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A New Rhodamine B Hydrazide Hydrazone Derivative for Colorimetric and Fluorescent "Off-On" Recognition of Copper(II) in Aqueous Media

Lijun Tang,* Jiaojiao Guo, and Nannan Wang

*Department of Chemistry, Liaoning Key Laboratory for the Synthesis and Application of Functional Compounds, Bohai University, Jinzhou 121013, China. *E-mail: ljtang@bhu.edu.cn*
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A new Rhodamine B hydrazide hydrazone **1** has been synthesized and investigated as a colorimetric and fluorescent "off-on" sensor for the recognition of Cu^{2+} in CH_3CN/H_2O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution. Sensor **1** displayed highly selective, sensitive and rapid recognition behavior toward Cu^{2+} among a range of biologically and environmentally important metal ions. Sensor **1** bind Cu^{2+} *via* a 1:1 stoichiometry with an association constant of 1.92×10^6 M^{-1}, and the detection limit is evaluated to be 7.96×10^{-8} M. The Cu^{2+} recognition event is reversible and is barely interfered by other coexisting metal ions.

Key Words : Rhodamine B derivative, Copper(II) recognition, Fluorescent, Colorimetric

Introduction

In the past two decades, the development of highly selective and sensitive artificial chemosensors for the detection of transition and heavy metal ions has attracted considerable attention.[1] The optical detection methods such as UV-vis and fluorescence spectroscopy still remain the most frequently used modes due to their high sensitivity and easy operability.[2]

Copper(II) ion, as the third adequate element after iron and zinc, plays a critical role in many fundamental physiological processes in organisms. It is well known that it serves as a catalytic cofactor for a variety of metalloenzymes such as superoxide dismutase, cytochrome c oxidase and tyrosinase. However, unregulated overloading of copper can result in severe neurodegenerative diseases such as Alzheimer's or parkinson's diseases.[3] In recent years, Cu^{2+} is also suspected to cause infant liver damage.[4] Thus, the highly selective, sensitive and rapid recognition of Cu^{2+} by chemosensors in aqueous media is imperative. Consequently, considerable efforts have been devoted to the development of probes for Cu^{2+} recognition.[5]

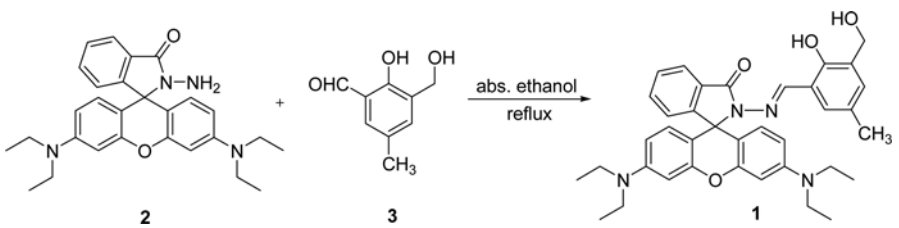
Rhodamine-based dyes are ideal candidates for constructing chemosensors for specific heavy and transition metal ions owing to their excellent spectroscopic properties such as long-wavelength emission, high fluorescence quantum yield and large molar extinction coefficient.[6] The rhodamine spirolactam form is basically colorless and non-fluorescent, while its metal induced ring-opened amide form has pink color and usually displays strong fluorescence. Recently, detection of metal ions by using of rhodamine spirolactam ring-opening process has been well reviewed.[7]

Herein, we report the synthesis and photophysical properties of a new rhodamine B based sensor **1**, which exhibits highly selective, sensitive and rapid Cu^{2+} recognition behavior via colorimetric and fluorescent detection modes.

Experimental

Apparatus and Reagents. Unless otherwise stated, all the solvents were of analytical grade from commercial sources and used as received. Column chromatography was performed on silica gel (200-300 mesh). UV-vis absorption spectra were measured on a SP-1900 spectrophotometer (Shanghai, China). Fluorescence measurements were performed on a Sanco 970 CRT spectrofluorometer (Shanghai, China). ^{1}H-NMR and ^{13}C NMR spectra were recorded on a Bruker Advance-600 MHz spectrometer. High resolution mass spectrum was measured on an Agilent 1200 time-of-flight mass spectrometer (Bruker, micrOTOF-Q). pH measurements were made with a Model PHS-25B meter. Compound **2**[8] and **3**[9] were prepared according to literature method.

