Spectrochimica Acta Part A 81 (2011) 14–20

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Rhodamine-based derivatives for Cu^{2+} sensing: Spectroscopic studies, structure-recognition relationships and its test strips

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ARTICLE INFO

Article history:
Received 24 February 2011
Received in revised form 10 May 2011
Accepted 16 May 2011

Keywords:
Spectroscopy
Rhodamine
Cu^{2+} detection
Structure-recognition relationships
Test strips

ABSTRACT

A rhodamine spirolactam/2-hydrazinopyridine derivative was synthesized and characterized, which exhibited high selectivity to Cu^{2+} over other metal cations. The Cu^{2+} recognition of this rhodamine derivative could be detected by fluorescence spectra, absorption spectra and an obvious color change which was observed easily by naked-eyes. The binding of this rhodamine derivative to Cu^{2+} is instantaneous and sensitive. Moreover, a linear relationship was found between the fluorescence intensity at 575 nm from 0.5×10^{-6} M to 3.0×10^{-6} M of Cu^{2+} concentration, and the limit of detection (LOD) was at low concentration of 2.11×10^{-8} M, this would benefit for the establishment of standard working curves in practical Cu^{2+}detection. Additionally, we synthesized rhodamine spirolactam/2-aminomethylpyridine derivative and rhodamine spirolactam/phenylhydrazine derivative as analogs for elucidate the structure-recognition relationships. Finally, we prepared the test strips of rhodamine spirolactam/2-hydrazinopyridine derivative for practical chromogenic the Cu^{2+} detection.

1. Introduction

The development of increasingly selective and sensitive methods for the determination of heavy metal ions is currently receiving considerable attention [1–4]. Among the various detection methods available, UV–vis and fluorescence spectroscopy still remain the most frequently used modes for the recognition of physiologically and environmentally important analytes due to their high sensitivity and easy operational use.

Cu^{2+} plays important roles in biological and environmental processes. The development of Cu^{2+} sensing materials with high selectivity and sensitivity is attracting increasing attention in analytical, biological and environmental sciences. However, most of the early-reported Cu^{2+} chemosensors are fluorescence-quenching types [5–10]. Factors such as the interference of coexisting metal ions, the convenient portability and the rapidly response still limited the practical application of the Cu^{2+} chemosensors. For the consideration of more sensitive detection, many fluorescent "Turn-on" chemosensors have been developed [11–17]; for the improvement of the water solubility, some water-soluble chemosensors were synthesized and employed in aqueous media [18,19]. So, developing new chemosensors or their derived sensing materials for rapid and convenient Cu^{2+} detection are still highly demanded.

Rhodamine-based compounds were found to be good fluorescent "Turn-on/off" switches. Since a ring opening-closing conversion (Scheme 1) between non-fluorescent (colorless) rhodamine spirolactam form and highly fluorescent (red) rhodamine ring-opening form could be induced by metal cations [20], based on the mechanism, many rhodamine derivated fluorescent chemosensors/chemodosimeters had been developed for the detection of various metal ions [21–28]. Dujols firstly utilized a rhodamine B hydrazide as a chemodosimeter for Cu^{2+} sensing in aqueous solution [29], after that, some metal-chelating moieties bearing rhodamine derivatives [30,31] were developed as a sort of highly selective Cu^{2+} chemodosimeters. Zhao [32] synthesized a Cu^{2+} selective chemodosimeter by incorporating a thiosemicarbazide moiety with rhodamine B, Chen [33] reported a chemosensor bearing rhodamine-binaphthyl groups to detect Cu^{2+}, Zeng [34] given a tripodal rhodamine receptor as Cu^{2+} and Hg^{2+} chemosensor, and Yu's research group [35] compared several carbohydrazone rhodamine derivatives for their Cu^{2+} recognition properties. These progresses were listed in Table 1. Although number of rhodamine-based Cu^{2+} chemosensors had been developed, the sensitivity and efficiency for Cu^{2+} detection should be further improved. On the other hand, how to rationally design a preferred molecular

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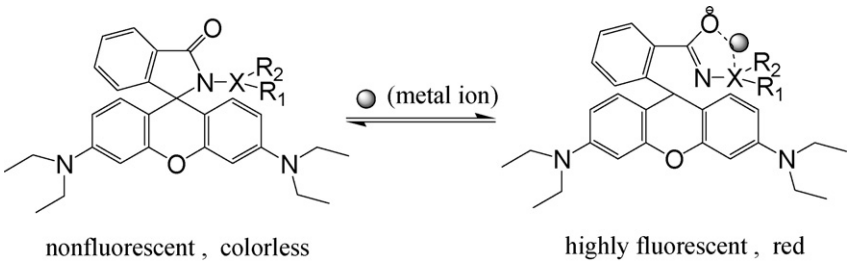
doi:10.1016/j.saa.2011.05.016



Scheme 1. Metal cations induced ring opening mechanism for rhodamine-based chemosensors.

structure for Cu^{2+} sensing is still a challenge. Therefore, design and synthesis of highly efficient rhodamine-based Cu^{2+} chemosensors and elucidate their structure-recognition relationship are desirable.

In this work, we designed and synthesized a rhodamine spirolactam/2-hydrazinopyridine derivative **1** as a chemosensor, which showed highly selectivity and sensitivity for Cu^{2+} over other coexistent metal cations in aqueous media. The fluorescent "Turn-on" process could be easily detected by means of spectroscopy or directly observed by naked eyes. Factors such as concentration dependence, pH effect, response time and coexisted metal ions that influenced Cu^{2+} sensing were also investigated and discussed. Additionally, comparing to most of the researches reported, which only demonstrated the recognition properties of the chemosensors themselves, two other analogs of rhodamine spirolactam/2-aminomethylpyridine derivative **2** and rhodamine spirolactam/phenyl hydrazine derivative **3** were synthesized in this work for the investigation of structure-recognition relationships (Scheme 2). Derivatives **2**, **3** showed much less fluorescence sensitivity to Cu^{2+} than that of **1** and these were benefits for rational design of more advanced chemosensors. For practical application, paper-made test strips were successfully prepared for aqueous Cu^{2+} detection.

