Highly sensitive optical chemosensor for the detection of Cu^{2+} using a rhodamine B spirolatam

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Abstract. Highly sensitive colorimetric chemosensor molecule **RHN** for selective detection of Cu^{2+} in mixed CH$_3$CN aqueous media was designed and prepared by incorporating the well-known rhodamine fluorophore and a terdentate O$_2$N binding unit into one molecule. The chemosensor **RHN** showed not only a reversible, selective, and sensitive absorbance enhancement response to Cu^{2+}, but also a strong colour development against the colourless blank during the sensing event, a feature that would facilitate 'naked-eye' detection.

Keywords. Chemosensor; Cu^{2+}; colorimetric; rhodamine B spirolatam.

1. Introduction

Chemosensors that convert molecular recognition into highly sensitive and easily detected signals have been actively investigated in recent years.[1–3] A number of chemosensors for metal ions have been reported to be capable of correlating metal ions concentration with changes in spectroscopic characteristics.[4–16] Since Cu^{2+} is a significant environmental pollutant and an essential trace element in biological systems, it is highly desirable to design and synthesize novel sensors for the measurement and detection of copper ion.[17–24] Among the sensors for Cu^{2+} ever reported, much attention has been drawn to the design of fluorescent sensors for the detection of copper ions due to the nondestructive, quick, and sensitive advantages.[25–30] However, due to the paramagnetic nature of the copper ion, some sensors often undergo fluorescence quenching upon the Cu^{2+} binding,[21–30] subsequently compromising the sensitivity of the sensors. On the other hand, the absorbance change or the colour variation on the selective binding of target analyte is a convenient method for the design of high sensitive chemosenors for copper ions. This colour change coupling with

the sensitivity and selectivity of the system for Cu^{2+} can be available for the simple 'naked eye' sensing of copper ion in aqueous media. From the viewpoint of sensitivity, an alternative way to convert absorbance signal into fluorescence signal can be exploited by fluorescence inner filter effect (IFE), which is useful for an optical chemical sensor with enhanced sensitivity in light of the absorbance changes alone due to the translation of the absorbance of absorber into the exponential changes in the fluorescence signals.[31–34]

Known by their excellent spectroscopic properties of large molar extinction coefficient and high fluorescence quantum yield, rhodamine-based dyes are excellent candidates for the design of some sensors with high sensitivity.[35–43] Based on the understanding of the sensing mechanism of rhodamine-based molecular senors,[39] we decided to investigate other binding sites in the dyes toward the selective sensing of Cu^{2+}. The idea was to find a dye that would respond in an analogous manner to the presence of Cu^{2+} showing a sensitive absorbance response to make the naked eye detection easier. Herein, we describe a new rhodamine-based chemosensor **RHN**, which shows a reversible, selective, and sensitive absorbance enhancement response to Cu^{2+} in a mixed aqueous environment. The improvement in

the colour response, the limit of quantification, and the reversibility in the sensing of the molecular sensor will make this approach a very promising one for the analysis of Cu^{2+} in water sample.

2. Experimental

2.1 *Materials and methods*

Unless otherwise stated, materials were obtained from commercial suppliers and were used without further purification. Rhodamine B hydrazide is prepared according to the literature method.[37] The NMR spectra were recorded on a DRX 400 Bruker spectrometer at 298 K with TMS as internal standard. The ESI-MS spectra were performed on a LCQ system (Finnigan MAT, USA) using CH_3OH–CH_3CN as the mobile phase. All pH measurements were made with a Model pHS-3C pH meter (Shanghai, China). UV/vis spectra were obtained on a Shimadzu UV-2501PC UV-vis recording spectrophotometer.

Absolute CH_3CN and doubly distilled deionized water were used throughout the experiments. The different ions stock solutions, including Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Al^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Ag^+, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Bi^{3+}, NH_4^+, SCN^-, IO_3^-, ClO_3^-, $Cr_2O_7^{2-}$, CrO_4^{2-}, BiO_3^-, MoO_4^{2-}, NO_2^-, NO_3^-, Cl^-, Ac^- and SO_4^{2-} were prepared in doubly distilled deionized water, respectively. The sensor **RHN** stock solution (10^{-5} M) was prepared in acetonitrile aqueous buffer solution (CH_3CN : H_2O, 40 : 60 (v : v), HAc-NaAc (0·04 M), pH 5·0).

