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ORIGINAL PAPER

A Rhodamine-Based "Off-On" Colorimetric and Fluorescent Chemosensor for Cu(II) in Aqueous and Non-aqueous Media

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Abstract A new rhodamine derivative (RhB-NSal) bearing an electron-withdrawing group $–NO_2$ at the 5-position of 2-hydroxyphenyl moiety was synthesized and its sensing behaviors for Cu^{2+} in acetonitrile and aqueous acetate-buffer/acetonitrile (2/3, *v*/*v*, pH 4.8) media were investigated. In each medium, significant absorption and fluorescence enhancements accompanied by an instant color change from colorless to pink were observed for RhB-NSal upon addition of Cu^{2+}. RhB-NSal binds with Cu^{2+} forming a 1:1 stoichiometric complex with an association constant of 6.72 (±0.03) × 10^4 M^{-1} and 4.23 (±0.03) × 10^4 M^{-1}, respectively. RhB-NSal displayed high selectivity for Cu^{2+} over possibly competitive metal ions except that Fe^{3+} and Bi^{3+} ion can respectively bring about a little interference in absorption and fluorescence with its sensing for Cu^{2+}. In dry acetonitrile, pronounced enhancements in the absorbance and emission of RhB-NSal were induced by Cu^{2+}, with a detection limit of 0.49 μM, exhibiting higher sensitivity than that of a known analogue bearing no substituent on its phenol ring, RhB-Sal. In aqueous solution, RhB-NSal displayed likewise a high selectivity but a lower sensitivity for Cu^{2+} than that in acetonitrile, with a detection limit of 14.98 μM, still more sensitive than RhB-Sal in absorption. By virtue of these properties, RhB-NSal could be used as an "Off-On" fluorescent and colorimetric chemosensor for Cu^{2+} in acetonitrile medium, and be developed to be a promising candidate of "Off-On" eye-naked chemosensor for Cu^{2+} in a weakly acidic aqueous medium.

Keywords Chemosensor · Copper (II) · Electronic effect · Rhodamine derivative · Spectroscopic response

Introduction

The rhodamine-based framework has attracted considerable attention in the fabrication of chemosensors or chemodosimeters owing to its known excellent spectroscopic properties [1–4] and ease of modification [5]. The spirolactam ring of a rhodamine molecule may be opened due to a metal ion chelation, protonation or a chemical reaction, which accordingly results in the remarkable variation of the molecule in its absorption and fluorescence. Based on this mechanism, many rhodamine derivatives have been developed as colorimetric and fluorescent chemosensors or chemodosimeters for a wide variety of metal ions and other interested species during these years [5–15]. Among the metal ions, copper (II) is a typical environmentally- and biologically-relevant metal ion which plays a pivotal role in the field of biology, chemistry, environment and clinical diagnosis, etc. In this regard, it is of great significance to construct more sensitive chemosensors or chemodosimeters for detecting the trace amount of Cu^{2+} which possibly exists in different media. Hereunto, a number of rhodamine-based Cu^{2+} chemosensors have been designed and constructed [16–23].

A salicylaldehyde rhodamine derivative which bears no substituent on its phenol ring, RhB-Sal, has been proven to be able to sense Cu^{2+} in neutral aqueous media by virtue of its selective and sensitive fluorescence enhancement [16]. In our previous works, rhodamine-based analogues, RhB-pMOSal [24] and RhB-Im [25], were developed as "off-on" fluorescent

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chemosensors for selectively sensing Cu^{2+} in aqueous solution and acidic aqueous solution respectively, but their sensitivity are not satisfactory. In this paper, the performance of a newly synthesized rhodamine compound, RhB-NSal, to sense Cu^{2+} in weakly acidic aqueous solution and dry acetonitrile media was comparatively examined. In the molecule of RhB-NSal, an electron-withdrawing group $–NO_2$ was introduced to the 5-position of the 2-hydroxyphenyl moiety (Scheme 1), which allowed us to further examine its electronic effect on the sensing performance. It was found that RhB-NSal responded to Cu^{2+} selectively, sensitively and reversibly in its absorption and fluorescence in acetonitrile medium over other metal ions. In weakly acidic aqueous solution, RhB-NSal displayed a high selectivity but lower sensitivity. Compared with RhB-Sal, the introduction of $5\text{-}NO_2$ group favored the sensing sensitivity of RhB-NSal for Cu^{2+} in both media.