2. Experimental

2.1. Instruments and materials

The ^1H NMR spectra were recorded at 400 MHz on a Varian Gemin-400. All absorption spectra were recorded in Pgeneral TU-1901 UV–Vis spectrometer. All fluorescence measurements were carried out on Hitachi F-4500 spectrophotometer. All pH values were measured with a Model pHS-3C pH meter (Shanghai, China). Thin layer chromatography (TLC) was carried out using silica gel F254, and column chromatography was conducted over silica gel (200–300 mesh), both of which were purchased from the Qingdao Ocean Chemicals (Qingdao, China). Doubly distilled water was used for all experiments. The metal ions are perchlorate salts of Ag^+, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ca^{2+}, Na^+, $Ni^{2+}$and Mg^{2+}, which were purchased from Sinopharm Chemical Reagent Beijing Co., compounds **1**, **2**, **3** were synthesized and characterized by the method showed below. All of other chemicals used here were analytical reagents and used as received.

2.2. Synthesis of 1

To a solution of rhodamine B (1.0 g, 2.3 mmol) in dry 1,2-dichloroethane (8.0 mL), phosphorus oxychloride (1.1 g, 6.9 mmol) was added dropwise at room temperature within 5 min. After being refluxed for 4 h, the reaction mixture was cooled and concentrated under vacuum to give rhodamine B acid chloride. The crude product was used directly in the next reaction without purification.

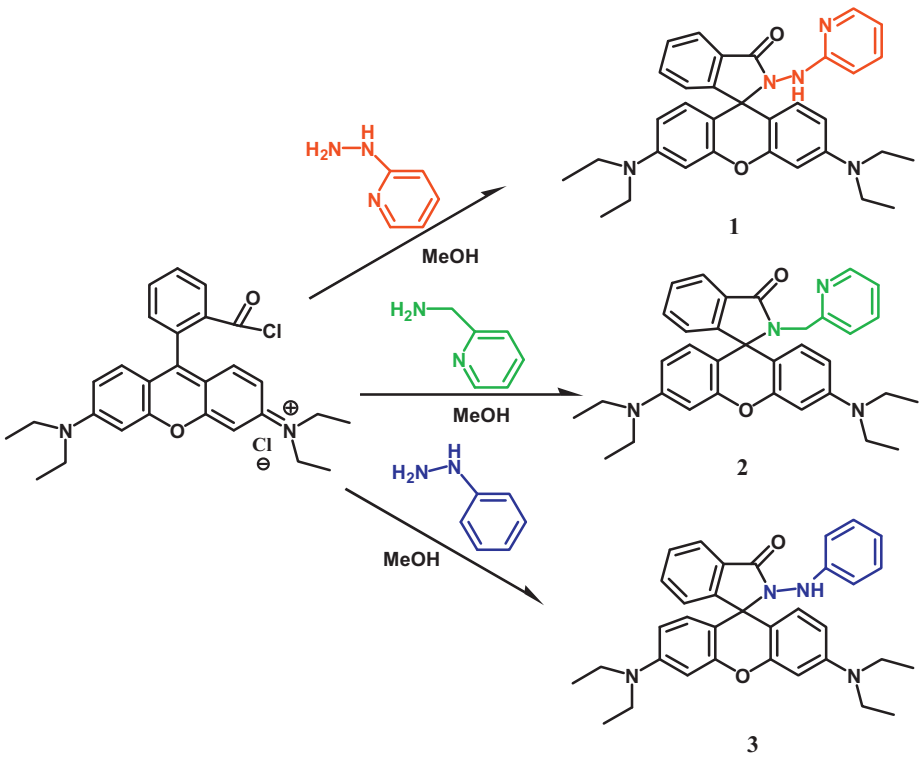
The crude rhodamine B acid chloride in last step was dissolved in dry acetonitrile (10 mL), and added dropwise to a solution of 2-hydrazinopyridine (4.6 mmol, 0.67 g) in dry acetonitrile (6.0 mL) containing triethylamine (8.0 mL). After stirring for 4 h at room temperature, the mixture was concentrated under vacuum and the crude product was purified by column chromatography (eluant: ethyl acetate/Petroleum ether; v/v = 1/20) to give compound **1** as a white solid in 70% yield. ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.13–1.16 (t, 12H, J = 7.2 Hz, NCH_2CH_3), 3.29–3.35 (q, 8H, J_1 = 6.8 Hz, J_2 = 7.2 Hz, NCH_2CH_3), 6.24–6.29 (m, 5H, ArH), 6.50–6.57 (m, 3H, ArH), 7.14–7.18 (t, 1H, J = 7.2 Hz, ArH), 7.26–7.28 (m, 1H, ArH), 7.54–7.63 (m, 2H, ArH), 7.96–7.97 (d, 1H, J = 4.0 Hz, ArH), 8.02–8.04 (d, 1H, J = 8.0 Hz, ArH); MS (ESI) m/z: 534.3 ($M + H^+$, 100%).

