ORIGINAL ARTICLE



A Colorimetric and Fluorescent Indicator for Hg²⁺ Detection Based on Cinnamamide Group-Containing Rhodamine Derivative

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Abstract

A colorimetric and fluorescent indicator based on cinnamamide group-containing rhodamine derivative was synthesized for the detection of Hg^{2+} . The rhodamine B and cinnamamide were connected via ethylenediamine as a bridging molecule through a condensation reaction to obtain a colorimetric and fluorescent indicator for the detection of Hg^{2+} in H_2O -EtOH (4:1, ν/ν). The indicator was excellent in the selectivity of Hg^{2+} and was almost unaffected by other common ions such as Na⁺, K⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Cr³⁺. The Hg^{2+} -containing aqueous solution turned from colorless to red within 7 min after the addition of the indicator, and had an absorption peak at 564 nm in UV-vis, which implies a significant colorimetric phenomenon. Their characteristic peaks varied with the Hg^{2+} content, and they reached a linear relationship at low concentrations. The binding stoichiometry proved to be 1:1. The lowest detection limit was 4.1×10^{-7} mol/L, ranging from acidic to neutral.

Keywords Rhodamine derivative \cdot Hg²⁺ detection \cdot Colorimetric indicator \cdot Fluorescent indicator \cdot Aqueous solution

Introduction

Heavy-metal pollution has become a serious problem in the world. Mercury (Hg) is derived from chemical industries such as chloro-alkali industry and the synthesis of mercuric compounds, and it has been confirmed that mercury has certain damage to the nervous system, immune system and internal organs [1, 2]. For this reason, scientists have provided several Hg²⁺ detection methods, usually including atomic absorption spectroscopy (AAS) [3], inductively coupled plasma mass spectrometry (ICP-MS) [4], etc. Despite the high selectivity and accuracy, these large devices cannot overcome the required special staff and time-consuming disadvantages. Therefore, there is an urgent need to find a quick, simple and economical method to detect mercury in the environment.

In recent years, a large number of colorimetric and fluorescent indicators have been reported for the detection of Hg^{2+} [5–10]. Rhodamine B (RB) as a colorimetric and fluorescent dye has good photostability, long excitation wavelength and high colorimetric and fluorescence quantum yield [11, 12]. The spirocyclic structure of lactam in RB has attracted great interest among researchers. The recognition of functional groups can achieve synergistic effects through the reaction of carbonyl groups with some metal ions. The fluorescence intensity of RB changes from low to high during the opening and closing of the lactam ring. In this way, certain metal ions can be selectively recognized.

The reaction of suitable polyamine with benzoic acid, phthalic anhydride, sulfosalicylic acid, phthalic acid and organic acid anhydride is based on the typical synthesis of rhodamine derivatives Hg^{2+} colorimetric and fluorescent probe [13–16]. In order to compensate for the lack of selectivity of Hg^{2+} , insufficient hydrophilicity and quantum yield of RB, it is possible to modify the RB derivatives after the condensation reaction. Most studies have focused on modifying the main molecular structure to design colorimetric and fluorescent probes, which focus on the detection of certain metal ions, particularly in all aspects where Hg^{2+} detection capability is prominent [17–21]. However, there are still problems with probe selectivity and sensitivity or environmental friendliness.

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In this paper, a colorimetric and fluorescent probe based on cinnamamide containing RB has been synthesized to detect Hg^{2+} . The structures, colorimetric and fluorescence capabilities were characterized by ¹H-NMR, UV-vis and fluorescent spectra. The effects of pH value, binding mode and external ion interference on its selectivity performance were discussed in detail.

Experimental

Materials and Instruments

¹H-NMR spectra were recorded on a Bruker DMX500 with CDCl₃ as the solvent and TMS as an internal standard record under 400 MHz. FT-IR spectra were obtained on a Bruker HYPERION 2000. The fluorescent spectra were received from Hitachi F-2500 with an excitation wavelength of 500 nm. UV-vis spectra were measured on a Hitachi U-3100 at a scan speed of 300 nm/min and a range from 500 nm to 800 nm.

