

<Research Paper>

Design and Synthesis of Novel Rhodamine-based Chemosensor Probe Toward Cu²⁺ Cation

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Abstract: Nowadays, fluorescent rhodamine chemosensors have attracted a worldwide interest due to its ability to selectively detect heavy and transition metal cations. Due to the importance in environmental and biological toxic effects, the developments of fluorescent chemosensors have been received considerable attention in recent. Especially, a rhodamine-based chemosensor probes have been proved to be useful by exhibiting the efficient “off-on” fluorescence switching toward selected metal cations. This fluorophore can undergo the transformation from non-fluorescent and colorless spirolactam derivative to fluorescent ring-open form. In this study, a new fluorescent chemosensor was synthesized using rhodamine B through two-step procedures, and its selectivity and related optical property were characterized. Selectivity and sensitivity was found toward Cu²⁺ guest molecules and then related optical properties of rhodamine B based fluorescent chemosensor compound were characterized using discussed. In addition, computational calculation was used to determine the HOMO/LUMO values.

Keywords: rhodamine, fluorescence, chemosensor, divalent copper(Cu²⁺), HOMO/LUMO

1. Introduction

Heavy metal cations have crucial role in a wide range of chemical and biological processes. Among the transition metal cations, divalent copper (Cu²⁺) is an essential trace element for many physiological systems and it plays a catalytic cofactor for a variety of transcriptional event^{1,2}. Conversely, under overloading concentration, copper exhibits severe toxic problem and can cause neurodegenerative diseases (e.g., Alzheimer's and Wilson's diseases)³⁻⁵.

Thus, there is an urgent need for effective, simple and real-time monitoring system. Abused amounts of copper cause a great prejudicial effect for human health and the environment. In this regard, the design and synthesis of novel chemosensors which can detect heavy metal cations with selectivity and sensitivity were highly worthy. These chemosensors may provide a less labor-consuming and a simple technical alternative to determine hazardous guest molecules.

It could be effectively applied in biological environmental systems⁶⁻⁸.

Due to the good stability of Schiff base ligands with Cu²⁺ to form complexes, rhodamine Schiff base ligands have been attracted a great attention in recent years⁹. On the basis of well-known ring-closed spirolactam (colorless, non-fluorescent) to ring-opened amide (colored, fluorescent) reaction, rhodamine derivatives have been extensively considered with an ideal model, that have been commonly utilized to prepare fluorophore materials for various metal cations¹⁰⁻¹².

In this context, we have prepared new designed chemosensor probe using rhodamine B hydrazide and indole-3-carboxaldehyde. The obtained sensor showed clear selectivity and sensitivity toward Cu²⁺ with compared to other hazardous metal cations. This finding might be utilized into various monitoring system and platform for environmental demands.

2. Experimental

2.1 Measurement

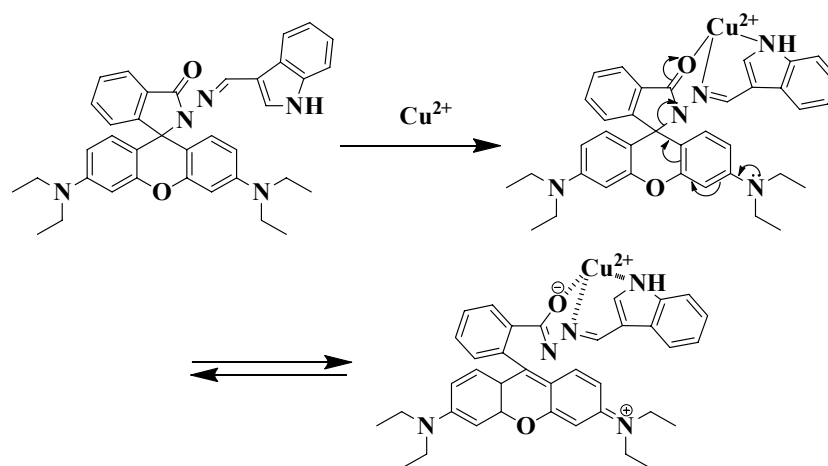
All the reagents and solvents, used for synthesis of rhodamine B based probe 1, were purchased from Aldrich and used without further purification.