Synthesis of Sensor 1. Compound **2** (1.0 g, 2.2 mmol) and **3** (0.382 g, 2.3 mmol) were dissolved in 30 mL of absolute ethanol and the mixture was heated to reflux for 6 hours. After the reaction completed, the solvent was rotary



Scheme 1. Synthesis of sensor **1**.

evaporated, the residue was purified by column chromatograghy with ethyl acetate-petroleum ether (1:2, v/v) as eluent, affording 0.932 g of **1** as pale yellow solid. Yield: 70.4%. mp 193.5-194.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.74 (s, 1H, Ar-OH), 8.85 (s, 1H, CH=N), 7.93 (d, 1H, 7.2 Hz, Phen-H), 7.62-7.58 (m, 4H, Phen-H), 7.17 (s, 1H, Phen-H), 7.11 (d, 1H, J = 7.2 Hz, Phen-H), 6.86 (s, 1H, Phen-H), 6.46-6.44 (m, 2H, Xanthene-H), 6.35 (d, 2H, J = 7.8 Hz, Xanthene-H), 5.01 (t, 1H, J = 4.8 Hz, CH_2OH), 4.40 (d, 2H, J = 4.8 Hz, CH_2OH), 3.31 (dd, 8H, J = 6.6 Hz, NCH_2CH_3), 2.18 (s, 3H, Phen-CH_3), 1.07 (t, 12H, J = 6.6Hz, NCH_2CH_3). ^{13}C NMR (150 MHz, DMSO-d_6, 298 K) δ 163.97, 153.02, 152.63, 152.25, 151.71, 149.10, 134.58, 130.87, 130.09, 129.69, 129.38, 128.57, 128.07, 128.02, 124.28, 123.57, 117.13, 108.89, 104.81, 97.99, 65.89, 57.98, 44.11, 21.38, 12.82. HRMS (ESI+) *m/z*, calcd for $C_{37}H_{41}N_4O_4$ $[M+H]^+$ 605.3128, found 605.3117.

Results and Discussion

Absorption Properties. Sensor **1** was facilely synthesized by condensation of Rhodamine B hydrazide **2** and 2-hydroxy-3-(hydroxymethyl)-5-methyl-Benzaldehyde (**3**) as depicted in Scheme 1. Preliminary absorption spectra studies demonstrated that sensor **1** (10 μM) in CH_3CN/H_2O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution has a good selectivity to Cu^{2+}. A solution of sensor **1** is colorless and shows no absorption in the visible region. Upon addition of Cu^{2+} (1.0 equiv to **1**), a new strong absorption band centered at 554 nm was formed. Concomitantly, the solution color changed from colorless to pink-red. These phenomena correspond to the Cu^{2+} binding induced ring-opening process of the spirolactam form of **1**.[7a] However, in the presence of other metal ions including Hg^{2+}, Ag^+, Pb^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Mg^{2+}, K^+ and Na^+ (1.0 equiv. for each), the absorption spectra of **1** solution did not show any obvious change (Fig. 1). It is note worthy that sensor **1** behaved a very high selectivity for Cu^{2+} over the potential competitors such as Zn^{2+}, Hg^{2+}, Cd^{2+} and Fe^{3+}.[10] The fact that only Cu^{2+} can induce the immediate color change suggests that sensor **1** can serve as a highly selective naked-eye visible probe for Cu^{2+} recognition.

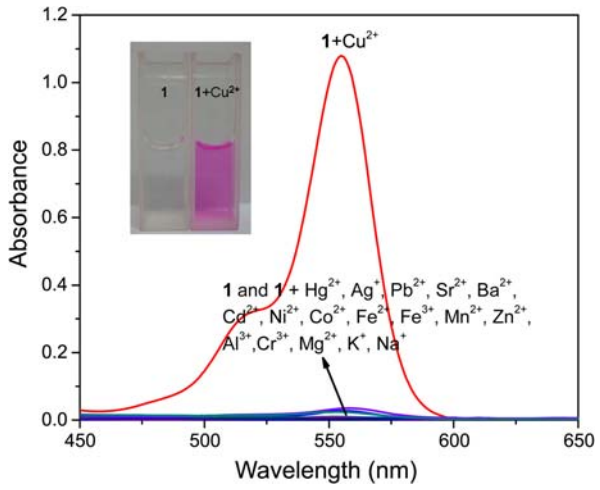


Figure 1. The absorption spectra of **1** (10 μM) in CH_3CN/H_2O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution upon addition of 1.0 equiv of various metal ions. Inset: Color changes of **1** solution before and after addition of Cu^{2+}.

