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Highly selective and sensitive fluorescent probe for the rapid detection of mercury ions

Zujun Xu,^a Wenxiu Shi,^a Chengjun Yang,^a Jing Xu,^a Huapeng Liu,^a Jing Xu^{*b} and Baocun Zhu^{id}^b

Mercury (Hg) is one of the major toxic heavy metals, harmful to the environment and human health. Thus, it is significantly important to find an easy and quick method to detect Hg²⁺. In this study, we designed and synthesized a simple fluorescent probe with excellent properties, such as high sensitivity and selectivity, rapid response, and outstanding water solubility. When Hg²⁺ (5 μM) was added to the probe solution, it exhibited a very large fluorescent enhancement (about 350-fold stronger than the free probe) with the help of hydrogen peroxide (H₂O₂). Probe HCDC could quantitatively detect Hg²⁺ in the range of 0–10 μM using the fluorescence spectroscopy method and the detection limit was measured to be about 0.3 nM (based on a 3σ/slope). Analytical application was also studied, and the probe HCDC exhibited excellent response to Hg²⁺ with the addition of H₂O₂ in real water samples. So, our proposed probe HCDC provided a practical and promising method for determining Hg²⁺ in the environment.

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1. Introduction

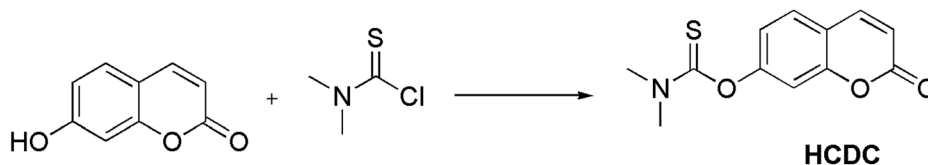
Heavy metal ion pollution has become a global environmental problem.^{1–3} As a common metal element, mercury (Hg) is extremely toxic and shows a great threat to the environment and human health due to its bioaccumulation, durability, and easy absorption by aquatic organisms.^{4–10} Once the mercury enters the organism, it will lead to the dysfunctions of the central nervous systems, the brain, and the kidneys.^{11–14} As we all know, the maximum contaminant level of mercury in drinking water is 2 ppb (0.01 μM) which was set by the Environment Protection Agency (EPA).^{15–18} It can be seen that mercury could pose a serious threat to humans at very low concentrations. Thus, developing a convenient, rapid, highly selective and sensitive method for the determination of Hg²⁺ is vitally important.

Nowadays, several classical methods have been applied to determine Hg²⁺ including electrochemical analysis, atomic absorption/emission spectroscopy, electrochemical analysis, and inductively coupled plasma mass spectrometry.^{19–23} Though these methods are selective and accurate to detect ions, most of them require long time, expensive instruments, and tedious sample pre-treatment.^{24–26} Thus, the techniques of fluorescent probes were widely studied due to the advantages of highly selective, operational simplicity,

and relatively low-cost.^{27–33} Many fluorescent probes for detecting Hg²⁺ have been reported; however, most of them were still limited by the disadvantages of low sensitivity, bad water solubility, and long response time.^{34–38} So, a simple water-soluble fluorescent probe for determining Hg²⁺ with high sensitivity and quick response became our primary task.

Very recently, we have reported several carbonothioate-based fluorescent probe for detecting Hg²⁺. These probes respectively chose the 2-(2'-hydroxyphenyl)benzothiazole (HBT),³⁹ dichlororesorufin,⁴⁰ and seminaphthorhodafluor⁴¹ as the fluorophores, and a carbonothioate moiety was used as the recognition receptor. All these showed quick response and high selectivity for detecting Hg²⁺. In connection with our continuing research, we designed a new probe HCDC (Scheme 1) for detecting Hg²⁺ with superior properties in this paper, and the property of probe HCDC was compared with other reported studies in Table 1. A dimethylthiocarbamic ester group and 7-hydroxycoumarin were respectively chosen as the recognition receptor and the fluorophore. We assumed that the thioester group changed to ester moiety in the presence of Hg²⁺, and the rapid cleavage of ester group was achieved with the help of H₂O₂. As a result, this restored the blue fluorescence of 7-hydroxycoumarin. The experimental results demonstrated that this probe possessed the advantages of (1) a very low detection limit of 0.3 nM (on the basis of 3σ/slope); (2) quicker response to Hg²⁺; (3) excellent water solubility, selectivity and anti-interference; (4) practical applicability in real water samples.

