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A novel highly selective colorimetric and "turn-on" fluorimetric chemosensor for detecting Hg²⁺ based on Rhodamine B hydrazide derivatives in aqueous media



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ARTICLE INFO	A B S T R A C T
Keywords:	A novel chemical sensor SD based on Rhodamine B hydrazide derivative was designed and synthesized, which disclosed bath colorization and "ware are" fluoreneous area for U_{2}^{++} in DMSO (U_0) (1 + 0 + (x)) solution
Chemosensor Colorimetric Fluorescence Hg ²⁺ Test strip DFT	displayed both colorimetric and "turn-on" fluorescence responses for Hg^{-1} in DMSO/H ₂ O (1 : 9, V/V) solution. In the presence of mercury ions, the sensor SD showed an obvious change in color from colorless to distinct rose red by naked eyes and rapidly provided bright orange fluorescence under UV lamp in the aqueous solution. The detection limit on fluorescence response of SD to Hg^{2+} was as low as 2.71×10^{-7} M. Compared to other metal ions such as Fe ³⁺ , Co ²⁺ , Ni ²⁺ , Cd ²⁺ , Pb ²⁺ , Zn ²⁺ , Cr ³⁺ , Cu ²⁺ , Ag ⁺ , Ca ²⁺ and Mg ²⁺ , SD could only selectively recognizes Hg^{2+} . The results of the Job's plot and infrared spectra analysis suggested that the combined stoi- chiometry between SD and Hg^{2+} was 1:1. Notably, SD could be used as molecular switch controlled by EDTA and Hg^{2+} cyclically. In addition, SD -based test strips were produced which could be used as a convenient and efficient Hg^{2+} test kits. SD also could qualitatively detect Hg^{2+} in tap water.

1. Introduction

With the development of science and technology, mercury compounds are widely used in electrical, medical, military, casting and other fields [1-3]. Mercury is liquid at room temperature and has toxicity, volatility and bioaccumulation along the trophic chain [4,5]. Mercury vapor formed by volatilization is highly toxic and easily enters the human body through the respiratory tract, causing sever diseases like deafness, vision lost, insanity, paralysis coma and Alzheimer's [6-8]. In addition, the same highly toxic mercury-containing compounds are even more difficult to prevent. After the waste liquid containing mercury enters the ecosystem, mercury with a strong migration and low degradability lead to mercury pollution [9-11]. Mercury was listed as one of the priority pollutants according to the U.S. EPA [12,13]. The maximum U.S. EPA limit for allowable levels of Hg^{2+} ions in drinking water is 2 ppb [14].

Fluorescent sensors have become powerful tools for the detection and imaging of trace ions in the environment, food and biological samples [15–17]. There have been many reports of mercury ion probes in recent years [18,19]. However, since mercury ions are generally used as quenchers due to the spin-orbit coupling effects and have sulphophile, most of the probes have problems such as high detection limit [20,21], poor water solubility [22-26] and toxicity of Sulfur [27-30].

Therefore, designing dual-channel probes with naked eye recognition and turn-on fluorescent signal in aqueous solution has attracted more attention.

Our team is also very interested in such probes [31-39]. We have found that designing probe based on Rhodamine B is ideal. Rhodamine B has good spectral characteristics, such as high fluorescence quantum yield, large molar extinction coefficient, high stability, etc [40-43]. In addition, favourable water solubility extends the application range of these probes [44]. We designed a Rhodamine B hydrazide-based probe **SD** for higher selectivity, sensitivity and reversibility of Hg²⁺. After adding Hg^{2+} , based on the open-ring process of the spirolactam of the probe **SD**, the color of the probe changed from colorless to purplish red, and the fluorescence changed from off to on at $\lambda_{ex} = 540$ nm. The detection limit of mercury ions in probe SD is down to 2.71×10^{-7} M. Recyclable projects can also be implemented by adding EDTA. Compared with other reported Hg^{2+} sensors (in **S1**), sensor **SD** has excellent sensitivity and water solubility and has been fabricated into test strips for practical testing. In addition, SD could be used to qualitatively detecting Hg²⁺ in tap water samples. The mechanism of the process was verified by ¹H NMR, UV-vis and other spectroscopy methods, and DFT calculation was performed.