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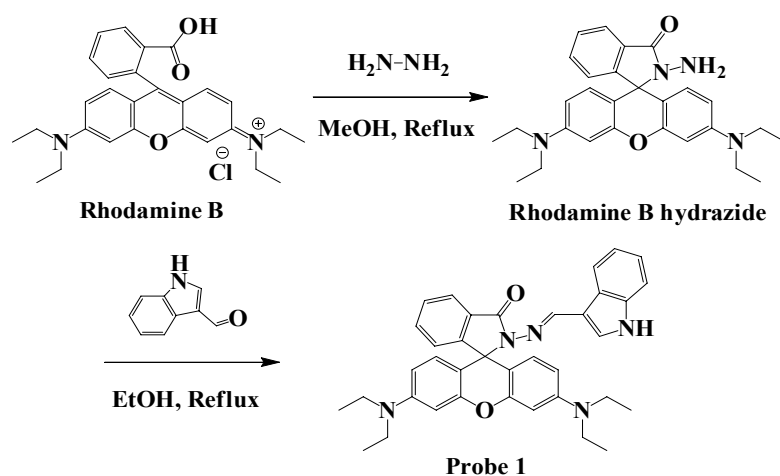
Absorption and fluorescence spectra of the prepared novel probe 1 were measured with an Agilent 8453 spectrophotometer and a Shimadzu RF-5301PC fluorescent spectrophotometer, respectively. $^1\text{H-NMR}$ spectra were recorded with AVANCE III 600 spectrometer operated at 600MHz NMR. HRMS data was recorded on a JEOL M Station [JMS-700]. HOMO/LUMO calculation and modelling simulation proceed with DMol³ of *Material Studio 4.3*.

2.2 Design of probe 1

A novel rhodamine probe 1 was synthesized from the reaction of rhodamine B hydrazide, followed by reaction with indol-3-carboxaldehyde for the detection toward Cu^{2+} . For this design concept, complex mechanism of probe 1 for Cu^{2+} was proposed in Scheme 1.



Scheme 1. The binding reaction of probe 1 with Cu^{2+}



Scheme 2. Synthesis of rhodamine B based probe 1

Cu^{2+} was likely bound to N atom of indol-3-carboxaldehyde group as well as another N atom of the Schiff base and O atom of carbonyl group in a spirolactam moiety of rhodamine B. As a result, the spirolactam form of rhodamine B could be broken and consequently the typical pink fluorescence of rhodamine-based chemosensor would be detected¹³.

2.3 Synthesis of probe 1

Rhodamine B hydrazide was synthesized according to the literature procedure¹⁴. Rhodamine B hydrazide (0.46g, 1mmol) was dissolved in 20mL of absolute ethanol. Addition of indole-3-carboxaldehyde (0.17g, 1.2mmol) to the reaction mixture was refluxed for 8h. The solution was then concentrated and allowed to stand at room temperature through overnight.

The precipitate was filtered the following day, washed several times with cold ethanol, recrystallized from MeCN and dried under reduced pressure to afford probe 1 (0.40g) with 68% yield as a light yellow solid (Scheme 2). Mp = 246-249°C. ¹H-NMR (600MHz, CDCl₃, ppm), the following chemical shifts were recorded: 11.48 (1H, s), 9.41 (1H, s), 7.87 (1H, d, *J*=7.153), 7.70 (1H, d, *J*=2.824), 7.68 (1H, s), 7.56-7.62 (2H, m), 7.33 (1H, d, *J*=8.904), 7.16 (1H, d, *J*=7.341), 7.10 (1H, t, *J*=7.341), 6.93 (1H, t, *J*=7.341), 6.44 (2H, d, *J*=2.259), 6.41 (1H, s), 6.39 (1H, s), 6.34 (1H, d, *J*=2.259), 6.32 (1H, d, *J*=2.259), 3.27-3.31 (8H, q, *J*=6.964), 1.05 (12H, t, *J*=6.694). EI⁽⁺⁾-HRMS (*m/z*): [M+H]⁺ calcd. for C₃₇H₃₇N₅O₂: 583.2947, found 583.2917.