^aSchool of Mathematics and Statistics, Ludong University, Yantai, China^bSchool of Water Conservancy and Environment, University of Jinan, Shandong Provincial Engineering Technology Research Center for Ecological Carbon Sink and Capture Utilization, Jinan, China. E-mail: 1426461661@qq.com; Fax: +86 531 82769235; Tel: +86 531 82769235



Scheme 1 Synthesis of probe HCDC.

2. Experimental

2.1 Materials and general methods

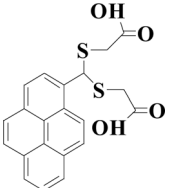
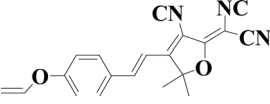
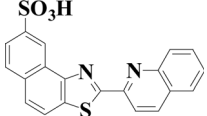
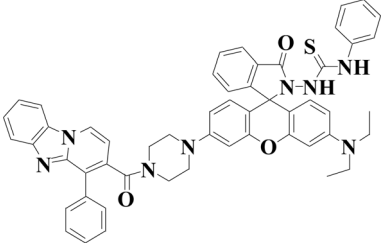
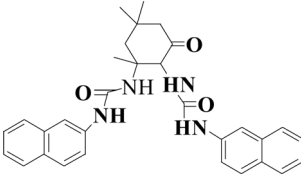
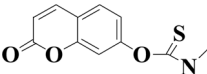
All the chemicals were gained from commercial suppliers and were used without further purification. The Sartorius Arium 611DI system can produce the ultra-pure water which was used during the entire process of the experiment. Column chromatography using Silica gel 200–300 mesh from the Qingdao Haiyang Chemical Co was used to purify the reaction product. The ^1H NMR and ^{13}C NMR were all

recorded on the Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $\text{DMSO}-d_6$, TMS as internal standard). Fluorescence spectra were gained from a Horiba FluoroMax-4 spectrophotometer. All the fluorescence spectra were uncorrected. Probe **HCDC** was prepared according to the previous paper.⁴²

2.2 General procedure for analysis

Stock solutions of probe **HCDC** (1 mM) were prepared in ethanol. And the parent stock solutions (1 mM) of Hg^{2+} , other

Table 1 Comparison of Fluorescent Probes for Hg^{2+}

Probe	LOD (nM)	Solution (v/v)	Time (min)	References
	1.8	Ethanol : PBS = 2 : 1	30	34
	10	$\text{C}_2\text{H}_5\text{OH}$: water = 1 : 1	40	35
	34	CH_3CN : water = 1 : 1	—	36
	18.8	$\text{C}_2\text{H}_5\text{OH}$: water = 2 : 8	20	37
	100	DMSO : H_2O = 9 : 1	30	38
	0.3	Aqueous solution	10	This work



