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Fluorescence Chemosensory Determination of Cu²⁺ Using a New Rhodamine–Morpholine Conjugate

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Abstract: A new rhodamine-B carbonyl-morpholine derivative (denoted as RECM) was prepared by a two-step synthesis procedure. The employed method allowed a lactam ring development of rhodamine-B and ethylenediamine to demonstrate a facile amide bond formation. The obtained RECM was confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry analysis. RECM was formed to detect copper ion (Cu^{2+}) due to its problematic toxicity features in aquatic ecosystems. It showed a high selectivity toward Cu^{2+} in comparison with some environmentally relevant alkali, alkaline earth, and transition metal cations at 50 μM in acetonitrile. Moreover, non-fluorescent RECM showed fluorescence intensity and UV-Vis absorbance increases in the presence of Cu^{2+} with high linear dependent coefficients ($R^2 = 0.964$ and $R^2 = 0.982$ respectively) as well as a color change from colorless to pink owing to the ring opening of the rhodamine spirolactam form. Binding capability experiments presented a clear 1:1 stoichiometry of RECM–Cu^{2+} complex with the binding constant (K_a) as 2.25×10^4 M^{-1}. The calculation of limits of detection (LOD) was 0.21 μM based on the linear regression method, which is below the maximum contaminant level goal (MCLG) value of Cu^{2+} (1.3 ppm equals to 20.46 μM) in drinking water. These characteristics make the RECM a promising candidate for the real-time detection of toxic Cu^{2+} in environmental monitoring applications.

Keywords: chemosensor; copper; rhodamine-B derivative; environmental monitoring

1. Introduction

Potentially toxic elements (PTEs) have attracted a lot of interest from environmental forums due to their adverse effects on ecosystems and human health [1]. Some transition metal ions such as copper play various roles in living systems as a nutrient trace element [2,3]. However, high intake levels of copper lead to neurodegenerative diseases and sclerosis-related disorders for humans [2–4]. Toxicity, environmental mobility, and complex chemical forms of copper in addition to its undesirable effects on human health have brought about a great deal of effort to develop various copper ion (Cu^{2+}) detection and removal techniques in the environment.

Traditional and standard methods to detect and analyze PTEs are complicated, mainly due to their time-consuming analysis, sample pretreatment process, costly instruments, and the fact that they

are unable to be used in the field [1,5]. There has therefore been a large amount of devoted effort to develop rapid, inexpensive, disposable, and simple sensing strategies for PTEs leading to reach a real-time, on-site, and selective analysis [1,6,7].

A fluorescent or colorimetric sensor detects and measures a physical parameter and converts it into an optical signal, which is subsequently read by an observer or an instrument [8]. Fluorescent chemosensors include two main parts: a receptor which is mainly responsible for perceiving the analyte, and a fluorophore, which converts the perception events into optical signals [9]. Rhodamine dyes have been extensively used as "off-on" chemosensors (fluorescence "off" spirolactam to fluorescence "on" ring-opened amide equilibrium) which create a pink-colored and orange fluorescent response to the presence of a specific metal cation as a result of the spirocycle moiety opening [10]. This is, to the best of our knowledge, first suggested by Xie's group [11], in that in the Cu^{2+} promoted ring-opening reaction, the carbonyl O atom and amine N atom in the hydrazide moiety of a spirolactam-based rhodamine derivative are the most electron-rich centers, causing a higher selectivity toward this cation. Recent reports introduced various rhodamine-hydrazide-based moieties which have been applied as Cu^{2+} chemosensors combined with several different receptors such as *N*-(2,4-dinitrophenyl) [12], naphthyridine groups [13], a tetraphenylethene (TPE) group [14], an introduced ferrocene unit into a salicylaldehyde rhodamine-hydrazine platform [15], and a 1,8-naphthalimide group [16].

The main drawback of synthesizing Cu^{2+} optical chemosensors could be complicated synthesis procedures, the high cost of starting materials, and the lack of selectivity towards intervening competitors such as Zn^{2+} and Ni^{2+} due to their close chemical behaviors to Cu^{2+} [17]. Herein, we presented a new rhodamine-B derivative, RECM, which has an "O–N–O" coordination site (two O atoms of carbonyl and one N atoms of methylamide), acts as a Cu^{2+} selective fluorescent sensor and demonstrates a strong "orange" fluorescence upon binding with Cu^{2+} in the presence of other metal cations. In fact, the addition of Cu^{2+} can lead to a visual color change observation. Our major motivation for this study was the fabrication of a highly sensitive and selective Cu^{2+} optical chemosensor using inexpensive starting materials and a conventional two-step synthesis, which can act as a fluorescent enhanced "turn-on" Cu^{2+} sensor and "naked-eye" indicator.

2. Materials and Methods

2.1. Reagents and Solutions

High purity rhodamine-B and ethylenediamine (EDA) were purchased from Sigma–Aldrich (USA) and ChemLab (Belgium), respectively. 4-morpholine carbonyl chloride and trimethylamine (Et$_3$N) were purchased from Merck (Germany). Solvents used (including ethanol (EtOH), methylene chloride (MC), and methanol (MeOH)) were of analytical reagent grade and purchased from Merck. Acetonitrile (MeCN) was of analytical reagent grade and purchased from Daejung (Korea). Solutions of Na^+, Cd^{2+}, Ca^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, and Zn^{2+} were prepared from nitrate salts; solutions of K^+, Co^{2+}, Hg^{2+}, and Fe^{3+} were prepared from chloride salts; and solution of Ag^+ was prepared from sulfate salt (all the metal ion salts were purchased from NewChem (England), Merck, and Scharlau (China)). Sodium chloride (NaCl) saturated solution and deionized water (DIW) were used throughout the experiments. Thin layer chromatography (TLC) was carried out using silica gel 60G F254 and column chromatography was done using silica gel 60 (both were purchased from Merck). All the reagents were used without further purification.