2.2 *Synthesis of compound* **RHN**

Rhodamine hydrazide (0·46 g, 1 mmol) and 2-hydroxy-1-naphthaldehyde (0·17 g, 1 mmol) were dissolved in 20 mL absolute ethanol. The mixed solution was refluxed with stirring for *ca.* 6 h, then the reaction mixture was cooled (concentrated to 10 mL) and allowed to stand at room temperature overnight. The precipitate was filtered and washed 3 times with 10 mL ethanol. After drying under reduced pressure, the reaction afforded compound **RHN** (0·336 g, yield: 55%). FT–IR: 1710·63 (*m*), 1618·06 (*s*), 1517·78 (*m*), 1311·42 (*s*), 1261·28 (*w*), 1220·78 (*w*), 1116·64 (*m*), 819·64 (*w*), 750·21 (*w*). ESI-MS mass spectra: 611·2 (cald. for $[1+H]^+$, 611·30) ^{1}H NMR: 0·990(12H, *t*, 17·0 Hz), 3·252 (8H, *m*, 8·0 Hz), 6·332 (2H, *d*, 10·5 Hz), 6·446 (4H, *d*, 10·5 Hz), 7·055 (1H, *d*, 11·5 Hz), 7·130 (1H, *d*, 9 Hz), 7·305 (1H, *t*, 18 Hz), 7·426 (1H, *t*, 19·5 Hz), 7·566 (1H, *d*, 9 Hz), 7·626 (1H, *t*, 19·5 Hz), 7·776 (1H, *t*, 14·5 Hz), 7·813 (2H, *m*, 18·5 Hz), 7·923 (1H, *d*, 9 Hz), 9·474 (1H, *s*).
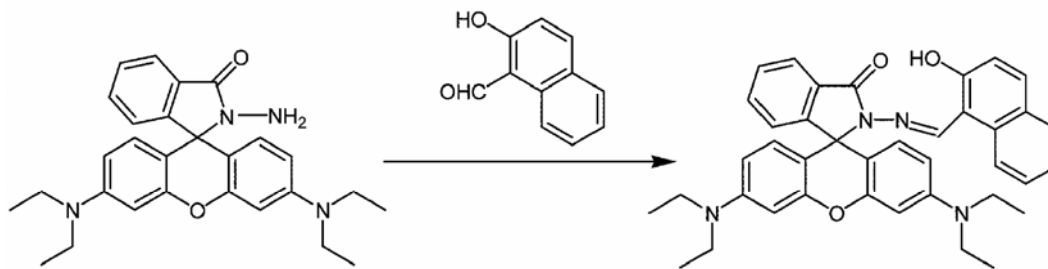
2.3 *X-ray crystallography*

Suitable crystals were selected for single-crystal X-ray diffraction structural analysis and the data were collected on a Siemens SMART-CCD diffractometer with graphitemonochromatized MoKa radiation ($\lambda = 0.71073$ Å), using the SMART and SAINT programs.[44] Forty-five frames of data were collected at 298 K with an oscillation range of 1_/frame and an exposure time of 10 s/frame. Indexing and unit cell refinement were based on all observed reflections from those 45 frames. The structures were solved by direct method and refined on F^2 by full matrix least-squares methods with SHELXTL version 5·1.[45] Anisotropic thermal parameters were refined for non-hydrogen atoms. Hydrogen atoms were localized in their calculated positions and refined by using the riding model. Crystallographic data and parameters for data collection and refinement of the complex are summarized in table 1.

3. Results and discussion

Rhodamine hydrazide was synthesized according to the literature procedure.[37] **RHN** was synthesized by treating rhodamine hydrazide with 2-hydroxy-1-naphthaldehyde with a high yield (scheme 1). By slowly evaporating the acetonitrile solution containing **RHN**, the single crystal suitable for X-ray diffraction was obtained in 65% yield. The structure analysis unambiguously revealed the unique spirolactam-ring formation (figure 1).