Experimental

Reagents and Chemicals

Rhodamine B was purchased from TDI, and 5-nitrosalicylaldehyde was from Sigma-Aldrich. Silica gel (300–400 mesh) used for thin-layer and column chromatography was obtained commercially from Qingdao Ocean Chemicals (Qingdao, China). Acetonitrile was distilled over CaH_2, and then stored over 4 Å molecular sieves prior to use. All metal salts used in the spectroscopic experiments were obtained from Shanghai Chemical Reagent Corporation (Shanghai, China). Two sets of stock solutions of single metal ion (1 mM) including Li^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Bi^{3+} and Cr^{3+} were prepared from their chloride salts except for Pb^{2+} and Ag^+ from nitrate salt, and Al^{3+}, Cu^{2+}, Mn^{2+}, Ni^{2+} from sulfate salt using acetonitrile and aqueous acetate-buffer/acetonitrile (2/3, *v*/*v*, pH 4.8) solution as solvent, respectively. The stock solution of RhB-NSal (100 μM) was prepared in dry acetonitrile, and two sets of the working solutions were prepared by diluting respectively the stock solution to 10 μM with dry acetonitrile and the aqueous acetate buffer/acetonitrile (2/3, *v*/*v*, pH 4.80, acetate, 20 mM) solution as well. The component acetonitrile in the buffer medium was used as a solubilizing reagent for RhB-NSal. All chemicals involved in the experiments were of analytical grade and used without further purification.

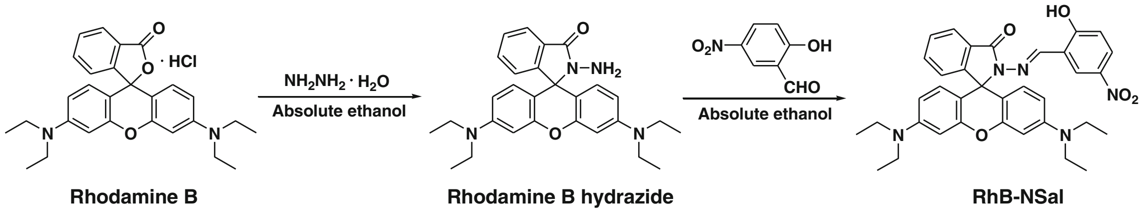
Apparatus

The electronic absorption spectra were obtained using a Shimadzu UV-2450 spectrophotometer and the steady-state fluorescence emission spectra were recorded on a JASCO FP-6500 spectrophotometer equipped with a thermostated cell compartment. A quartz cuvette of 1.0 cm optical path length was used throughout the whole spectroscopic measurements. The pH measurements were carried out on a PHS-3C Exact Digital pH meter. The FTIR spectra were recorded on a NEXUS 670 spectrometer using KBr pellets. The mass spectra were obtained on an LCQ electron spray mass spectrometer (ESMS, Finnigan). NMR spectra were measured on a Bruker DRX-500 spectrometer with tetramethylsilane (TMS) as an internal standard and $CDCl_3$ as a solvent. All of the measurements were carried out at 298.0 ± 0.2 K. Elementary analysis for C, H, N was performed on a Vario EL cube elementar.

General Procedures

The sensing behaviors of RhB-NSal for various metal ions were examined in both aqueous and non-aqueous media by electronic absorption and fluorescence spectroscopy. The UV–vis and fluorescence titrations were performed by transferring aliquots of each metal stock solution into 3 mL corresponding RhB-NSal (10 μM) working solution in a quartz cuvette. After each addition, the solution was fully mixed and equilibrated for *ca.* 2 min. The UV–vis absorption spectra were recorded within 450–600 nm, and the fluorescence spectra were taken in the scope of 540–650 nm with excitation light of 520 nm. The slit width of the excitation and the emission was both set at 5 nm. All experiments were run in triplicate, and the average values were adopted.

