Scientific paper

A Dansyl-Rhodamine Based Fluorescent Probe for Detection of Hg^{2+} and Cu^{2+}

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Abstract

A novel fluorescent probe based on dansyl-appended rhodamine B was developed. The probe can selectively recognize and sense Hg^{2+} and Cu^{2+} from other common metal ions by showing unique fluorescence and absorption characteristics. In MeCN/HEPES buffer solution, the probe gives a ratiometric fluorescent response to Hg^{2+}, which was ascribed to the fluorescence resonance energy transfer from dansyl moiety to the ring-opened rhodamine B moiety, while the presence of Cu^{2+} causes fluorescence quenching. Beside the fluorescence change, the presence of Cu^{2+} and Hg^{2+} can induce intensive absorption at about 555 nm, which resulted in a color change from colorless to pink.

Keywords: Rhodamine; Fluorescent probe; Copper ion; Mercury ion

1. Introduction

Copper and mercury are among the most ubiquitous of toxic heavy and transition metals in our environment due to their widespread use in industrial production and daily life. Copper overload is linked to brain damage and oxidative stress although it is an essential trace element.[1] Additionally, copper is highly toxic to aquatic organisms and ecosystems. Mercury is a highly toxic element. All humans are exposed to some level of mercury because it diffuses globally through atmospheric circulation. In freshwater and marine food webs, the bioaccumulation of mercury is so efficient that the mercury levels in top predators, for example shark, may be thousands of times higher than in the surrounding water.[2] Therefore, the development of fluorometric or colorimetric probes for the fast and convenient detection of Cu^{2+} and Hg^{2+} in organisms and environment has attracted considerable attention.[3–11] In recent years, considerable efforts have been made to take advantage of rhodamine dyes in constructing fluorometric probes for metal ions,[12–16] and a number of excellent fluorescent probes for Cu^{2+} or Hg^{2+} have been reported.[17–18] However, the probes that can sense two or more mental ions separately were rarely reported.[19–21]

In the present work, a novel fluorescent probe (**RD**) based on dansyl-appended rhodamine B was designed and synthesized for detection of Hg^{2+} and Cu^{2+} (Scheme 1). In MeCN/HEPES buffer solution, it can give a ratiometric fluorescent response to Hg^{2+} and a fluorescent quenching response to Cu^{2+}. Meanwhile, the probe can give colorimetric responses to Cu^{2+} and Hg^{2+}.

2. Experimental

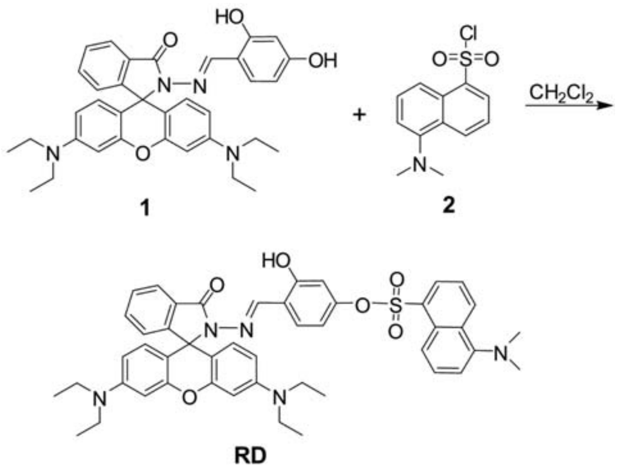
2. 1. Reagents and Apparatus

All chemicals were purchased from commercial suppliers and used as received without further purification. Rhodamine B derivative (compound **1** in Scheme 1) were prepared according to the published procedure.[22] Analytical grade acetonitrile and deionised water were used in all spectral measurements. The samples of metal ions were prepared by dissolving the corresponding metal nitrates in water except the sample of Hg^{2+}, which was obtained by dissolving mercuric sulfate in dilute nitric acid (0.1 mol/L).