An optimized CH_3CN/water (40 : 60, v : v) mixed media of **RHN** was selected for the spectral investigation. To investigate the influence of the different acid concentration on the spectra of compound **RHN** and find a suitable pH span in which compound **RHN** can selectively detect Cu^{2+} efficiently, the acid titration control experiments were performed. As shown in figure 2, the absorption titration curve of free **RHN** did not show obvious characteristic colour of rhodamine between pH 5·0 and 9·0, suggesting that spirolactam tautomer of **RHN** was insensitive to the pH changes in this range, as is still revealed in the solid state. Under the optimized con-
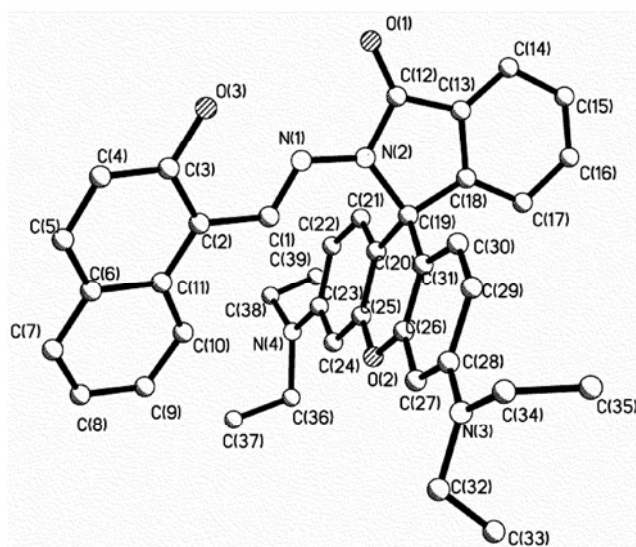
Scheme 1. Synthesis route of compound **RHN**.

Table 1. Crystallographic data and refinement parameters for complex **RHN**.

Crystal	**RHN**
Empirical formula	$C_{39}H_{39}N_4O_4$
Formula mass	627·74
Colour, habit	Colourless, block
Crystal dimensions (mm)	$0·3 \times 0·2 \times 0·2$
Crystal system	Monoclinic
Space group	Pc
Z	2
a (Å)	10·630(6)
b (Å)	11·960(7)
c (Å)	15·278(9)
α (°)	90
β (°)	107·712(9)
γ (°)	90
Temperature (K)	296(2)
Volume (Å³)	1850·3(18)
D (Mg m⁻³)	1·127
Radiation	$MoK\alpha$ ($\lambda = 0·71073$ Å)
Absorption coeff. (μ) (mm⁻¹)	0·074
F_{000}	666
Observed reflections	8785
Independent reflections	4217
Data/restraints/parameters	4217/2/428
Maximum shift/error	0·00
Goodness-of-fit on F^2	1·007
Final R induces [$I > 2\sigma(I)$]	0·0949
R induces (all data)	0·1206

Figure 1. The crystal structure of sensor **RHN** with the atom labelling. Selected bond distance: C(1)–N(1) 1·274(9), N(1)–N(2) 1·387(8), C(28)–N(3) 1·366(8), C(26)–O(2) 1·405(7), C(19)–N(2) 1·508(8), C(12)–N(2) 1·394(8), C(1)–C(2) 1·472(10), C(3)–O(3) 1·366(10), C(12)–O(1) 1·240(9).

ditions, the reaction between sensor **RHN** and Cu^{2+} is so rapid that it can be effectively completed within seconds. Furthermore, the absorption spectra of complex **RHN**-Cu^{2+} remained intact even for days, suggesting that the complex **RHN**-Cu^{2+} is quite stable in the solution.

