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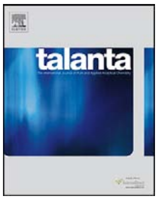

Rhodamine-based ratiometric fluorescence sensing for the detection of mercury(II) in aqueous solution

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ABSTRACT

Novel ratiometric fluorescent silica nanoparticles with high selectivity towards Hg^{2+} were synthesized for the detection of Hg^{2+}. Hg^{2+} promoted the ring opening of spirolactam in the rhodamine moiety grafted onto the silica nanoparticles, resulting in a change in the fluorescence intensity. The fluorescence intensity was proportional to the Hg^{2+} concentration, and the detection limit (S/N = 3) for Hg^{2+} was found to be 2.59×10^{-9} mol L^{-1} with a linear range from 0.4 to 8×10^{-7} mol L^{-1}. In addition, the morphology of the silica nanoparticles, the effects of pH and co-existing substances, and the reversibility were investigated. The proposed approach was successfully applied to the determination of Hg^{2+} in water samples.

1. Introduction

Mercury is one of the most dangerous and ubiquitous of pollutants [1]. Its contamination is widespread and arises from a variety of natural sources [2], and it causes serious environmental and health problems because it can easily pass through skin, respiratory, and gastrointestinal tissues into the human body, where it damages the central nervous and endocrine systems [3]. Due to the high toxicity of mercury, considerable attention has been devoted to explore new fluorescence chemosensors [4–8] for analyzing Hg^{2+} with sufficient selectivity. However, most of them suffer from cross-selectivity towards other metal cations, simultaneous drifts or fluctuations of individual signals [9]. Thus, it becomes more important to establish an accurate ratiometric fluorescence approach with good selectivity, low detection limit, easy usage and good reproducibility.

Since the ratio value of fluorescence intensity contributes more reliable results compared to the absolute intensity, in this study ratiometric fluorescence was selected and applied. In application, however, the number of available organic dyes with the suitable excitation wavelength and distinguishable emission spectrum is limited, which restricts the universality of the present ratiometric fluorescence approaches. There have been many studies of quantum dots (QDs) in fluorescence sensing since their emission is wave-tunable by simply controlling the size and using a wide excitation wavelength. We therefore chose QDs to provide the fluorescence background in the ratiometric fluorescence approach. In the study, QDs were immobilized in silica nanoparticles, which provided an effective matrix to avoid the effects of the surrounding environment, for example, the quenching effect caused by heavy metal cations could be effectively minimized. The well-known process that the spirolactam [10] was conversed to ring-opening structure of rhodamine derivatives [11,12] was employed for the selective analysis of Hg^{2+}. In the study, we selected rhodamine 6G (Rh6G) as a fluorescent reagent, and it was grafted onto the surfaces of silica nanoparticles to compose QDs@SiO_2-Rh6G nanoparticles as shown in Scheme 1. In addition, a series of experiments characterizing the sensing approach based on the QDs@SiO_2-Rh6G nanoparticles were carried out, including morphological measurement of the nanoparticles, the reproducibility and reversibility of the sensing approach, and the effects of pH and co-existing substances. The proposed sensing approach was successfully applied to the determination of Hg^{2+} in water samples.

2. Experimental

2.1. Chemicals and instruments

Rhodamine 6G, tetraethoxysilane (TEOS) and 3-aminopropyltetramethoxysilane (3-APTMS) were purchased from Sigma–