2.3. Synthesis of 2

To a 100 mL flask, rhodamine B acid chloride (0.6 g, 1.3 mmol) was dissolved in 30 mL methanol. Excess of 2-aminomethylpyridine (0.36 g, 3.4 mmol) was then added dropwise with vigorous stirring at room temperature within 20 min. Then the mixture was heated to reflux for 24 h. The solution changed from dark purple to light orange. Then the mixture was cooled down and the solvent was removed under reduced pressure. 1 M HCl (50 mL) was added to dissolve the residue, then 1 M NaOH (70 mL) was added slowly with stirring until the pH of the solution reached pH = 9–10. The resulting precipitate was filtered and washed 3 times with 15 mL of water, and then dried in a vacuum drying oven to afford **2** as a pink solid (0.35 g, yield 51%). IR (KBr) ν: 2967, 2963, 1686, 1634, 1615, 1514, 1466, 1421, 1376, 1219, 1120, 817, 747, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.15 (t, 12H, J = 7.0 Hz, NCH_2CH_3), 3.27–3.32 (q, 8H, J_1 = 7.2 Hz, J_2 = 8.0 Hz, NCH_2CH_3), 4.50 (s, 2H, CH_2-Pyridine), 6.06–6.07 (d, 1H, J = 4.0 Hz, ArH), 6.08–6.09 (d, 1H, J = 4.0 Hz, ArH), 6.27–6.30 (m, 4H, ArH), 6.83–6.86 (m, 1H, ArH), 7.07–7.09 (d, 1H, J = 8.0 Hz, ArH) 7.12–7.14 (m, 1H, ArH), 7.28–7.32 (m, 1H, ArH), 7.45–7.50 (m, 2H, ArH), 7.98–8.01 (m, 1H, ArH), 8.18–8.20 (m, 1H, ArH); MS (EI) m/z: 533 ($M + H^+$, 100%).

Table 1
The comparison of the properties of current rhodamine-based Cu^{2+}chemosensors.

Sensors	Linear range	Limit of detection	Response time	Working pH
Lit [29]	2.0×10^{-8}–2×10^{-6} M	2.0×10^{-8} M	2 min	7.0
Lit [31]	2.5×10^{-8}–3.3×10^{-6} M	2.5×10^{-8} M	–	7.0
Lit [32]	2.0×10^{-7}–4.0×10^{-6} M	7.8×10^{-7} M	–	7.0
Lit [34]	6.0×10^{-7}–1.0×10^{-4} M	3.0×10^{-7} M	–	6.8
Lit [35]	8.0×10^{-7}–1.0×10^{-5} M	3.0×10^{-7} M	1 min	7.1



Scheme 2. Synthesis and structures of the rhodamine derived compounds: chemosensor 1 and its analogs 2 and 3.

2.4. Synthesis of 3

To a 100 mL flask, rhodamine B acid chloride (0.6 g, 1.3 mmol) was dissolved in 30 mL methanol. Excess of phenyl hydrazine (0.36 g, 3.4 mmol) was then added dropwise with vigorous stirring at room temperature. Then the mixture was heated to reflux for 48 h. The solution changed from dark purple to light orange. Then the mixture was cooled and solvent was removed under reduced pressure. 1 M HCl (50 mL) was added to dissolve the residue, then 1 M NaOH (70 mL) was added slowly with stirring until the pH of the solution reached pH = 9–10. The resulting precipitate was filtered and washed 3 times with water, and the crude product was purified by column chromatography (eluant: ethyl acetate/petroleum ether; v/v = 4/1) to give compound **3** 0.31 g as a white solid (yield: 45%). IR (KBr) ν 3434, 3261, 2968, 2926, 1704, 1613, 1515, 1355, 1224, 1118, 816, 789, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.13–1.17 (t, 12H, J = 8.0 Hz, NCH_2CH_3), 3.31–3.37 (q, 8H, J_1 = 6.8 Hz, J_2 = 7.2 Hz, NCH_2CH_3), 6.26–6.27 (d, 1H, J = 2.4 Hz, ArH), 6.29–6.30 (d, 1H, J = 2.4 Hz, ArH), 6.50–6.51 (d, 2H, J = 2.4 Hz, ArH), 6.75–6.77 (d, 2H, J = 2.4 Hz, ArH), 7.02–7.03 (m, 1H, ArH), 7.14–7.18 (m, 3H, ArH), 7.25–7.27 (m, 2H, ArH), 7.49–7.50 (m, 2H, ArH), 8.36–8.37 (m, 1H, ArH).

2.5. Absorption and fluorescence spectroscopy measurements

Rhodamine derivatives **1**, **2** and **3** was dissolved in ethanol to prepare the stock solution with a concentration of 10 mM, then diluted with 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) buffer solution for spectroscopy measurement. The perchlorate salts of Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Li^+, Na^+, K^+, Cs^+, Fe^{3+}, Fe^{2+}, Cd^{2+}, Cr^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, Zn^{2+}, Ag^+ and Co^{2+} were dissolved in distilled water to prepare the stock solution with a concentration of 10 mM. The spectra were recorded in Pgeneral TU-1901 UV–Vis and Hitachi F-4500 spectrophotometer respectively.

3. Results and discussion

3.1. UV–vis absorption and fluorescence studies of 1 to metal ions

The perchlorate alts of Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Li^+, Na^+, K^+, Cs^+, Fe^{3+}, Fe^{2+}, Cd^{2+}, Cr^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, Zn^{2+}, Ag^+ and Co^{2+} ions were used to evaluate the metal ion binding properties of compounds **1**, **2**, **3** in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1 pH = 7.0) solution. The fluorescence spectra were obtained by excitation of the rhodamine fluorophore at 540 nm. As we expected, compound **1** (5 μM) in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) solution was colorless and weakly fluorescent (ϕ = 0.012) due to the existence of non-conjugated spirolactam structural form of **1** (Fig. 1a and b). After addition of the perchlorate salts of the metal ions (50 μM) to the solution of **1**, a 17-fold significant fluorescence (Fig. 1a) and absorbance (Fig. 1b) enhancement (ϕ = 0.22) could be observed with addition of 10 equiv of Cu^{2+} (Fig. 1), and a slightly fluorescence enhancement (2-fold) was observed after addition of Fe^{3+}, and other metal cations did not induce such spectroscopic changes. The fluorescent "turn-on" of **1** in the presence of Cu^{2+}, accompanied an obvious purple color change could be observed by naked-eyes in aqueous solution, while other metal ions did not show such color change, indicated the high selectivity of **1** to Cu^{2+} among the metal cations (Fig. 2).

