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Hg^{2+}-selective fluorescent probe based on rhodamine 6G and its application in aqueous media

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ABSTRACT

Based on rhodamine 6G, a highly sensitive and selective fluorescent sensor R6G1 for Hg^{2+} detection had been designed and prepared. It was synthesized through the well-known reaction that thiourea derivatives with amine could easily be transformed into guanidine derivatives with the promotion of Hg^{2+}. By coordination with Hg^{2+}, R6G1 exhibited high sensitivity and selectivity over other metal ions in aqueous systems. Furthermore, fluorescence titration experiments established the well-fitted linearity function of the fluorescent intensity with the concentration of Hg^{2+} in aqueous solution. The results showed that R6G1 provided high water solubility and high selectivity toward Hg^{2+} but no significant response toward other competitive cations and anions. It was suggested that the chemosensor would find its application in environmental field requiring rapid and accurate Hg^{2+} ion analysis.

Keywords: Rhodamine; Fluorescent probe; Hg^{2+}; Synthesis; Spectral properties

1. Introduction

Mercury is considered a highly toxic and hazardous element on earth [1]. Some human activities, such as the combustion of solid waste and the gold mining, have caused a fast increase in Hg^{2+} level in the environment. Ionic mercury can be converted into neurotoxic methylmercury in the natural environment [2–4]. Therefore, there is an urgent need for the determination of Hg^{2+} ion in the environment as well as in

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industrial waste. Fluorescence probe has attracted much attention because of its simplicity and sensitivity. Fluorometric methods can reach a much lower detection limit, and the indicators involved are widely available [5–7]. Among numerous indicators, rhodamine-based dyes have been widely used as fluorophoric and chromophoric probes for their large molar extinction coefficients, high fluorescence quantum yields, and long absorption and emission wavelength [8]. Herein, a new rhodamine 6G-based derivative R6G1 was synthesized as fluorescence chemosensor for Hg^{2+} ions (Fig. 1). It was designed through a well-known reaction by transformation of thiourea derivatives with amine into guanidine derivatives with the promotion of Hg^{2+}. Compared with other Hg^{2+} fluorescent chemosensors, R6G1 showed high water solubility and high selectivity toward Hg^{2+} but no significant response toward other competitive cations and anions in environment.

2. Materials and methods

2.1. Instruments and reagents

Mass spectrometry data were obtained with a LC/Q-TOF MS spectrometer. ^{1}H-NMR spectrum was recorded on a Bruker AVIII-400 spectrometer operating at 400 MHz where chemical shifts were indicated by δ values in ppm (in $CDCl_3$, TMS as internal standard). Fluorescence measurements were performed on a VARIAN CARY Eclipse Fluorescence Spectrophotometer, and the slit width was 2.5 nm for excitation and 5 nm for emission. Ultraviolet measurements were performed on Agilent 8453 UV–visible Spectroscopy System. All pH measurements were made with a METTLER TOLEDO FE20. The chemicals used in this work were all of analytical grade and used without further purification. Deionized water was used throughout the experiments. All stock chemicals solutions were freshly prepared.

2.2. Preparation of metal ions stock solutions

Solutions (0.01 M) of the salts of $MgCl_2$, $CaCl_2$, $NiCl_2$, $MnCl_2$, KCl, NaCl, $ZnSO_4$, $CoCl_2$, $CdCl_2$, $BaCl_2$, $FeCl_2$, $HgCl_2$, $CuSO_4$, $FeCl_3$, and $CrCl_3$ were prepared in water with buffer in pH 7.15 (0.02 M Tris–HCl buffer). Solution (0.01 M) of the salt of $Pb(NO_3)_2$ was prepared in pure water.

2.2.1. Test solutions

A known volume of 0.5 ml of R6G1 buffer solutions: R6G1 (0.1 mM acetonitrile) was added in an appropriate aliquot of each metal stock. The solution was diluted to 5 mL with Tris–HCl buffer before measuring at wavelength 510 nm.