The binding ratio between RhB-NSal and Cu^{2+} ion was determined by Job's plot using the data of UV–vis absorption spectra, and was verified by Benesi-Hildebrand method [26]. In the determination, the total concentration of Cu^{2+} and RhB-NSal was kept at 10 μM and the molar ratio of Cu^{2+} was changed from 0 to 1.0. Each solution was prepared by mixing appropriate aliquots of the corresponding RhB-NSal and Cu^{2+}



Scheme 1 Synthesis of RhB-NSal

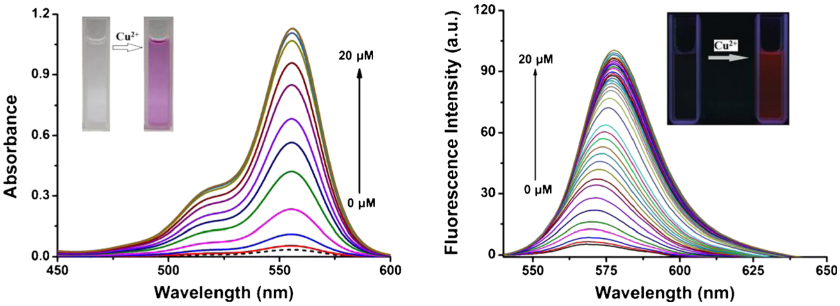


Fig. 1 Variations of the absorption (*left*) and fluorescence (*right*) spectra of RhB-NSal (10 μM) in dry acetonitrile in the presence of different amount of Cu^{2+}

working solution, and diluting to 5 mL with dry acetonitrile and aqueous acetate buffer, respectively.

Synthesis

RhB-NSal was synthesized according to the reported procedures [24, 25] using rhodamine B and 5-nitrosalicylaldehyde as raw materials (Scheme 1). An excessive 5-nitrosalicylaldehyde (0.1 g, 0.6 mmol) was added into 20 mL absolute ethanol containing rhodamine B-hydrazide (0.23 g, 0.5 mmol), and the resultant mixture was stirred vigorously for 24 h at room temperature. After the reaction completed, the yellow precipitate was collected by centrifugation and then dried in vacuum. Further purification by silica gel column chromatography (eluent: petroleum ether/ethyl acetate (4/1, *v*/*v*) containing 1 % (*v*/*v*) triethylamine) afforded 0.27 g yellow powder (yield: 73.3 %) with a melting point of 232-234 °C. ESI-MS for $C_{35}H_{35}N_5O_5$ in the acetate-buffered acetonitrile solution (pH 4.80): m/z, $[M + H^+]^+$, 606.68, found 606.58; $[2\ M + H^+ + Na^+]^{2+}$, 1,235.36, found 1,235.50. (Supporting Information Fig. S1). ^1H NMR ($CDCl_3$): δ (ppm) 1.18 (12H, t, $NCH_2\underline{CH_3}$), 3.36 (8H, q, $N\underline{CH_2}CH_3$), 6.28 (2H, d, Xanthene-H), 8.08 (1H, d, Ar-H), 8.9 (1H, s, N = C-H), 12.06 (1H, s, Phen-OH) (Supporting Information Fig. S2). Elemental analysis. Found (calcd.) (%): C, 74.34 (74.28); H, 7.43 (7.42); N, 11.55 (11.56). FT-IR spectra (ν, cm^{-1}): 3,477 (s), 2,970 (m), 2,929 (m), 1,695 (m), 1,614 (s), 1,515 (s), 1,372 (m) (Supporting Information Fig. S3).

Results and Discussion

Spectroscopic Response of RhB-NSal to Cu^{2+} in Dry Acetonitrile and Aqueous Acetate Media