In order to investigate the dependence of the absorption and fluorescence intensities to the concentration of Cu^{2+}, we carried out absorption and fluorescence titration experiments by the addition of increasing amount of Cu^{2+} into the aqueous solution of **1**. As shown in Fig. 3a, the fluorescence intensity of **1** (5 μM) at 578 nm was found increased gradually with the increasing of the concentration of Cu^{2+} from 0.5×10^{-6} M to 3.0×10^{-6} M and then reached a platform, indicated a complete complex formation of **1** and Cu^{2+}, as shown in Fig. 3b, the absorption titration is in accordance with the result of fluorescence titration. More importantly, a linear relationship between the fluorescence intensity of **1** and Cu^{2+} concentration

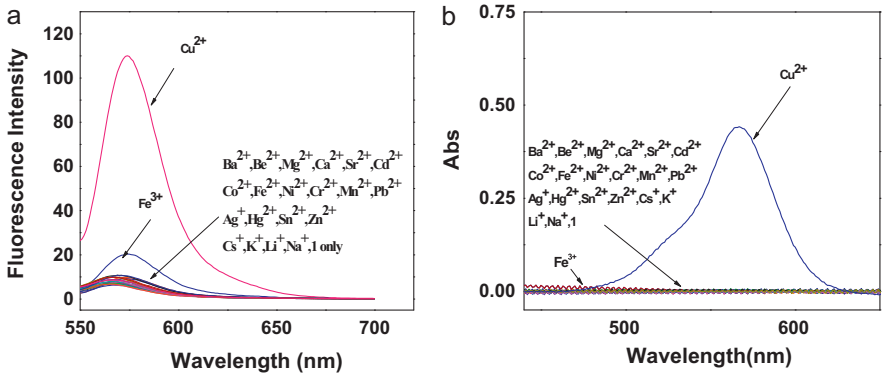


Fig. 1. Fluorescence spectra (a) and absorption (b) of **1** (5 μM) in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) solution in the presence of various metal ions (50 μM) Other ions: Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Li^{+}, Na^{+}, K^{+}, Cs^{+}, Fe^{3+}, Fe^{2+}, Cd^{2+}, Cr^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, Zn^{2+}, Ag^{+} and Co^{2+}.

Fig. 2. Photographs of 10 μM **1** after addition of 50 μM metal ions in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) solution, from left to right: Cu^{2+}, Fe^{3+}, Fe^{2+}, Pb^{2+}, Hg^{+}, Cr^{2+} and Ni^{2+}.

was found with the Cu^{2+} concentration in the range from 0.5×10^{-6} to 3.0×10^{-6} M (Fig. 4), with a detection limit [36] of 2.11×10^{-8} M (based on $S/N = 3$). The results indicate that **1** could be employed to detect micromolar lever concentration of Cu^{2+} in aqueous solution, additionally, the linear relationship between **1** and Cu^{2+} (from 0.5×10^{-6} to 3.0×10^{-6} M) benefits for the establishment of standard working curves in practical Cu^{2+}detection.

3.2. Binding constant and stoichiometry

In order to investigate the binding capability of **1** to Cu^{2+}, Job's plot (**1** and Cu^{2+} with a total concentration of 3.0 μM) was utilized to determine the binding stoichiometry of the **1**-Cu^{2+} complex. It revealed that **1** and Cu^{2+} formed a complex in 1:1 stoichiometry (as

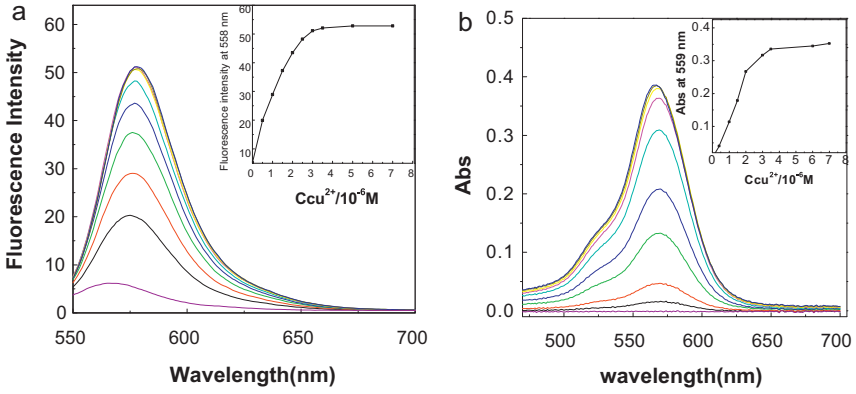


Fig. 3. Fluorescence spectra (a) and absorption (b) of **1** (5 μM) in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) solution in the presence of various amount of Cu^{2+}. Inset: absorbance at 559 nm (a) and fluorescence intensity at 578 nm (b) in increasing of Cu^{2+} concentration.

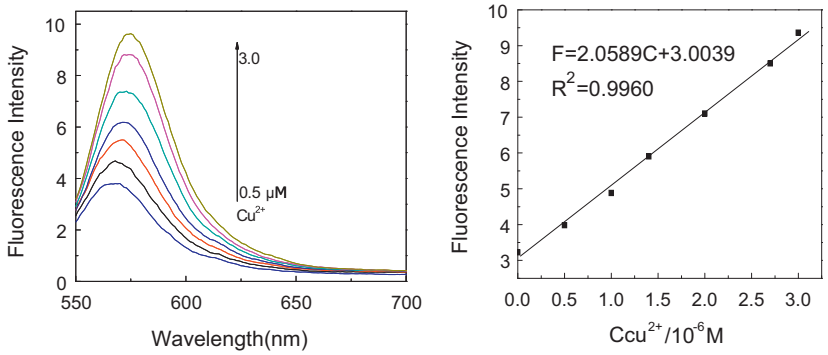


Fig. 4. Linear relationships between **1** (1 μM) and Cu^{2+} concentration (from 0 to 3.0 μM) by a plot of fluorescence intensity as a function of Cu^{2+} concentration.

