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# A new $Cu^{2+}$ -induced color reaction of a rhodamine derivative *N*-(3-carboxy)acryloyl rhodamine B hydrazide

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A new rhodamine derivative, *N*-(3-carboxy)acryloyl rhodamine B hydrazide (CARB), has been synthesized, and its unusual spectroscopic reaction with  $Cu^{2+}$  has been investigated. The derivative exhibits a rapid and reversible non-fluorescent absorption upon coordination to  $Cu^{2+}$ , which is a rather unusual phenomenon for rhodamine B derivatives. Stoichiometric measurements using the Job's method and the molar ratio method reveal that one CARB molecule combines two  $Cu^{2+}$  ions, and the two  $Cu^{2+}$  ions play different roles: one opens the spirocyclic structure and the other quenches the fluorescence of the xanthene moiety. This reaction mechanism is supported by a comparative study on the model compound *N*-acryloyl rhodamine B hydrazide as well as by the density functional theory calculations. Furthermore, the absorption response of CARB is highly selective for  $Cu^{2+}$  over other common ions, which implies that CARB may be used as a colorimetric probe for the rapid visual detection of  $Cu^{2+}$ .

*N*-(3-carboxy)acryloyl rhodamine B hydrazide, reaction mechanism, colorimetric probe, visual detection, copper, *N*-acryloyl rhodamine B hydrazide

# 1 Introduction

Rhodamine dyes, like fluorescein dyes, are fluorochromes that belong to the family of xanthenes. Because rhodamine dyes have many advantages such as low cost, long-wavelength absorption/emission, high molar absorption coefficient, high quantum yield, and photostability [1–10], they have been widely used as a molecular platform for the design of new spectroscopic probes. Moreover, rhodamine derivatives are nonfluorescent and colorless when existing in spirocylic forms, whereas their ring-opening structures would give rise to strong fluorescence emission and pink color [1–8]. Based on the transformation between these two forms, considerable efforts have been directed toward the development of novel spectroscopic off-on-type probes by introducing a suitable recognition unit for various analytes, such as Hg<sup>2+</sup> [3, 4], Fe<sup>3+</sup> [9], Cu<sup>2+</sup> [10–12], ClO<sup>-</sup> [5, 13], Cr<sup>3+</sup> [14], and Pb<sup>2+</sup> [15]. On the other hand, some of the rhodamine derivatives are dependent on the reaction conditions. For example, a simple change in the medium or solvent system may result in switching the selective response from one analyte to another, thereby enabling the same rhodamine probe to be used for different detections [1, 16, 17]. The above measures have updated a number of traditional colorimetric methods in terms of selectivity and simplicity. Among the rhodamine probes reported for Cu<sup>2+</sup>, however, the prolonged reaction time and cross-sensitivities toward other metal ions remain to be problematic.

Copper(II) is known to be an essential trace element in

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biosystems and is an important environmental pollutant at high concentrations [18]. Therefore, determination of  $Cu^{2+}$  has attracted wide-spread interests. In particular, colorimetric measurements of  $Cu^{2+}$  have received extensive attention, because such detections have the advantage of simplicity [19], reliability, and visualization. So far, many probes have been proposed for the colorimetric sensing of  $Cu^{2+}$  [20–23].

In connection with a program of investigating new spectroscopic probes, a series of rhodamine probes have been developed in our laboratory [3, 5, 24-27]. In the present study, we report the synthesis of a new rhodamine derivative, N-(3-carboxy)acryloyl rhodamine B hydrazide (CARB; Scheme 1), and its unusual spectroscopic reaction with Cu<sup>2+</sup>. The derivative displays a rapid and reversible non-fluorescent absorption upon coordination to  $Cu^{2+}$ , which is rather rare for rhodamine B derivatives. This unusual reaction mechanism has been investigated by a comparative study on the model compound N-acryloyl rhodamine B hydrazide (ARB, Scheme 1) as well as by the density functional theory calculations. Moreover, compared with many of the reported rhodamine probes for Cu2+, CARB exhibits an improved overall performance in terms of fast reaction time and good selectivity, which makes it to be of great potential use in the rapid visual detection of  $Cu^{2+}$ .