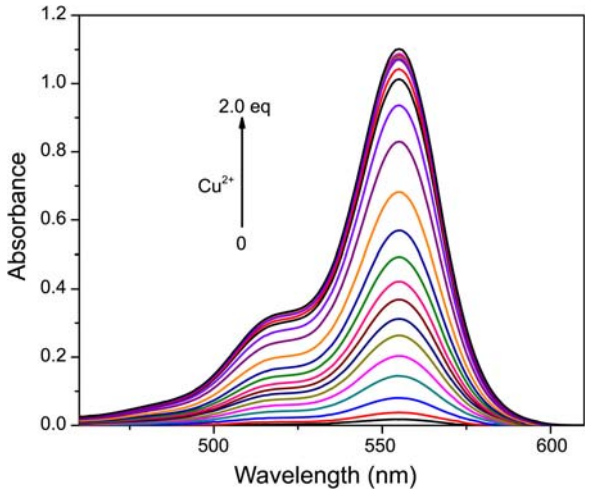


Figure 2. Changes in absorption spectra of **1** (10 μM) in CH_3CN/H_2O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution upon addition of Cu^{2+} (0 to 2.0 equiv).

Absorption spectra titration of **1** solution with different amount of Cu^{2+} was then carried out and the results were shown in Figure 2. Upon incremental addition of Cu^{2+} (0 to 2.0 equiv), the new absorption band centered at 554 nm increased gradually, the absorbance reached saturation when 1.0 equiv of Cu^{2+} was added. Further increasing of Cu^{2+} amount could not cause noticeable absorption intensity changes. Based on the molar ratio method,[11] our titration results strongly support the 1:1 binding stoichiometry of **1** and Cu^{2+}.

Fluorescence Properties. Solution of sensor **1** itself (10 μM) in CH_3CN/H_2O (1:1, v/v, HEPES 10 mM, pH = 7.0) shows a very weak fluorescence at 590 nm, which indicating the predominance of its spirolactam form. The characteristic peak of the 9-carbon of **1** near 66 ppm in the ^{13}C NMR spectrum also supports this consideration.[12] Addition of 1.0 equiv. of Cu^{2+} ion resulted in a remarkable fluorescence enhancement at 590 nm. Whereas, upon addition of other metal ions such as Hg^{2+}, Ag^+, Pb^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Mg^{2+}, K^+ and Na^+ (1.0 equiv. for each), no significant changes in the fluorescence emission of sensor **1** was occurred (Fig. 3). These facts further indicate that sensor **1** has high selectivity and sensitivity to Cu^{2+}.

The fluorescence titration of **1** (10 μM) by using of different Cu^{2+} amounts (0 to 5.0 equiv) was then conducted. As shown in Figure 4, the fluorescence intensity at 590 nm increased gradually upon incremental addition of Cu^{2+}. The emission reached saturation when 1.0 equiv. of Cu^{2+} was added, which corresponding to a 9.4-fold fluorescence enhancement. Comparing to the similar structure Cu^{2+} sensor, salicylaldehyde rhodamine B hydrazone,[12a] the fluorescence

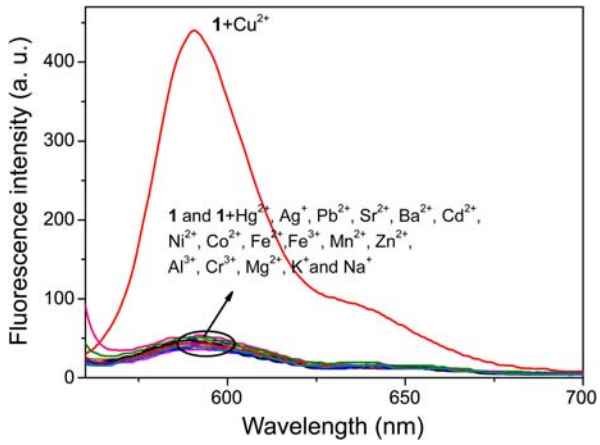


Figure 3. Fluorescent spectra of **1** (10 μM) in CH_3CN/H_2O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution upon addition of 1.0 equiv metal ions (Hg^{2+}, Ag^{+}, Pb^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Mg^{2+}, K^{+} and Na^{+}). λ_{ex} = 530 nm.