2.2. Instrumentation and Apparatus

NMR spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Bruker DRX-400 MHz instrument (USA) in chloroform (CDCl$_3$) using tetramethylsilane (TMS) as the internal standard; chemical shifts are recorded in ppm. Mass spectra were recorded on a Shimadzu GC-14A gas chromatograph (Japan). Melting points were determined on an Electrothermal 9100 melting point apparatus (England). UV-Vis absorption spectra were measured on a Unico 4802 UV-Vis double beam spectrophotometer (China)

in micromolar concentrations. Steady-state fluorescence spectra were measured on a Cary Eclipse fluorescence spectrophotometer (USA). All measurements were performed at room temperature and repeated three times.

2.3. Synthesis of the Chemosensor RECM

2.3.1. Synthesis of Rhodamine-B-lactam-ethylenediamine (RE)

RE was synthesized according to the literature [10,18–21]. In a typical synthesis, in a 50 mL round-bottom flask, rhodamine-B (1 g, 2.09 mmol) was dissolved in 30 mL fresh dry EtOH. EDA (2.5 mL, 37.44 mmol) was then added dropwise under vigorous stirring at room temperature. This solution was then refluxed for 36 h until it lost its red color. After the reaction was completed, the solution was cooled down to room temperature and the solvent was evaporated under vacuum. DIW (30 mL) was added to the residue and the resulting mixture was extracted with MC (2 × 30 mL). The collected organic phase was washed two times with DIW and dried with anhydrous Na_2SO_4. The solvent was evaporated under vacuum to get a pale pink product which was further separated and purified by silica gel column chromatography (MC: MeOH = 97:3, *v/v*) afforded 0.45 g (44%) of RE as a foamy solid. M.p. 216–221 °C; ^{1}H NMR (400 MHz, CDCl3): δ (ppm) = 7.93–7.91 (m, 1H), 7.49–7.44 (m, 2H), 7.12–7.10 (m, 1H), 6.46 (s, 1H), 6.44 (s, 1H), 6.39 (d, *J* = 2.8 Hz, 2H), 6.30 (d, *J* = 2.4 Hz, 1H), 6.28 (d, *J* = 2.8 Hz, 1H), 3.35 (q, *J* = 7.2 Hz, 8H), 3.22 (t, *J* = 6.8 Hz, 2H), 2.46 (t, *J* = 6.4 Hz, 2H), 1.18 (t, *J* = 6.8 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 168.78, 153.49, 153.28, 148.84, 132.47, 131.13, 128.66, 128.07, 123.84, 122.79, 108.17, 105.50, 97.71, 65.06, 44.35, 43.52, 40.80, 12.59; ESI-MS m/z^+ (%) calculated for $C_{30}H_{36}N_4O_2$: 484.64, found 484.