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2. Experimental

2.1. Materials and physical methods

All reagents and solvents were reagent grade at analytical grade without further purification. Fresh double distilled water was purified by standard methods and was used throughout the analytical experiment. Perchlorate salt of cations (Cu²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cd²⁺, Pb²⁺, Zn²⁺, Cr³⁺, Hg²⁺, Ag⁺, Ca²⁺ and Mg²⁺) were purchased from Alfa-Aesar Chemical Reagent Co. and stored in a vacuum desiccator. UV-vis spectra were recorded on a Shi-madzu UV-2550 spectrometer. Fluorescence spectra were obtained by using a Shimadzu RF-5301 Fluorescence Spectrometer equipped with a xenon lamp. Melting points were measured on an X-4 digital melting-point apparatus. Electrospray ionization mass spectra (ESI-MS) was conducted on an Agilent 1100 LC-MSD-Trap-VL system.¹H NMR and ¹³C NMR were recorded on a Mercury-400BB at 400 MHz spectra, and samples were dissolved in DMSO-d₆. ¹H chemical shifts are reported in ppm downfield from tetramethyl silane (TMS, δ scale) with the solvent resonances as internal standards. Melting points were measured on an X-4 digital meltingpoint apparatus. Electrospray ionization mass spectra (ESI-MS) was conducted on an Agilent 1100 LC-MSD-Trap-VL system. Infrared spectra were performed on a Digilab FTS-3000 FTIR spectrophotometer.

2.2. Synthesis of intermediate rhodamine B hydrazide [45]

Rhodamine B hydrazide was prepared by a one-step condensation reaction of Rhodamine B (0.96 g, 2 mmol) and hydrazine hydrate (1 ml, 0.02 mol) in methanol (30 ml). The reaction was refluxed for 3 h at 65 °C in air. After the reaction was completed, it was cooled to room temperature. The pH was adjusted to 8 with NaOH solution and a lot of solid precipitate appeared. Then washed three times with distilled water to give a pale pink compound in 83% yield, melting point: 214–216°C.

2.3. Synthesis of SD

Rhodamine B hydrazide (0.46 g, 1 mmol) and 2-Hydroxy-5-methoxybenzaldehyde (0.15 g, 1 mmol) was dissolved in EtOH (30 mL) and refluxed for 6 h. After completion of the reaction, the mixture was cooled to room temperature, and the precipitate was washed with distilled water. Then filtered the mixture and recrystallized the precipitate with DMF-H₂O to give a crimson compound in 79% yield. As



Fig. 1. Single-crystal X-ray structure of sensor SD.

shown in Scheme 1. Melting point > 300°C. ¹H NMR (CDCl₃, 400 MHz, ppm) d: 10.36 (s, 1 H), 9.24 (s, 1 H), 7.97 (m, 1 H), 7.53 (m, 2 H), 7.18 (d, J = 7.2 Hz, 1 H), 6.78 (d, J = 2.6 Hz, 2 H), 6.63 (d, J = 2.2 Hz, 1 H), 6.48 (m, 4 H), 6.26 (dd, J = 8.9, 2.5 Hz, 2 H), 3.71(s,3 H), 3.32 (q, J = 7.0 Hz, 8 H), 1.15 (t, J = 7.0 Hz, 12 H). (Fig. S2). ¹³C NMR (DMSO- d_6 , 100 MHz, ppm) d: 163.90, 153.34, 152.51, 151.72, 149.46, 148.99, 134.41, 129.47, 128.16, 123.52, 119.79, 118.97, 117.77,112.27, 108.57, 105.56, 97.76, 66.14, 55.82, 44.13, 12.87 (Fig. S3). m/z (ES +) calcd. for: [C₃₆H₃₈N₄O₄] ⁺ = 591.29, found: 591.3724 (Fig. S4). The structure of sensor SD was further confirmed by single-crystal X-ray. Diffraction the single crystal of probe suitable was obtained by using DMF solvent diffusion method for X-ray crystal-lography. The distance of H (3A)… N (4) was 1.914 nm (Fig. 1).