Rhodamine B (AR), ethylenediamine (AR), methanol (AR), dichloromethane (AR), cinnamic acid (AR), thionyl chloride (AR), N,N-Dimethylformamide (AR), triethylamine (AR), sodium bicarbonate (AR), absolute ethanol (AR), copper (II) sulfate pentahydrate (AR), iron (III) chloride hexahydrate (AR), sodium chloride (AR), potassium nitrate (AR), magnesium nitrate hexahydrate (AR) and zinc nitrate (AR), were all purchased from Sinopharm Chemical Reagent Co., Ltd. Different stock solutions of Na⁺, K⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺ were prepared by dissolving the corresponding salts. The above materials were utilized without further treatment.

The standard solution of Hg²⁺ was purchased from Keli Technology Company in Analysis and Test Center, Guangzhou.

Synthesis of Cinnamoyl Chloride (Intermediate a)

Cinnamic acid (1 g) was dissolved in thionyl chloride (10 mL) in a 50 mL reaction flask. Subsequently, DMF (0.1 mL) was added dropwise to the solution. The mixture was heated to flux for 2 h and then thionyl chloride was removed by vacuum distillation. A light yellow powder was obtained and named as cinnamoyl chloride (Intermediate A). The product was sealed and stored under dry and cool condition.

Synthesis of RB - Ethylenediamine Derivative (Intermediate B)

RB (5 g) was dissolved in ethanol (50 mL) in a 250 mL reaction flask. Ethylenediamine (10 mL) was dropped into the solution with stirring. Under nitrogen protection, the reaction was carried at 75-85 °C for 4 h. The solvent was removed

after the flask was cooled to room temperature. The light brown solid was extracted with dichloromethane (30 mL) for 3 times. The organic phase was dried over anhydrous sodium sulfate for 12 h, and then an orange solid can be obtained after rotary evaporation. The RB- ethylenediamine derivative (intermediate B) can be prepared by chromatography using methanol / dichloromethane (1:10, v/v) as the developing agent. Intermediate B was stored under dry and dark conditions.

Synthesis of RB - Ethylenediamine - Cinnamamide Derivative (Probe 1)

Intermediate B (2.0 g) was dissolved in ethanol (50 mL) in a 250 mL reaction flask. Subsequently, triethylamine (1.0 mL) and sodium bicarbonate (0.5 g) were added while Intermediate A (1.0 g) dissolved in ethanol (10 mL) was added dropwise to the 250 mL reaction flask. After the reaction was allowed to proceed at room temperature under a nitrogen atmosphere for 24 h, the organic solvent was evaporated, and the product was extracted twice with dichloromethane (50 mL). The organic phase was washed three times with water and combined. Then, the organic phase was dried over anhydrous sodium sulfate for 5 h (Fig. 1).

After removing anhydrous sodium sulfate by filtration, the resulting yellow product was washed for three times with cold ethanol, and then a brown solid named Probe 1 was prepared by chromatography using methanol / dichloromethane (1:15, v/v) as developing agent. The product was stored in dry, dark and sealed conditions.

The ¹H-NMR spectra of Probe 1 is given in Fig. 2: δ 7.83 (*s*, 1H, imine), 7.60 (*s*, 1H, benzene), 7.37 (*t*, 2H, benzene), 7.02 (*s*, 1H, benzene), 6.30 (*d*, 2H, methyne), 6.28 (*m*, 5H, benzene), 6.24 (*d*, 4H, benzene), 3.18 (*d*, 8H, methylene), 2.87 (*dd*, 4H, methylene), 2.32 (*s*, 2H, benzene), 1.12(*t*, 12H, methyl).

The multiple states appearing at 2.87 correspond to the hydrogen n, o on the methylene of ethylenediamine, while the multiple peaks at 7.83 correspond to hydrogen a on the imine group, and the ratio of hydrogen to methyl is 1:12. It shows that RB has reacted with ethylenediamine, and the terminal amino group has then reacted with cinnamoyl chloride, which confirms the structure of the RB derivative.

The FT-IR spectra of Probe 1 are shown in Fig. 3(a). The characteristic absorption peaks of RB can be clearly observed at wavenumbers of 1600 cm^{-1} and 1250 cm^{-1} . Typically, the characteristic absorption peaks of the diamines are generally around 3300 cm^{-1} , 2900 cm⁻¹ and 900 cm⁻¹. However, due to the large number and high intensity of the absorption peaks of the rhodamine itself, the diamine's peaks have been covered to some extent. Therefore, another characterization should be performed to prove the generation of Probe 1.