2.3.2. Synthesis of RE–Carbonyl Morpholine (RECM)

RECM was synthesized according to the literature [22,23]. In a 50 mL round-bottom flask, RE (0.096 g, 0.20 mmol) was dissolved in 20 mL fresh dry MC. Et_3N (0.030 g, 0.30 mmol) was then added under stirring at room temperature. After cooling the solution to 0 °C in an ice bath, a solution of 4-morpholine carbonyl chloride (0.045 g, 0.30 mmol) in 5 mL fresh dry MC was added dropwise over 10 min. The solution was then stirred for 2 h at room temperature. After the reaction was completed, the solvent was evaporated under vacuum and the residue dissolved in 25 mL MC. The resulting mixture was extracted with saturated NaCl solution (2 × 25 mL) and the collected organic phase was dried on anhydrous Na_2SO_4. The solvent was evaporated under vacuum to get a pale yellow product which was further separated and purified by silica gel column chromatography (MC:MeOH = 98:2, *v/v*) afforded 0.07 g (60%) of RECM as a foamy solid. M.p. 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.91–7.89 (m, 1H), 7.49–7.44 (m, 2H), 7.11–7.07 (m, 1H), 6.47 (s, 1H), 6.44 (s, 1H), 6.39 (d, *J* = 2.4 Hz, 2H), 6.29 (d, *J* = 2.4 Hz, 1H), 6.27 (d, *J* = 2.8 Hz, 1H), 3.72–3.69 (m, 4H), 3.38–3.41 (m, 4H), 3.35 (q, *J* = 7.2 Hz, 8H), 3.04–3.00 (m, 2H), 3.28–3.32 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 170.32, 157.99, 153.96, 153.28, 148.93, 132.83, 130.31, 128.38, 128.16, 123.93, 122.74, 108.24, 104.55, 97.77, 66.70, 66.61, 66.09, 47.24, 44.33, 43.88, 42.64, 40.78, 12.61; ESI-MS m/z^+ (%) calculated for $C_{35}H_{43}N_5O_4$: 597.76, found 597. The synthesis of compounds RE and RECM were conducted as the synthetic steps shown in Figure 1.
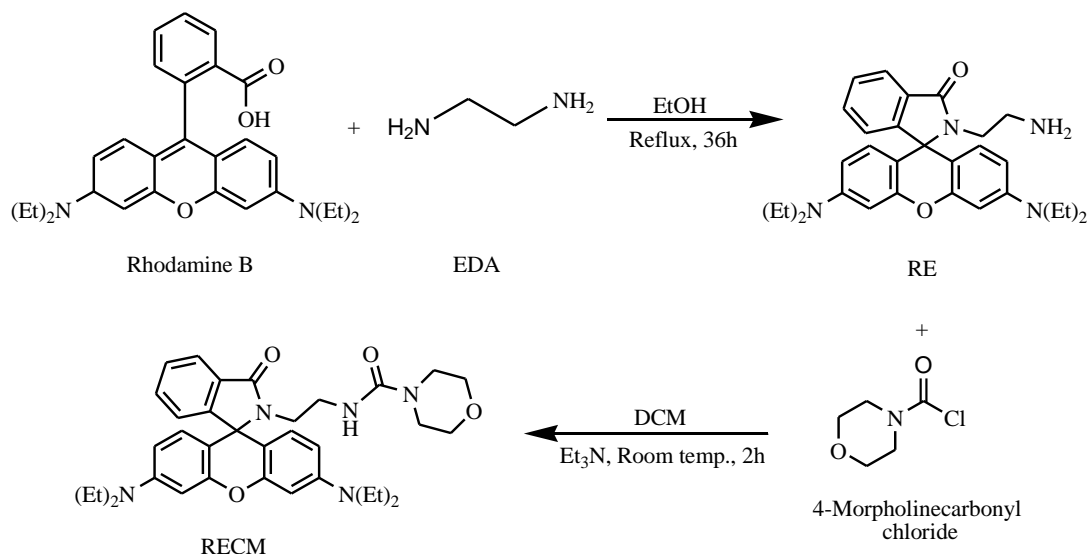
Figure 1. Synthesis route of compounds: rhodamine-B-lactam-ethylenediamine (RE) and RE–carbonyl morpholine (RECM).

2.4. Measurement Procedures

2.4.1. Selectivity Measurements

For the most part, two main approaches could be adopted in order to examine a chemosensor's selectivity. The first method consists of measuring the sensor's optical responses to the analyte in the presence of each sole intervening competitor; however, in the second one, the sensor's optical responses to the analyte are measured in the presence of all of the interfering elements. In this study, we took the first approach to assess the possible interferences precisely. The solutions of different alkali, alkaline earth, and transition metal cations including K^+, Ag^+, Na^+, Cd^{2+}, Ca^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}, Fe^{3+}, and Cu^{2+} (1.0×10^{-2} M) were prepared by dissolving the required amounts of nitrate/chloride/sulfate salts in absolute CH_3CN. These solutions were diluted one more time to get the standard stock solutions of metal cations (5.0×10^{-4} M). In addition, the standard stock solution of RECM (5.0×10^{-4} M) was prepared by dissolving 0.03 g of the compound in 100 mL CH_3CN. Each time in selectivity experiments, 0.5 mL RECM (5.0×10^{-4} M) was added to a 5 mL volumetric flask followed by adding 0.3 mL different metal cation solutions (5.0×10^{-4} M) using a micropipette to give 30 μM of metal cations. After adjusting the final volume with CH_3CN, the resulting solutions were shaken for 5 s and placed at room temperature for 120 min. A blank solution of RECM was prepared without Cu^{2+} under the same conditions. Finally, each resulting solution filled a quartz optical cell and measured on the UV-Vis spectrophotometer and fluorescence spectrophotometer (excitation wavelength was performed at 530 nm and emission wavelength collected from 530 to 650 nm; the excitation and emission slits were 2.5 and 5 nm, respectively).

2.4.2. Spectral Measurements

The binding studies of chemosensor RECM were carried out in absolute CH_3CN. Each time in titration experiments, 0.5 mL RECM (5.0×10^{-4} M) was added to a 5-mL volumetric flask followed by adding the required amount of Cu^{2+} (5.0×10^{-4} M) gradually using a micropipette to get the considered Cu^{2+} concentrations. After adjusting the final volume with CH_3CN, the blank and resulting solutions were prepared to be measured on the UV-Vis spectrophotometer and fluorescence spectrophotometer as previously.

2.4.3. Job Plot Measurements

The different concentrations of Cu^{2+} including 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, and 80 μM were prepared by taking the required amounts from the standard stock solution of Cu^{2+} (5.0×10^{-4} M) using a micropipette and transferring to a 5-mL volumetric flask. In addition, different concentrations of RECM including 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, and 20 μM were prepared by taking the required amounts from the standard stock solution of RECM (5.0×10^{-4} M) using a micropipette and adding to the volumetric flasks in the way that the total concentrations of Cu^{2+} and RECM in each flask, was equivalent to 100 μM. After adjusting the final volume with CH_3CN, the resulting solutions were prepared to be measured on a fluorescence spectrophotometer as previously.

3. Results and Discussion

3.1. Selectivity

The new rhodamine-B-based derivative, RECM, was synthesized through a facile two-step synthesis as detailed in Section 2 to obtain a pale yellow solid. Its structure was fully confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectra.