To evaluate the sensing potential of RhB-NSal for Cu^{2+} ion possibly existing in complex media, the spectroscopic response of RhB-NSal to free Cu^{2+} ion in dry acetonitrile and aqueous acetate buffer/acetonitrile (2/3, *v*/*v*, pH 4.80) was firstly investigated by absorption and fluorescence spectroscopy. As shown in Fig. 1, the free RhB-NSal in dry acetonitrile exhibited weak absorbance and fluorescence emission (λ_{ex}=520 nm) due to the unique spirolactam form [2, 3]. The characteristic absorption band lies in the range of 450–600 nm with a λ_{max} of 555 nm (ε=3.71 × 10^4 L mol^{-1} cm^{-1}). Upon addition of Cu^{2+} ion into the solution, the absorption enhanced instantly and a new peak with the maximum wavelength at *ca*. 517 nm occurred. Concomitantly, the solution color became pink from colorless instantaneously (Fig. 1 inset). The fluorescence intensity of the solution similarly enhanced with the increasing amount of Cu^{2+}. When the concentration of Cu^{2+} approached 10 μM, approximate 45 and 15-fold enhancements in the absorbance (at 555 nm) and emission intensity (at 575 nm) were measured respectively, accompanied by a red shift of the maximum emission wavelength from 568 to 575 nm. The detection limit was determined from the fluorescence titration data according to a classical method [27] and was found to be 0.49 μM, which was lower than those of many reported Cu^{2+} chemosensors [25, 28–30]. As for a known analogue bearing no substituent

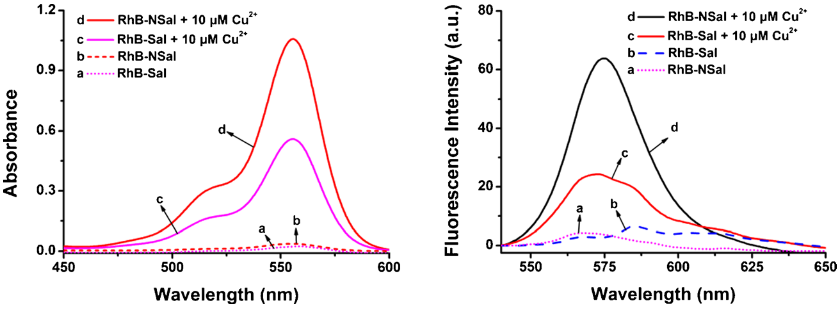
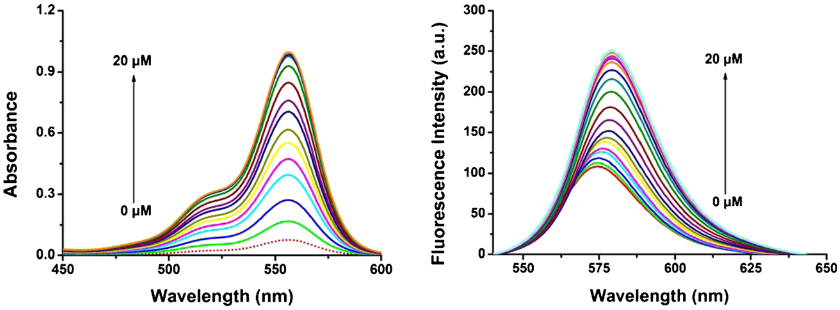


Fig. 2 Spectral comparison between RhB-NSal (10 μM) and RhB-Sal (10 μM) in absorption (*left*) and fluorescence emission (*right*) in acetonitrile upon addition of equivalent Cu^{2+}

Fig. 3 Variations of the absorption (*left*) and fluorescence (*right*) spectra of RhB-NSal (10 μM) in aqueous acetate-buffer/acetonitrile (2/3, *v*/*v*, pH 4.8) solution in the presence of different amount of Cu^{2+}



on its phenol ring, RhB-Sal, only *ca* 23 and 9-fold enhancements at its maximum absorbance and emission were estimated, respectively, which indicates that RhB-NSal is more sensitive than RhB-Sal toward Cu^{2+} under the same conditions (Fig. 2). Also, the results suggest that the additional 5-nitro group favors the sensing capability of RhB-NSal for Cu^{2+} ion, which could be ascribed to the enhancement of the binding capacity of RhB-NSal to Cu^{2+} due to the electron-attracting effect of nitro group.