Under the optimized conditions, the absorption spectra of **RHN** with the different Cu^{2+} concentration were recorded, respectively. As shown in figure 3a, the absorption spectra of **RHN** (10 μM) in CH_3CN/water ($CH_3CN : H_2O$, 40 : 60, v : v, 0·04 M

HAc-NaAc, pH 5·0) buffer solution exhibited only a very weak band above 500 nm, which was ascribed to the spirolactam form of molecule **RHN**. Upon the addition of Cu^{2+}, the absorption spectra showed a new maximum absorption wavelength at 556 nm ($\varepsilon = 75000$ M⁻¹ cm⁻¹), which can be ascribed to the ring-opened tautomer of **RHN**. The absorbance of **RHN** at 556 nm increased linearly with lower concentration of Cu^{2+} (between 1 and 10 μM). Since the linear response toward Cu^{2+} was established, it was of interest to determine the limit of quantification for the detection of Cu^{2+}. Once the solutions of the colourless compound **RHN** were titrated with Cu^{2+}, as can be seen in figure 3b, the dye has a linear response to increasing amounts of low Cu^{2+} concentration (between 5 and 50 nM), establishing that the system has a limit of quantification down to ~0·32 ppb.[46]
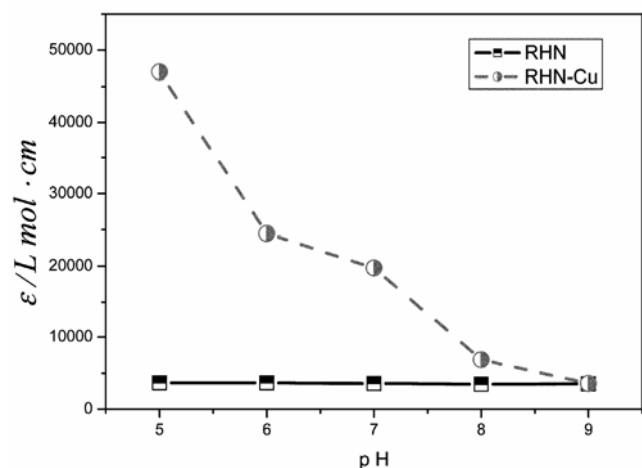
Figure 2. Absorbance at $\lambda_{max} = 556$ nm of **RHN** (10 μM) in CH$_3$CN/water (CH$_3$CN : H$_2$O, 40 : 60, v : v) mixed media *vs* different acid concentration before and after addition of 10 μM Cu^{2+}.
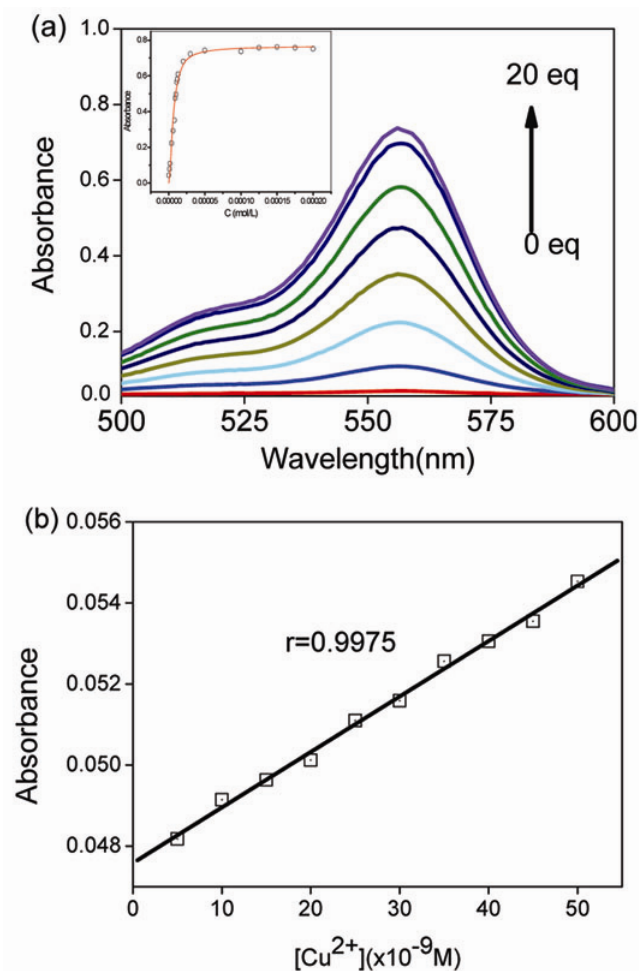

Figure 3. (**a**) The UV-vis spectra of **RHN** (10 μM) upon the addition of Cu^{2+} (0~20 equiv). Inset: absorbance at 556 nm as a function of Cu^{2+} concentration, red line represents a best fitting. (**b**) Absorbance ($\lambda_{max} = 556$ nm) of **RHN** (10 μM) as a function of the Cu^{2+} concentration (5~50 nM).