The fluorescence responses of RECM (50 μM in CH_3CN) toward the addition of various competitive cations (30 μM in CH_3CN) were recorded at 530 nm as the excitation wavelength (Figure 2).
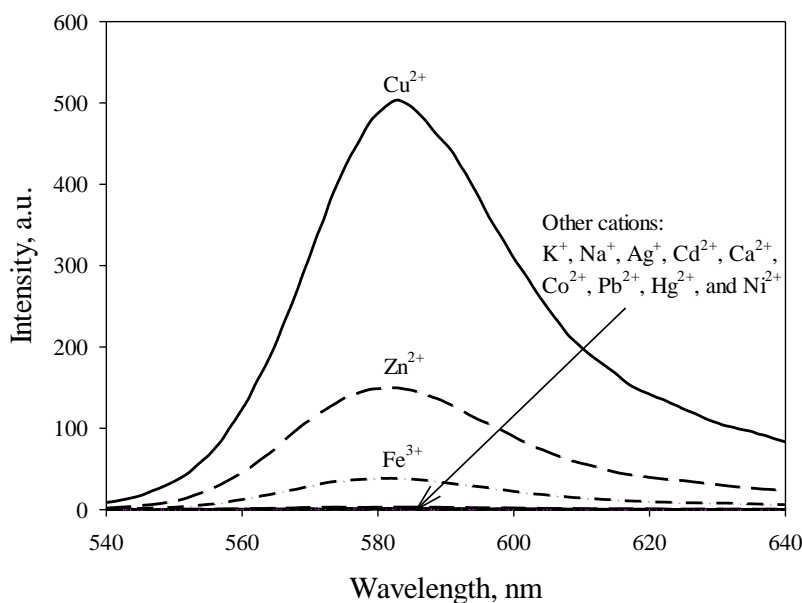

Figure 2. Fluorescence spectra (λ_{ex} = 530 nm) of RECM (50 μM) in CH_3CN in the presence of various competitive cations (30 μM).

Its fluorescence intensity was slightly influenced by the addition of some common alkali and alkaline earth cations (K^+, Na^+, and Ca^{2+}), and transition cations (Ag^+, Cd^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}, and Fe^{3+}), respectively. However, a remarkably enhanced orange fluorescence for RECM was observed upon addition of 30 μM Cu^{2+} in CH_3CN to the solution. Figure 3 confirms this, showing UV-Vis responses of RECM (50 μM in CH_3CN) upon addition of common mentioned interfering cations at 400–600 nm. Its absorbance spectra clearly shows that much smaller spectral changes (Zn^{2+} and Fe^{3+}) or no significant spectral changes (K^+, Ag^+, Na^+, Cd^{2+}, Ca^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, and Ni^{2+}) can be observed through the addition of cations (30 μM in CH_3CN), indicating that RECM exists as a spirolactam-closed form. In contrast, RECM abruptly changed from colorless to pink upon addition of 30 μM Cu^{2+} in CH_3CN to the solution indicating that the equilibrium shifted to a ring-opened amide,

which is consistent with that of fluorescence spectra, and indicating RECM can serve as a "naked-eye" chemosensor for Cu^{2+} in CH_3CN media. Also, visual color changes of RECM in the presence of Cu^{2+} and other competitive cations are shown in Figure 3 (inset).
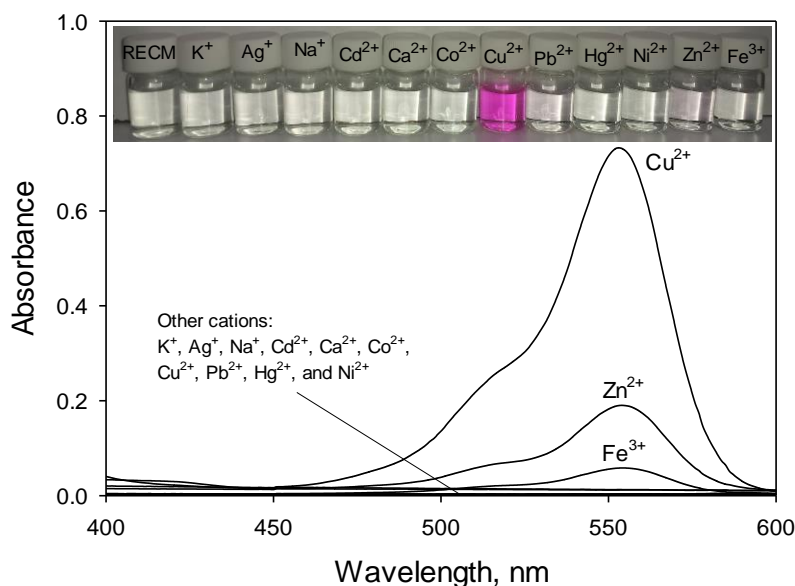

Figure 3. Absorbance spectra of RECM (50 μM) in CH_3CN in the presence of various competitive cations (30 μM), Inset: Visual color changes of RECM (50 μM) in CH_3CN in the presence of various competitive cations (30 μM).