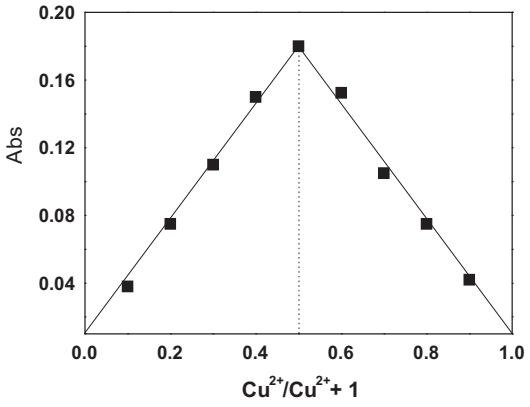


Fig. 5. Job's plot of **1** and Cu^{2+} in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0), (total concentration of **1** and Cu^{2+} was kept at 3.0 μM, absorbance was recorded at 558 nm).

shown in Fig. 5). On the base of the 1:1 stoichiometry, we calculated the value of the stability constant for the complex by nonlinear curve fitness [37] as following expression. Here Y and Y_0 are the absorbance of the solution of **1** in the presence and absence of Cu^{2+}, C_M and C_L are the concentrations of Cu^{2+} and **1**; Ks is the stability constant. According to titration data, the stability constant between **1** and Cu^{2+} was determined as $Ks = 3.84 \times 10^5$ ($r^2 = 0.9958$).

$$Y = Y_0 + \frac{Y_{\lim} - Y_0}{2}\left\{1 + \frac{C_M}{C_L} + \frac{1}{K_S C_L} - \left[\left(1 + \frac{C_M}{C_L} + \frac{1}{K_S C_L}\right)^2 - 4\frac{C_M}{C_L}\right]^{1/2}\right\}$$

3.3. Influence of pH value on Cu^{2+} detection

In many cases, the solution pH strongly affects the spectroscopic properties or responses of chemosensors [38]. Therefore, the influence of pH on the response of **1** to Cu^{2+} was also investigated in this work. As depicted in Fig. 6, no significant absorbance change at 558 nm of **1** (10 μM) could be observed (bottom line) in the pH ranging from 4.0 to 10.0, suggesting that the spirolactam form was stable within this pH range. However, in the presence of Cu^{2+} (100 μM), an obvious absorbance change at 558 nm of **1** was observed under different pH values range from 4.0 to 10.0 (Fig. 6, upper line). The absorbance at 558 nm decreased drastically with the decrease of pH

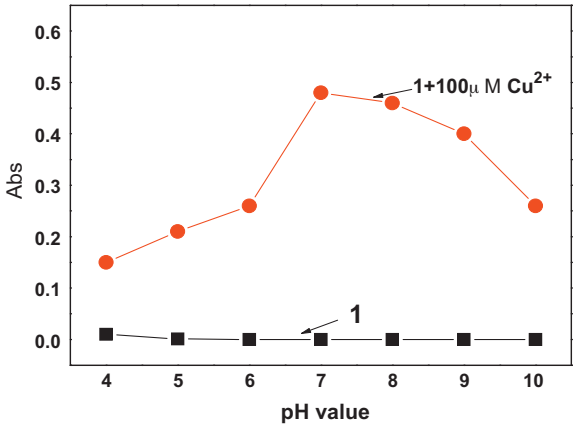


Fig. 6. Absorbance of **1** (10 μM) in Tris–HCl (10 mM) aqueous buffers of different pH in the absence (bottom line, at 558 nm) and presence (top line, at 558 nm) of 100 μM Cu^{2+}. The change of absorbance for **1** alone is quite mild in pH 5.0–10.0 range.

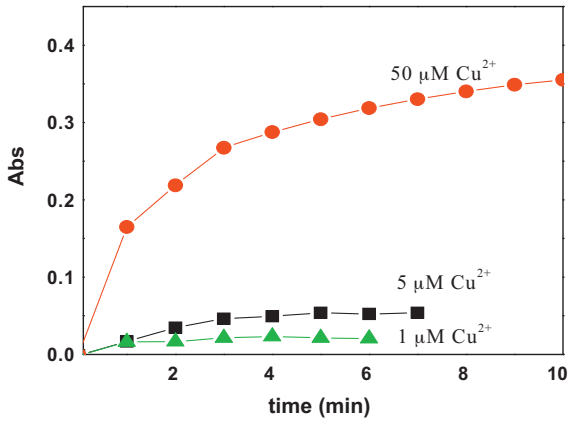


Fig. 7. Absorbance at 558 nm of **1** (5 μM) in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) with addition of different concentration of Cu^{2+}(50 μM, 5 μM and1 μM).

value from 7.0 to 4.0, due to the dissociation of coordinated Cu^{2+} by the compatible protonation on pyridine and hydrazine moiety; the highest absorbance response was observed after addition of Cu^{2+} around pH 7.0, then decreased under higher pH from 8.0 to 10.0, due to the formation of Cu $(OH)_2$ under higher pH value and result in the decrease of the absorbance.

