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A potential visual fluorescence probe for ultratrace arsenic (III) detection by using glutathione-capped CdTe quantum dots

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ABSTRACT

The interaction between mercaptoacetic acid (MA)-capped CdTe QDs, MA-capped CdTe/ZnS QDs or glutathione (GSH)-capped CdTe QDs with As(III) was studied using fluorescence spectrometry. As (III) has a high-affinity to reduced-GSH to form As(SG)$_3$, and the emission of the GSH-capped CdTe QDs ($\lambda_{em.}$ = 612 nm) is quenched effectively. Thus, a novel fluorescence spectrometric method was developed for As (III) determination by using GSH-CdTe QDs. Under optimal conditions, the quenched fluorescence intensity (F_0/F) increased linearly with the concentration of As (III) ranging from 5.0×10^{-6} to 25×10^{-5} mol L^{-1}. The limit of detection (3σ) for As (III) was found to be 2×10^{-8} mol L^{-1}. This method is potentially useful in visual detection of As (III) under irradiation of the ultraviolet light.

1. Introduction

Many arsenic species are of high toxicity and intrigue lots of research concerns [1]. Since inorganic species As (III) is more stable than As (V) under slightly reducing conditions or low pH, up to 10% of total arsenic exists as As (III) in uncontaminated surface and deep ocean waters. The median lethal dose (LD$_{50}$) for As (III) in rats is lower than 14 mg/kg, which is one of the most toxic arsenic species [2,3]. There are many evidences to support that some cancers [4–6] and other diseases are caused by ingesting arsenic. Such evidence has led the U.S. Environmental Protection Agency (EPA) to lower the present drinking water standard for arsenic from 50 to 5 μg L^{-1} [7]. To monitor ultratrace arsenic, atomic fluorescence spectrometry (AFS) is the best choice in many cases [8,9]. However, AFS is an in-laboratory technique and can hardly be used for field analytical chemistry. In addition to AFS, molecular fluorescence spectrometry also offers advantages of high sensitivity and convenience, especially when visual detection is possible. Recently, the exploration of systems capable of sensing and recognizing biomacromolecules [10,11] and inorganic ions [12–15] based on quantum dots (QDs) is a topic of considerable interest, since QDs offer size-controllable fluorescence properties, high fluorescence quantum yields and good stability against photobleaching. Glutathione (GSH) is a tripeptide that protects cells from toxins such as heavy metals and free radicals. This process could be interfered strongly with the presence of As (III) [16], and this interference has been used to study the active sites of enzymes containing sulfhydryl groups [17]. GSH is also a very good candidate ligand for QDs synthesis, and high quantum yield GSH-capped CdTe QDs can be directly prepared in an aqueous system without any post-preparative treatment [18,19]. GSH-capped QDs are very stable in water solution and have high biocompatibility; and they have been used in biological imaging [19,20] and ions/molecules detection [21,22]. The QDs fluorescence spectrometric method is based on the fluorescence quenching of GSH-QDs by the interaction between analytes and GSH ligands. For example, GSH-capped CdZnSe QDs are applied for ultrasensitive detection of Pb^{2+} [21]. Because the K_{sp} value of Pb–S is much lower than that of Zn–S and comparable to that of Cd–S, the fluorescence intensity of GSH-capped CdZnSe QDs is selectively reduced in the presence of Pb^{2+}.

We here prepared three kinds of water-soluble QDs, and compared the change of fluorescence intensity (quenching) in the presence of As (III). Arsenic (III) has a high affinity to reduced-glutathione to form As(SG)$_3$ [23,24], and the fluorescence of GSH-QDs is reduced significantly in the presence of As (III). The experimental results confirmed that GSH-capped CdTe QDs ($\lambda_{em.}$ = 612 nm) could be used as a sensitive fluorescence probe for the detection of ultratrace As (III). This method is potentially useful for visual detection of As (III) in samples under the UV irradiation.