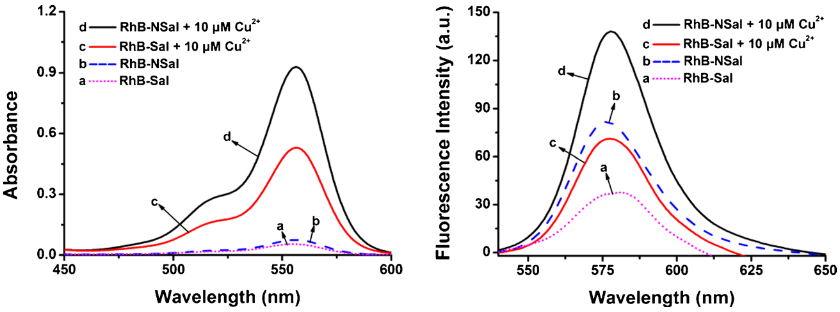
The spectroscopic response of RhB-NSal to Cu^{2+} in aqueous media was further examined. A weakly acidic aqueous solution with a pH value of 4.80 maintained by acetate buffer was selected in case of the hydrolysis of possibly coexisting highly-valent metal ions. As shown in Fig. 3, free RhB-NSal in the aqueous acetonitrile solution exhibited weak absorption but medium strong emission (λ_{ex}=520 nm), which suggests part intramolecular opening of the spirolactam ring due to the effect of H^+ ion [31]. The characteristic absorption band also lies in the scope of 450–600 nm with a λ_{max} of 556 nm ($\varepsilon = 7.52 \times 10^4$ L mol^{-1} cm^{-1}). A similar fluorogenic and chromogenic response toward Cu^{2+} in this medium was observed for RhB-NSal. Approximately 12 and 1.7-fold enhancements in the maximum absorbance (at 556 nm) and emission (at 578 nm) of RhB-NSal (10 μM) were respectively estimated when equivalent Cu^{2+} was added into the solution. The detection limit was calculated according to the same method to be 14.98 μM. Moreover, the maximum emission wavelength underwent a slight red shift with the increase of Cu^{2+} as shown in Fig. 3, which could be ascribed to the rigidity enhancement of RhB-NSal molecule due to Cu^{2+} coordination [6]. Obviously, RhB-NSal exhibits lower sensitivity for Cu^{2+} in aqueous solution than that in dry acetonitrile.

In comparison to RhB-Sal, the same amount of Cu^{2+} induced more remarkable absorption increase of RhB-NSal in aqueous media, a case similar to that in dry acetonitrile. However, the difference of fluorescence enhancement between RhB-NSal and RhB-Sal was hardly observed under the same conditions (Fig. 4). The results suggest that the Cu^{2+} ion-induced fluorescence enhancement of RhB-NSal has been impaired by the higher background resulted by H^+ ion in the solution [32]. This may be the reason why most of rhodamine chemosensors reported in literature were applied in neutral aqueous or non-aqueous media so as to circumvent such spectroscopic interference.

Binding Interaction Between RhB-NSal and Cu^{2+} in Dry Acetonitrile and Aqueous Acetate Media

All above spectroscopic results suggest that Cu^{2+} ion binds with RhB-NSal, resulting in the spirolactam ring of RhB-NSal open, and concomitantly forming RhB-NSal-Cu(II) complex. Similar to the previously reported rhodamine analogues, RhB-Sal [16] and RhB-pMOSal [25], RhB-NSal could provide carbonyl oxygen, imino nitrogen, and phenol oxygen as coordination atoms to bind Cu^{2+}. Compared with RhB-Sal, the electron-attracting 5-nitro group of RhB-NSal facilitated the dissociation of hydroxyl proton on the phenol ring, thus enhancing its complexation ability with Cu^{2+}, which could accordingly contribute to its fluorescence response to Cu^{2+} ion. As for the molecule of RhB-pMOSal, the electron-donating 4-methoxy group on the phenol ring may enhance the conjugation effect of imino group –C = N–, and likewise improve the binding capacity of imino nitrogen to Cu^{2+}. In view of these results, it could be anticipated that the

Fig. 4 Spectral comparison between RhB-NSal (10 μM) and RhB-Sal (10 μM) in absorption (*left*) and fluorescence emission (*right*) in aqueous acetate solution upon addition of 10 μM Cu^{2+}