To date, various Cu^{2+}-rhodamine-based chemosensors were developed which involved some negligible interferences of competitive cations [24–27]. On the periodic table of elements, the three transition cations Cu^{2+}, Zn^{2+} and Ni^{2+} lie in the same period and are next to each other [26]. The differentiation of their photophysical properties is too difficult, due to their similar coordinating ability and the high proximity of their values of ionic radius and charge density [28]. As shown in Figures 2 and 3, among various competitive cations, only Zn^{2+} and Fe^{3+} displayed 86 and 21-fold enhancements, respectively, in fluorescence intensity at the same concentration. Importantly, the fluorescent signal of RECM to Cu^{2+} hardly can be interfered by the other cations with similar concentration. A number of recent studies revealed similar results to this study, owing to reporting Cu^{2+} chemosensors composed of one or more carbonyl O atoms and amine N atoms in the hydrazide moiety of a spirolactam-based rhodamine derivative [12,14,29]. Thus, it is self-evident that oxygen and nitrogen donor atoms could be introduced into the structure of a Cu^{2+} chemosensor in order to eliminate interference caused by intervening competitors such as Fe^{2+}, Fe^{3+}, Ni^{2+}, and Zn^{2+}. The presented results of this work demonstrated that only Cu^{2+} could give a high fluorescence intensity and strong absorbance together with no considerable fluorophore-cation interaction of other mentioned cations, and RECM offered an excellent selectivity toward Cu^{2+}.

3.2. Optical Sensing Properties

Fluorescence and absorbance changes of RECM upon gradual titration with Cu^{2+} were examined. The fluorescence titration of RECM (50 μM in CH_3CN) was performed with a Cu^{2+} concentration range of 1–100 μM in CH_3CN. As shown in Figure 4a, fluorescence intensity increases upon addition of increasing concentrations of Cu^{2+} up to 55 μM, and gives a linear response within the range of 1–55 μM ($R^2 = 0.965$) (Figure 4a, inset), but decreases upon addition of more than 55 μM (Figure 4b). The limit of detection (LOD) of RECM was calculated based on the fluorescence titration. To that end, the emission intensity of RECM without Cu^{2+} was measured by 10 times and the standard deviation

of blank measurements was determined. The LOD of RECM was found to be 0.21 μM based on the definition by IUPAC ($LOD = 3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements and m is the slope between intensity versus sample concentration) [30] which is less than the maximum contaminant level goal (MCLG) of Cu^{2+} (1.3 ppm equals to 20.46 μM) in drinking water recommended by the US Environmental Protection Agency (USEPA) [31]. RECM shows an acceptable efficiency in terms of LOD in comparison with several types of research which have been done into the designation of optical chemosensors to detect and determine Cu^{2+} in aqueous media over the past decades. Kim et al. [32] designed and synthesized a new simple rhodamine-B derivative equipped with the 4-nitrosalicylaldimine-based receptor (RB-HN) which exhibits selective optical recognition for Cu^{2+} over commonly-interfering metal cations in DMSO–H_2O media. RB-HN demonstrates a color change from yellow to pink upon gradual titration with Cu^{2+} and a 1:1 RB-HN–Cu^{2+} complex mode in the binding stoichiometry. The LOD of the RB-HN for Cu^{2+} was 0.47 μM. Kaur et al. [33] designed a phenothiazine-based optical probe, which exhibits a significant color change from orange to blackish blue in the presence of Cu^{2+} based on soft-soft metal interactions with the S and N atoms of the probe. Selective and sensitive responses of the probe toward Cu^{2+} over other interfering metal cations took about 70 s and the LOD was determined to be 0.3 μM. Erdemir et al. [34] demonstrated two novel design of anthracene-based optical sensors (AOC and ATC) for Cu^{2+} prepared in one step procedures. The LODs of AOC and ATC were found as 1.09 μM and 1.19 μM respectively, and the stoichiometry of their complexes with Cu^{2+} was determined to be 1:1. However, various optical probes have been developed which allow detecting the presence of Cu^{2+} with lower LODs and can be successfully applied as selective and sensitive chemosensory towards Cu^{2+} in aqueous media [26,35].
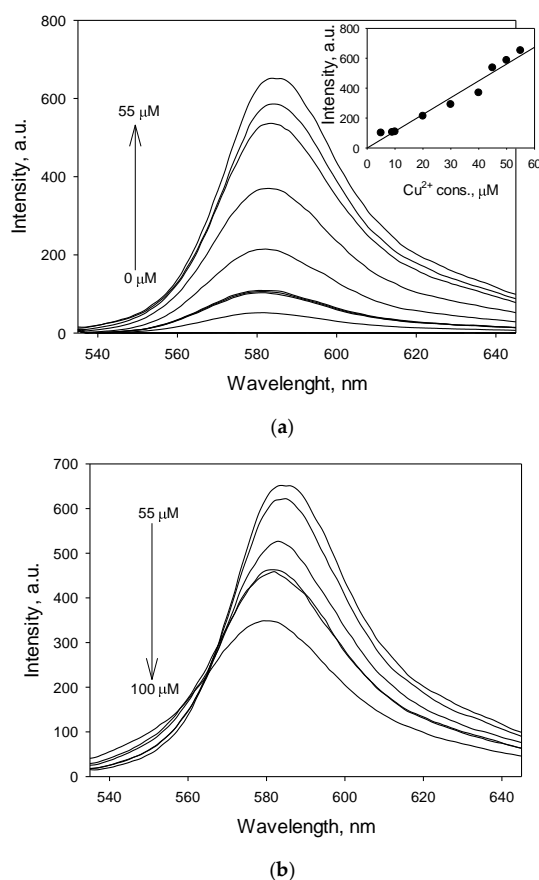

(a)

(b)

Figure 4. Fluorescence spectra (λ_{ex} = 530 nm) of RECM (50 μM) in CH_3CN in the presence of (**a**) 0–55 μM and (**b**) 55–100 μM of Cu^{2+}, respectively. Inset: Linear fluorescence response of RECM (50 μM) in CH_3CN within the Cu^{2+} concentration range of 1–55 μM.