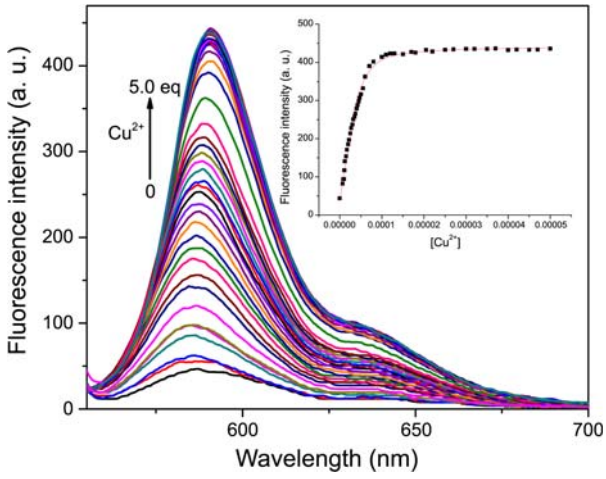


Figure 4. Fluorescence emission changes of **1** upon addition of Cu^{2+} (0-5.0 equiv) in CH_3CN/H_2O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution.

intensity enhancement is largely improved, which implies that the paramagnetic Cu^{2+} quenching effect is greatly inhibited by the simple modification of salicylaldehyde moiety. Further increasing of Cu^{2+} concentration did not induce significant fluorescence emission changes. These results reveal that sensor **1** behaves a good sensitivity toward Cu^{2+} and bind Cu^{2+} through a 1:1 stoichiometry. The result of nonlinear least-squares fitting of the titration profiles (inset in Fig. 4) employing a 1:1 binding mode equation[13] also strongly support the 1:1 interaction between **1** and Cu^{2+}, and the association constant K_a was calculated to be 1.92×10^6 M^{-1}.

Competitive Studies. For an effective sensor, the high selectivity for the target analyte over potentially competitive species is required.[14] Hence, the competition experiments in the presence of potentially competitive metal ions were conducted by UV-vis absorption and fluorescence spectroscopy. Except of Cu^{2+}, other metal ions (1.0 equiv for each) do not

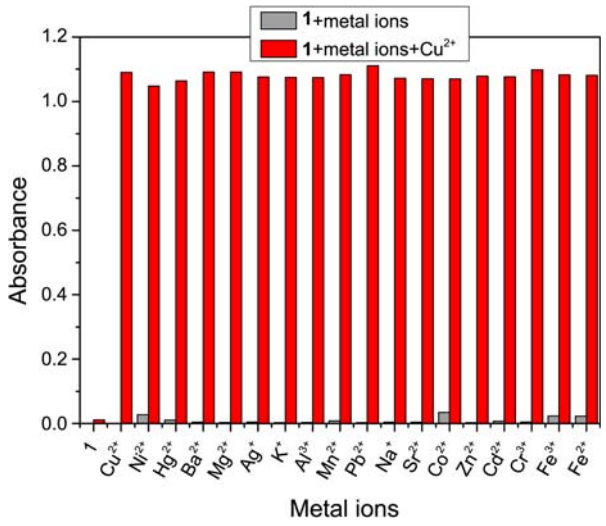


Figure 5. The absorption responses of **1** (10 μM) at 554 nm to various metal ions. The gray bars represent the absorption of **1** in the presence of 1.0 equiv of miscellaneous metal ions, the red bars represent the absorption of the above solution upon further addition of 1.0 equiv. of Cu^{2+}.

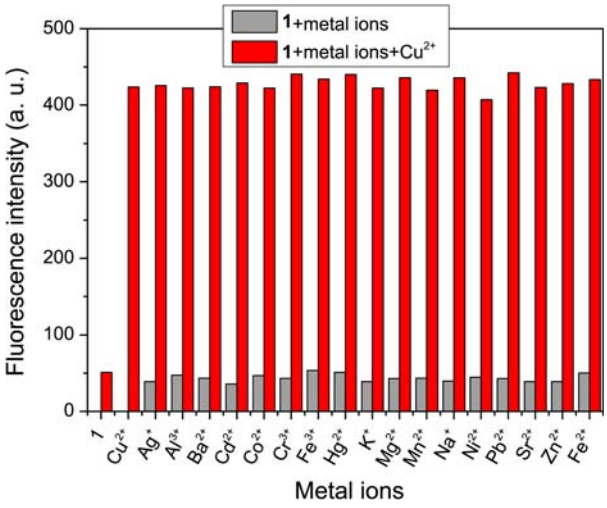


Figure 6. The fluorescence changes of **1** (10 μM) at 590 nm to various metal ions. The gray bars represent the fluorescence intensity of **1** in the presence of 1.0 equiv of miscellaneous metal ions, the red bars represent the fluorescence intensity of the above solution upon addition of 1.0 equiv of Cu^{2+}.