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2. Experimental

2.1. Reagents

Tellurium powder, cadmium chloride ($CdCl_2{\cdot}5H_2O$), sodium borohydride ($NaBH_4$), mercaptoacetic acid (MA), zinc chloride ($ZnCl_2$) and sodium sulfide (Na_2S) were purchased from Kelong Reagent Co., Chengdu, China. Arsenic standards were provided by Analysis Center of Tsinghua University, China. Reduced L-glutathione was purchased from Sigma–Aldrich. All chemicals were of analytical grade and used without further purification. All solutions were prepared with water purified by a Millipore Milli-Q Purification System.

2.2. Apparatus

Fluorescence measurements were conducted with a Thermo Electron Varioskan spectral multimode reader using 384-well fluorescence plates. UV/vis absorption spectra were recorded by using a SHIMADZU UV/Vis-2450 spectrophotometer. PHS-3E pH Meter (Shanghai Feile Co., Ltd.) was used in the preparation of buffers. Fluorescence imaging was carried out with a gel image analysis system (Beijing Liuyi Instrument Factory, WD-9413A) equipped with a 365 nm reflected UV source.

2.3. Synthesis of MA-CdTe, MA-CdTe/ZnS QDs and GSH-CdTe QDs

MA-capped CdTe QDs and MA-capped CdTe/ZnS QDs were prepared according to a method described previously [25]. Briefly, $NaBH_4$ was used to react with tellurium powder at a molar ratio of 2:1 in 10 mL water to prepare fresh NaHTe. 100 mL of $CdCl_2$ (0.1 mM) and MA (25 μL) were mixed in a three-neck round bottle, and was then adjusted to pH 10.0 with 1.0 M NaOH at room temperature. The solution was degassed with pure N_2 for 30 min. Under vigorous magnetic stirring, the freshly prepared NaHTe solution was injected. The final molar ratio of Cd:Te:MA was fixed at 1:0.3:3. Then the precursor solution was heated to reflux under N_2 protection for 60 min. Afterward, cold ethanol was added and MA-CdTe QDs were precipitated out by centrifugation. MA-capped CdTe/ZnS QDs were prepared by a similar procedure. When the CdTe precursor was heated to reflux for 30 min, Zn^{2+} (10 nM) and S^{2-} (10 nM) were added slowly and simultaneously. After 30 min, the products were separated by the addition of cold ethanol and centrifugation.

GSH-capped CdTe QDs were synthesized following a method reported by Ying et al. [19] with minor modification. Typically, 0.1 mmol of $CdCl_2$ solution and 0.1 mmol of GSH (reduced L-glutathione) were mixed in 100 mL water and the pH of the solution was adjusted to 8.0 with 1.0 M NaOH solution under magnetic stirring. Under pure N_2 protection, 200 μL of freshly prepared NaHTe solution was injected, and then the mixture was heated to reflux under N_2 protection. Aliquots of the sample were taken at different time intervals to record their fluorescence spectra. The obtained QDs were precipitated with cold ethanol, and the precipitate was separated by centrifugation and re-dissolved in water. The precipitation process was repeated for three times in order to eliminate free glutathione ligands and salts in the GSH-CdTe QDs colloids.

2.4. Fluorescence experiment

In previous work, it was noted that pH change of the medium could result in pronounced fluorescence change of QDs. Therefore, QDs stock solutions were prepared in Tris–HCl buffer solution (0.1 M, pH 7.4). In a typical procedure, an amount of 100 μL of the buffer-diluted QDs solution was mixed with 1400 μL of cations (dispersed in Tris–HCl buffer) of varied concentrations in a 3 mL Eppendorf (Ep) tube. Then, the mixed solution was mixed thoroughly on a vortex mixer. After 10 min, 100 μL solutions were added in the square hole on the 384-well fluorescence plate. The fluorescence spectra were recorded from 450 nm to 750 nm and the fluorescence intensity of the solution was recorded at the maximum emission wavelength with the excitation wavelength at 370 nm.