3. Result and Discussion

3.1 Selectivity of probe 1

Selectivity of probe 1 toward various metal cations was investigated by UV-Vis spectroscopy in the MeCN solution and the results are shown in Figure 1. In absorption monitoring, however, none of the metal cations showed any discernible spectral absorption with selective point of view. In this regard, selectivity of probe 1 with various metal cations was not satisfactory to be utilized.

Figure 2 explains the fluorescent turn-on response of probe 1 with various metal cations. When 10μM of each metal cations was mixed with probe 1, there were no considerable changes in fluorescent emissions with other metal cations except Cu²⁺ which showed a clear fluorescent emission signal. With the addition of Cu²⁺ to the probe 1, fluorescence intensity of probe 1 displayed a spectacular increase and its wavelength was maximized at 578 nm. These results indicate that probe 1 showed a significant selective function toward Cu²⁺. In this regard, probe 1 is utilized as an appropriate chemosensor toward Cu²⁺ detection.

To observe selectivity for Cu²⁺ over other metal cations, the affinity competition of probe 1 for various metal cations was investigated.

As shown in Figure 3, fluorescent enhancement caused by the mixture of Cu²⁺ with other metal cations was similar to that caused by Cu²⁺ only.

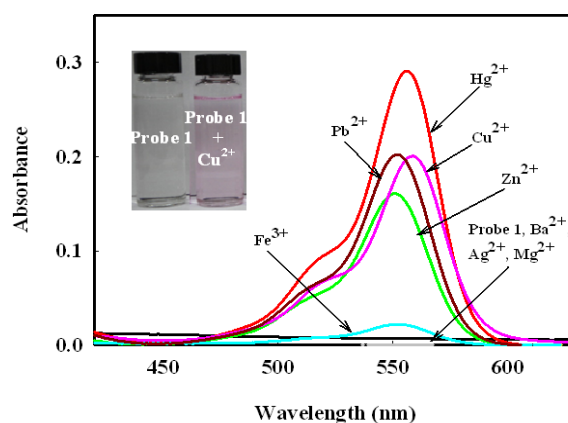


Figure 1. Absorption spectra of probe 1 (10μM) upon the addition of various metal cations (10μM) in MeCN. Inset: the photograph of the color change before and after the addition of Cu²⁺ to a solution of probe 1.

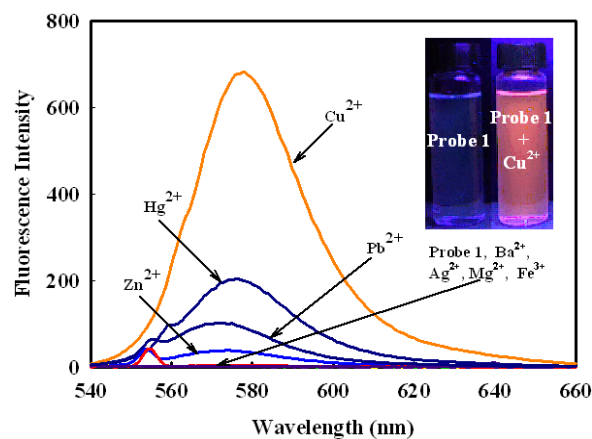


Figure 2. Emission spectra of probe 1 (10μM) upon the addition of various metal cations (10μM) in MeCN. Inset: the photograph of the fluorescence change before and after the addition of Cu²⁺ to a solution of probe 1.

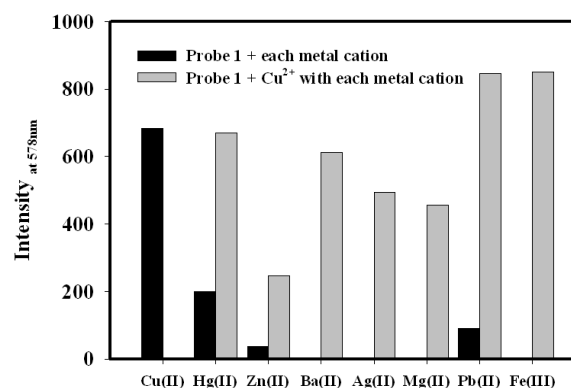


Figure 3. Fluorescence response of probe 1 to Cu²⁺ or to various metal cations (the black bar portion) and to the mixture of other metal cations with Cu²⁺ (the gray bar portion) in MeCN.