3. Results and discussion

A series of recognition experiments were carried out to **SD** in DMSO/H₂O (v/v = 1:9) solution. In the UV-vis experiment, when 50 equiv. of Hg²⁺ were added to **SD** (2.0×10^{-5} M), a broad absorption band appeared at 565 nm, while the absorption band at 363 nm was weakened (Fig. 2). No significant spectral changes occurred after the addition of other cations to **SD**. It was also observed by the naked eye that the **SD** solution changed from colorless to distinct rose red after the addition of Hg²⁺. In the fluorescence experiment, only when 50 equiv. of Hg²⁺ was added, **SD** (2.0×10^{-5} M) in DMSO/H₂O (v/v = 1:9) solution at the excitation wavelength of 540 nm was significant



Scheme 1. Synthetic procedures for receptor SD.



Fig. 2. Absorbance spectra of SD $(2.0 \times 10^{-5} \text{ M})$ in DMSO/ $H_2O(1:9, v/v)$ in the presence of Hg^{2+} and other ions: Fe³⁺, Co²⁺, Ni²⁺, Cd²⁺, Pb²⁺, Zn²⁺, Cr³⁺, Cu²⁺, Ag⁺, Ca²⁺ and Mg²⁺ (50 equiv.). Inset: The photograph showing the color change of SD solution in DMSO/ $H_2O(1:9, v/v)$ after addition of Hg^{2+} and other ions at room temperature.

Fig. 3. Fluorescence intensity of SD $(2.0 \times 10^{-5} \text{ M})$ in DMSO/H₂O (1:9, v/v) in the presence of Hg²⁺ and other ions: Fe³⁺, Co²⁺, Ni²⁺, Cd²⁺, Pb²⁺, Zn²⁺, Cr³⁺, Cu²⁺, Ag⁺, Ca²⁺ and Mg²⁺ (50 equiv.). Inset: The photograph showing the color change of SD solution in DMSO/H₂O (1:9, v/v) after addition of Hg²⁺ and other ions at room temperature under UV light.



Titration experiments with mercury ions were carried out at room

temperature to investigate the change in the response of SD $(2.0 \times 10^{-5}M)$ to the ring opening of rhodamine derivatives in DMSO/ H_2O (v/v = 1:9) solution. As shown in Fig. 4, mercury ion (0.1 M) were added dropwise to adjust the concentration to 0–21.7 equiv., which was observed that the absorption peak at 368 nm gradually weakened while the absorption peak at 565 nm increased. When 31.2 equivalents of



Fig. 4. Absorbance spectra titration of **SD** $(2.0 \times 10^{-5} \text{M})$ in the presence of different concentration of Hg²⁺ (0.0–21.7 equiv.) in DMSO/H₂O (v/v = 1:9).



Fig. 5. Fluorescence intensity titration of **SD** $(2.0 \times 10^{-5}$ M) in the presence of different concentration of Hg²⁺ (0.0-31.2 equiv.) in DMSO/H₂O (v/v = 1:9).

 Hg^{2+} (0.1 M) were added, when excited at 540 nm, the fluorescence of **SD** was significantly enhanced at 588 nm (Fig. 5).

In order to further verify the selectivity of **SD**, anti-interference tests of various metal ions (Fe^{3+} , Co^{2+} , Ni^{2+} , Cd^{2+} , Pb^{2+} , Zn^{2+} , Cr^{3+} , Cu^{2+} , Ag^+ , Ca^{2+} and Mg^{2+}) were carried out. As shown in Fig. 6, in the absence of mercury ions, the **SD** solution containing various cations did not cause significant spectral changes, but all of the solutions showed significant spectral changes after the addition of Hg²⁺. The results in

а



Fig. 7. The reversibility of fluorescence of SD controlled by alternating addition of EDTA and Hg^{2+} .

the UV-vis spectrum and the fluorescence spectrum indicated that the sensor **SD** could be an effective probe for distinguishing Hg^{2+} .