produce significant absorption (Fig. 5) and fluorescence (Fig. 6) intensity changes. Nevertheless, upon addition of Cu^{2+} (1.0 equiv to **1**) to the solution containing **1** and other metal ion, drastic increases in absorption (Fig. 5) and fluorescence emission (Fig. 6) were observed. From the results, it is inferred that the UV-vis and fluorescence Cu^{2+} recognition processes by **1** are barely interfered by other coexisting metal ions, therefore, sensor **1** exhibits an excellent anti-jamming ability.

Determination of Binding Stoichiometry. To further determine the binding stoichiometry between receptor **1** and Cu^{2+}, continuous variation method (Job's plot)[15] with a total concentration of $[Cu^{2+}]+[\mathbf{1}]$ as 10 μM was employed (Fig.

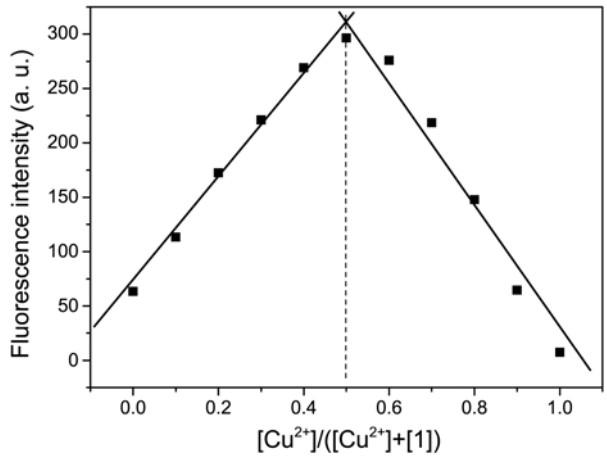


Figure 7. Job's plot monitored at 590 nm, the total concentration of $[Cu^{2+}]+[\mathbf{1}]$ was 10 μM.

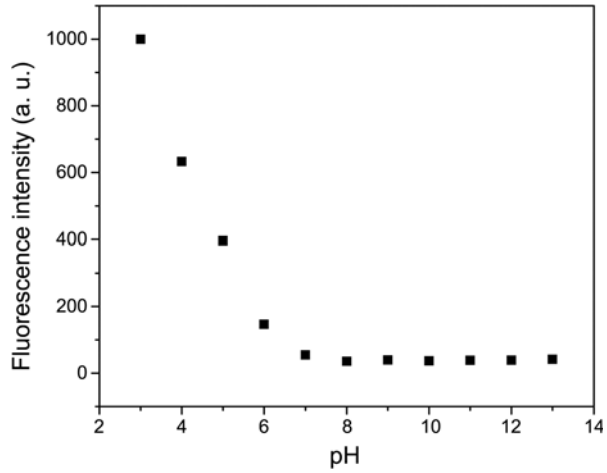


Figure 8. Effect of pH on the fluorescence at 590 nm of **1** (10 μM) in CH_3CN/H_2O (1:1, v/v).

7). The tested solution exhibited maximum fluorescence intensity when the molar fraction of Cu^{2+} is 50%, which indicating the 1:1 binding stoichiometry of **1** and Cu^{2+}.

Effect of pH. Furthermore, the effect of pH on the fluorescence of **1** in CH_3CN/H_2O (1:1, v/v) was investigated. The acid-base titration experiments showed that the fluorescence intensity almost keep constant at pH greater than 7.0 (Fig. 8). This result means **1** is suitable for detection of Cu^{2+} at near neutral pH conditions.

Study on Reversibility. The chemical reversible binding behavior of **1** to Cu^{2+} was verified by absorption spectra (Fig. 9). Ethylene diamine tetraacetic acid disodium salt ($EDTANa_2$) was selected as the chelating reagent due to its high affinity to Cu^{2+}.[16] Accordingly, a HEPES buffered CH_3CN/H_2O (1:1, v/v, pH = 7.0) solution composed of **1** (10 μM) and Cu^{2+} (10 μM) was titrated with $EDTANa_2$. As expected, addition of $EDTANa_2$ resulted in a significant absorption decrease at 554 nm and the solution turned into its original colorless state when excess $EDTANa_2$ was added. These results reveal that the binding of **1** and Cu^{2+} is reversible rather than a cation-catalyzed reaction.[17]