^1H and ^{13}C NMR spectra were recorded on a Bruker-400 spectrometer. ESI-MS spectra were performed on a Bruker Esquire HCT mass spectrometer. Fluorescence spectra were taken on a Hitachi F-7000 fluorescence spectrometer. UV-vis absorption spectra were measured on a TU-1901 spectrophotometer. The pH values were determinated on a PHS-3C digital pH meter.

2. 2. Synthesis of the Probe RD

Under –25 °C, rhodamine B derivative **1** (0.69 g, 1.2 mmol), dansyl chloride (0.35 g, 1.3 mmol) and triethylamine (0.2 ml, 1.4 mmol) were combined in dichloromethane (20 mL) and then stirred for 6 h. After the solvent was evaporated under reduced pressure, the crude product was purified by column chromatography (petroleum ether/ethyl acetate, 1:1, *v:v*) to give **RD** as a red solid (0.59 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.00 (s, 1H), 9.10 (s, 1H), 8.57 (d, 1H, *J* = 8.0 Hz), 8.39 (d, 1H, *J* = 8.0 Hz), 8.04 (t, 1H, *J* = 7.2 Hz), 7.94 (d, 1H, *J* = 7.2 Hz), 7.64 (t, 1H, *J* = 8.0 Hz), 7.52 (m, 2H), 7.41 (t, 1H, *J* = 8.0 Hz), 7.23 (d, 1H, *J* = 8.0 Hz), 7.15 (d, 1H, *J* = 7.2 Hz), 6.92 (d, 1H, *J* = 8.0 Hz). 6.42 (m, 5H), 6.36 (s, 1H), 6.23 (d, 2H, *J* = 8.0 Hz), 3.31 (q, 8H, *J* = 6.8 Hz), 2.90 (s, 6H), 1.14 (t, 12H, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.2, 159.6, 153.5, 151.9, 151.4, 151.3, 150.6, 149.1, 133.6, 132.1, 132.0, 131.2, 131.0, 129.95, 129.86, 129.8, 129.0, 128.6, 128.0, 124.2, 123.3, 123.0, 119.4, 117.7, 115.7, 112.8, 110.6, 108.1, 105.2, 97.9, 66.5, 45.4, 44.3, 12.6. ESI-MS *m/z*: 810.4 $[M+H]^+$; calcd for $C_{47}H_{48}N_5O_6S$, 810.3 (Fig.S1-3).



Scheme 1. Synthesis of **RD**

3. Results and Discussion

3. 1. Fluorescence Responses to Hg^{2+} and Cu^{2+}

The fluorescence changes of **RD** were investigated in MeCN/H_2O solution (9/1, 10 mM HEPES buffer, pH 7.2) upon addition of a wide range of metal ions including Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Ag^+, Pb^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Fe^{2+}, and Fe^{3+}. As shown in Fig. 1, **RD** alone displayed the emission of the dansyl moiety around 545 nm when excited at 370 nm. Upon addition of Hg^{2+}, the emission at 545 nm decreased, and in the meantime the fluorescence of the rhodamine moiety near 580 nm was observed. Besides Hg^{2+}, the presence of Cu^{2+} has a great impact on the **RD** fluorescence, which was quenched by the addition of Cu^{2+}. The fluorescence changes were visible to the naked eye: the addition of Hg^{2+} causes a fluorescence color change from green to orange, while the addition of Cu^{2+} leads to fluorescence disappearance (Fig. 1 inset). Except Hg^{2+} and Cu^{2+}, no tested metal ions show any significant effect on the fluorescence spectrum of **RD.**

The fluorescence titrations of **RD** with Hg^{2+} and Cu^{2+} were carried out by the addition of increasing amount of Hg^{2+} or Cu^{2+}, respectively, to **RD** solution. As shown in Fig. 2, upon gradual addition of Hg^{2+} the significant fluorescence enhancement at 580 nm, and mean-