Fig. 1 Synthesis routine of (**a**) Intermediate A; (**b**) Intermediate; (**c**) Probe 1



The fluorescent spectra of Probe 1 and RB are given in Fig. 3(b). Compared to RB, Probe 1 does not show a fluorescent peak at 580 nm in the excitation wavelength. Probe 1 did not fluoresce under irradiation with a fluorescent lamp, indicating that a lactam structure was generated in Probe 1.

Results and Discussions

High Selectivity for Hg²⁺

To test whether Probe 1 has any response to external ions, it is added to a standard solution with ordinary metal ions (Na⁺, K⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Cr³⁺, Hg²⁺, the concentration of each

Fig. 2 ¹H-NMR spectra of Probe 1

metal ion was 0.5 mmol/L). At the same time, Intermediate B was also dropped into the above solution with various ions for comparative experiments. From the results, there was no significant change in color or fluorescence after Intermediate B was mixed with common metal ions. When Probe 1 was mixed with Hg^{2+} , the solution containing Hg^{2+} turned intense pink. However, the rest did not show any significant changes. The result is shown in Fig. 4(a).

By naked-eye observation, we also performed UV-vis spectra analysis using all of the above samples to determine if each metal ion had a combination effect with Probe 1. The results are given in Fig. 4(b), and it was found that in the Hg²⁺-containing solution, the peak intensity of Probe 1 at 564 nm increased significantly, which represents a strong connection between Probe 1 and Hg²⁺. In other samples, although the





Fig. 3 a FT-IR and (b) Fluorescent spectra of Probe 1

peak intensity at 564 nm also increased, their peak intensity wad 90% lower than that generated by Hg^{2+} . It can reflect the results of the color, while those containing Hg^{2+} can be easily identified by observation. The UV-vis results indicate that the strong combination between Probe 1 and Hg^{2+} allows Probe 1 to be used for sensitive Hg^{2+} detection without interference from other common metal ions.

Time and pH Response

In order to measure the sensitivity of Probe 1, 0.5 mL of a solution in a certain concentration of Hg^{2+} was prepared and mixed with 10 mL of Probe 1 by ultrasonic vibration. The intensity of the characteristic UV-vis peak was measured at 0–10 min intervals after the addition of Probe 1. The results

are shown in Fig. 5(a), from which we can learn that the highest intensity reached 7 min after the addition of Probe 1, and then there was a little change. This proves that Probe 1 has a relatively long detecting time and is suitable for non-real-time monitor.

As for pH response, 10 mL of Probe 1 and 10 parts each containing 2 mL Hg^{2+} solution with different pH values (pH = 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) were mixed and the fluorescence intensity was measured after 10 min. From Fig. 5(b), it can be concluded that when the pH of the system is in the range of 2 to 7, the solution has better absorption intensity than the sample with pH greater than 8. This means that Probe 1 has a predominant binding nature to Hg^{2+} under neutral and acidic conditions, mainly because Hg^{2+} is not stable under alkaline conditions. The above results indicate that the

Fig. 4 a Photograph of Probe 1 (0.1 mmol/L) mixed in H₂O-EtOH (4:1, ν/ν , 0.1 mmol), b UV-vis spectra of Probe 1 with A-H, and (c) UV-vis spectra of Probe 1 only with Hg²⁺. A-H: Na⁺, K⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Cr³⁺, Hg²⁺



detection of Hg^{2+} by Probe 1 is suitable for acidic or neutral conditions.

Binding Form

The binding stoichiometry of Probe 1 with Hg^{2+} can be studied using the Benesi-Hildebrand equation and Job's plot model [22–24].

$$\frac{1}{A-A_0} = \frac{a}{a-b} \cdot \left[\frac{1}{K[M]} + 1\right] \tag{1}$$

In Formula (1), K represents the complexation constant between the fluorescent probe and the metal ion; A_0 represents the UV adsorption of Probe 1 without the metal ion, and Arepresents the UV adsorption of Probe 1 with the metal ion. [M] represents the concentration of added metal ion, where a



Fig. 5 a UV-Vis spectra and (b) fluorescent intensity of Probe 1 with Hg^{2+}

and b are constants. The complexation constant can be calculated by fitting a curve from $1/(A-A_0)$ to 1/[M].