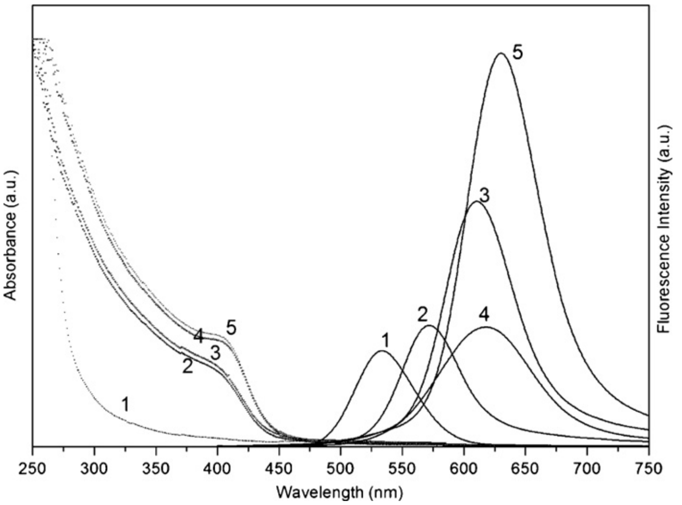

Fig. 1. UV/vis absorption (dotted lines) and fluorescence emission spectra (solid lines) of different capped QDs: (1) MA-CdTe, $\lambda_{max(em.)} = 533$ nm; (2) MA-CdTe/ZnS, $\lambda_{max(em.)} = 619$ nm; (3) GSH-CdTe (30 min), $\lambda_{max(em.)} = 572$ nm; (4) GSH-CdTe (60 min), $\lambda_{max(em.)} = 612$ nm; and (5) GSH-CdTe (90 min), $\lambda_{max(em.)} = 631$ nm. $\lambda_{ex.} = 370$ nm.

2.5. Selectivity experiment

The following inorganic salts were used for the cation selectivity experiment: sodium chloride, calcium nitrate, magnesium chloride, aluminum nitrate, nickel acetate tetrahydrate, chromium trichloride, manganese nitrate, zinc acetate, copper nitrate, iron (III) chloride hexahydrate, iron (II) sulfate heptahydrate and cadmium chloride. Firstly, salt stock solutions of 10 mM were prepared. Subsequently, salt solutions of 10 μM were prepared from the 10 mM stock solutions by serial dilution with Tris–HCl solution (pH 7.4).

3. Results and discussion

3.1. Fluorescence properties of different QDs

Compared with traditional organic fluorescence dyes, semiconductor QDs offer size- and composition-tunable emission from visible to infrared region, large absorption coefficients across a wide spectral range, and high level of brightness and photostability [26]. Fig. 1 displays the UV/vis absorption and fluorescence emission spectra ($\lambda_{ex.} = 370$ nm) of the prepared QDs. The diameters of QDs were calculated from the wavelength of the first excitonic absorption peak of the corresponding sample according to Peng's method [27]. The obtained QDs under different synthetic conditions exhibited broad absorption with narrow and symmetrical emission peaks in the region from 533 nm to 631 nm.

3.2. Interaction of As (III) with MA-CdTe, MA-CdTe/ZnS and GSH-CdTe QDs

To compare the interaction between the QDs and As (III), the relative fluorescence intensity of 10 μM MA-CdTe ($\lambda_{max} = 533$ nm), MA-CdTe/ZnS ($\lambda_{max} = 619$ nm), GSH-CdTe ($\lambda_{max,30min} = 572$ nm, $\lambda_{max,60min} = 612$ nm, and $\lambda_{max,90min} = 631$ nm) QDs in response to