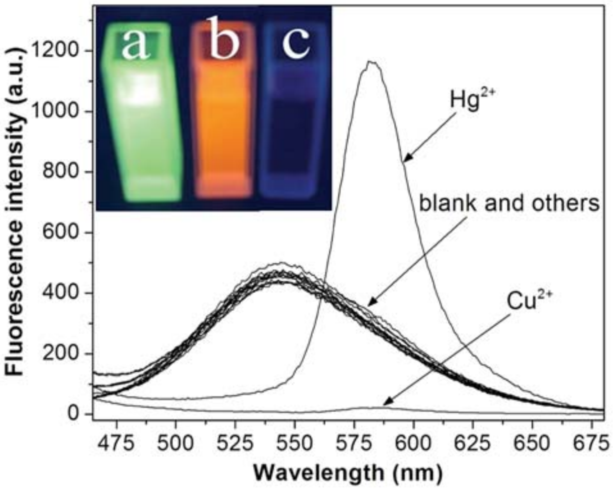


Figure 1. Fluorescence spectra of **RD** (10 μM) upon addition of various cations (10 μM) in MeCN-H_2O solution (9/1, 10 mM HEPES buffer, pH 7.2) when excited at 370 nm. Inset: the fluorescence photographs of **RD** in the absence (a) and presence of Hg^{2+} (b) and Cu^{2+} (c) in dark-box ultraviolet analyzer when excited at 365 nm.

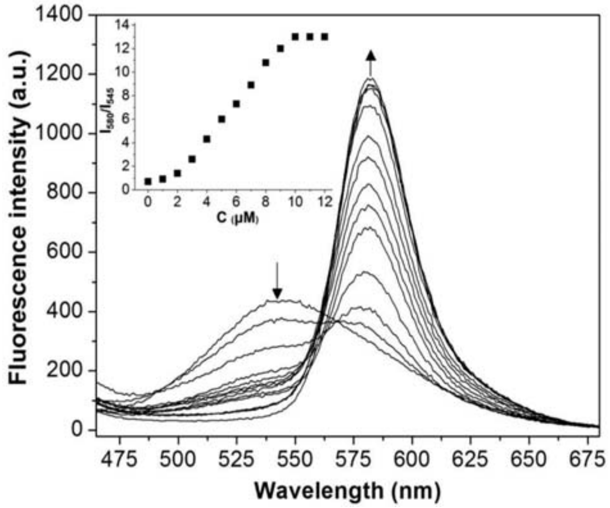


Figure 2. Fluorescence titration spectra of **RD** (10 μM) with Hg^{2+} (0–12 μM). Inset: the fluorescent intensity ratio (I_{580}/I_{545}) as a function of Hg^{2+} concentration.

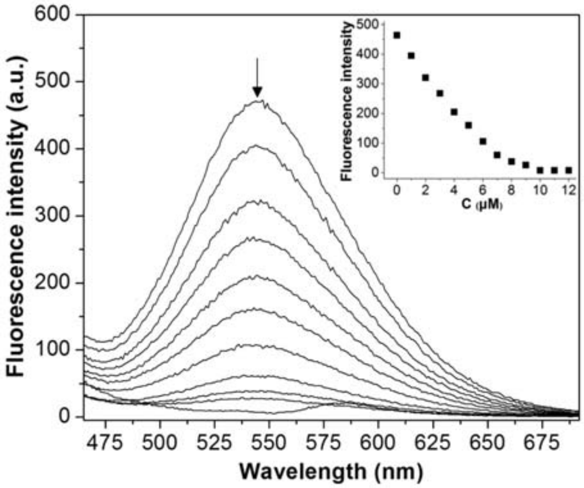


Figure 3. Fluorescence titration spectra of **RD** (10 μM) with Cu^{2+} (0–12 μM). Inset: the fluorescent intensity at 545 nm as a function of Cu^{2+} concentration.