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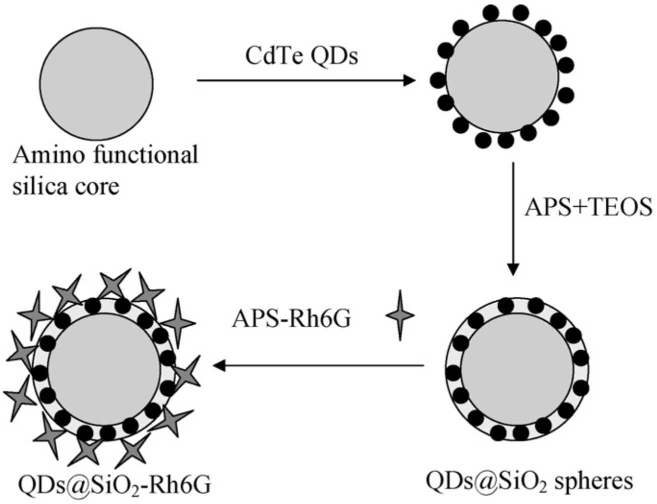


Scheme 1. Synthetic procedure to obtain QDs@SiO$_2$-Rh6G spheres.

Aldrich (Milwaukee, WI, USA). Phosphate buffer solution (PBS, pH 7.0) and other reagents used were of analytical reagent grade without further purification. All aqueous solutions were prepared with pure water from a Millipore Autopure WR600A system (Millipore, Ltd., USA).

2.1.1. *Characterization of particles*

The fluorescence spectra of QDs@SiO$_2$-Rh6G nanoparticles were recorded with an F-4500 spectrofluorometer (Hitachi, Japan), and their morphology was examined with a Tecnai F30 (FEI Co. USA) transmission electron microscopy (TEM). Sample preparation was carried out by placing a drop of the freshly prepared colloidal solution on a carbon-coated copper grid and allowing the solution to evaporate.

2.2. *Preparation of QDs@SiO$_2$-Rh6G*

2.2.1. *Silica cores*

Silica cores were prepared by a typical Stober-based synthesis method [13]. To prepare the silica cores, TEOS (1.0 mL) and anhydrous ethanol (5.3 mL) were added to a clean glass reaction vessel containing anhydrous ethanol (20 mL), ammonium hydroxide (28%, 1.9 mL) and pure water (1.63 mL). The mixture was stirred for 24 h. To modify the silica surface with amino groups, 3-APTMS (0.20 mL) was added to the above solution and stirred for another 24 h. After the reaction, the prepared samples were centrifuged at 13000 rpm for 10 min to collect the silica cores. The cores were further washed with ethanol and pure water by centrifugation and decantation several times to remove the unreacted chemicals. The obtained silica particles were dried in a desiccator before use.

2.2.2. *QDs@SiO$_2$ spheres*

CdTe-QDs were synthesized by microwave irradiation according to the literature method [14]. Then, 0.5 mL CdTe-QDs were precipitated by the addition of pure ethanol. The resultant precipitate was centrifuged and washed with ethanol several times. The precipitate was then redispersed in 2 mL pure water and mixed with silica cores (10 mg) modified with amino groups in 2 mL pure water and 0.5 mL PBS (pH 7.0). The reaction mixture was stirred for 8 h at room temperature in the dark. The resulting QDs@SiO$_2$ spheres were isolated by centrifugation and washed with pure water to remove the excess QDs. After this procedure, silica spheres coated with CdTe-QDs were further coated with an outer layer of silica in water/ethanol (1:4, 5 mL) containing 15 μL APTS and 10 μL TEOS [15–17]. After the hydrolysis and condensation of the silanes on the surface of the composite spheres (24 h), an outer silica shell was formed. The obtained silica nanoparticles were isolated by centrifugation and redispersed in 5 mL pure water.

2.2.3. *Synthesis of QDs@SiO$_2$-Rh6G*

Rhodamine 6G (49.8 mg) in 5.0 mL ethanol was mixed with 0.20 mL 3-APTMS. The reaction solution was refluxed for 6 h. After cooling to room temperature, the solvent was then evaporated. The resulting solid was diluted with 5.0 mL anhydrous toluene, and then 0.5 mL of the obtained solution was added to QDs@SiO$_2$ spheres (5 mL) and stirred in reflux conditions under N$_2$ for 24 h. The collected solid was washed several times with methylene chloride, acetone and pure water to rinse away any surplus chemicals. After this, the solid was redispersed in 6 mL pure water.