2.3. Synthesis of R6G1

Compound 2 was synthesized according to the literature method [8]. The reaction mixture of rhodamine 6G and ethylenediamine was refluxed for 3 h until the color turns to light yellow. The product was obtained by recrystallization from acetonitrile to give 749 mg of compound 2 (white solid) in 78.4% yield. Afterward, to synthesize the mercury ion recognition probe, the compound 2 (550 mg) and Lawesson's reagent (250 mg) were dissolved in dry toluene. The mixture was refluxed for 6 h under N_2 atmosphere. After the removal of toluene, the residue was dissolved in dichloromethane and purified by column chromatography with ethyl acetate/petroleum ether (volume ratio 1:3) as eluant to afford R6G1 in 26% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.14 (d, 1H, $J = 2.4$ Hz), 7.51 (d, 1H, $J = 1.6$ Hz), 7.50 (d, 1H, $J = 3.6$ Hz), 7.07 (m, 1H), 6.35 (s, 2H), 6.10 (s, 2H), 3.61 (t, 2H, $J = 5.6$ Hz), 3.20 (m, 4H), 2.61 (t, 2H, $J = 6.0$ Hz,), 1.87(s, 6H), 1.32 (t, 6H, $J = 7.2$ Hz,); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 168.8, 153.9, 151.8, 147.6, 132.6, 131.4, 128.5, 128.2, 124.0, 123.0, 118.2, 106.3, 96.7, 65.2, 44.1, 40.9, 38.5, 16.9, 14.9 $[M + H]^+$: 473.2.

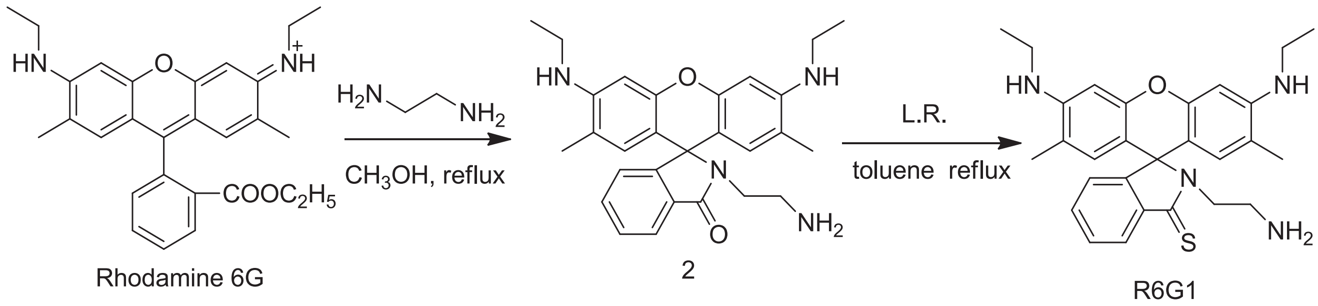

Fig. 1. Synthetic route of R6G1.

3. Results and discussion

3.1. Selectivity of R6G1 with different metal ions

The selectivity of R6G1 with various metal ions was conducted in Tris–HCl buffer. Upon addition of Hg^{2+}, R6G1 showed an apparent fluorescence enhancement. Different from Hg^{2+}, other metal ions, such as Cr^{3+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Pb^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Zn^{2+}, Ba^{2+}, K^+ and Ni^{2+}, did not induce any spectral response under identical conditions in aqueous solution (Fig. 2). Only Fe^{3+} and Cu^{2+} induced very small spectral changes. The fluorescence enhancement of R6G1-Hg^{2+} suggested that R6G1 might serve as a "naked eye" probe for Hg^{2+} in environment.

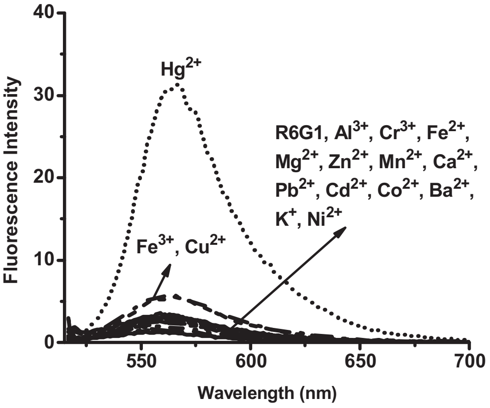

Fig. 2. Fluorescent emission change of R6G1 (5 μM) upon addition of 40 equiv metal ions of Fe^{3+}, Al^{3+}, Cr^{3+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Co^{2+}, Pb^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Zn^{2+}, Ba^{2+}, K^+, and Ni^{2+} in 0.02 M Tris–HCl.

3.2. Coordination of Hg^{2+} with R6G1

To investigate the interaction of R6G1 and Hg^{2+}, a fluorescence titration experiment was carried out to determine the binding affinity for Hg^{2+} in 5 μM solution of R6G1 in Tris–HCl buffer. From the Fig. 3, an increase in fluorescence intensity of R6G1 could be observed with the addition of Hg^{2+} and saturated after addition of about 5×10^{-6} M of Hg^{2+}. The interaction of Hg^{2+} with R6G1 fits well with a 1:1 binding model under the concentration range.