while, the gradual fluorescence disappearance at 540 nm were observed, which should be ascribed to the fluorescence resonance energy transfer (FRET) from dansyl moiety to the ring-opened rhodamine moiety.[23–26] Therefore, **RD** as a ratiometric fluorescent probe for Hg^{2+} was established. Fig. 3 shows the gradual fluorescence quenching along with the increase of Cu^{2+} concentration. Consequently, **RD** can serve only as a fluorescence-quenching probe for Cu^{2+}. The detection limits of **RD** for Hg^{2+} and Cu^{2+} were calculated to be 2.22×10^{-8} mol/L and 4.35×10^{-8} mol/L, respectively, with the equation DL = 3δ/S (δ: the standard deviation of the blank solution; S: the slope of the calibration plot, Fig. S4–5).

3. 2. Colorimetric Signaling of Hg^{2+} and Cu^{2+}

RD alone shows no absorption in the visible light region, so it is colorless. Upon addition of Cu^{2+} and Hg^{2+} separately, strong absorption appeared at 552 nm and 558 nm, respectively, along with a color change from colorless to pink. Obviously, the addition of Cu^{2+} induced a stronger absorption and thicker pink color than that of Hg^{2+}. Excluding Cu^{2+} or Hg^{2+}, all the other metal ions have no effect on the UV/vis spectrum of **RD** (Fig. 4). UV/vis absorption titration with Cu^{2+} and Hg^{2+} were recorded respectively in Fig. 5 and 6. The absorbance of **RD** was found to increase gradually with the increasing concentration of metal ions. In addition, a linear relationship between the absorbance and metal ions concentration was observed (Fig. 5 and 6, inset). This implies the possibility of quantitative determination of Cu^{2+}/Hg^{2+} by spectrophotometry. The colorimetric detection limits of **RD** for Hg^{2+} and Cu^{2+} were calculated to be 4.17×10^{-7} mol/L and 1.36×10^{-7} mol/L, respectively, with the equation DL = 3δ/S (δ: the standard deviation of the blank solution; S: the slope of the calibration plot Fig. S6–7).

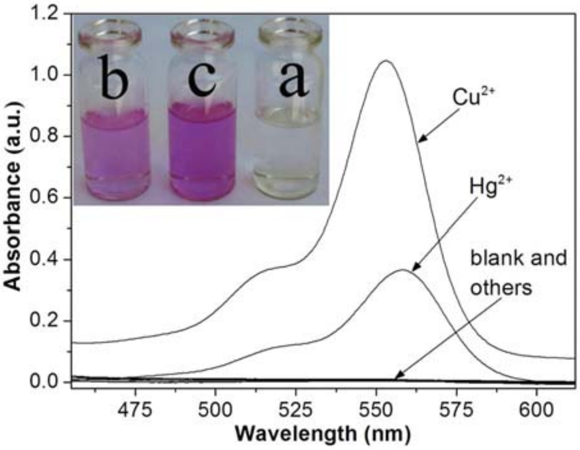


Figure 4. UV-vis spectra of **RD** (10 μM) upon addition of various cations (10 μM) in MeCN-H_2O buffer solution. Inset: Colors of **RD** solution before (a) and after the addition of Hg^{2+} (b) or Cu^{2+} (c) under natural light.

