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We herein designed a ratiometric fluorescence probe through hybridizing fluorescein isothiocyanate-doped silica nanoparticles with CdTe/CdS quantum dots and demonstrated its efficiency for visual detection of Hg$^{2+}$. The color of the solution gradually turned from red to green along with the increase of Hg$^{2+}$ concentration.

Mercury, is considered highly toxic and widespread pollutant, and it exists in a variety of different forms (metallic, ionic, and as a part of organic salts and complexes). Mercuric ion (Hg$^{2+}$), as one of the most stable inorganic forms of mercury, can accumulate in organisms and interact with the thiol groups in protein to cause serious threat to human health. Along with the widespread use of mercury in industry, the mercury contamination (especially Hg$^{2+}$) of the natural environment has attracted more and more attention. Thus, the development of rapid, sensitive and selective methods for the reliable detecting of Hg$^{2+}$ is highly demanded and of great significance.

Thus far, various efficient and reproducible methods, including atomic absorption/fluorescence spectrometry, high-performance liquid chromatography, surface enhanced Raman scattering, inductively coupled plasma mass spectroscopy, circular dichroism spectra and voltammetric have been developed for the determination of Hg$^{2+}$. Unfortunately, those methods are usually complicated, time-consuming, and costly. In contrast, fluorescent chemodosimeters have become a powerful tool for sensing trace amounts of Hg$^{2+}$ owing to their simplicity, high sensitivity, and short responsive time. However, most of the present chemosensors respond to Hg$^{2+}$ by decreasing or increasing the fluorescence intensity, this single intensity-based sensing is frequently compromised by some other factors, such as the probe concentration, instrumental efficiency, and environmental effects in complex real samples. On the contrary, methods based on ratiometric fluorescence could eliminate most of the ambiguities by self-calibration of two or more different emission bands. Besides, the signal variation of the ratiometric fluorescence probe was easier to be distinguished by the naked eye.

Currently, ratiometric fluorescence detection were mainly based upon dual-emission fluorescence nanoparticles, which were readily obtained by combining two different fluorophores in one nanoparticle, one fluorophore as reference and another as a signal report unit. The key point for the integration of two different fluorophores was how to preserve their own emission properties during the complicated building-up process. Silica, a versatile and biocompatible material for coating layers on nano-objects, has been widely used for encapsulating various fluorescence components together and fabricating multicolor fluorescence nanoparticles. To date, dyes and quantum dots (QDs)-based dual-emission silica nanoparticles have gained significant attention and developed as ratiometric fluorescence probes for metallic ion or small molecule sensing. It was worth mentioning that although the fluorescence signal in silica core was superior, the emission properties of the response fluorophore on the surface of the silica tended to suffer a great destruction due to photobleaching or covalent linking with a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling scheme.

With these insights, here we presented a simple method to prepare dual-emission silica nanoparticles for ratiometric fluorescence detection of Hg$^{2+}$. As illustrated in Scheme 1, fluorescein
(FITC)-doped silica nanoparticles were first synthesized using a modified Stöber method. Then, a three-layer polyelectrolyte film, consisting of poly(sodium 4-styrene-sulfonate) (PSS) layer sandwiched between two layers of poly(allylamine) hydrochloride (PAH) was deposited on the surface of FITC-doped silica nanoparticles. Finally, the negatively charged N-Acetyl-L-cysteine (NAC)-capped CdTe/CdS QDs were chemisorbed on the nanoparticles through electrostatic interaction. Notably, the resultant nanoprobes constructed by this method have several features that make it particularly attractive of Hg\(^{2+}\): (1) the silica nanoparticles protected the reference dye (FITC) from being directly exposed to the environmental oxygen, and thus greatly enhanced the photostability of the entrapped dye; (2) the response to CdTe/CdS QDs was directly chemisorbed on the surface of nanoparticles, which effectively decreased the loss of fluorescence via covalent linking with an EDC coupling strategy; (3) the NAC molecules which served as a strong chelating reagent for Hg\(^{2+}\), making the rapid and selective detection of Hg\(^{2+}\) possible; (4) in the presence of different amounts of Hg\(^{2+}\), the nanoprobes displayed continuous color changes from red to green due to the quenching of the red fluorescence of QDs which can be clearly observed by the naked eye. The experimental conditions, selectivity and sensitivity of this ratiometric fluorescence nanosensor to Hg\(^{2+}\) were carefully studied. The feasibility of the dual-emission silica nanoparticles for Hg\(^{2+}\) determination in real water samples was also investigated.