The absorbance titration of RECM (50 μM in CH$_3$CN) was conducted with a Cu^{2+} concentration range of 9–100 μM in CH$_3$CN. The absorbance spectra exhibit significant enhancement upon addition of increasing concentrations of Cu^{2+} up to 45 μM (Figure 5a), which is confirmative suggesting the delocalization xanthene moiety and spirolactam formation of RECM. In addition, the absorbance response increases linearly within the range of 1–45 μM (R^2 = 0.958) (Figure 5a, inset). Visual color changes of RECM in the presence of Cu^{2+} are also shown in Figure 5a (inset). In contrast, absorbance decreases upon addition of more than 45 μM (Figure 5b).
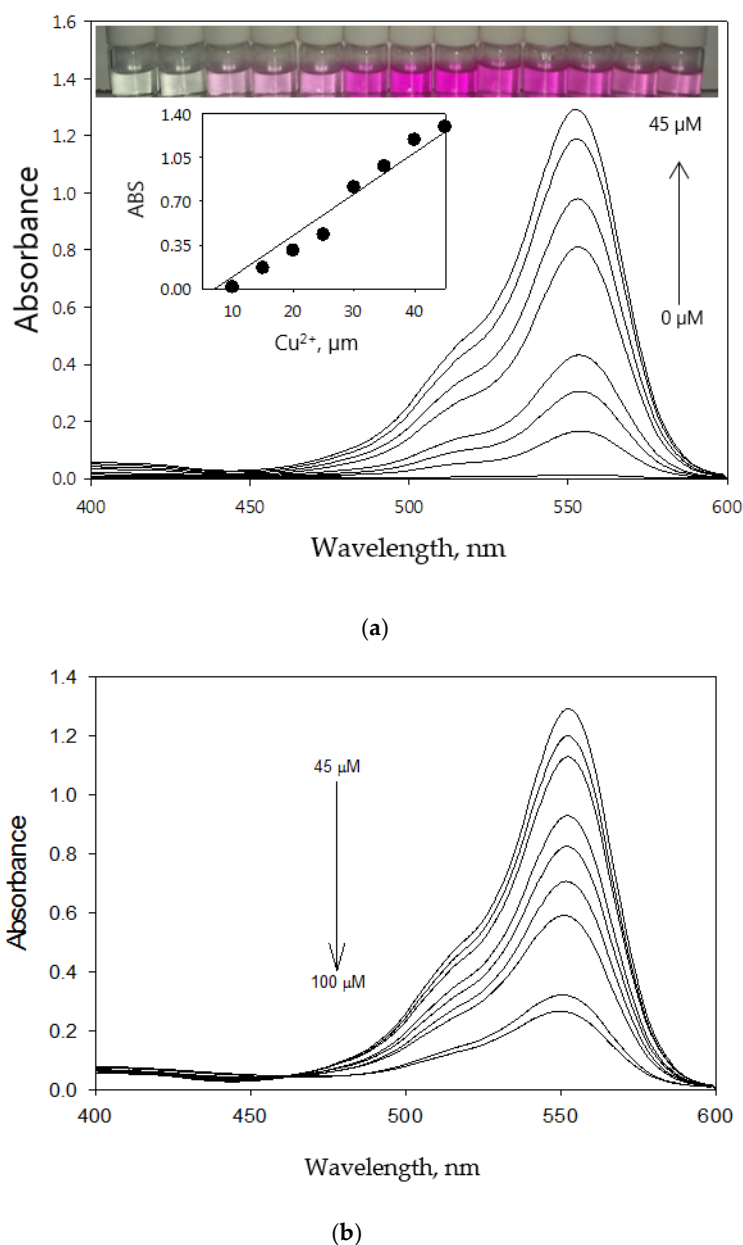

(a)

(b)

Figure 5. Absorbance spectra of RECM (50 μM) in CH$_3$CN in the presence of (**a**) 0–45 μM and (**b**) 45–100 μM of Cu^{2+}, respectively, Inset: Linear absorbance response of RECM (50 μM) in CH$_3$CN within the Cu^{2+} concentration range of 1–45 μM.

The fluorescence and absorbance decreased upon addition of more than 55 μM and 45 μM Cu^{2+} respectively, that might be respected to the paramagnetic nature of Cu^{2+} [27,36,37]. Paramagnetic square-planar or tetragonally distorted metal ions such as Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, and Pd^{2+}

are able to quench the fluorescence of optical chemosensors which is presumptively undesirable for analytical purposes [38]. The paramagnetic effect is basically the result of an enhanced electronic states' mixing (considered as a formally forbidden intersystem crossing) of a ligand by means of the inhomogeneous magnetic field of the paramagnetic ion [39]. For this reason, classical probes for Cu^{2+} and other strongly paramagnetic metal ions are usually based on the quenching of the fluorescence [40]. Shao's group [41] designed a highly selective Cu^{2+} fluorescent sensor has based on a spiropyran derivative, which combines the characteristics of metal binding and signal transduction in cationic recognition. Their study revealed that lower concentrations of the chemosensor in the system resulted in the weak blank fluorescence signal value, while at relatively higher concentrations of the chemosensor, the fluorescent response could not be quenched completely even if a high concentration of Cu^{2+} was added to the solution. In our study, RECM and Cu^{2+} binding did open the spirolactam ring. Nevertheless, the fluorescence of the ring-opened amide forming upon increasing Cu^{2+} concentrations was partially quenched by the cation [24]. A possible answer would be a relative lack of sufficient concentration of the fluorescent sensor to cause the recognition process to be assured of avoiding the paramagnetic effect. From a signal detection viewpoint, the quenching behavior of the probe, however, is not a preferable fluorescence enhancement response [5,24].