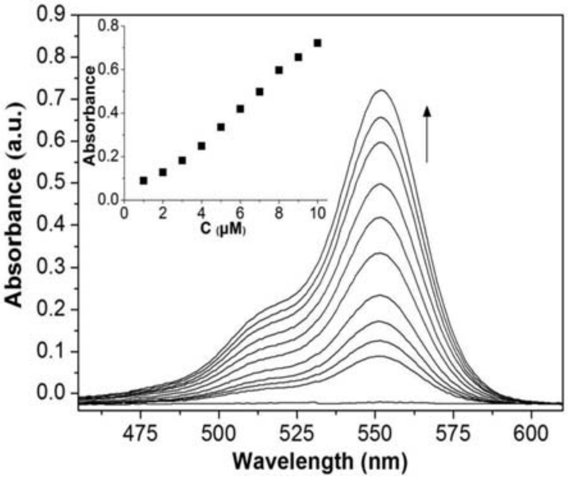


Figure 5. UV-vis spectra of **RD** (10 μM) in the presence of different concentrations of Cu^{2+} (0–10 μM) in MeCN-H_2O buffer solution. Inset: absorbance as a function of Cu^{2+} concentration.

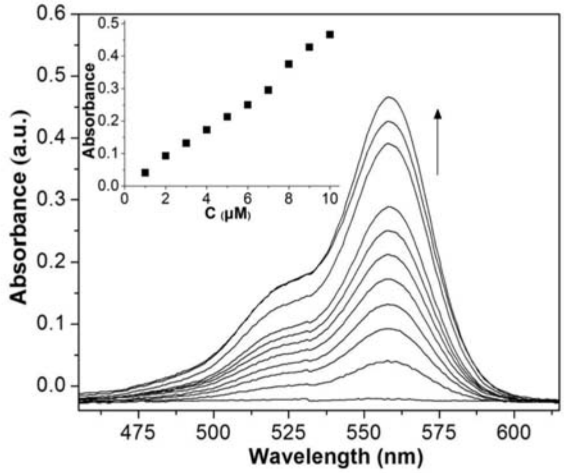


Figure 6. UV-vis spectra of **RD** (10 μM) in the presence of different concentrations of Hg^{2+} (0–10 μM) in MeCN-H_2O buffer solution. Inset: absorbance as a function of Hg^{2+} concentration.

3. 3. Effect of pH and Selectivities for Hg^{2+} and Cu^{2+}

It is well known that the spiroring-opening of rhodamines can be induced by H^+ besides metal ions, so it is necessary to investigate the pH effect on the fluorescence change of **RD**. The fluorescence intensity ratios (I_{580}/I_{545}) of **RD** in the absence and presence of Hg^{2+} at different pH values were shown in Fig.7. When pH>6.0, the fluorescence of **RD** without any interference was observed. When Hg^{2+} was added, the fluorescence intensity ratios were maintained at maximum value under pH<8.0. When pH>8.0, the ratiometric fluorescent responses rapidly disappeared. Therefore, the probe can be used in the pH range from 6.0 to 8.0. The pH value of 7.2 was chosen for all the spectral measurements in this work.

For evaluating the effects of common metal ions on the selectivity of **RD** for Hg^{2+}/Cu^{2+}, competition experiments were carried out by measuring the fluorescence spectra of **RD** in the presence of testing ions and various interfering metal ions. As can be seen from Fig. 8, the highly selective response of **RD** to Hg^{2+} was observed in the presence of various interfering metal ions except Cu^{2+} which can partially quench the Hg^{2+}-induced fluorescence. The interactive effect between Hg^{2+} and Cu^{2+} was examined by measuring fluorescence of **RD** upon successive addition of Cu^{2+}, Hg^{2+} and then Cu^{2+}. As shown in Fig. 9, the presence of Cu^{2+} induces a fluorescence quenching at 545 nm. The subsequent addition of Hg^{2+} causes a new fluorescence enhancement at 580 nm, which decreases following the addition of Cu^{2+}.