The result demonstrated that probe 1 has higher binding affinity toward Cu^{2+} than other various metal cations. Thus, this sensor can be used to detect Cu^{2+} , even though any target environmental samples have various metal cations.

3.2 Sensitivity of probe 1

UV-Vis titration absorption spectra of different mole concentrations of Cu^{2+} with probe 1 in MeCN solution is depicted in Figure 4. The probe 1 exhibits extremely weak absorption in the visible range in the absence of Cu^{2+} due to probe 1 exists in the form of the ring-closed spirolactam structure in the solution. However, the addition of Cu^{2+} to the probe 1 solution leads to a dramatic change in the visible range and a distinct new peak at 559 nm was observed.

Meanwhile, the colorless solution of probe 1 rapidly turned into pink: suggesting the formation of the ring-opened amide form of probe 1 upon Cu^{2+} binding. In this regard, probe 1 exhibits a significant Cu^{2+} -induced absorption. From inset figure, one can see that the absorption intensity increased with increasing Cu^{2+} concentration up to $10\mu\text{M}$ and then remained as a constant. This finding indicates the titration curve to be assumed a 1:1 stoichiometry and this binding mode of probe 1- Cu^{2+} complexes was also supported by a Job's plot¹⁵.

For the measurement of Job's plot method, various molar ratios between probe 1 and Cu^{2+} (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0) in MeCN were prepared. Total concentration of probe 1+ Cu^{2+} complex was kept at $100\mu\text{M}$. The relationship between maximum absorption peaks versus different mole fractions is shown in Figure 5. Maximum absorption peak was reached when the molar fraction was 0.5. In this regard, these results indicate a 1:1 ratio for probe 1- Cu^{2+} complex, in which Cu^{2+} was bound with a probe 1. And this behaviour provides a good opportunity to detect Cu^{2+} with high sensitivity.

With further investigations, Figure 6 shows a variation of fluorescent spectra as a function of Cu^{2+} concentration which was measured to evaluate its sensing behaviour by means of fluorescent titration in MeCN.

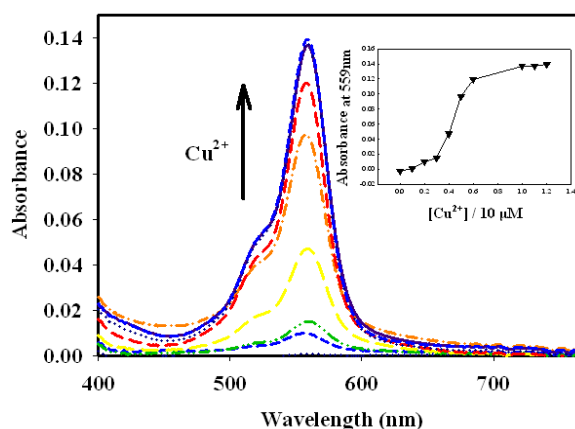


Figure 4. Absorption spectra of probe 1 ($10\mu\text{M}$) with addition increasing concentration (0~1.4equiv.) of Cu^{2+} in MeCN. Inset: absorbance at 559 nm of probe 1 as a function of Cu^{2+} concentration.

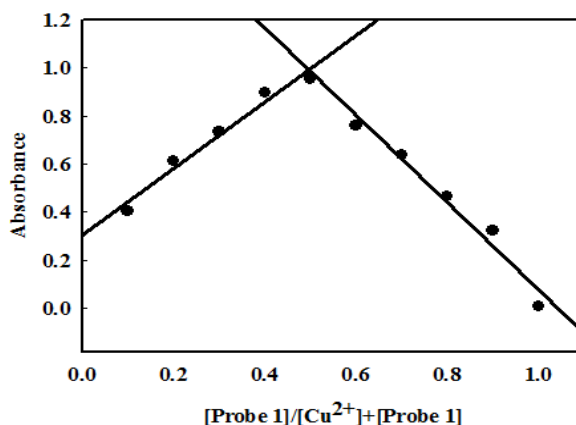


Figure 5. Job's plot analysis of probe 1 with Cu^{2+} in MeCN. Total concentration of probe 1+ Cu^{2+} was kept constant at $100\mu\text{M}$.