In order to further study the practicality of **SD** for Hg^{2+} mixed aqueous solution, the effect of pH on **SD** was studied by fluorescence spectroscopy. As shown in **Fig. S5**, Hg^{2+} was added to the **SD** solution at different pH values, and the results showed that **SD** could work in the pH range of 4.0–9.0.

With further investigation, after adding EDTA to **SD**-Hg²⁺ in DMSO/H₂O (v/v = 1:9) solution, the fluorescence intensity of the solution returned to the original state of **SD**. This indicated that the reversibility of the binding process between Hg²⁺ and **SD** can be demonstrated (Fig. 7). Since EDTA has a strong affinity to Hg²⁺, when EDTA complexed to Hg²⁺, it leads to de-metallization of the sensor **SD** and regeneration of the spironolactam ring, which caused the fluorescence quenched. The fluorescence of **SD** can be repeated as an "OFF-ON-OFF" switching process multiple times.

As shown in Fig. 8, the changes in fluorescence intensity of **SD** with Hg^{2+} were almost linear. As calculated on the basis of $3S_B/S$ (where S_B is the standard deviation of the blank solution and S is the slope of the calibration curve) [46], the detection limit was down to 2.71×10^{-7} M, indicating that **SD** can detect low concentration of Hg^{2+} in practical applications. To determine the combined stoichiometry between **SD** and Hg^{2+} , job's plot was drawn (Fig. 9). When the molar fraction of Hg^{2+} was 0.5, the fluorescence intensity reached an extreme value, indicating that the stoichiometric ratio of **SD** and Hg^{2+} was 1:1.

To confirm the complexation mechanism between **SD** and Hg^{2+} , the ¹H NMR titration spectrum in DMSO- d_6 was investigated (Fig.10). **SD** showed two single peaks at 10.36 ppm and 9.01 ppm, corresponding to



Fig. 6. (a) Absorbance spectra changes for **SD** $(2.0 \times 10^{-5} \text{ M})$ in presence of selected metal ions in DMSO/H₂O (v/v = 1:9) solution. (b) Fluorescence intensity changes for **SD** $(2.0 \times 10^{-5} \text{ M})$ in presence of selected metal ions in DMSO/H₂O (v/v = 1:9) solution. The red bars represent the intensity of **SD** in the presence of other cations (Fe³⁺, Co²⁺, Ni²⁺, Cd²⁺, Pb²⁺, Zn²⁺, Cr³⁺, Cu²⁺, Ag⁺, Ca²⁺ and Mg²⁺). The green bars represent the change that occurs after addition of Hg²⁺ to the previously solutions, respectively.



Fig. 8. Fluorescence detection limit spectra of SD in DMSO/H₂O (v/v = 1:9) solution upon addition of an increasing concentration of Hg²⁺.



Fig. 9. Job's plots of SD and Hg^{2+} .



Fig. 11. Comparison between the FT-IR spectral data for SD and SD-Hg²⁺ complex.

-OH and -CH = N, respectively. After Hg^{2+} was added, the -CH = N signal downfield shifted to 9.86 ppm and the -OH signal upfield shifted to 9.21 ppm. In addition, the proton peak in the benzene ring also had a splitting phenomenon due to the change of electron density caused by the ring-opening of the spirolactam of the SD. The ring-opening was also confirmed in the Fourier transform infrared spectrum (Fig.11). After the addition of Hg^{2+} , the absorption peak of carbonyl band in the spironolactone ring disappeared at 1691 cm^{-1} while a new absorption peak of amide carbonyl appeared at $1589\,\mathrm{cm}^{-1},$ indicating that the spironolactam ring was opened. Based on these results, Scheme 2 can be used to describe the possible reaction mechanism between **SD** and Hg^{2+} .