2.2.4. *Detection of Hg^{2+}*

Different amounts of Hg^{2+} in aqueous solution were added to clean, calibrated glass test tubes containing the above QDs@SiO$_2$-Rh6G solution (0.2 mL) and 1 mL PBS. Then the solution was diluted to the mark with water and mixed thoroughly.

3. Results and discussion

3.1. *Characterization of the prepared silica spheres*

Generally, the silica core size could be controlled by changing the amount of ammonium hydroxide in the reaction mixture

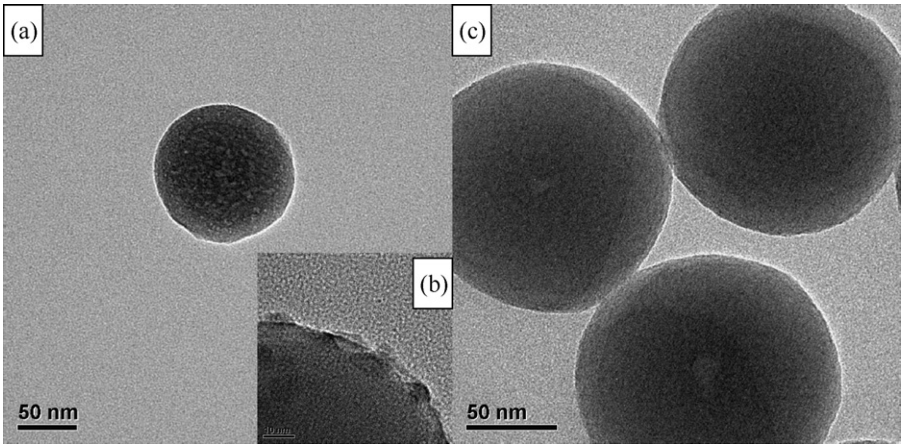


Fig. 1. Morphological observations of nanoparticles: (a) TEM image of silica cores; (b) HRTEM image of silica cores assembled with CdTe-QDs; (c) TEM image of QDs@SiO$_2$-Rh6G.

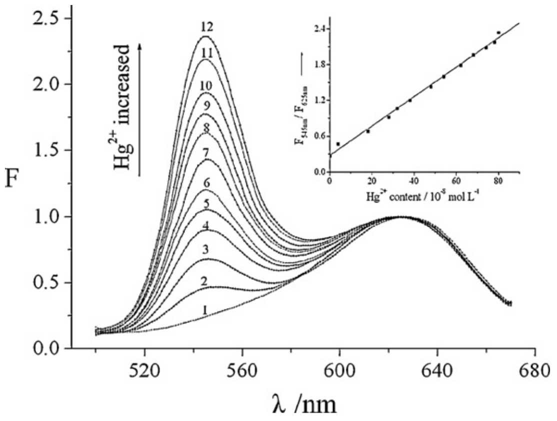


Fig. 2. Response of QDs@SiO$_2$-Rh6G towards various concentrations of Hg^{2+}. Inset: correlation between the intensity ratio and the added amounts of Hg^{2+} ions.

[13]. In the experiment, high-resolution transmission electron microscopy (HRTEM) was used for the characterization of the silica spheres. As shown in Fig. 1a, the diameter of the silica cores modified with amino groups was about 100 nm. A typical HRTEM image of the QDs@SiO$_2$ spheres is shown in Fig. 1b. It can be observed that many CdTe-QDs were dispersed on the surface of each silica core. The TEM image of the targeted QDs@SiO$_2$-Rh6G as shown in Fig. 1c indicates that the QDs@SiO$_2$-Rh6G in aqueous solution was well-dispersed without aggregation. The thickness of the outer shell could be controlled by the amount of TEOS added [15–17].