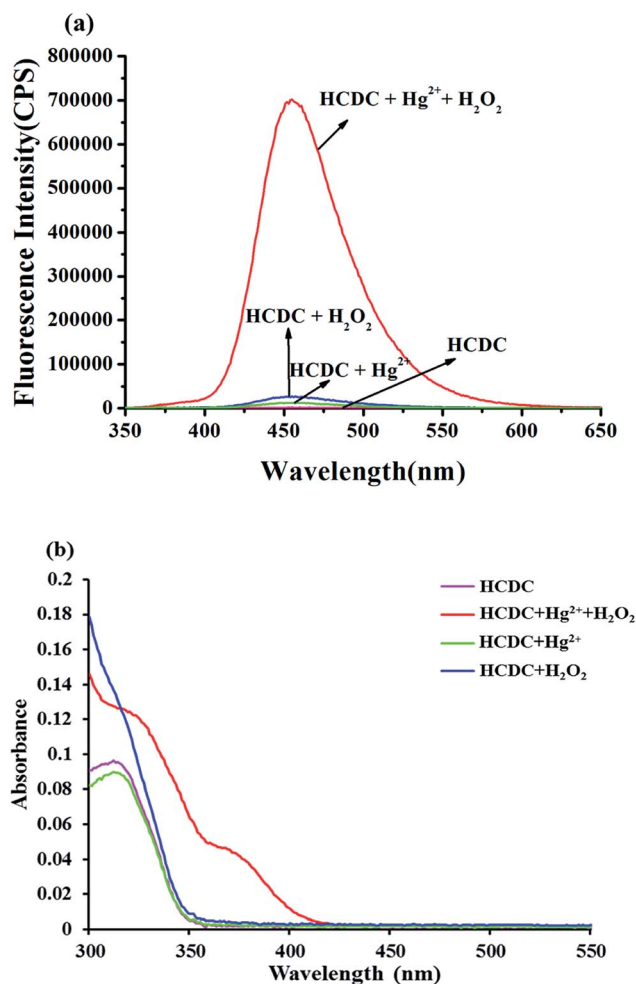


Fig. 1 Fluorescence spectra (a) and absorption spectra (b) of probe HCDC (5 μM) toward Hg^{2+} (5 μM) and H_2O_2 (3 mM) under the ultrapure water containing HEPES (5 mM, pH 7.4).

metal ions and common anions species (10 mM) were prepared in ultrapure water. The test solution contained 50 μL of the probe stock solution and moderate amount of each stock solution, and eventually diluted to 10 mL in a test tube with 100% ultrapure water containing HEPES (5 mM, pH 7.4).

Before Hg^{2+} and other ions were added to the test solution, H_2O_2 (3 mM) was added. All the tests were conducted at room temperature (25 $^\circ\text{C}$).

2.3 Determination of the detection limit

According to the previous paper,^{43,44} the method of fluorescence titration was used to calculate the detection limit. The fluorescence emission spectrum of probe HCDC was measured by fifteen times in the absence of Hg^{2+} in order to achieve the standard deviation of blank measurement. To gain the slope, the fluorescence intensity at 455 nm was plotted as a concentration of Hg^{2+} . Therefore, the formula for calculating the detection limit is described as following:

$$\text{Detection limit} = 3\sigma/k$$

where σ is the standard deviation of the five spectrums among the fifteen spectrums which peaks were nearest, k is the slope between the Hg^{2+} concentration *versus* the fluorescence intensity.

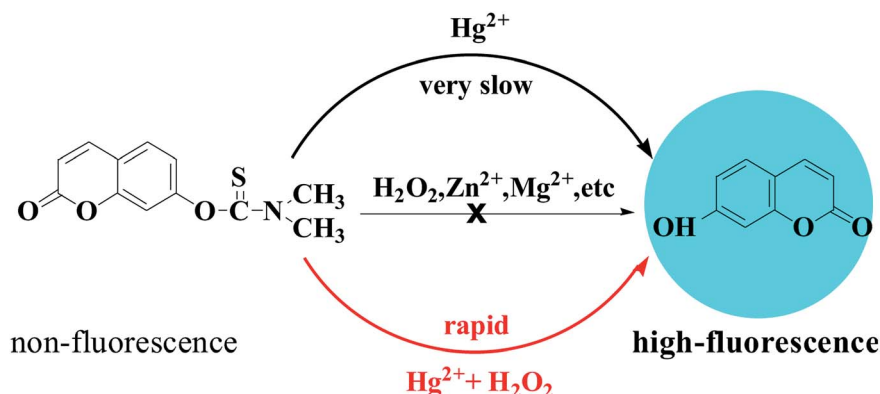
2.4 Analytical application

Three water samples were chosen to test the analytical application of the probe HCDC. One water sample was collected from Jia Zi Lake, University of Jinan, China, and the other two samples were respectively collected from the Xiu Jiang River and Yellow River, at Jinan, China. All the samples needed to filter through filter paper before the measurement.

3. Results and discussion

3.1 Characteristic spectra

In this paper, the fluorescence and absorption spectra of probe HCDC was shown as Fig. 1. In the absence of Hg^{2+} and H_2O_2 , the probe solution exhibits a very weak fluorescence emission peak at 455 nm. When only Hg^{2+} was added to the probe solution, the reaction was very slow. And, the reaction was not carried out when only the H_2O_2 was added. Once Hg^{2+} and



Scheme 2 Reaction Mechanism of probe HCDC for Hg^{2+} with the help of H_2O_2 .