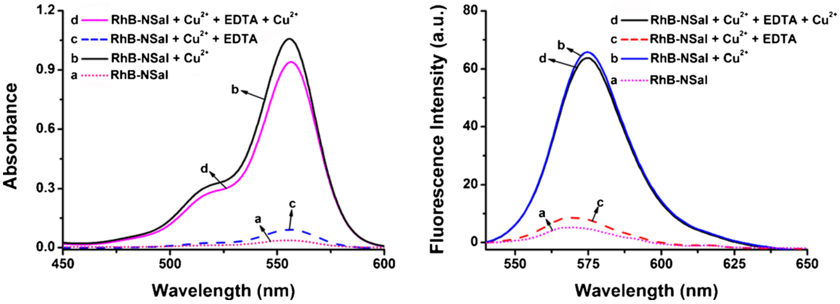


Fig. 5 Chemical reversibility of Cu^{2+}-mediated absorbance (*left*) and fluorescence (*right*) changes of RhB-NSal in dry acetonitrile (**a**) free RhB-NSal (10 μM); (**b**) a + Cu^{2+} (10 μM); (**c**) b + EDTA (10 μM); (**d**) c + Cu^{2+} (10 μM)

substituents which could reinforce the binding capacity of the coordination atoms on a rhodamine molecule may enhance its spectroscopic response to a metal ion. However, the H^+ ion in acidic aqueous medium may in most cases act as the same role as a metal ion resulting in the spirocycle of a rhodamine molecule open, and thus increase its emission background noise [32, 33], which lowers to some extent the sensing sensitivity of the rhodamine chemosensor.

The binding ratio between RhB-NSal and Cu^{2+} in their complex in both dry acetonitrile and aqueous acetate media was examined by Job's method, respectively. Fitting of the Job's plot afforded a 1:1 stoichiometry for the complex (Fig. S4), which was verified by the Benesi-Hildebrand method [26]. Based on this result, the association constant (*Ka*) of RhB-NSal with Cu^{2+} was determined using the Benesi-Hildebrand equation. As shown in Fig. S5, the plotting of $1/(A–A_0)$ versus $1/[Cu^{2+}]$ showed satisfactory linear curves in both media, which confirmed the 1:1 binding ratio between RhB-NSal and Cu^{2+} in the RhB-NSal-Cu(II) complex. The association constants (*Ka*) were calculated from the curve slope to be $6.72 (\pm 0.03) \times 10^4$ M^{-1} and $4.23 (\pm 0.03) \times 10^4$ M^{-1} in dry acetonitrile and aqueous media, respectively. The value of the former is some higher than the latter though they are not significantly different, which is in agreement with the higher sensitivity of RhB-NSal for Cu^{2+} in dry acetonitrile than that in aqueous solution.

Moreover, the chemical reversibility, one of the properties of a chemosensor, for the Cu^{2+}-mediated spectroscopic responses of RhB-NSal in each medium was examined. As shown in Fig. 5, when equivalent chelator, EDTA, was introduced into the mixture of RhB-NSal (10 μM) and Cu^{2+} (10 μM) in dry acetonitrile, the pink solution immediately turned almost colorless, accompanied by an instant attenuation of its emission intensity. To this solution was reversely added Cu^{2+} ion, the spectroscopic responses gradually recovered. The similar circumstances were also observed for RhB-NSal and Cu^{2+} in the aqueous medium (Fig. S6). These results indicate that the binding of RhB-NSal with Cu^{2+} is a chemically reversible process, and the compound RhB-NSal may be served as a potential recyclable chemosensor for Cu^{2+} ion in aqueous or non-aqueous media by means of fluorescent and colorimetric responses.

Sensing Selectivity of RhB-NSal for Cu^{2+} in Dry Acetonitrile and Aqueous Acetate Media

Selectivity is a basic requirement for a compound to be used as a chemosensor. To evaluate the sensing selectivity of RhB-NSal for Cu^{2+} in a complex environment, the spectroscopic interference of possibly competitive metal ions including Li^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Ag^+, Zn^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, Bi^{3+}, Cr^{3+}, Mn^{2+}, Ni^{2+} and Pb^{2+} was investigated in dry acetonitrile and aqueous acetate media, respectively. As demonstrated in Fig. 6 and 7, an instant color change from colorless to pink and *ca* 11-fold absorption increase were observed for RhB-NSal (10 μM) solution upon addition of equivalent Cu^{2+}. The equivalent Fe^{3+} led to a faint pink color and *ca* 2.5-fold absorption enhancement of the solution. Other tested