Probe 1 was added to a solution containing various concentrations of Hg^{2+} . As shown in Fig. 4(c), it can be observed

Table 1 The absorbance of Probe 1 (0.1 mmol/L) with Hg²⁺ (0.5–4 equiv.) in solution (H₂O-EtOH, 4:1, ν/v)

No.	Relative content of Hg ²⁺	Maximum absorption intensity
1	0.05	0.007236
2	0.1	0.014652
3	0.2	0.028896
4	0.3	0.041004
5	0.4	0.058068
6	0.5	0.073116
7	0.8	0.096324
8	1	0.140592
9	1.5	0.220092
10	2	0.279972
11	2.5	0.351156
12	3	0.407820
13	3.5	0.495216
14	4	0.561852



Fig. 6 Fluorescent intensity of Probe 1 vs. Hg^{2+} concentration in H_2O -EtOH (4:1, v/v) and the proposed binding form

that with the increase of Hg^{2+} , the absorbance at 564 nm becomes stronger due to the ring-opening effect of Hg^{2+} in Probe 1 caused by the lactam structure. It means the combination of Probe 1 and Hg^{2+} . The specific data is given in Table 1. In order to determine the stoichiometry, several combination models were tried. Finally, 1:1 in stoichiometry was accepted for the fitting. From the data in Table 1, it can be seen that the fitted line is very close to the linear regression equation, and the correlation coefficient is 0.99971.

The fitted curve of the fluorescence intensity of Probe 1 and Hg^{2+} is shown in Fig. 6. The total concentration of Probe 1 and Hg^{2+} was maintained at 2.0×10^{-4} mol/L. It can be seen that when the concentration of Hg^{2+} is equal to 0.5 equiv., there is a maximum fluorescence intensity at 564 nm, which also indicates that the binding form of Hg^{2+} and Probe 1 follows a stoichiometric ratio of 1:1. The proposed binding form is also given.



Fig. 7 UV-vis absorbance of Probe 1 mixed with various metal ions (Na⁺, K⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Cr³⁺) in H₂O-EtOH (4:1, ν/ν) (wavelength: 564 nm)

Detection Limit and Anti-Interference

To find the detection limit (DL) of Probe 1, the excess Probe 1 was added to the solution with low concentration of Hg²⁺. According to the formula $DL = 3S/\rho$ [17], where S represents the standard deviation of the blank test, ρ represents the slope of the line (ratio of relative intensity to sample concentration), and the detection limit can be calculated to be 4.13×10^{-7} mol/L, which means that Probe 1 is still valid in very low concentrations of Hg²⁺ treated water samples.

The specific procedure for determining whether other metal ions interfere with the detection of Probe 1 is as follows: 8 parts (2 mL each) solution (H₂O-EtOH, 4:1, v/v) were mixed with Hg²⁺ (1 mmol/L) with X (1 mmol/L, X can be Na⁺, K⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Cr³⁺, respectively), respectively, then Probe 1 (2 mL, 0.1 mmol/L) was added to the solution. Their absorbance in the UV-vis spectra after 10 min were compared. As shown in Fig. 7, it can be inferred that the absorbance becomes stronger in the presence of other metal ions. All samples containing Hg²⁺ and foreign ions have stronger absorption than samples containing only foreign ions, indicating that Probe 1 can be used to detect Hg²⁺ in samples containing various other ions.

Conclusions

In summary, we have synthesized a colorimetric and fluorescent indicator based on rhodamine derivatives containing cinnamamide group that can be used to detect Hg²⁺ in aqueous solutions. The detection limit of this indicator is 4.13×10^{-7} mol/L, which can respond within 7 min. It has excellent anti-interference performance to other common ions (Na⁺, K⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Cr³⁺) in H₂O-EtOH solution (1/4, v/v). We assume that these studies on RB- ethylenediamine cinnamamide derivatives provide a promising prospect for the detection of Hg²⁺ in aqueous solutions.

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