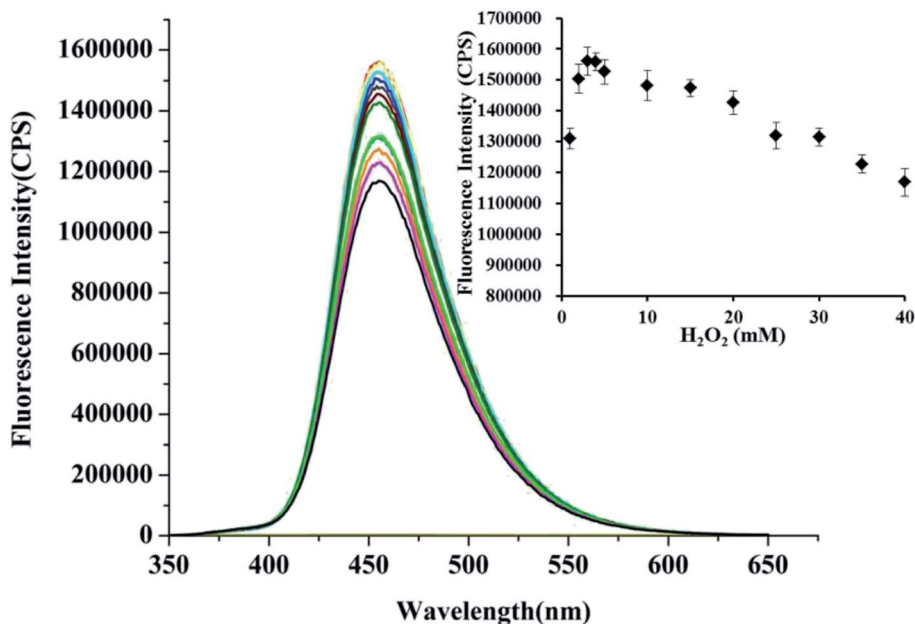


Fig. 2 Fluorescence spectra of probe HCDC (5 μM) in the presence of Hg^{2+} (5 μM) and different concentrations of H_2O_2 (final concentration: 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40 mM) under HEPES (5 mM, pH 7.4) aqueous solution. Excitation wavelength = 330 nm. Error bar = RSD ($n = 3$).

H_2O_2 were all added to the probe HCDC solution, the maximum fluorescence emission peak exhibits a very large enhancement at 455 nm (Fig. 1a). The fluorescence intensity peak of the probe solution containing Hg^{2+} and H_2O_2 was about 350-fold stronger than that of the blank probe solution. Meanwhile, in the absorption spectra, with the addition of Hg^{2+} and H_2O_2 , the probe solution exhibited a new absorption peak at 364 nm (Fig. 1b). The result showed that Hg^{2+} could generate the cleavage of an ester group in the presence of H_2O_2 (Scheme 2).⁴⁰

3.2 Effect of H_2O_2

Different concentrations of H_2O_2 was respectively added to the solution of probe HCDC (5 μM) and Hg^{2+} (5 μM) in the aqueous solution which contained HEPES (5 mM, pH 7.4). It was shown in inset of Fig. 2, the fluorescence intensity was increased first and then decreased with the increasing concentrations of H_2O_2 (the range of 1 to 40 mM). The enhancement of fluorescence reached the maximum when the concentration of H_2O_2 was 3 mM. The results implied that the low concentration of H_2O_2 (1 to 3 mM) could promote the cleavage of ester group and amplify the response of probe HCDC to Hg^{2+} . But when the concentration is over 3 mM (4 to 40 mM), H_2O_2 will inhibit the response of probe HCDC to Hg^{2+} . Thus, we chose 3 mM H_2O_2 as the amplification reagent in the detection of Hg^{2+} .

3.3 Time dependence of detecting Hg^{2+}

Response time is a significant element to test the effect of the probe. So, the response time of probe HCDC was investigated under the analytical conditions which were mentioned above. The result was shown in Fig. 3. When only Hg^{2+} was added to