In order to better confirm the proposed mechanism of **SD** and Hg^{2+} , DFT calculations were performed at B3LYP / 6–311 g (2d, p) level [47]. When **SD** was complexed with Hg^{2+} , the -CN bond of the rhodamine spironolactone ring was destroyed, which promoted the coordination between the carbonyl oxygen of the ionophore and the mercury ion. Compared to the π electrons in LUMO, the π electrons in the HOMO of



Fig. 10. ¹H NMR spectra of SD and in the presence of different amounts of Hg^{2+} in DMSO- d_6 at room temperature.



Fig. 12. The DFT computed HOMO and LUMO diagram of SD and SD-Hg $^{2+}$ system.

SD-Hg²⁺ system was transferred to mercury ion, and the energy gap (ΔE) between them was 0.049 au and 0.036 au, respectively, indicating that the coordination of **SD** with Hg²⁺ reduced the HOMO- LUMO energy gap and stabilizes the system (Fig.12). These calculations were consistent with the experimental results.

4. Application

In order to study the application value of sensor **SD** in real life, the test strips were prepared by immersing the filter papers in **SD** (0.1 M) DMSO/H₂O (1:9, v / v) solution and then exposing them into air to dry them. As shown in Fig. 13, when Hg²⁺ was added to the test papers, a remarkable color change could be observed under visible light and ultraviolet light. Therefore, **SD** test papers had excellent application value in the detection of Hg²⁺.

To further demonstrate that SD can detect Hg^{2+} concentrations in



Fig. 13. Photographs of **SD** (0.1 M) on test strips (a) only **SD**, (b) after immersion into water solutions with Hg^{2+} , (c) only **SD** under UV lamp, (d) after immersion into water solutions with Hg^{2+} under UV lamp, at room temperature and irradiation under UV light at 365 nm.





Fig. 14. linear fluorescence response of **SD** (0.1 M) in water samples. The response (F) is normalized to the emission of the free sensor (F_0).

water environments, we added 0–5 ppb of Hg^{2+} to the **SD** (0.1 M) solution. At a concentration of 2 ppb (the maximum U.S. EPA limit for allowable levels of Hg^{2+} ions in drinking water), the fluorescence emission intensity of **SD** increased by 3.2 times (Fig. 14). Under the experimental conditions, the fluorescence intensity of the **SD** solution was almost proportional to the amount of Hg^{2+} ($R^2 = 0.99$), indicating that **SD** can distinguish the safety and toxicity levels of inorganic mercury in drinking water.

The practicability of the probe **SD** was demonstrated by analysing the water sample. The content of Hg^{2+} in tap water was measured by fluorimetry under the same conditions, and the tests was repeated three times, as shown in Table 1. The detected Hg^{2+} concentration was close to the added concentration. The recovery rate was about 100%, and the relative standard deviation (RSD) of the three measurements were less than 4.20%, which indicates that it is feasible and accurate to determine the Hg^{2+} in the environmental sample using **SD**.

5. Conclusions

In summary, we designed and synthesized a simple and effective sensor **SD**, which could selectively recognize Hg^{2+} through dualchannel within the pH range of 4–10. The detection limit of fluorescence response of **SD** to Hg^{2+} was as low as 2.71×10^{-7} M based on the basis of $3S_B/S$. The fluorescence process could be reversed by adding Hg^{2+} and EDTA, and the switching process could be repeated several times without a large fluorescence loss. By studying the ¹H NMR and IR spectra, the proposed reaction mechanism between **SD** and Hg^{2+} was due to the ring opening of the spironolactone ring of rhodamine. In addition, the **SD**-based test strips were prepared which could be used as a colorimetric and fluorescence measurement tool for rapid detection of Hg^{2+} . The qualitative detection of mercury ions in tap

Table 1

determination of Hg^{2+} in the tap water.

Sample	${\rm Hg}^{2+}{\rm added}$ concentration / (µM)	Hg^{2+} found concentration /(μM)	recovery rate /%	RSD / (%, n = 3)
Tap water	2	1.97	98.5	4.2
	5	5.12	102.4	3.5
	10	10.21	102.1	3.3

water successfully verified the feasibility of **SD** in practical applications. We believe that **SD** as a Hg^{2+} sensor makes it more conspicuous for its potential application.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jphotochem.2019.04. 031.

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