3.3. Response Time

To investigate the response time of RECM with Cu^{2+}, absorbance was recorded on RECM (50 μM in CH_3CN) reacting with Cu^{2+} (30 μM in CH_3CN) in 0–120 min after mixing together. The absorbance increases immediately and stables within 110 min (Figure 6).
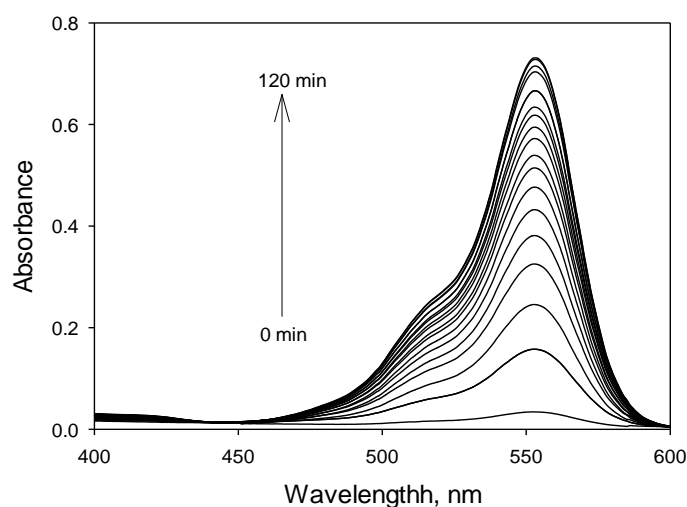

Figure 6. Absorbance spectra of RECM (50 μM) in CH_3CN in the presence of 30 μM of Cu^{2+} in 0–120 min after mixing together.

According to this result, all the prepared samples were placed at room temperature for 120 min after shaking for 5 s and before analyzing with the instruments. Su et al. [42] developed a convenient optical sensor towards Cu^{2+} using polydihydroxyphenylalanine nanoparticles (PDNPs). PDNPs fluorescence-quenching increased and became constant after 10 min. Ghaedi et al. [43] demonstrated the application of a novel optode based on a central composite designation and its efficiency towards Cu^{2+} in aqueous media. The response time to maintain a constant fluorescence intensity as the result of the complexation of optode and Cu^{2+} was 6.9 min. Some other recent works reported various Cu^{2+} optical chemosensors with shorter response times [33,44]. Sensors with faster response times and higher selectivities are preferable in monitoring ultra-trace levels of toxic metal ions [9]. From this point of view, the new synthesized rhodamine-B derivative (RECM) shows a weak efficiency in terms of response time and needs to be optimized in order to possess more rapid optical responses.

3.4. Binding Capability

To evaluate the binding stoichiometry of the RECM–Cu^{2+} complex and the binding constant (K_a), the job plot method and Benesi–Hildebrand correlation were used, respectively. In the job plot method, total concentrations were kept at 100 μM and the molar ratio was given by [Cu^{2+}]/([Cu^{2+}] + [RECM]) which measured from 0.18 to 0.80 using the fluorescence spectrophotometer. As shown in Figure 7, the maximum fluorescence intensity of the molar ratio was 0.5 that means a 1:1 RECM–Cu^{2+} complex mode in the binding stoichiometry.
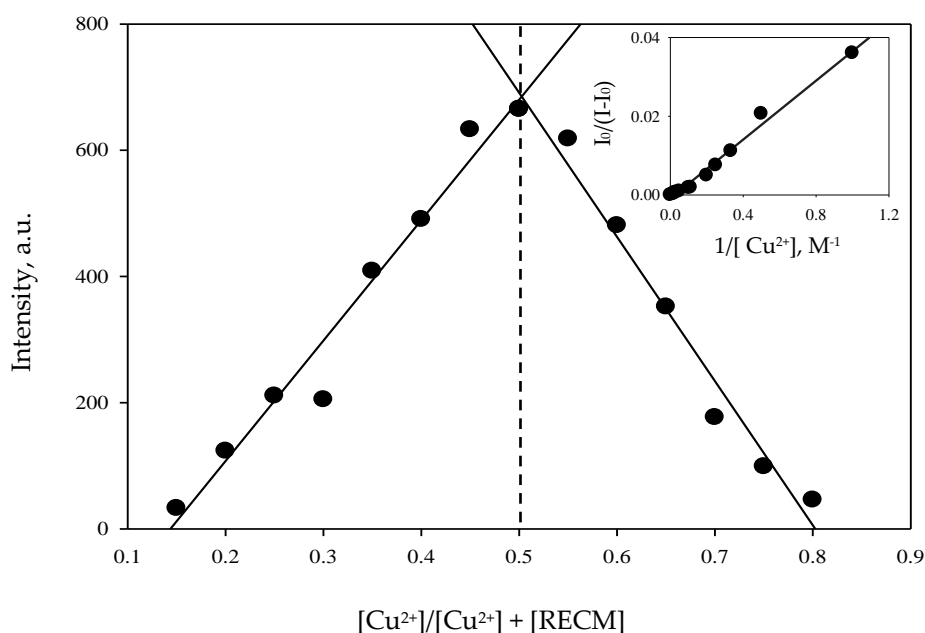