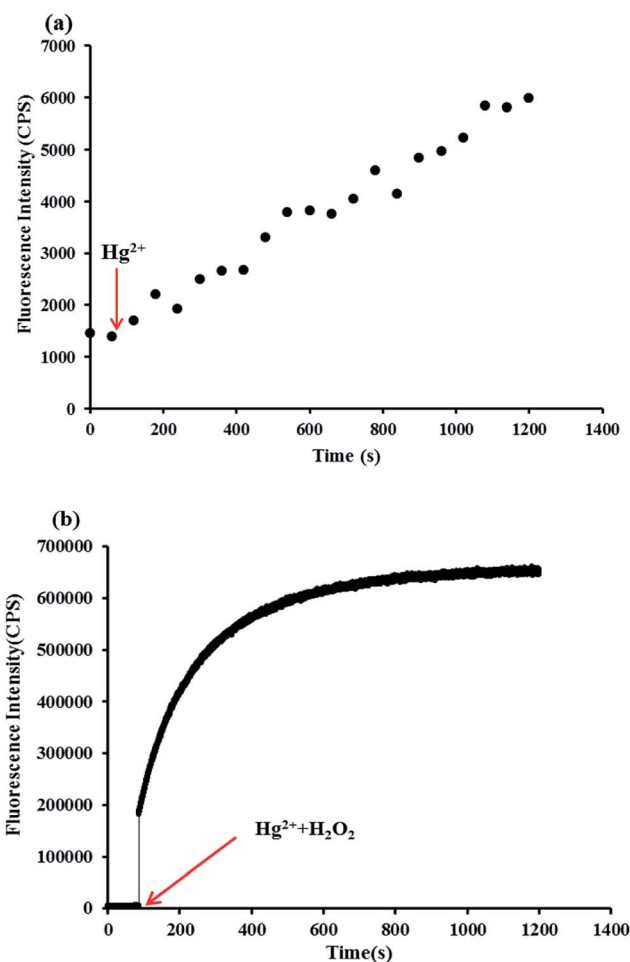


Fig. 3 Reaction time for probe HCDC (5 μM) with the addition of only Hg^{2+} (5 μM) (a), and Hg^{2+} (5 μM) and H_2O_2 (3 mM) (b).



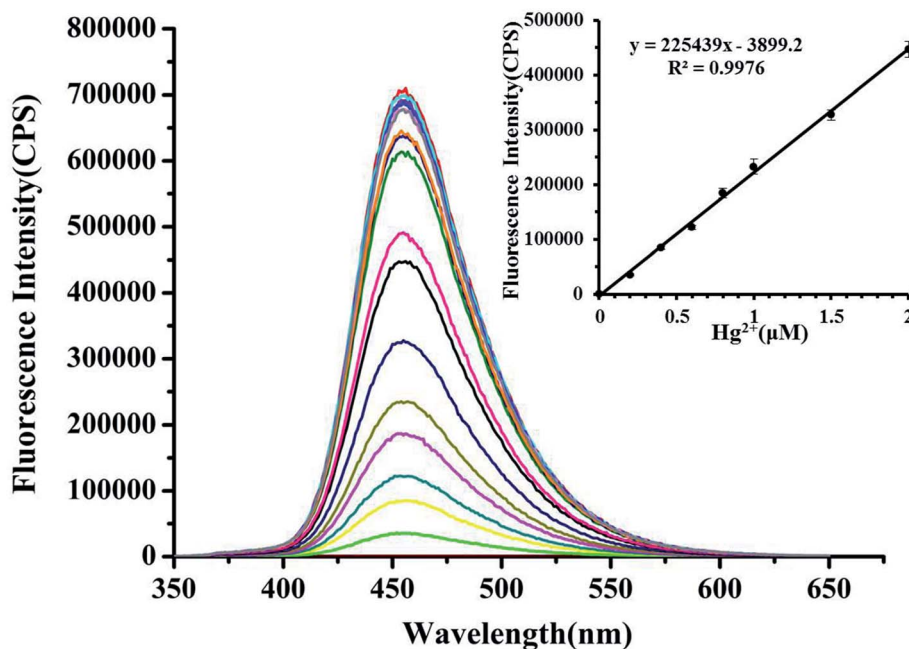


Fig. 4 Fluorescence spectra of probe HCDC (5 μM) toward the various concentrations of Hg^{2+} (final concentration: 0, 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10 μM) and H_2O_2 (3 mM). The fluorescence intensity at 455 nm of probe HCDC increased with the increasing concentrations of Hg^{2+} . Excitation wavelength = 330 nm, emission wavelength = 455 nm. Conditions: in HEPES (5 mM, pH 7.4). Solution: ultrapure water. Error bar = RSD ($n = 3$).

the probe solution, the reaction was very slow. While, the fluorescence intensity at 455 nm was increased as soon as Hg^{2+} and H_2O_2 were added, and increased gradually until the reaction time exceeded 10 min. Thus, all the measurements of the spectrum were carried out 10 minutes after the addition of Hg^{2+} . The results showed that the probe HCDC could provide a rapid analytical method for detecting Hg^{2+} .