The nonlinear fitting of the titration curve has also indicated a 1:1 stoichiometry for the R6G1-Hg^{2+} complex. This binding mode was supported by the data of Job's plots evaluated from the absorption spectra of R6G1 and Hg^{2+} with a total concentration of 10 μM (Fig. 4). According to the 1:1 model, a stability constant (K_a) of 106 (Log $K_a = 5.91$) was obtained for the R6G1 binding with Hg^{2+}. The results mean that R6G1 might be of some practical use in the environmental analysis.

3.3. Competition experiments

Among the competition experiments of Hg^{2+} with other metal ions, there are some interference in Cu^{2+}, Al^{3+}, Cr^{3+}, and Fe^{3+}, which afford fluorescence enhancement upon the subsequent addition of Hg^{2+}. Fortunately, the enhancement did not influence the recognition for Hg^{2+} in environment. As shown in Fig. 5, competition experiments of Hg^{2+} with other metal ions imply that R6G1 has more coordination tendency and selectivity to Hg^{2+}.

Anions play the fundamental roles of in many chemical and biological processes. Here, the influence of anions on R6G1 was also examined. Fig. 6 displays the fluorescence responses of R6G1 to the presence of various environmental relevant anions. Except I^-, the Hg^{2+}-induced fluorescence response remains unaffected in the background of 40 equiv of other anions. These results indicate that R6G1 is a good candidate as a fluorescent probe using in environment.

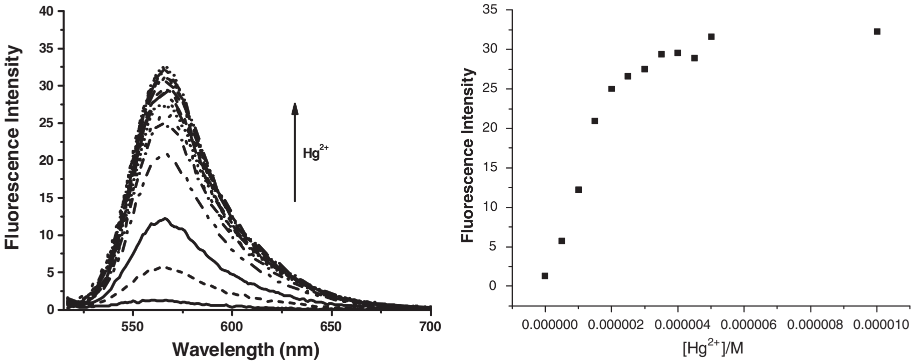

Fig. 3. Fluorescence titration spectra of R6G1 (5 μM) upon addition of Hg^{2+} in 0.02 M Tris–HCl buffer.

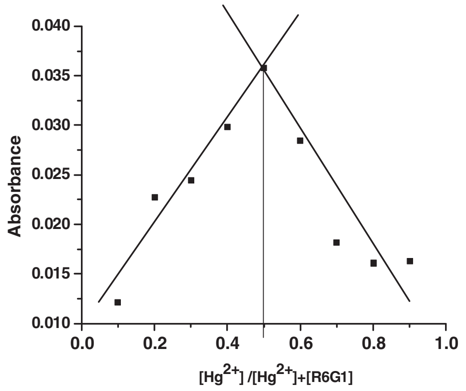

Fig. 4. Job's plot of UV absorption changes of R6G1.

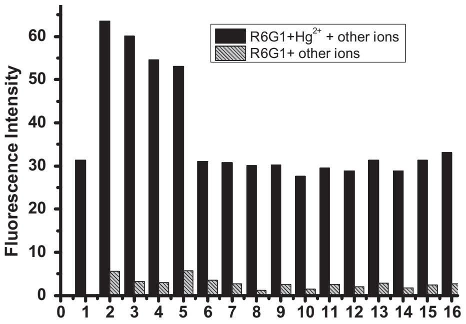

Fig. 5. Fluorescence response of R6G1 (5 μM)–200 μM of Hg^{2+} in 0.02 M Tris–HCl buffer containing 200 μM of various metal ions.

Notes: (1) Hg^{2+}, (2) Cu^{2+}, (3) Al^{3+}, (4) Cr^{3+}, (5) Fe^{3+}, (6) Fe^{2+}, (7) Mg^{2+}, (8) Zn^{2+}, (9) Mn^{2+}, (10) Ca^{2+}, (11) Pb^{2+}, (12) Cd^{2+}, (13) Co^{2+}, (14) Ba^{2+}, (15) K^+, (16) Ni^{2+}.

