+} also exhibited the similar optical response (bar E and F in Fig. 6) as the ratiometric fluorescence QDs probe suspensions containing Ag^{+} (1.0 μM) or Cu^{2+} (1.0 μM) alone. These results suggest that the ratiometric fluorescence QDs probe as a potential Hg^{2+} probe has a high selectivity and that the co-presence of other common metal ions produce a negligible effect on the optical response.

3.6. Possible mechanism

In order to determine the nature of PL quenching of green QDs by Hg^{2+}, the time-resolved fluorescence spectroscopy was performed. As the probe consisted of two differently sized QDs (*i.e.* red and green QDs), the fluorescence lifetimes were studied by monitoring two emission positions. In the absence of Hg^{2+}, the excited state of green and red emitting QDs decayed tri-exponentially with an average lifetime of 25.54 and 29.46 ns, respectively (the detailed fluorescence lifetime data were comprised in Table S1). After adding Hg^{2+} into the probe suspension, the lifetime of green QDs was substantially decreased from 25.54 to 6.49 ns, while red QDs sustained the relatively constant lifetime (Fig. 7). It has been established that a decrease in the fluorescence lifetime of QDs can be observed when an electron or hole acceptor is adsorbed onto the semiconductor surface [46]. Thus, the decreased exciton average lifetime for green QDs in the ratiometric fluorescence QDs probe implied that the added Hg^{2+} was absorbed onto the surface of green QDs due to the existing dangling bonds on QDs surface. This hypothesis was also supported by UV–vis absorption spectroscopy. The first excitonic absorption peak of original green-emitting CdTe/CdS QDs was shifted to longer wavelength by 10 nm in the presence of Hg^{2+} (Fig. S7). The similar absorption behaviors were also observed in previous reports [47–49]. The adsorption of Hg^{2+} on green QDs surface changed the surface states of QDs and thus facilitated the electron transfer or the other non-radiative electron/hole recombination annihilation. As a result, the PL of QDs decreased remarkably. In contrast, the preservation of the original lifetime for red QDs upon adding Hg^{2+} was contributed to the protection of outer compact silica shell that prevented the potential disruption from the external environments. As a result, the intrinsic recombination of electron-hole pair of QDs was not affected and the constant PL intensity and lifetime were observed.

Table 2
Application of the ratiometric fluorescence QDs probe for Hg^{2+} detection in biological fluids.

Samples	Added (nM)	Measured (nM)	Recovery (%)	RSD[a] (%)
FBS (diluted 50 times)	50.0	55.7	111.4	4.4
	100.0	95.8	95.8	1.8
FBS (diluted 100 times)	50.0	52.4	104.8	3.4
	100.0	94.5	94.5	2.6
Human urine-1	50.0	51.8	103.8	5.1
	100.0	101.4	101.4	2.9
Human urine-2	50.0	49.1	98.2	2.4
	100.0	102.5	102.5	4.1

[a] Relative standard deviations were calculated with $n=5$.

3.7. Detection of Hg^{2+} in real samples

To demonstrate the practicability of the ratiometric fluorescence QDs probe for the detection of Hg^{2+}, the detection performance was evaluated in biological fluids. Diluted FBS and human urine samples were used as the carriers for ratiometric fluorescence QDs probe and Hg^{2+}. It should be noted that Hg^{2+}, as a highly toxic metal ions, exists in biological fluids at extremely low concentration. The Hg^{2+} content in these real samples was established by spiking Hg^{2+} at different concentration levels. Without contamination of Hg^{2+}, sample solutions with dispersed ratiometric fluorescence QDs probe emitted a yellow green fluorescence color as observed in pure water. Upon spiking Hg^{2+} at different concentrations, a visible and evident fluorescence color transition could be observable. The corresponding optical response (I_{535}/I_{618}) was compared with the standard working plot to evaluate the utility of the ratiometric fluorescence QDs probe for Hg^{2+} detection in real samples and the results were summarized in Table 2. It was found that the obtained Hg^{2+} concentration from working plot was in good agreement with that added. It should be noted that many cations ions and/or nutrient components are present in FBS and human urine. Our results show that the presence of these co-existing components in these biological fluids has a negligible effect on the optical response of the ratiometric fluorescence QDs probe and almost does not interfere with the visual detection of Hg^{2+}. This fully clarifies the practicability of the dual-emission ratiometric QDs probe for the visual detection of Hg^{2+}.

4. Conclusions

In summary, we have developed a quantum dots-based dual-emission ratiometric fluorescence probe for the visual detection of Hg^{2+}. The ratiometric fluorescence probe was facilely constructed by the use of two differently sized CdTe/CdS QDs corresponding to green and red fluorescence colors, respectively. The resulting QDs nanocomposite exhibited a dual-emission feature. Due to direct exposure to exterior environment, the outer green QDs were very sensitive to Hg^{2+} and the green fluorescence was gradually quenched with the increase of Hg^{2+} concentration. In contrast, red QDs embed in silica nanoparticles can maintain the constant fluorescence intensity. Thus, the continuous increase of Hg^{2+} content in the dual-emission QDs suspension changed the fluorescence intensity ratios of two sized QDs at two emission wavelengths and induced simultaneously the evolution of fluorescence color of probe solution, which facilitated the visual detection of Hg^{2+}. The ratiometric fluorescence QDs probe showed a linear relationship between fluorescence intensity ratios and Hg^{2+} concentration and the LOD was down to 3.1 nM. This ratiometric fluorescence assay exhibits the high selectivity and sensitivity to Hg^{2+} and has been successfully used to determine the Hg^{2+} content in biological fluids.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.036.

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