3.4 Quantification of Hg^{2+}

As shown in Fig. 4, the continuous enhancement of fluorescence intensity at 455 nm can be seen with the continuous addition of Hg^{2+} (the range of 0 to 10 μM). And, there was a good linearity ($y = 225439x + 3899.2$, $R^2 = 0.998$) between the Hg^{2+} concentrations of 0–2 μM and the fluorescence intensity with a detection limit of 0.3 nM (based on $3\sigma/\text{slope}$). The detection limit of the probe HCDC was far lower than the standard of Hg^{2+} (10 nM) in drinking water which set up by the Environmental Protection Agency (EPA). The above results showed that the probe HCDC could detect Hg^{2+} quantitatively by the fluorescence spectrometry method with excellent sensitivity.

3.5 Selectivity to Hg^{2+}

High selectivity is also an important factor for an excellent probe. The selectivity of HCDC toward Hg^{2+} was evaluated under the above-mentioned conditions. The effect of the metal ions (Mg^{2+} , Zn^{2+} , Ni^{2+} , Ca^{2+} , Na^+ , K^+ , Pb^{2+} , Cd^{2+} , Cr^{6+} , Fe^{2+} , Fe^{3+} , Sn^{2+}) and the common anions species (NO_2^- , NO_3^- , HCO_3^- , H_2PO_4^- , F^- , CO_3^{2-} , Cl^- , SO_4^{2-}) on fluorescence spectra of probe HCDC were investigated. Firstly, Hg^{2+} ,

other metal ions and common anions species were respectively added to the solution, and only Hg^{2+} resulted in a considerable fluorescence enhancement (Fig. 5a and c). The results exhibited that HCDC possesses high selectivity toward Hg^{2+} .

The interference of the metal ions and common anions species on the detection of Hg^{2+} was also studied. Even though the metal ions and common anions species were respectively existed in the solution, the probe HCDC also had a good response to Hg^{2+} (Fig. 5b and d). These studies clearly indicated that the probe HCDC could be used for the selective detection of Hg^{2+} without interference from anions.

3.6 Analytical application

Based on the above results, the probe HCDC owned the excellent properties containing a very low detection limit, quicker response, excellent water solubility, selectivity and anti-interference. So, we attempted to investigate the practical application of the probe for the selective detection of Hg^{2+} in three real water samples. The results were shown in Table 2. At first, there were no Hg^{2+} in three water samples, and then 2 μM , 5 μM , or 10 μM Hg^{2+} was respectively added to the solution of real water (5 mM, HEPES, pH 7.4) containing 5 μM probe HCDC and 3 mM H_2O_2 . Every sample was repeatedly configured and tested for 3 times. Therefore, we obtained a good method for detecting Hg^{2+} in the environment.