3.2. *Characteristics of the Hg^{2+} sensing nanoparticle*

As shown in Fig. 2, the maximal emission wavelength of QDs@SiO$_2$-Rh6G was at 545 nm. With an increase of Hg^{2+} concentration, the fluorescence intensity of QDs@SiO$_2$-Rh6G increased. Experimental results revealed that there was a linear dependence of fluorescence intensity on the concentration of Hg^{2+}, and the linearity was found to be from 0.4 to 8×10^{-7} mol L^{-1}, with a correlation coefficient of 0.997 (inset in Fig. 2). The detection limit was found to be 2.6×10^{-9} mol L^{-1}. Compared to the previous published methods [18–20] for Hg^{2+} (limits of detection about 1 μM), the approach described in this paper was much lower. Thus the new method could be used for high-sensitivity Hg^{2+} determination.

3.3. *Effect of pH*

For practical application, the pH effect on the detection sensitivity for the method was studied. The effects of pH on the nanoparticles were measured and evaluated. As shown in Fig. 3, in an acid medium (pH < 5.29), ring opening of rhodamine derivatives took place causing the fluorescence intensities of QDs@SiO$_2$-Rh6G at 545 nm to increase, because of the strong protonation of the rhodamine [21]. When the pH was beyond 5.29, no significant ring-opening phenomenon could be observed. However, in the presence of Hg^{2+}, the fluorescence of QDs@SiO$_2$-Rh6G recovered and its intensity obviously increased. The fluorescence intensity remained constant in the pH range from 5.29 to 9.18. In this region, Hg^{2+} induced a remarkable fluorescence OFF–ON change. Thus, PBS (pH 7.0) was selected and applied in this study.

3.4. *Reversibility*

In the determination of specific analytes, the reversibility of the chemical sensing approach employed is an important aspect. In this work, the reversibility of the system established was studied by the introduction of iodide anion (I$^-$) as shown in Fig. 4. When 0.2 mL prepared QDs@SiO$_2$-Rh6G solution was exposed to 8.0×10^{-7} mol L^{-1} Hg^{2+}, the fluorescence emission at 545 nm was increased (Fig. 4(1)). The result can be explained that Hg^{2+} induced the spirolactam ring of rhodamine derivatives open. However, fluorescence disappeared upon addition of 4 equiv of KI to the Hg^{2+} (Fig. 4(2)). This phenomenon could be attributed to the stronger binding ability of the iodide anion (I$^-$) toward Hg^{2+} [22,23], inducing the spirolactam of the rhodamine derivatives to close again. When excess Hg^{2+} was added to the system, Hg^{2+} reacted with the spirolactam again because I$^-$ had been reacted out. The spirothiolactone ring opening took place and fluorescence could be produced again (Fig. 4(3)). Therefore, the results showed that spectral sensing was reversible.

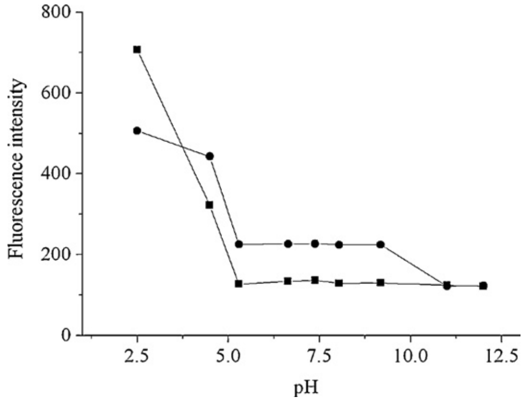


Fig. 3. Fluorescence intensity of QDs@SiO$_2$-Rh6G (–■–) and their sensing responses (–●–) for Hg^{2+} in solutions of different pH.