3.4. Response time of 1 to Cu^{2+}

It is known that a short response time is preferred for a high efficient chemosensor [30]. Herein, the time dependence of absorption change for **1** to Cu^{2+} was investigated by recording the change of absorbance at 558 nm against times under different concentrations of Cu^{2+}. As shown in Fig. 7, the results revealed that the response time of **1** to Cu^{2+} increased with the increasing of Cu^{2+} concentration. The absorbance at 558 nm of **1** (5 μM) increased rapidly and almost reached a platform within 10 min after addition of 50 μM of Cu^{2+} (line a), while slow response was observed at lower Cu^{2+}concentration less than 5 μM (lines b and c). Moreover, the short response time of **1** to Cu^{2+} (>50 μM) demonstrates the possible application of **1** in real-time Cu^{2+} detection.

3.5. Selectivity of 1 to Cu^{2+} in the presence of other coexisting metal cations

Selectivity is a very important parameter for evaluating the performance of a chemosensor. The interference of coexist metal ions to Cu^{2+} detection was investigated by UV–vis absorption spectra. As shown in Table 2, no significant absorbance changes of **1**/Cu^{2+} solution could be observed in the presence of the coexist metal ions

Table 2
Absorbance at 558 nm of **1** (5 μM) in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) at the presence of 50 μM (10 equiv) Cu^{2+} and 150 μM other metal ions.

Cu^{2+} + metal ions	Absorbance (at 558 nm)
$Cu^{2+} + Ca^{2+}$	0.440
$Cu^{2+} + Na^{+}$	0.441
$Cu^{2+} + Pb^{2+}$	0.438
$Cu^{2+} + Fe^{2+}$	0.440
$Cu^{2+} + Cd^{2+}$	0.435
$Cu^{2+} + Zn^{2+}$	0.438
$Cu^{2+} + Hg^{2+}$	0.441
$Cu^{2+} + Mg^{2+}$	0.445
$Cu^{2+} + Ag^{+}$	0.430
$Cu^{2+} + Fe^{3+}$	0.450
$Cu^{2+} + Ni^{2+}$	0.440
Cu^{2+}	0.442

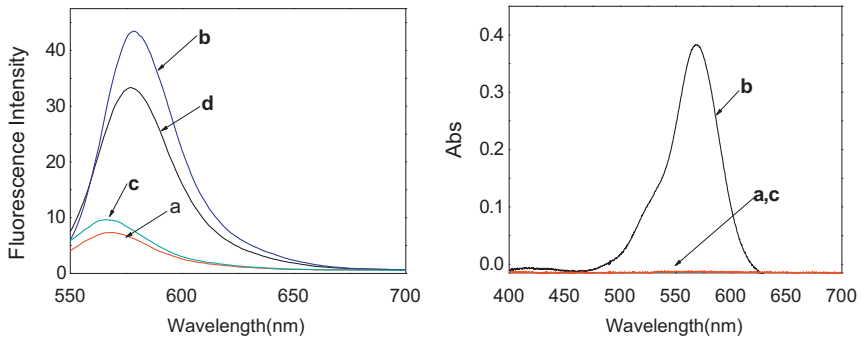


Fig. 8. (a) Fluorescence spectra in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) solutions. Line a: 5 μM **1**. Line b: 5 μM **1** + 5 μM Cu^{2+}. Line c: 5 μM **1** + 5 μM Cu^{2+} + 1 mM EDTA, line d: 5 μM **1** + 5 μM Cu^{2+} + 1 mM EDTA + 1.1 mM Cu^{2+} (Excitation at 540 nm). (b) Absorption spectra in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1, pH = 7.0) solutions. Line a: 5 μM **1**. Line b: 5 μM **1** + 5 μM Cu^{2+}. Line c: 5 μM **1** + 5 μM Cu^{2+} + 1 mM EDTA.

(150 μM, 3 times to Cu^{2+}), indicating that **1** exhibits high selectivity to Cu^{2+} over other metal ions. These results showed that **1** has remarkable sensing selectivity to Cu^{2+}, which made its suitable of practical Cu^{2+} detection.

3.6. Sensing mechanism

For further understanding of the interaction between **1** and Cu^{2+}, we studied the chemical reversibility of the binding of **1** to Cu^{2+} in buffered solution. As shown in Fig. 8a, the enhancement of the fluorescence spectra at 558 nm of **1** induced by Cu^{2+} could be almost completely quenched with the addition of excess EDTA, due to the strong coordination capability of chelating agent EDTA to Cu^{2+} (line c). Moreover, after the addition of excess Cu^{2+} to the above mixed solution, the fluorescence intensity at 558 nm could recover to 80% of the original ones. These results indicated that the binding of **1** to Cu^{2+} is a chemically reversible coordination rather than a metal cation-catalyzed reaction, and **1** was not cleaved in the process of Cu^{2+} binding [29]. On the other hand, UV–vis spectra also illustrated the coordination is chemically reversible (Fig. 8b), as seen by naked-eyes, the purple color of the mixed solution of **1**/Cu^{2+} disappeared instantly upon the addition of excess EDTA, similar to many rhodamine spirolactam-based fluorescent chemosensors reported formerly [35], the fluorescence enhancement of **1** upon addition of Cu^{2+} could be rationalized by the metal ion induced spirolactam ring-opening mechanism.