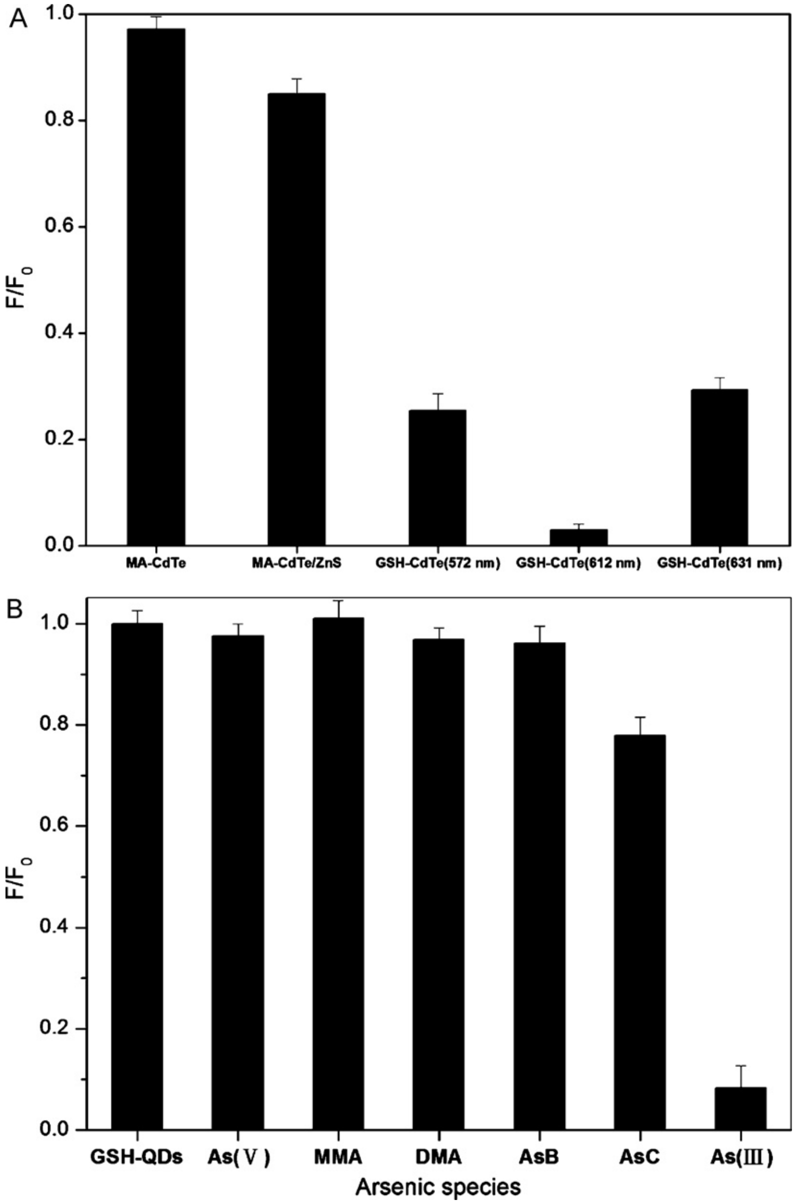

Fig. 2. (A) Response of 10 μM MA-CdTe (λ_{max} = 533 nm), MA-CdTe/ZnS (λ_{max} = 619 nm), GSH-CdTe ($\lambda_{max,30min}$ = 572 nm, $\lambda_{max,60min}$ = 612 nm, $\lambda_{max,90min}$ = 631 nm) in the presence of 10 μM As (III) solution. $\lambda_{ex.}$ = 370 nm, where F_0 and F are the fluorescence intensity of the QDs in the absence and presence of As (III); and (B) effects of 10 μM GSH-CdTe QDs fluorescence with 10 μM different arsenic species. ($\lambda_{ex./em.}$ = 370/612 nm).

10 μM As (III) was investigated, respectively. Fig. 2A shows that MA capped CdTe or CdTe/ZnS QDs have no response to As (III), but the GSH-CdTe fluorescence quenched obviously in the presence of As (III). Furthermore, the effect of As (III) on the fluorescence quenching varied with GSH-CdTe QDs prepared with different refluxing times. The GSH-CdTe (612 nm) refluxing within 60 min was quenched by As (III) with the highest sensitivity. Therefore, GSH-CdTe (612 nm) QDs was selected as a sensitive fluorescent probe for As (III) detection in the following experiments.

There are four valence states of arsenic (−3, 0, +3, and +5) with varied toxicity. Fig. 2B shows the relative fluorescence intensity of GSH-CdTe QDs in the presence of the same concentration of As (III), As (V), methylarsinic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC). As (III) has the strongest influence on the QDs fluorescence, and the rest five species have no significant impact on the QDs fluorescent intensity.