3.5. *Effects of co-existing substances*

The ability of the method established above to selectively detect metal ions is an important requirement for practical applications. Therefore, changes in the fluorescence properties of QDs@SiO$_2$-Rh6G caused by co-existing metal ions were investigated in the study. When the tolerated limit for a foreign substance was taken as the largest amount yielding a relative error of less than 10% for the determination of 6.0×10^{-7} mol L^{-1} Hg^{2+}, no obvious effects could be found in the presence of 5.0×10^{-3} mol L^{-1} K$^+$, Na$^+$; 5.0×10^{-4} mol L^{-1} Ca^{2+}, Ba^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}; 1.0×10^{-4} mol L^{-1} Ni^{2+}, Co^{2+}, Al^{3+} or Fe^{3+}; and 2.0×10^{-5} mol L^{-1}, Ag$^+$, Cu^{2+}, Pb^{2+}, or Cd^{2+}. This high selectivity of the synthetic nanoparticles was in accordance with previous reports [23–26], revealing that the spiro-

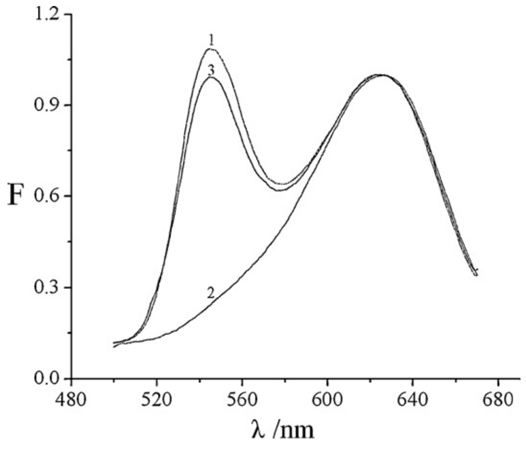
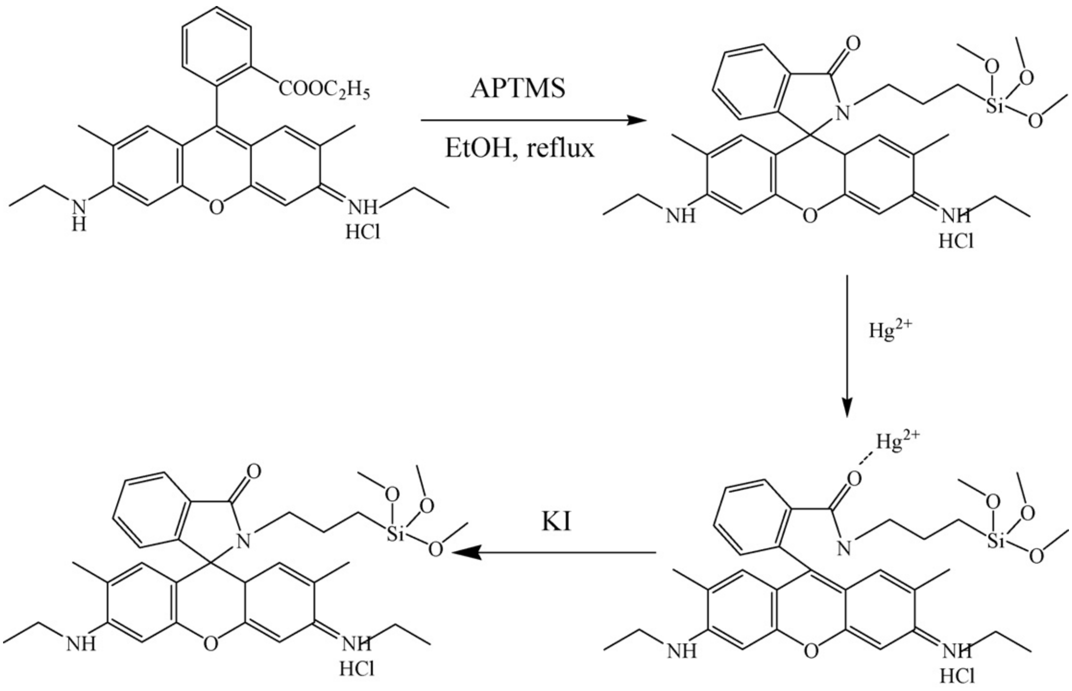