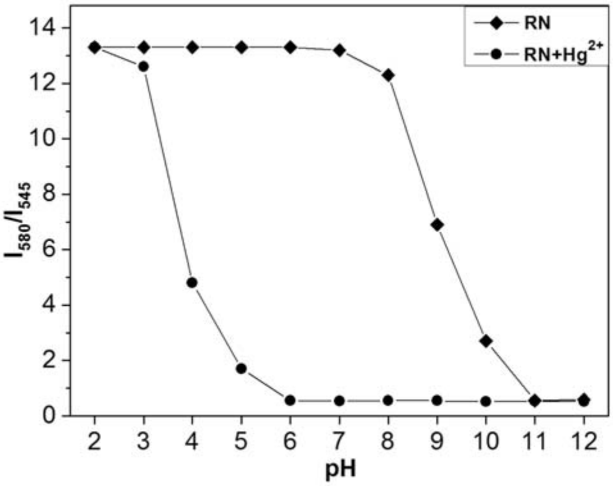


Figure 7. pH-dependent fluorescence intensity ratios of probe **RD** (10 μM) in the absence and presence of Hg^{2+} (10 μM).

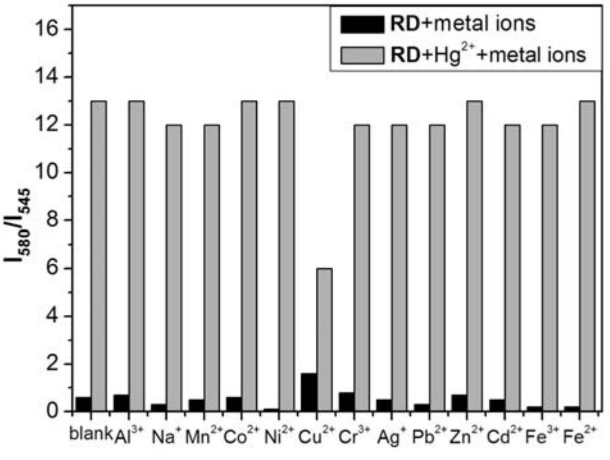


Figure 8. Selective responses of **RD** (10 μM) to 10 μM Hg^{2+} in the presence of 10 μM various other ions.

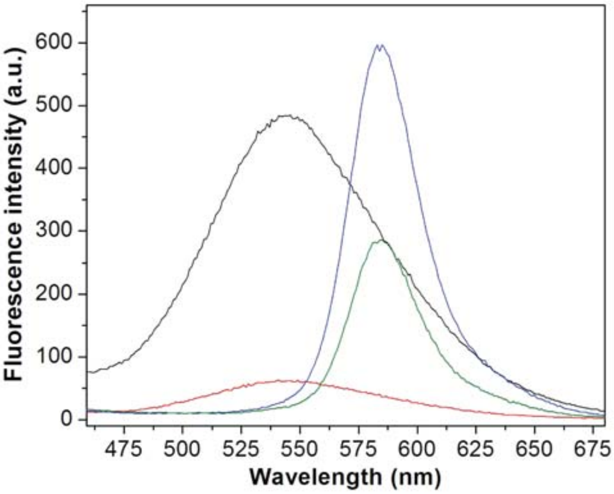


Figure 9. Fluorescence spectra of 10 μM **RD** (black) upon successive addition of 8 μM Cu^{2+} (red), 8 μM Hg^{2+} (blue) and then 8 μM Cu^{2+} (green).

3. 4. Binding Mode and Signaling Mechanism

As is well known, the rhodamine-based probes give turn-on fluorescence responses to specific metal ions by way of coordination between them and the subsequent spirolactam ring-opening. To determine the stoichiometry between Hg^{2+}/Cu^{2+} and **RD**, Job's plots were employed by using the absorbance as functions of molar fraction of Hg^{2+}/Cu^{2+}. For both metal ions, the maximum absorbance appeared when molar fractions are 0.5, indicating 1:1 stoichiometry for **RD-**Hg^{2+} and **RD-**Cu^{2+} complexes (Fig. S8). The binding mode of **RD-**Cu^{2+} complex was further illuminated by ESI-MS testing of **RD** in the presence of 1.2 equiv of $Cu(NO_3)_2$. As seen in Fig. 10, a peak with *m/z* 871.3 was observed, which was assigned to [**RD**-H+Cu]$^+$ (calcd. 871.2). The observed and calculated isotopic patterns agree well with each other (Fig. 10, inset). The presence of [**RD**-H+Cu]$^+$ suggests the deprotonation of the phenolic hydroxyl upon the coordination of **RD** with Cu^{2+}. Based on above discussion, the coordination structure of **RD**-Cu^{2+} complex was proposed as shown in Fig. 10 inset. It is regrettable that the peak corresponding to