This binding mode was supported by the data of Job's plots[47] evaluated from the absorption spectra of **RHN** and Cu^{2+} with a total concentration of 10 μM (figure 4). The absorption spectra of **RHN** and Cu^{2+} in CH$_3$CN/water (CH$_3$CN : H$_2$O, 40 : 60, v : v, 0·04 M HAc-NaAc, pH 5·0) were measured. As expected, the result obtained from the Job's plot unambiguously indicated the formation of a 1 : 1 complex between **RHN** and Cu^{2+}. The nonlinear fitting of the titration curve assuming a 1 : 1 stoichiometry for the **RHN**-Cu^{2+} complex yields a good fitting re-
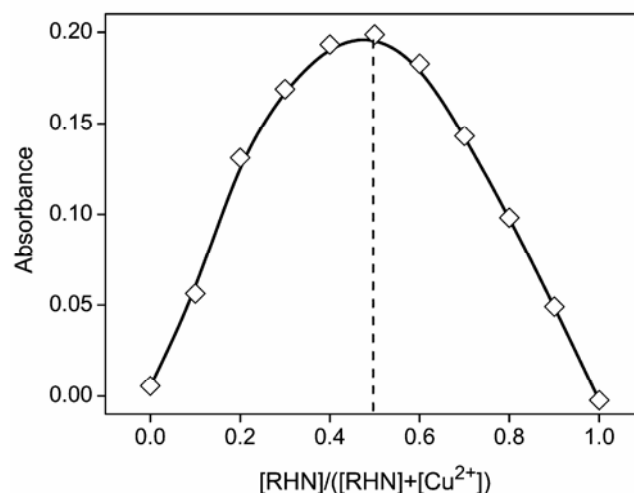

Figure 4. Job's plot evaluated from the absorption spectra of **RHN** and Cu^{2+} with the total concentration of 10 μM, indicating the 1 : 1 stoichiometry for **RHN**-Cu^{2+} complex.
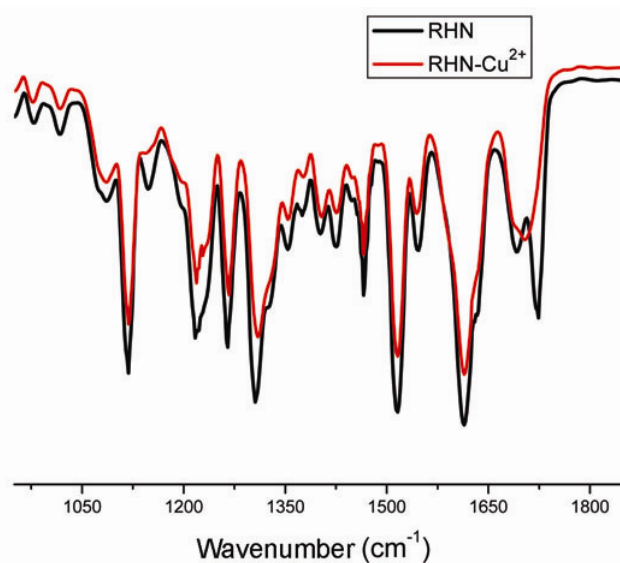
Figure 5. IR spectra of compound **RHN** and **RHN**-Cu^{2+} complex