3.3. Selective fluorescence quenching of GSH-CdTe QDs by As (III)

The purified GSH-CdTe QDs were dispersed in Tris–HCl buffer solution (0.1 M, pH 7.4) and the same amount of metal cation solutions (10 μM) was added respectively. The fluorescence intensities of these solutions were measured as shown in Fig. 3. It was found that the fluorescence intensity of GSH-CdTe had the strongest response to As (III) but not other metal ions such as Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Ni^{2+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Cu^{2+} and Fe^{3+}. Furthermore, the quenching effect of As (III) on fluorescence emission of GSH-CdTe QDs was found to be concentration dependent. Therefore, this selective response toward As (III) enabled us to use GSH-CdTe QDs as a selective fluorescence probe for As (III) detection.

3.4. Limit of detection for As (III)

Fig. 4 shows that greater fluorescence quenching was observed at lower GSH-CdTe concentration in the presence of the same As

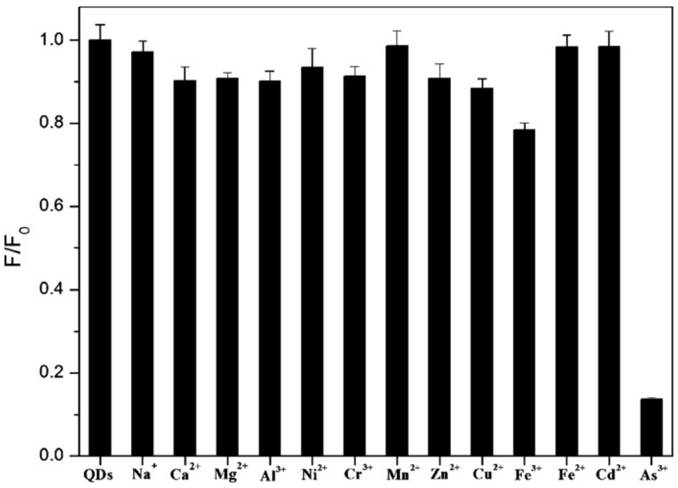

Fig. 3. Effect of 1 μM different ions on the relative fluorescence of 1 μM GSH-CdTe QDs ($\lambda_{\text{max},60\ \text{min}} = 612$ nm) when excited with 370 nm.

(III) concentration. A concentration of 5 nM was the minimum QDs concentration for As (III) detection with reasonable signal-to-noise ratio (S/N > 3). Further, the reproducibility of the system was evaluated by five replicates of the 5 nM GSH-CdTe QDs in the presence of As (III) ions of varied concentrations, with relative standard deviation ranging from 1.2% to 1.9% (see Fig. 5).

The linearity of the proposed method for As (III) detection was studied with different amounts of QDs, as shown in Fig. 6. The fluorescence quenching was described by the Stern–Volmer equation,

$$\frac{F_0}{F} = 1 + K_{\text{sv}}[C]$$

where F_0 and F are the fluorescence intensities of the QDs in the absence and presence of As (III), respectively, and K_{sv} is the Stern–Volmer quenching constant and $[C]$ is the concentration of the As (III). When 10 nM QDs was used, the linear relationship of the equation plot was the best, with a linear correlation coefficient $R^2 = 0.999$. The relation between $1/K_{\text{sv}}$ and QDs concentration suggested that we could achieve lower detection limit with lower QDs concentration (see the inset in Fig. 4). The limit of detection was evaluated using three standard deviations of 11 measurements of the blank/slope of the calibration curve, and was found to be 20 nM for As (III) in the presence of 10 nM GSH-CdTe QDs under the optimized condition.