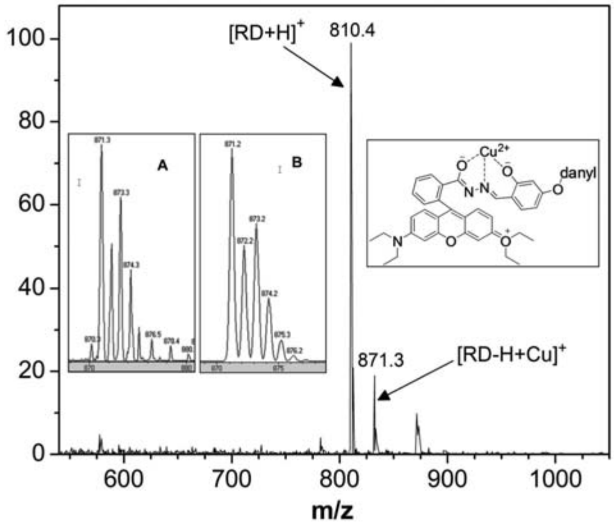


Figure 10. ESI-MS spectrum of **RD** in the presence of $Cu(NO_3)_2$. Inset: observed (A) and calculated (B) isotopic patterns for [**RD**-H+Cu]$^+$, and the proposed coordination pattern of **RD**-Cu2+ complex.

RD-Hg^{2+} complex was not observed, but it can be infered that Hg^{2+} should coordinate with **RD** just like Cu^{2+}. The Cu^{2+}-induced fluorescence quenching should be ascribed to the fact that paramagnetic Cu^{2+} is the most notorious fluorescence quencher. [27–30]

4. Conclusion

In summary, a new probe based on dansyl-appended rhodamine B, which is capable of detecting Cu^{2+} and Hg^{2+}, has been developed. Free **RD** emits green fluorescence. The presence of Cu^{2+} can cause the green fluorescence quenching. Upon addition of Hg^{2+}, an orange fluorescence enhancement and meanwhile the disappearance of the green emission were observed, which was ascribed to the fluorescence resonance energy transfer from dansyl moiety to the ring-opened rhodamine B moiety. Additionally, the probe can give colorimetric responses to Cu^{2+} and Hg^{2+} by producing intense absorptions at 552 nm and 558 nm, respectively, which resulted in a color change from colorless to pink. Other common metal ions including heavy and transition metal ions have no effect on the fluorometric and colorimetric responses to Hg^{2+} and Cu^{2+}.

5. Acknowledgements

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6. References

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Povzetek

Razvili smo novo fluorescenčno senzorsko probo, osnovano na rodaminu B s pripeto danzilno skupino. Proba lahko selektivno prepoznava in zaznava Hg^{2+} in Cu^{2+} ione preko edinstvenih fluorescenčnih in absorpcijskih lastnosti, ne pa tudi ostalih običajnih kovinskih ionov. V pufrski raztopini MeCN/HEPES daje proba raciometrični fluorescenčni odgovor na Hg^{2+}, ki ga pripisujemo fluorescenčnemu resonančnemu prenosu energije z danzilne skupine na odprti obroč rodamina B, medtem ko prisotnost Cu^{2+} povzroči dušenje fluorescence. Poleg sprememb v fluorescenci prisotnost Cu^{2+} in Hg^{2+} povzroči tudi intenzivno absorpcijo pri približno 555 nm, zaradi česar se barva spremeni iz brezbarvne v rožnato.