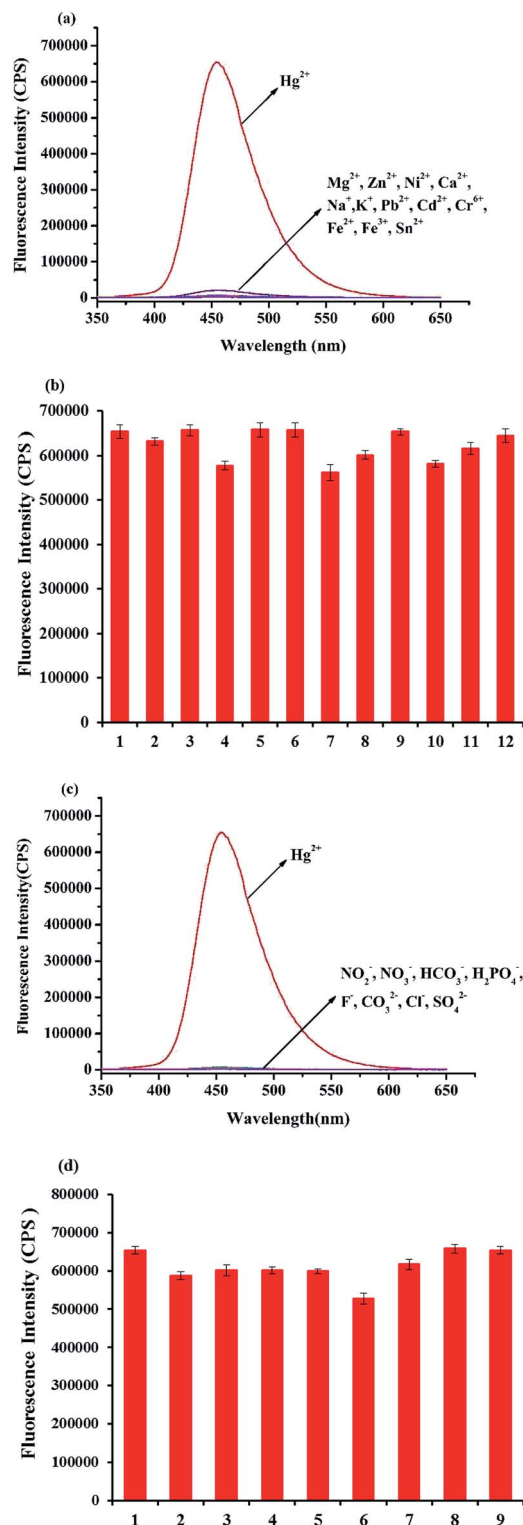


Fig. 5 (a) Fluorescence responses of probe HCDC (5 μM) toward Hg^{2+} (5 μM), and other metal ions (100 μM). (b) Fluorescence responses of probe HCDC toward Hg^{2+} and in presence of other metal ions. (1) Hg^{2+} ; (2) Mg^{2+} ; (3) Zn^{2+} ; (4) Sn^{2+} ; (5) Ca^{2+} ; (6) Na^+ ; (7) Fe^{3+} ; (8) Pb^{2+} ; (9) Cd^{2+} ; (10) Fe^{2+} ; (11) K^+ ; (12) Ni^{2+} . (c) Fluorescence responses of probe HCDC (5 μM) toward Hg^{2+} (5 μM), and common anions species (100 μM). (d) Fluorescence responses of probe HCDC toward Hg^{2+} and in presence of other common anions species (1) Hg^{2+} ; (2) NO_2^- ; (3) NO_3^- ; (4) HCO_3^- ; (5) H_2PO_4^- ; (6) F^- ; (7) CO_3^{2-} ; (8) Cl^- ; (9) SO_4^{2-} . The solution: in H_2O_2 (3 mM), HEPES (5 mM, pH 7.4) aqueous solution. Error bar = RSD ($n = 3$).

Table 2 Analysis results of Hg^{2+} in three real water samples ^a

Real water sample	Found Hg^{2+}	Addition Hg^{2+} (μM)	Found (μM)	Recovery (%)	RSD ($n = 3$) (%)
Sample A	No	2	1.82 ± 0.06	91.06	2.89
		5	4.52 ± 0.11	90.47	2.10
		10	9.61 ± 0.40	96.06	4.02
Sample B	No	2	1.85 ± 0.11	92.52	5.36
		5	4.97 ± 0.29	99.33	5.83
		10	10.11 ± 0.25	101.11	2.51
Sample C	No	2	2.24 ± 0.13	111.93	6.56
		5	5.15 ± 0.21	102.96	4.19
		10	9.73 ± 0.37	97.28	3.72

^a Sample A, lake water from JiaZi Lake, University of Jinan; sample B, from the Xiu Jiang River, at Jinan, China; sample C, from the Yellow River, at Jinan, China.

4. Conclusion

In a word, we have designed and synthesized a highly sensitive and selective probe for detecting Hg^{2+} by the fluorescence spectrum. The enhancement of fluorescence intensity of the probe solution containing Hg^{2+} and H_2O_2 was very significant, which could reach 350-fold stronger than that of the blank probe solution. There was a good linear relationship in the range of 0–2 μM Hg^{2+} , and the low detection limit was 0.3 nM (on the basis of $3\sigma/\text{slope}$). The probe showed excellent solubility in water. More importantly, the response of probe HCDC to Hg^{2+} in real water samples was also satisfactory. All these remarkable advantages of this new probe suggest that this probe will have excellent application prospect in the detection of mercury.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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