Fig. 6 Photographs of the color change of RhB-NSal (10 μM) in dry acetonitrile solution upon addition of equivalent metal ions

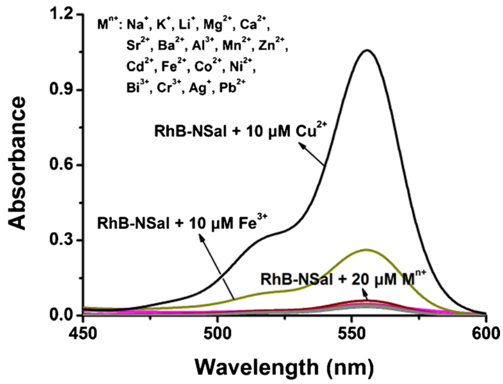


Fig. 7 Absorption changes of RhB-NSal (10 μM) in dry acetonitrile upon addition of each tested ion in concentration of 20 μM. M^{n+} represents the single metal ion tested

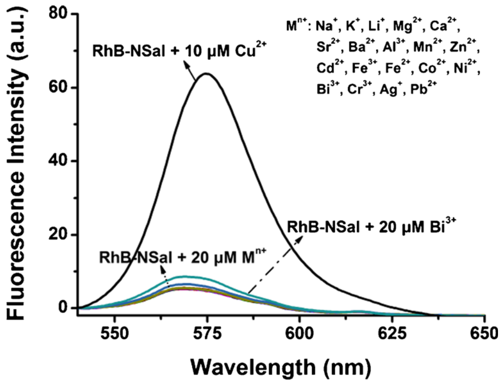


Fig. 9 Effect of single competitive metal ion (M^{n+}) on the fluorescence emission of RhB-NSal (10 μM) in dry acetonitrile solution. Cu^{2+}, 10 μM; Each of the tested metal ions, 20 μM

metal ions in concentration of 20 μM for each hardly impacted on the absorption of the RhB-NSal solution. These results indicate a high specificity of RhB-NSal for Cu^{2+} ion in dry acetonitrile. In an independent experiment, a substantial change in the absorption was similarly observed for 10 μM RhB-NSal in dry acetonitrile solution containing the mixed competitive metal ions when equivalent Cu^{2+} ion was added, which further proved the high selectivity of RhB-NSal for Cu^{2+} (Fig. 8).

The effect of various possibly competitive metal ions on the fluorescence response of RhB-NSal toward Cu^{2+} in dry acetonitrile solution was subsequently investigated. As shown in Fig. 9 and Fig. S7, the outcome satisfactorily corroborated the high selectivity of RhB-NSal for Cu^{2+} shown in the electronic absorption spectroscopy. Among the possibly competitive metal ions, Bi^{3+} ion was found to induce a tiny fluorescence enhancement of RhB-NSal (10 μM). All other metal ions had little effect on the fluorescence of RhB-NSal under the same conditions. In contrast, the equivalent Cu^{2+} resulted in a remarkable emission of RhB-NSal centered at 575 nm (*ca.* 15-fold) in addition to an obvious absorption enhancement shown above. An independent experimental result shown in Fig. 10 exhibited that the mixed competitive metal ions in concentration of 20 μM for each among triggered only *ca.* 3-fold fluorescence enhancement of RhB-NSal (10 μM) whereas the subsequent addition of 10 μM Cu^{2+} induced *ca.* 17-fold enhancement.

The high selectivity of RhB-NSal for sensing Cu^{2+} was likewise observed in acetate-buffered aqueous acetonitrile solution as shown in Fig. S8–S13, except that Fe^{3+} and Bi^{3+} ion, similar to the cases observed in acetonitrile medium, were respectively found to bring about some interference with its absorption and fluorescence. Such interference could be overcome by pre-lowering the solution acidity of the tested samples and then centrifugal separation by virtue of the property of facile hydrolysis of both highly-valent metal ions, which was evidenced in our experiments.