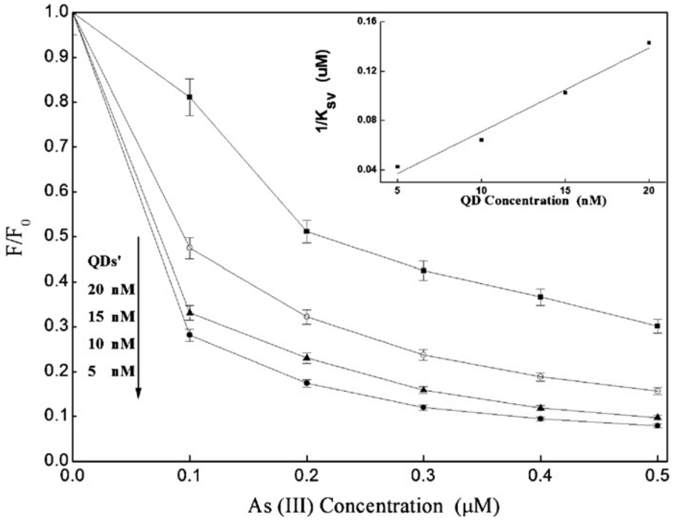

Fig. 4. Effect of As (III) ion concentration on the fluorescence intensity of different concentrations of GSH-CdTe ($\lambda_{\text{ex./em.}} = 370/612$ nm). The inset shows the linear relationship between $1/K_{\text{sv}}$ and concentration of QDs.

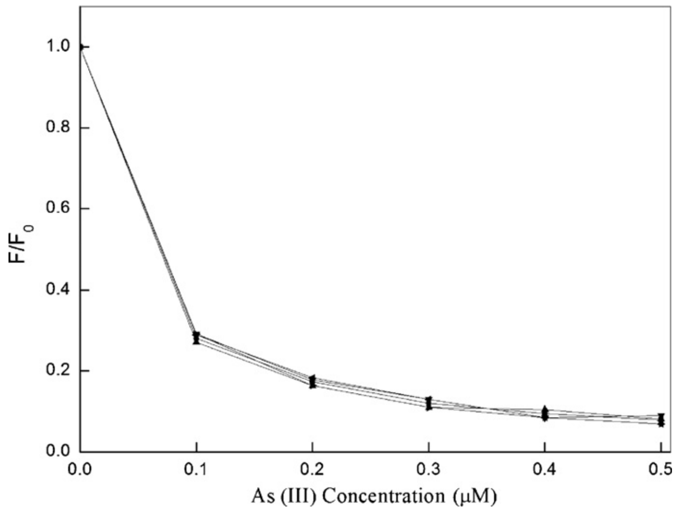

Fig. 5. Fluorescence quenching of five samples of 10 nM GSH-CdTe with varied As (III) concentration. Each point was repeated for five times.

3.5. Possible mechanism of quenching effect by As (III)

GSH as a sulfhydryl-containing tripeptide is an ideal capping agent for the CdTe QDs synthesis. The GSH capping layer is very important to guarantee the stability of the QDs. Therefore, the metal–GSH interaction may be an important parameter for the fluorescence quenching effect. GSH is identified as a compound which reacts with As (III) [16]:

$$\text{As}^{3+} + 3\text{GSH} \rightarrow \text{As(SG)}_3 + 3\text{H}^+$$

Therefore, we believed that the GSH capping layer was preferentially displaced from the surface of the CdTe QDs upon the binding of As (III). The displacement of GSH capping consequently created imperfection on the CdTe QDs surface, resulting in fluorescence quenching.

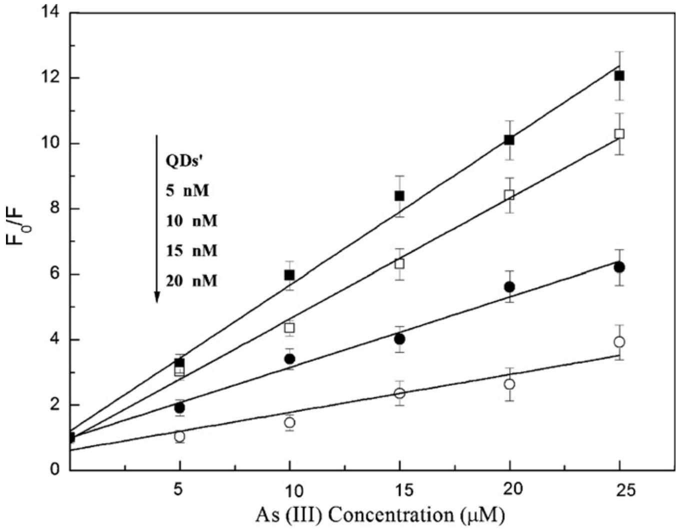

Fig. 6. Stern–Volmer's plots with varied concentrations of GSH-CdTe QDs.