Figure 7. The binding stoichiometry of the RECM–Cu^{2+} complex, according to the job plot method. Inset: the Benesi–Hildebrand plot result to evaluate K_a of the RECM–Cu^{2+} complex.

The 1:1 binding mode of the Benesi–Hildebrand correlation [45] is usually used to estimate the K_a as following:

$$\frac{I_F^0}{(I_F - I_F^0)} = \left(\frac{1}{f}\right)\left[\frac{1}{K_a[M]} + 1\right] \tag{1}$$

where I_F^0 and I_F are the fluorescence intensity of RECM at 584 nm in the absence and presence of Cu^{2+}, respectively. f is the fraction of the initial accessible fluorescence to the chemosensor and [M] is the Cu^{2+} concentration. The inset in Figure 7 shows the results of the Benesi–Hildebrand plot and K_a of RECM–Cu^{2+} complex in CH$_3$CN was found to be 2.25×10^4 M^{-1}. K_a can also be estimated by the ratio intercept/slope of the Benesi–Hildebrand plot's linear regression. The obtained K_a strongly supported RECM–Cu^{2+} 1:1 binding stoichiometry and showed a strong probe in comparison with some recently-reported work on Cu^{2+} fluorescent and colorimetric probes [5,16,46]. Most of the various Cu^{2+}-rhodamine-based chemosensors have already been developed and involve a coordination mode with 1:1 stoichiometry, which is the most possible binding mode of rhodamine-based chemosensors and Cu^{2+} [5,24,25,32]. However, there are few types of research demonstrating different molar ratios for newly-developed rhodamine-based derivatives and Cu^{2+}.

3.5. Structure and Complexation Mechanism

The first idea to consider RECM as a Cu^{2+} sensitive and selective fluorescent and colorimetric chemosensor was based on the spirocycle opening mechanism, which was the same as reported spirocycle rhodamine-based derivatives [5,10,16,24,25,36]. With this consideration, directly linked

chelating agents to rhodamine spirolactam moiety including one methylamide N and two carbonyl O atoms made a possible binding pocket of RECM toward Cu^{2+} and induced the spirolactam ring opening upon RECM and Cu^{2+} binding interaction. The strong chelating ability of RECM toward Cu^{2+} was responsible for both fluorescence and color changes and also led to obtaining the 1:1 binding stoichiometry.

The proposed mechanism of RECM–Cu^{2+} complexation along with the obvious OFF-ON optical signal is shown in Figure 8.
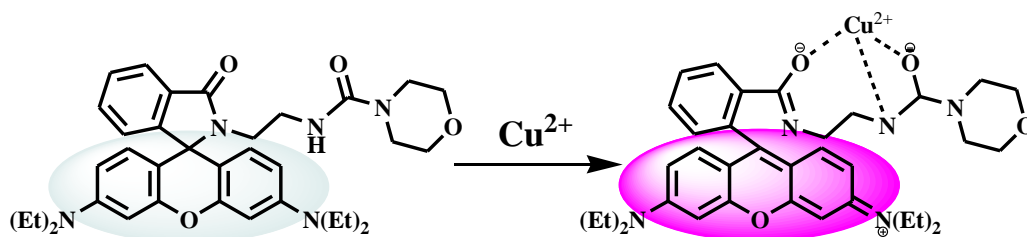

Figure 8. Proposed binding mode and Cu^{2+} sensing mechanism of RECM.

4. Conclusions

The new rhodamine-B-based derivative RECM was prepared using a conventional two-step pathway, including a lactam ring formation of rhodamine-B and ethylenediamine following by a facile amide bond formation. After it was structurally characterized, the optical responses toward Cu^{2+} were collected from both fluorescence intensity and absorbance spectra. As we expected, the fluorescence intensity of RECM was obviously enhanced upon addition of Cu^{2+} up to 55 μM with a high linearly dependent coefficient, confirmed with UV-Vis absorbance spectra. When plotting fluorescence intensity against Cu^{2+} concentration, we observed a slight decrease in fluorescence intensity at high cation concentrations, which might well be owing to paramagnetic quenching behavior of the analyte. To explore RECM selectivity toward Cu^{2+}, the competition experiments were carried out in the presence of some environmentally relevant alkali, alkaline earth, and transition metal cations at 50 μM. The fluorescence and absorbance spectra displayed no obvious interference in the fluorometric and colorimetric detection for Cu^{2+}. In conclusion, we report that the carbonyl-morpholine-structured rhodamine-B derivative, RECM, can serve as a sensitive and selective "naked-eye" chemosensor toward Cu^{2+} in acetonitrile in a 1:1 complex mode, and with LOD of 0.21 μM based on the linear regression method, which makes RECM sufficient to detect relevant concentrations of Cu^{2+} in the environmental monitoring applications.

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