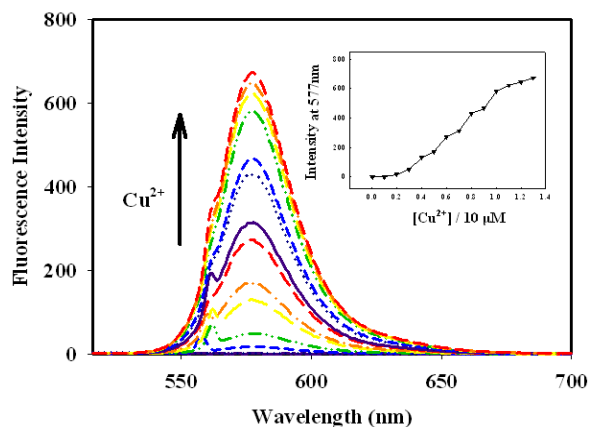


Figure 6. Fluorescence spectra of probe 1 ($10\mu\text{M}$) with addition increasing concentration (0~1.4equiv.) of Cu^{2+} in MeCN. Inset: emission intensities at 577 nm of probe 1 as a function of Cu^{2+} concentration.

With increasing Cu²⁺ concentration, new emission fluorescent intensity gradually increased at 577 nm. This increase in fluorescent intensity clearly demonstrated that probe 1 can serve as a highly sensitive chemosensor for Cu²⁺ detection.

3.3 Computational simulation of probe 1

We have computationally calculated to investigate electron distributions and HOMO/LUMO energy levels of probe 1. It has been simulated with *Material Studio 4.3 suite* program which is the quantum mechanical code using density functional theory (DFT).

Perdew-Burke-Ernzerhof (PBE) function of generalized gradient approximation (GGA) level with double numeric polarization basis set was used to calculate the energy level of the frontier molecular orbitals¹⁶⁻¹⁸.

As shown in Figure 7, electron distributions and its HOMO/LUMO energy levels of probe 1 were calculated. Related HOMO/LUMO values were calculated to be -4.372eV and -2.024eV respectively. In this regard, the ΔE value was finally calculated to be 2.348eV. Electron density distribution of the molecular skeleton reveals that at HOMO distribution of the probe 1, it was located essentially over the indole-3-carboxaldehyde moiety, while at LUMO state, it was mainly distributed over spirolactam moiety. This indicates that probe 1 has interesting property of intramolecular charge transfer system.

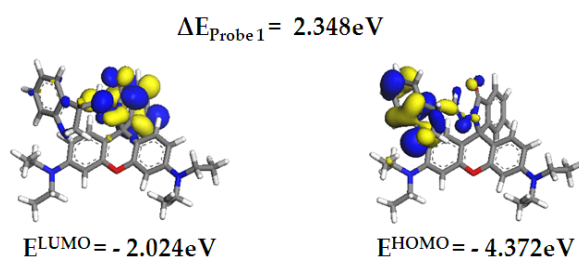


Figure 7. Electron distributions and HOMO/LUMO energy levels of probe 1.

4. Conclusions

In summary, a novel fluorometric probe 1 for Cu²⁺ detection based on the ring-opening of spirolactam was designed and synthesized via the nucleophilic addition reaction of indole-3-carboxaldehyde and rhod-

amine B hydrazide. Studies showed that probe 1 exhibited highly selective and sensitive to Cu²⁺ with compared to other metal cations such as Hg²⁺, Pb²⁺, Mg²⁺, Zn²⁺, Ag²⁺, Ba²⁺ and Fe³⁺. Binding configuration between probe 1 and Cu²⁺ was elucidated to 1:1 stoichiometry complex, which was determined with Job's plot method. HOMO/LUMO energy potential was calculated and movement of electron density distribution was simulated with computational simulation system.

Acknowledgments

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