To further illustrate the structure-recognition activity relationship of the chemosensor **1**, two analog compounds **2** and **3** were designed and synthesized. As shown in Scheme 2, we noticed that **1** bearing a 2-hydrazinopyridine moiety, in which a nitrogen atom attached to the 2-position of pyridine, **2** have a methylene-pyridine moiety, lack of a nitrogen atom attached to 2-position of pyridine, while **3** with a phenyl attached to hydrazine moiety instead of pyridine. The structure differences of **1**, **2** and **3** might affect their binding capability to Cu^{2+}. The comparison of the fluorescence intensity of **1**, **2** and **3** with addition of Cu^{2+} under the same concentration was shown in Fig. 9, a 17-fold florescence enhancement was observed in the solution of **1**, while only 3-fold and 4-fold fluorescence enhancement was observed in **2** and **3** solutions respectively. The results indicated that **1** have more effectively binding capability to Cu^{2+} than its analogs **2** and **3**. Due to the synergetic effect of the nitrogen atoms on pyridine ring and the hydrazo-attached to pyridine 2-position improve the Cu^{2+} coordination capability of **1**, while **2** and **3** did not have both of the nitrogen atoms, that resulted in much less coordination capability to Cu^{2+}. Associate to Yu′s reported derivative [31], which a more methane–carbon atom was linked between the pyridine 2-position and the hydrazo-rhodamine than that of **1** and also showed an effectively binding capability to Cu^{2+}, these structure activity

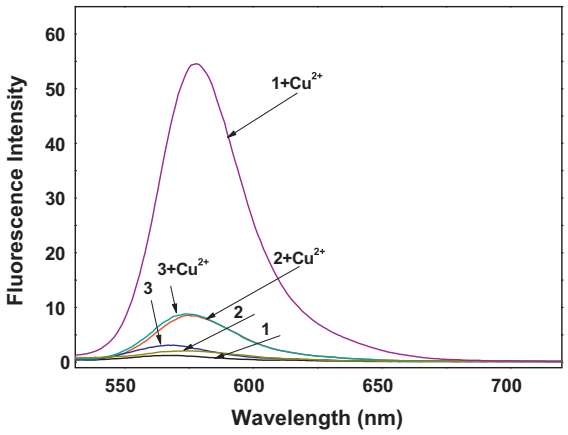


Fig. 9. Fluorescence spectra of 1, 2, 3 (2.5 μM) in buffered 10 mM Tris–HCl/EtOH (v/v = 1/1 pH = 7.0), in the absence and presence of 25 μM Cu^{2+}.

relationship of the neighbor groups/atoms of nitrogen atom further demonstrate that nitrogen atoms of pyridine 2-position and hydrazo-play very important roles for the selectivity/sensitivity of rhodamine-based chemosensors.

4. Fast detection of Cu^{2+} with the test strips of 1

For the requirement of practical application, an easily prepared and portable detection kit/strips should be taken into consideration, therefore, test strips were prepared by immersing filter papers into a EtOH/water (v/v = 1/1) solution of **1** (0.1 M) and then drying in air. The test strips made from **1** were directly uti-

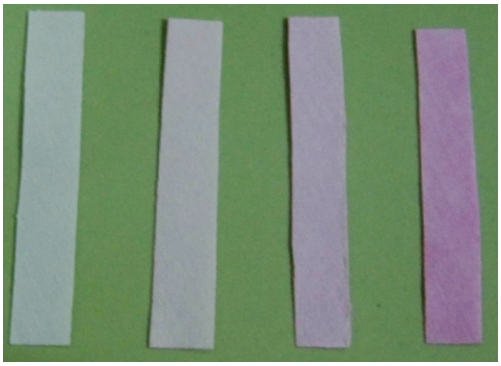


Fig. 10. Photographs of the test strips made from **1** for the detection of Cu^{2+} with different concentrations in EtOH/water (v/v = 1/1) solution. Left to right: 0, 1.0×10^{-4} M, 5.0×10^{-4} M, 5.0×10^{-3} M.

lized in the detection of Cu^{2+} in EtOH/water (v/v = 1/1) solution under different concentration. The obvious color change of **1** solution was observed only treated with Cu^{2+} solution, while other metal ions solutions do not induce such changes [36]. Moreover, the test strips were utilized for the sensing of different concentration of Cu^{2+}, as depicted in Fig. 10, the purple-red color of the test strips intensified from 0 to 1.0×10^{-4} M, and the colorimetric changes exhibited that the test strips made from **1** naked-eyes could be employed as portable tools for fast Cu^{2+} detection.

5. Conclusion

In summary, we synthesized and characterized a rhodamine-based derivative **1**. It exhibits high selectivity to Cu^{2+} over other metal cations. The remarkable fluorescence and absorption enhancement of **1** with addition of Cu^{2+} could be easily detected by spectroscopy, and the obvious color change also could be easily observed by naked-eyes. The binding of **1** to Cu^{2+} ($Ks = 3.84 \times 10^5$) is instantaneous and sensitive, which could be used to detect Cu^{2+} at a low concentration limit of 2.11×10^{-8} M. Moreover, we found a linear relationship between the fluorescence intensity at 575 nm and Cu^{2+} concentration (from 0.5×10^{-6} M to 3.0×10^{-6} M). Furthermore, the weak-response of **2** and **3** to Cu^{2+} elucidated the possible synergetic effect of the nitrogen atoms of hydrazo-, and the nitrogen atoms of pyridine ring is essential for the improvement of Cu^{2+} binding/sensing capabilities. Additionally, the chemically reversible binding of **1** to Cu^{2+} showed **1** could be served as a potential recyclable component in sensing materials. For practical application, the paper-made test strips of **1** could be employed as portable sensing tools for fast Cu^{2+} detection.

Acknowledgements

We thank the Science and Technology Innovation Foundation for the College Students of Beijing (no. B091000814); the National natural science foundation of China (NSFC no. 20972015) and the Natural Science Foundation of Beijing, (no. 2082016) for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.saa.2011.05.016.