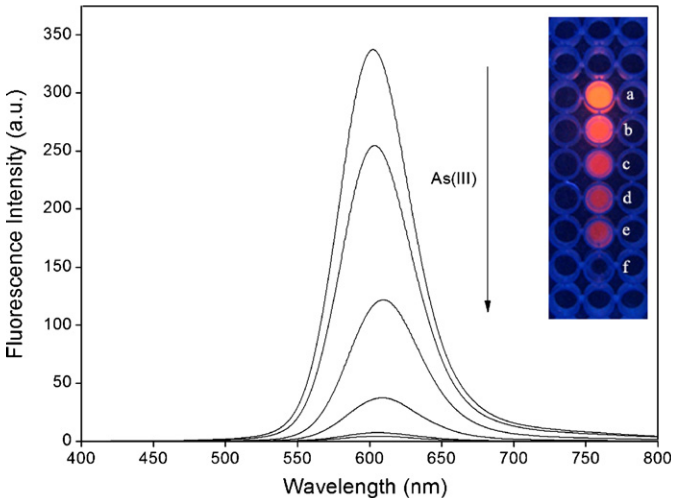

Fig. 7. Fluorescence spectra representing the quenching of As (III) with the concentration of 0, 5, 10, 15, 20, and 25 nM (from top to bottom, a → f), the inset corresponding to the color in a 96-well plate under the irradiation of a UV lamp ($\lambda = 370$ nm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Analytical results of water samples.

Sample	As (III) added ($\mu g\,L^{-1}$)	As (III) found ($\mu g\,L^{-1}$)[a]	Recovery (%)
Tap water 1	0	–	–
Tap water 2	5	4.9 ± 0.02	98
Tap water 3	10	9.7 ± 0.04	97
River water 1	0	–	–
River water 2	5	5.3 ± 0.03	106
River water 3	10	10.1 ± 0.04	101

[a] Mean of three determinations.

3.6. *Visual detection of As (III) in 96-well plates*

To simplify the detection procedure and model the portable As (III) detector, a 96-well plate was chosen as the reaction container instead of a colorimetric cuvette. Under the illumination of a UV lamp, this phenomenon can be visualized due to the excellent fluorescent property of the QDs. Fig. 7 shows the fluorescence spectra of 50 μL GSH-CdTe QDs (10 nM) quenched by the gradient concentration of As (III) in a 96-well plate. The fluorescence signals were read and analyzed simultaneously by the Varioskan multimode reader. Evidently, the GSH-QDs fluorescence emission decreased regularly with the increase in the concentration of As (III), that is, the colors of the samples gradually weakened under the UV ($\lambda = 370$ nm) irradiation.

3.7. *Preliminary application*

This method was applied to the determination of arsenic (III) in spiked tap water and river water samples (Table 1). Water samples were filtered through Millipore cellulose membrane filter (0.45 μm pore size). The pH of the samples was adjusted to 7.4 with buffer solution. Then the procedures given in Section 2.4 were applied to the final solutions. The results showed that the analytical recoveries of As (III) added to tap water and river water were ranged from 95% to 106%.

4. Conclusions

In summary, water soluble GSH-capped CdTe QDs have been demonstrated with highly sensitive fluorescence quenching property in the presence of As (III). The quenching effect may be rationalized by the competitive GSH capping layer binding between the CdTe QDs and the metal ions present in the solution. This method is potentially useful in visual detection of As (III) in field analytical chemistry.

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