Conclusion

In summary, the sensing behaviors of a newly-synthesized rhodamine derivative RhB-NSal for Cu^{2+} ion in dry

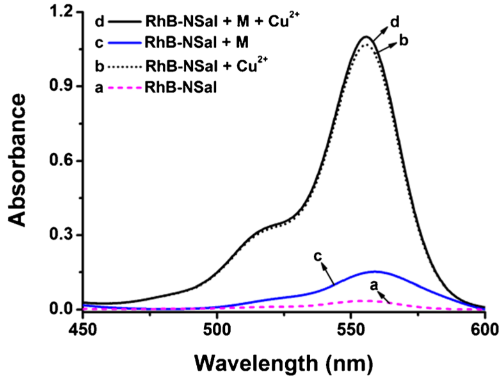


Fig. 8 Effect of the possibly competitive metal ions on the absorption of RhB-NSal in dry acetonitrile solution. RhB-NSal, 10 μM; Cu^{2+}, 10 μM; M represents the possibly competitive metal ions mixed except for Cu^{2+}, the concentration of each competitive ion among was 20 μM

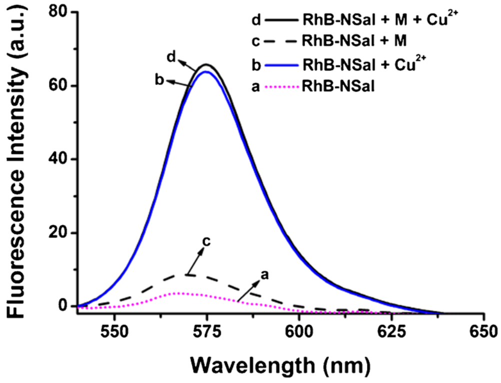


Fig. 10 Effect of the mixed competitive metal ions on the fluorescent sensing of RhB-NSal (10 μM) for Cu^{2+} (10 μM) in dry acetonitrile solution. M represents the mixed competitive metal ions except for Cu^{2+}, the concentration of each ion among was 20 μM

acetonitrile and weakly acidic aqueous acetonitrile solution (pH 4.80) were comparatively investigated. In each medium, the absorption and the fluorescence of RhB-NSal enhanced significantly, accompanied by an instant color change from colorless to pink upon addition of Cu^{2+} ion. RhB-NSal was expected to reversibly bind to Cu^{2+} in each medium, forming a 1:1 stoichiometric RhB-NSal-Cu(II) complex with an association constant of $6.72\ (\pm 0.03) \times 10^4\ M^{-1}$ and $4.23\ (\pm 0.03) \times 10^4\ M^{-1}$, respectively.

RhB-NSal displayed similar high selectivity for Cu^{2+} over co-existing metal ions except that Fe^{3+} brought about some absorption interference and Bi^{3+} led to a little fluorescence interference in each medium. Approximate 45- and 15-fold enhancements in dry acetonitrile whereas 12- and 1.7-fold enhancements in aqueous solution were estimated for RhB-NSal (10 μM) in its maximum absorbance and emission intensity when equivalent Cu^{2+} was added into each solution. The detection limits for Cu^{2+} were calculated respectively to be 0.49 and 14.98 μM. Clearly, the sensitivity of RhB-NSal for Cu^{2+} in acetonitrile is higher than that in aqueous solution. The H^+-resulted stronger spectral background may be responsible for the lower sensitivity in the latter medium. Moreover, RhB-NSal in acetonitrile exhibited higher sensitivity in absorption and fluorescence for Cu^{2+} than that of the analogue bearing no substituent on its phenol ring, RhB-Sal. In the aqueous solution, however, RhB-NSal displayed comparable fluorescence but more remarkable absorption response to Cu^{2+} under the same conditions. The electron-attracting 5-nitro group of RhB-NSal facilitates the dissociation of hydroxyl proton on the phenol ring, and thus enhances its binding capacity with Cu^{2+}, which may accordingly favor its spectroscopic response to Cu^{2+} ion. By virtue of the performance, RhB-NSal could be developed to be a promising "Off-On" fluorescent and colorimetric chemosensor for Cu^{2+} in acetonitrile and a candidate of eye-naked "Off-On" chemosensor for Cu^{2+} in a weakly acidic aqueous medium.

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