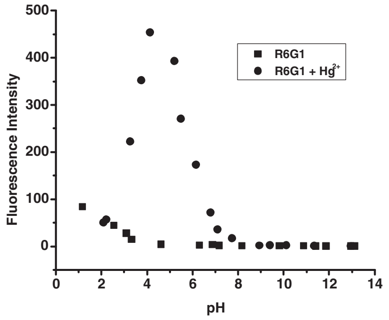

Fig. 7. pH-dependent fluorescence response of R6G1 and upon addition of Hg^{2+}.

3.4. pH effect of Hg^{2+} with R6G1

The pH-dependent fluorescence spectra of R6G1 and R6G1-Hg^{2+} have been investigated. Proton induced a reversible coloration in low pH for R6G1. Fig. 7 shows that R6G1 and R6G1-Hg^{2+} had stable fluorescence properties over a wide pH span, which suggests that R6G1 is suitable for application in environment and biological samples at a physiological pH value.

3.5. Possible mechanism

The possible mechanism for these fluorescent changes is shown in Fig. 8. According to some rhodamine-based chemsensors reported in the literature, it may be that Hg^{2+} coordinates with the corresponding part of R6G1 and induces the opening of the spiro-ring. This coordination is an irreversible process, which is supported by the fact that the color solution was identified as R6G3 by TLC and MS.

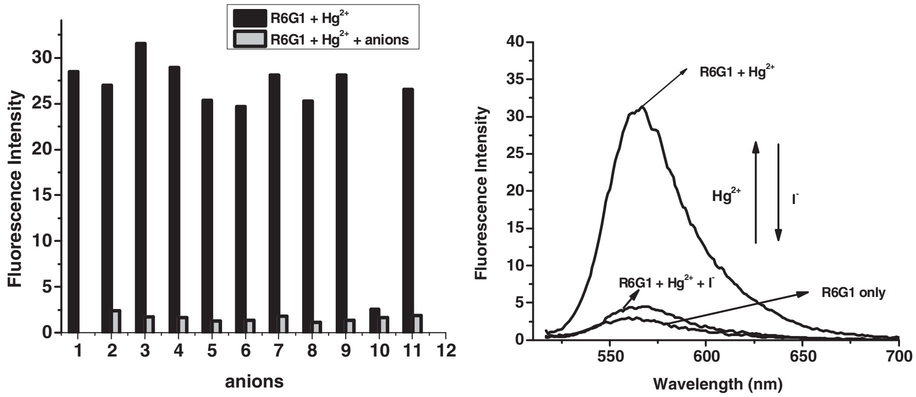

Fig. 6. (a) Fluorescence emission change of R6G1 (5 μM) upon addition of 40 equiv various anions and (b) fluorescence emission change of R6G1 (5 μM) upon addition of Hg^{2+} and I^-.

Notes: (1) Hg^{2+}, (2) H_2PO^{3-}, (3) NO^{2-}, (4) F^-, (5) MoO_4^{2-}, (6) HCO^{3-}, (7) SO_3^{2-}, (8) Br^-, (9) SO_4^{2-}, (10) I^-, (11) Cl^-.

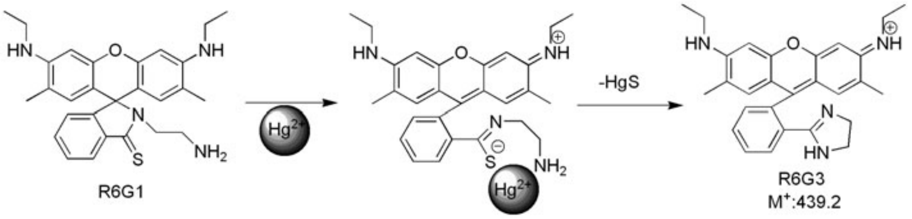

Fig. 8. Proposed Hg^{2+}-promoted ring opening of R6G1.

4. Conclusion

In summary, a new fluorescent sensor for Hg^{2+} has been synthesized based on rhodamine 6G. Upon addition of Hg^{2+}, a new absorbance and emission band with the maximum absorbance at 530 nm and emission wavelength at 560 nm appeared. More importantly, the results showed R6G1 with high water solubility and high selectivity toward Hg^{2+} but no significant response toward other competitive cations and anions. Moreover, reversibility of pH-dependent fluorescence is an excellent indication of the present probe to be used as chemical sensor [9]. This sensor will find its applications in a variety of fields requiring rapid and accurate Hg^{2+} ion analysis in environmental samples. Real wastewater trials would be an excellent indication of the applicability of the probe [9,10].

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