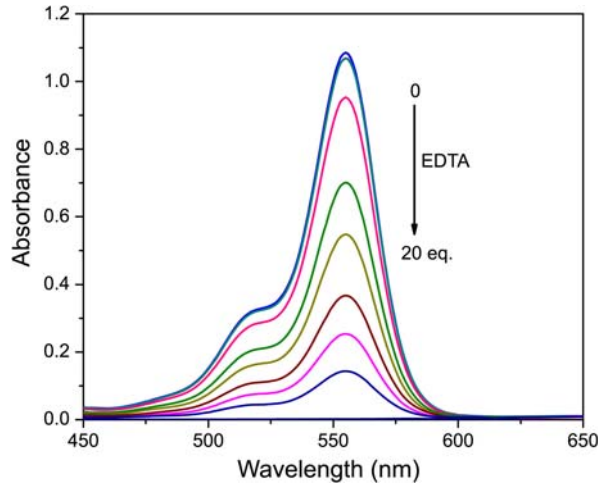


Figure 9. Changes in absorption spectra of **1** (10 μM) upon addition of $EDTANa_2$ in CH_3CN/H_2O (1:1, v /v, HEPES 10 mM, pH = 7.0) solution.

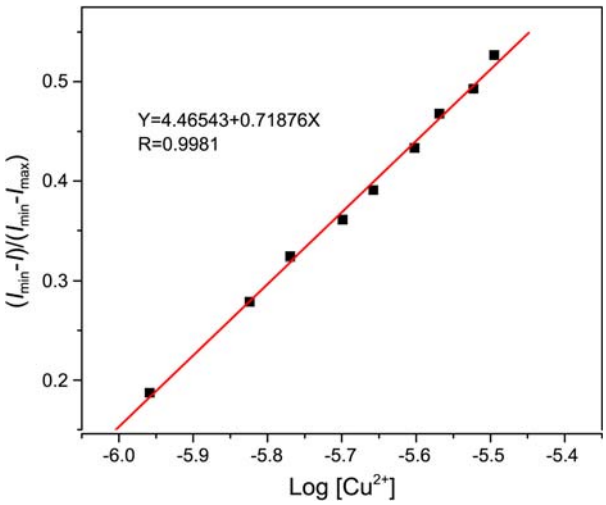


Figure 10. Normalized intensity of solution **1** (10 μM) *vs* $\log[Cu^{2+}]$ in the low Cu^{2+} concentration range 0.7×10^{-6} to 3.2×10^{-6} M.

Detection Limit Evaluation. To check its practical utility, the fluorescence detection limit of **1** for Cu^{2+} was evaluated. Based on the results of fluorescence titration experiment, the fluorescence intensities at 590 nm were normalized between the minimum and maximum intensity. Plotting of $(I_{min} - I)/(I_{min} - I_{max})$ versus $\log[Cu^{2+}]$ afforded a nice linear relationship (R = 0.9981), the point at which this line crossed the ordinate axis was regarded as the detection limit[18] and was calculated to be 7.96×10^{-8} M (Fig. 10), which is below or in the same range compared with some recently reported rhodamine B-based Cu^{2+} sensors.[12a,19] The value is lower than the limit of copper in drinking water (~20 μM) and the typical concentration of blood copper (15.7-23.6 μM) in normal individuals defined by the U.S. Environmental Protection Agency.[5b,20] Comparing with its parent structure, salicylaldehyde rhodamine B hydrazone,[12a] our sensor **1** also has the nano molar level detection ability even at 10 μM sensor concentration. These results indicate that sensor **1** is

sensitive enough to monitor Cu^{2+} concentration in water.

Conclusions

In summary, we have developed a new rhodamine B based derivative **1** as a colorimetric and fluorescent dual modes sensor for recognition of Cu^{2+}. Sensor **1** exhibits highly selective and sensitive absorption and fluorescence responses to Cu^{2+} in neutral 50% CH_3CN/H_2O buffered solution. The 1:1 binding stoichiometry of **1** and Cu^{2+} was proved by nonlinear-least squares fitting of titration profiles and Job's plot, the association constant and detection limit were calculated to be 1.92×10^6 M^{-1} and 7.96×10^{-8} M, respectively. The Cu^{2+} recognition process is reversible and is hardly interfered by other coexisting metal ions. The excellent selectivity and sensitivity of **1** to Cu^{2+} make it a promising sensor for Cu^{2+} detection in environmental and biological samples.

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