The architecture and optical properties of the ratiometric probe were studied in detail. Typical transmission electron microscopy (TEM) images showed that the obtained FITC-doped silica cores, with an average diameter of 98 nm, were nearly spherical and smooth (Fig. 1A). The change in zeta potential effectively indicated that PAH/PSS/PAH layers were successfully deposited on the cores by step (Fig. S1). After the chemisorption of negatively charged NAC-capped CdTe/CdS QDs (Fig. S2), the hybrid probe surfaces were comparatively rough (Fig. 1B). High-resolution transmission electron microscopy (HRTEM) images displayed numerous, individual, dark “QD islands” on the carrier silica cores (Fig. 1C), which indicated the QDs were distributed homogeneously on the silica core surfaces. The X-ray photoelectron spectroscopy (XPS) and fluorescence spectra of the ratiometric probe further confirmed the assembly of CdTe/CdS QDs on the silica core surfaces. Compared with the spectrum of the FITC-doped silica nanoparticles (Fig. 1D, curve a), the spectrum of dual-emission silica nanoparticles (Fig. 1D, curve b) shows several new peaks at 614.5, 568.6, 401.2, 164.6 and 36.5 eV, which correspond to Cd3p3, Te3d5, Cd3d5, S2p and Te4d, respectively. The amplified XPS from 200 to 0 eV was shown in Fig. S3. Meanwhile, the fluorescence spectrum (Fig. 1E) of the ratiometric probe (curve c) presents two peaks characteristic of the FITC-doped silica cores (curve a) and CdTe/CdS QDs (curve b), respectively. The red-shift of the fluorescence peak for the probe may be attributed to interparticle plasmon coupling caused by nanoparticle clusters.
For the dual-emission hybrid nanoparticles to be used as a ratiometric probe, photostability was an important factor to be investigated. After consecutive illuminations at 365 nm, the relative fluorescence intensity ratio ($F_{579}/F_{519}$) exhibited no apparent change with 120 min, implying the long-term photostability of the probe in aqueous solution (Fig. S4). In addition, we found the fluorescence intensity of each peak (675 and 519 nm) also has no significant change, which indicated the outstanding colloidal stability of the hybrid nanoparticles. The influence of pH (from 4.0 to 10.0) on the fluorescence intensity ratio of the ratiometric probe in the absence and presence of Hg$^{2+}$ were also examined (Fig. S5). The results suggested that the pH value was a prominent factor for the determination of Hg$^{2+}$ and the optimized pH was 7.4.

The response of the resultant ratiometric fluorescence probes toward different concentrations of Hg$^{2+}$ has been studied and the corresponding fluorescence spectra were shown in Fig. 2. In the absence of Hg$^{2+}$, two well-resolved emission peaks at 519 and 675 nm were observed under a single excitation at 365 nm, which can be attributed to the emission of FITC in the core and CdTe/CdS QDs on the surface of the silica nanoparticles, respectively. It was noted that the fluorescence intensity at 675 nm was highly sensitive to Hg$^{2+}$ and decreased as the concentration of Hg$^{2+}$ increased from 0 to 42 µM (Fig. 2A). The green fluorescence of FITC at 519 nm was also found decreased with the increasing of the Hg$^{2+}$ concentration, but the extent of decrease was much less than that of the red fluorescence. As shown in Fig. S6, there is a little spectral overlap between between the emission spectrum of CdTe/CdS QDs and the absorption spectrum of FITC-doped silica core, which indicated that a slight fluorescence resonance energy transfer (FRET) from QDs to FITC-doped silica might occur. Therefore, the decrease of the emission at 519 nm might mainly attribute to the decrease of the FRET, which resulted from the reduction of the fluorescence intensity of QDs upon addition of Hg$^{2+}$. Under optimal conditions, the fluorescence intensity ratio increased linearly with the concentration of Hg$^{2+}$ ranging from $1.0 \times 10^{-6}$ to $9.0 \times 10^{-4}$ M (Fig. 2B, inset) and the limit of detection for Hg$^{2+}$ was $3.4 \times 10^{-7}$ M based on the definition of three times the deviation of the blank signal (3σ). More importantly, the slight decrease of the fluorescence from CdTe/CdS QDs could result in distinguishable color changes under a UV lamp (Fig. 2C), confirming that the validity of the FITC doped silica core as an internal calibration and the visual detection of Hg$^{2+}$ by the naked eye is feasible. Moreover, the advantages of the ratiometric probe for visual detection of Hg$^{2+}$ is verified by the comparison with the single fluorescence quenching experiment, in which only a pure red NAC capped CdTe/CdS QD probe is employed for the detection of Hg$^{2+}$ (Fig. S7). Unlike the ratiometric probe, the color changes of the single fluorescence quenching of the red NAC capped CdTe/CdS QDs upon the addition of Hg$^{2+}$ are hard to observe (Fig. S8). The comparison clearly shows that the ratiometric fluorescence probe possesses higher sensitivity and reliability than a single fluorescence quenching probe for visual detection.