Figure 6. Photographs of **RHN** in CH$_3$CN/water (CH$_3$CN : H$_2$O, 40 : 60, v : v, 0·04 M HAc-NaAc, pH 5·0) in the presence of different concentration of Cu^{2+} (from left to right): [Cu^{2+}] = 0, 5, 10 and 50 μM, respectively.
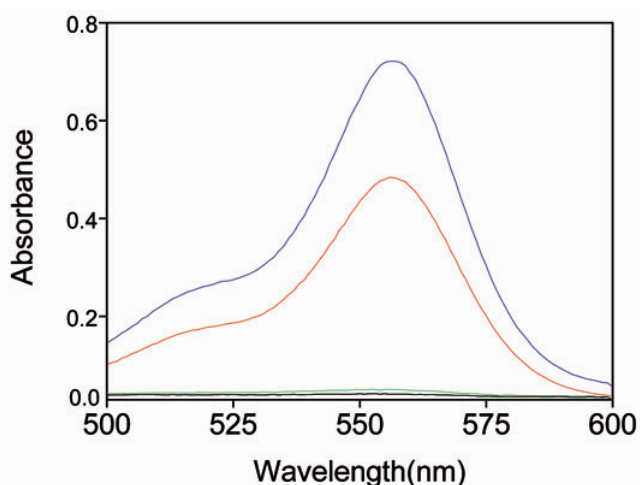
Figure 7. Reversible absorption response of **RHN** to Cu^{2+}. Black: 10 μM **RHN**; Red: 10 μM **RHN** and 10 μM Cu^{2+}; Green: 10 μM **1** and 10 μM Cu^{2+} and then addition of 400 μM EDTA (Na salt); Blue: 10 μM **RHN** and 10 μM Cu^{2+}, EDTA and then addition of 500 μM Cu^{2+}.

sult with association constant K_{ass} of 5·4 × 10^5 M^{-1},[48] further suggesting the 1 : 1 binding mode between the sensor **RHN** and metal ion.

Furthermore, IR spectra of **RHN** and **RHN-Cu^{2+}** were also checked respectively in KBr disks, and the results were shown in figure 5. In the IR spectra, the strong peak at 1722·21 cm^{-1} of the receptor **RHN**, which corresponds to the characteristic amide carbonyl absorption, was shifted to 1702·92 cm^{-1} for the Cu^{2+} compound, suggesting that a strong binding of the carbonyl group occurs with copper ion.

Accordingly, the titration solution exhibited an obvious and characteristic colour change from colourless to purple, indicating that sensor **RHN** can serve as a 'naked-eye' indicator for Cu^{2+} in CH$_3$CN aqueous media. Colour changes as signalling events have been widely used because it requires the use of

inexpensive equipment or no equipment at all as colour changes can be detected by the naked eye.[49–52] Interestingly, the addition of Cu^{2+} into the colourless solution of **RHN** generated a purple colour rapidly, while other ions of interest gave no visible change. With further investigations, even using a lower concentration (i.e. micromolar level) of Cu^{2+}, an obvious colour change was observed as shown in figure 6. The results suggested **RHN** can serve as a 'naked-eye' chemosensor selective for Cu^{2+} in buffered mixed aqueous media.

As with many reported rhodamine-based spirolactam sensors, the Cu^{2+} induced absorbance enhancement of **RHN** is most likely the result of the spiro ring-opening mechanism.[35–43] Herein, the chelation of Cu^{2+} with the carbonyl O, imino N, and naphthol O atoms results in the formation of the open-ring tautomer form of **RHN**. Furthermore, since the colour of **RHN-Cu^{2+}** disappeared immediately upon the addition of Cu^{2+} chelating agent EDTA, whereas excess Cu^{2+} would recover the signal of absorbance enhancement (figure 7). These results suggest that the response of **RHN** to Cu^{2+} is reversible rather than the cation-catalysed reaction.[35–37]

4. Conclusion

In conclusion, an optical chemosensor **RHN** from Rhodamine B (**RB**) by a two-step reaction was designed and synthesized, which displays a significant change of both colormetric response upon the Cu^{2+} binding. The detection limit for Cu^{2+} was found to be at nanomolar level via the absorbance enhancement. Thus, compound **RHN** may be considered as a potentially practical colorimetric chemosensor for selective naked-eye detection of Cu^{2+}.

Acknowledgement

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