References

[1] D.T. Quang, J.S. Kim, Chem. Rev. 110 (2010) 6280–6301.
[2] L.J. Tanga, F.F. Li, M.H. Liua, R.K. Nandhakumar, Spectrochim. Acta Part A 78 (2011) 1168–1172.
[3] Zh.Q. Hua, X.M. Wang, Y.C. Feng, L. Ding, H.Y. Lu, Dyes Pigments 88 (2011) 257–261.
[4] M. Kumar, N. Kumar, V. Bhalla, H. Singh, P.R. Sharma, T. Kaur, Org. Lett. 13 (2011) 1422–1425.
[5] H.S. Jung, P.S. Kwon, J.W. Lee, J.I. Kim, C.S. Hong, J.W. Kim, S.H. Yan, J.Y. Lee, J.H. Lee, T.H. Joo, J.S. Kim, J. Am. Chem. Soc. 131 (2009) 2008–2012.
[6] X. Dai, Y.X. Yang, X.F. Wang, R.L. Sheng, J. Chem. Res.-S 6 (2009) 356–358.
[7] J. Xie, M. Menand, S. Maisonneuve, R. Metivier, J. Org. Chem. 72 (2007) 5980–5985.
[8] H.L. Mu, R. Gong, Q. Ma, Y.M. Sun, E.Q. Fu, Tetrahedron Lett. 48 (2007) 5525–5529.
[9] Y.Q. Weng, F. Yue, Y.P. Zhong, B.H. Ye, Inorg. Chem. 46 (2007) 7749–7755.
[10] H.J. Kim, S.Y. Park, S. Yoon, J.S. Kim, Tetrahedron 64 (2008) 1294–1300.
[11] G.K. Li, Z.X. Xu, C.F. Chen, Z.T. Huang, Chem. Commun. (2008) 1774–1776.
[12] R. Martinez, A. Espinosa, A. Tarraga, P. Molina, Org. Lett. 7 (2005) 5869–5872.
[13] E.J. Jun, H.N. Won, J.S. Kim, K.-H. Lee, J. Yoon, Tetrahedron Lett. 47 (2006) 4577–4580.
[14] N.K. Singhal, B. Ramanujam, V. Mariappanadar, C.P. Rao, Org. Lett. 8 (2006) 3525–3528.
[15] X. Qi, E.J. Jun, L. Xu, S.J. Kim, J.S. Hong, Y.J. Yoon, J. Yoon, J. Org. Chem. 71 (2006) 2881–2884.
[16] R. Martinez, F. Zapata, A. Caballero, A. Espinosa, A. Tarraga, P. Molina, Org. Lett. 8 (2006) 3235–3238.
[17] G.K. Liu, Z. Xu, X.C.F. Chen, Z.T. Huang, Chem. Commun. (2008) 1774–1776.
[18] X.Q. Chen, M.J. Jou, H. Lee, S. Kou, J. Lim, S.W. Nan, S. Park, K.M. Kim, J. Yoon, Sens. Actuators B 137 (2009) 597–602.
[19] Y. Xiang, Z.F. Li, X.T. Chen, A.J. Tong, Talanta 74 (2008) 1148–1153.
[20] H.N. Kim, M.H. Lee, H.J. Kim, J.S. Kim, J. Yoon, Chem. Soc. Rev. 37 (2008) 1465–1472.
[21] Y.K. Yang, K.J. Yook, J. Tae, J. Am. Chem. Soc. 127 (2005) 16760–16761.
[22] D. Wu, W. Huang, C. Duan, Z. Lin, Q. Meng, Inorg. Chem. 46 (2007) 1538–1540.
[23] W.M. Liu, L.W. Xu, H.Y. Zhang, J.J. You, X.L. Zhang, R.L. Sheng, P.F. Wang, Org. Biomol. Chem. 7 (2009) 660–664.
[24] O.A. Egorova, H. Seo, A. Chatterjee, K.H. Ahn, Org. Lett. 12 (2010) 401–403.
[25] W.Y. Lin, L. Yuan, W. Tan, J.B. Feng, L.J. Long, Chem. Eur. J. 15 (2009) 1030–1035.
[26] M.H. Lee, H.J. Kim, S. Yoon, N. Park, J.S. Kim, Org. Lett. 10 (2008) 213–216.
[27] K.M.K. Swomy, S.K. Ko, S.K. kwon, H.N. Lee, C. Mao, J.M. Kim, K.H. Lee, J. Kim, I. Shin, J. Yoon, Chem. Commun. (2008) 5915–5917.
[28] W. Huang, C. Song, C. He, G. Lu, X. Zhu, C. Duan, Inorg. Chem. 48 (2009) 5061–5072.
[29] V. Dujols, F. Ford, A.W. Czarnik, J. Am. Chem. Soc. 119 (1997) 7386–7387.
[30] X. Zhang, Y. Shiraishi, T. Hirai, Org. Lett. 9 (2007) 5039–5042.
[31] Y. Xiang, A.J. Tong, P.Y. Jin, Y. Ju, Org. Lett. 8 (2006) 2863–2866.
[32] M.L. Zhao, X.F. Yang, S.F. He, L.P. Wang, Sens. Actuators B 135 (2009) 625–631.
[33] X. Chen, M.J. Jou, H. Lee, S. Kou, J. Lim, S.-W. Nam, S. Park, K.M. Kim, J. Yoon, Sens. Actuators B 137 (2009) 597–602.
[34] X. Zeng, L. Dong, C. Wu, L. Mu, S.F. Xue, Z. Tao, Sens. Actuators B 141 (2009) 506–510.
[35] Y. Zhao, X.B. Zhang, Z.X. Han, L. Qiao, C.Y. Li, L.X. Jian, G.L. Shen, R.Q. Yu, Anal. Chem. 81 (2009) 7022–7030.
[36] R. Sheng, P. Wang, Y. Gao, Y. Wu, W. Liu, J. Ma, H. Li, S. Wu, Org. Lett. 10 (2008) 5015–5018.
[37] R. Sheng, P. Wang, Y. Liu, J.X.S. Wu, S. Wu, Sens. Actuators B 128 (2008) 507–511.
[38] Y. Zhou, C. Zhu, X. Gao, X. You, C. Yao, Org. Lett. 12 (2010) 2566–2569.