To evaluate the selectivity of the nanoprobe to Hg$^{2+}$, we measured the fluorescence intensity ratio ($F_{579}/F_{519}$) in the presence of Hg$^{2+}$ and other representative metallic ions Ba$^{2+}$, Ca$^{2+}$, Cd$^{2+}$, Fe$^{3+}$, Mg$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, K$^+$, Na$^+$, Ag$^+$, and Cu$^{2+}$ under the same conditions. As displayed in Fig. 3, in the presence of other ions, the fluorescence intensity ratio did not show significant change compared with the dual-emission nanoparticles (blank), suggesting the good selectivity of the ratiometric fluorescence probe toward Hg$^{2+}$ over other metal ions. Furthermore, the nanoprobe still demonstrated an excellent selectivity in the presence of all possible interference ions. The fluorescence quenching mechanism of Hg$^{2+}$ in this case may be complex: both ion-binding and electron transfer can lead to the decrease of QD emission.$^{12}$ The red-shift from 675 to 685 nm in fluorescence spectra (Fig. 2A) is attributed to strong binding of Hg$^{2+}$ onto the surface of NAC capped-CdTe/CdS QDs.$^{10}$ On the other hand, like our previous work, the fast response of the QDs to Hg$^{2+}$ (with 2 min, Fig. S9) showed the selective fluorescence quenching could also arise from the stronger affinity of N atom (from NAC) to Hg$^{2+}$ through fast electron transfer process than other metallic ions.$^{17}$

### Table 1

Determination of Hg$^{2+}$ content in Hg$^{2+}$-spiked tap water and lake water samples using the proposed ratiometric probe

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added Hg$^{2+}$ concentration (µM)</th>
<th>Found Hg$^{2+}$ concentration (µM)</th>
<th>Recovery (%)</th>
<th>RSD (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>2.0</td>
<td>2.0</td>
<td>104.0%</td>
<td>4.9%</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>6.21</td>
<td>103.5%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Lake water</td>
<td>2.0</td>
<td>2.13</td>
<td>106.5%</td>
<td>5.1%</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>5.84</td>
<td>97.33%</td>
<td>3.4%</td>
</tr>
</tbody>
</table>

$^{1}$Cold-vapour atomic fluorescence spectrometry

These initial results indicated that the dual-emission hybrid nanoparticles could be developed as an effective ratiometric fluorescence probe for the sensing of Hg$^{2+}$. To further confirm its practicality in real samples, the ratiometric probe has been applied to

[Note: The image contains a graph showing fluorescence intensity ratio ($F_{579}/F_{519}$) for different concentrations of various metal ions in the presence or absence of Hg$^{2+}$, demonstrating the selectivity of the probe.]
determine the concentration of Hg²⁺ in real water samples spiked with different amounts of Hg²⁺ in both tap water and lake water. As shown in Table 1, it can be seen that the estimated recoveries and RSD of the measurements were in the range of 97.33-106.5% and 2.3-5.1%, respectively, which were satisfactory for quantitative assays performed in real samples. Moreover, the proposed method analysis results were comparable with the results obtained by CV-AFS suggesting that the as-prepared ratiometric fluorescence probe was reliable and practical.

Conclusions

In summary, a ratiometric fluorescence probe for Hg²⁺ has been designed and fabricated by chemisorption numerous CdTe/CdS QDs upon the surfaces of the FITC-doped silica spheres. The nanoprobe takes advantage of the high stability of the dye-doped silica, the superior fluorescence properties of QDs as well as the specificity of NAC toward Hg²⁺. The ratiometric fluorescence probe provided an effective platform for rapid and reliable detection of Hg²⁺ in solutions on the basis of the measurement of both ratiometric fluorescence intensity and fluorescence color changes. Additionally, the ratiometric fluorescence probe has been successfully applied for the determination of spiked Hg²⁺ in real water samples.

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