Fig. 4. Fluorescence spectra of 0.2 mLQDs@SiO$_2$-Rh6G + 8.0×10^{-7} mol L^{-1} Hg^{2+} (1); (1) + 3.2×10^{-6} mol L^{-1} KI (2); (2) + 4.0×10^{-6} mol L^{-1} Hg^{2+} (3).



Scheme 2. Hg^{2+}-induced ring opening and cyclization of rhodamine derivatives.

lactam of rhodamine derivatives had a response specific to Hg^{2+} over other prevalent elements in the environment.

3.6. *Precision and reproducibility of QDs@SiO$_2$-Rh6G*

To determine whether the intensity ratios have batch-to-batch reproducibility, average peak intensity ratios from five batches silica nanoparticles solutions prepared by the above method were compared. The standard deviation for these intensity ratios was calculated to be 4.6%, indicating acceptable fabrication reproducibility for the proposed approach. The precision of the sensing approach was evaluated by five replicate determinations, and the relative standard deviation (RSD, $n = 11$) was 3.9% at an Hg^{2+} concentration of 500 nmol L^{-1}.

3.7. *Sensing mechanism*

In this study, the preparation of rhodamine derivatives was performed according to the published procedure [23,24]. The obtained rhodamine molecule was in a spirolactam form, which selectively responds to the mercury ion over other metal ions [24–26]. The mechanism is briefly described in Scheme 2. In the presence of Hg^{2+}, the suspension shows an enhanced fluorescence emission centered at 545 nm. Yang et al. [25] and Lee et al. [27] indicate that the Hg^{2+} ion induces a highly conjugated rhodamine system via formation of opened-spirolactam to give a strong fluorescence emission. In this process, the Hg^{2+} ion takes part in the complexation with an oxygen atom of the rhodamine carbonyl group, and induces spirolactam ring opening, resulting in the enhancement of the fluorescence intensity at 545 nm. When KI is added, the opened-spirolactam is closed again, which can be attributed to the stronger binding ability of I^- than Hg^{2+} [22,23].

3.8. *Application on water sample analysis*

The method proposed was applied for the determination of Hg^{2+} in tap and lake water samples. The amount of soluble mercury ions (II) in the water could easily be determined using the proposed method. The RSD for the determination ranged from 1.4 to 4.8%. The recovery measured as the percentage difference between the mean concentrations found and the amounts added, ranged from 97.5 to 103.9%. Surface water samples obtained from Xiamen University Lake were filtered three times through qualitative filter paper before use. The determination results are presented in Table 1.

Table 1
Determination of Hg^{2+} in water samples with the rhodamine-based sensing method.

Sample	Added (10^{-7} mol L^{-1})	Found[a] (10^{-7} mol L^{-1})	RSD (%)	Recovery (%)
Tap water				
1	0	ND	–	–
2	1.6	1.66	1.4	103.9
3	2.6	2.60	2.1	100.1
4	3.2	3.28	4.8	102.5
Lake water				
1	0	0.80	1.7	–
2	4.0	3.90	4.3	97.5
3	4.6	4.53	2.8	98.5

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

4. Conclusions

In summary, we have fabricated a novel reversible ratiometric fluorescence sensing approach for Hg^{2+} determination. The silica nanoparticles displayed well-resolved dual fluorescence emission, with the Rh6G derivative at 545 nm and the CdTe-QDs at 625 nm. The QDs@SiO$_2$-Rh6G presented the advantages of high selectivity for Hg^{2+}, precision, reproducibility, selectivity and reversibility, and the experimental results indicated a good potential for application in the determination of Hg^{2+} in environmental water.

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