## 2 Experimental

#### 2.1 Apparatus

The absorption spectra were recorded in  $1 \times 1$  cm quartz cells on a TU-1900 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). pH Values were measured on a model HI-98128 pH-meter (Hanna Instruments Inc., USA). MALDI-TOF mass spectra were measured on a BIFLEX III mass spectrometer (Bruker, Inc.). Electrospray ionization mass spectra (ESI-MS) were measured on an LC-MS 2010A (Shimadzu, Japan) instrument. Elemental analyses were carried out on a Flash EA 1112 instrument. <sup>1</sup>H NMR spectra were measured on a Bruker DMX-400 spectrometer at 400 MHz in CD<sub>3</sub>OD.

## 2.2 Reagents

Rhodamine B, hydrazine hydrate (85%), maleic anhydride, and acryloyl chloride were purchased from Beijing Chemical Reagent Co and used as received. The solutions of Al<sup>3+</sup>, Ca<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Hg<sup>2+</sup>, Mg<sup>2+</sup>, Ni<sup>2+</sup>, and Sn<sup>2+</sup> were prepared from their chloride salts; the solutions of Ag<sup>+</sup>, Ba<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, K<sup>+</sup>, La<sup>3+</sup>, Pb<sup>2+</sup>, and Zn<sup>2+</sup> were prepared from their nitrate salts; the solutions of Fe<sup>2+</sup> and Mn<sup>2+</sup> were prepared from their sulphate salts; the solutions of CO<sub>3</sub><sup>2-</sup>, ClO<sup>-</sup>, HPO<sub>4</sub><sup>2-</sup>, and SiO<sub>3</sub><sup>2-</sup> were prepared from their sodium salts. The Palitzsch's buffer solution (0.2 mol/L) was prepared with H<sub>3</sub>BO<sub>3</sub>, NaCl, and Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> [28]. The stock solutions (1.0 mmol/L) of CARB and ARB were prepared with tetrahydrofuran (THF) as solvent. Distilled-deionized water was used unless otherwise noted. All other reagents were of analytical grade and used without further purification.

## 2.3 Synthesis of CARB and ARB

CARB was synthesized from rhodamine B via a two-step reaction (Scheme 1). First, rhodamine B hydrazide was prepared in 75% yield employing the previously reported procedure [5]. Then, rhodamine B hydrazide (456 mg, 1.0 mmol) was dissolved in dichloromethane (30 mL), to which a solution of maleic anhydride (490 mg, 5.0 mmol) in dichloromethane (10 mL) was added dropwise. The resulting solution was stirred at 35 °C for 2 h, and then the solvent was removed under reduced pressure to get a violet-red residue, which was purified by silica-gel column chromatography with ethyl acetate-petroleum ether (bp 60–90 °C) (1:9, v/v) as eluent, affording 194 mg of CARB (yield 35%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, 298 K)  $\delta$  7.94 (d, J=7.2 Hz, 1H), 7.61 (m, 2H), 7.12 (d, J=7.4 Hz, 1H), 6.60 (d, J=8.8 Hz, 2H), 6.42 (s, 2H), 6.38 (d, J=9.0 Hz, 2H), 6.27 (d, J=13.2 Hz, 1H), 6.02 (d, J = 13.0 Hz, 1H), 3.40 (g, J = 6.8 Hz, 8H), 1.33 (s, 2H), 1.18 (t, J=6.8 Hz, 12H). MALDI-TOF, m/z:



Scheme 1 Synthesis of CARB and ARB.

555.2  $[M+H]^+$ . Elemental analysis, calcd. for  $C_{32}H_{34}N_4O_5$ : C 69.30, H 6.18, N 10.10%; found, C 69.46, H 6.37, N 9.82%.

The model compound ARB was synthesized by treating rhodamine B hydrazide with acryloyl chloride (Scheme 1). Acryloyl chloride (450 mg, 5.0 mmol) was added dropwise to a solution of rhodamine B hydrazide (456 mg, 1.0 mmol) in dichloromethane (30 mL). The resulting solution was stirred at 0 °C for 2 h, and then treated with water (50 mL). The organic layer was separated, and the solvent was removed under reduced pressure to yield a violet-red residue, which was then purified by silica-gel column chromatography with methanol as eluent, affording 153 mg ARB (yield 30%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, 298 K)  $\delta$  7.94 (d, J= 7.3 Hz, 1H), 7.63 (m, 2H), 7.14 (d, J = 7.4 Hz, 1H), 6.58 (d, J = 8.8 Hz, 2H), 6.42 (s, 2H), 6.38 (d, J = 8.9 Hz, 2H), 6.20 (m, 2H), 5.64 (dd,  $J_1$ =8.8 Hz,  $J_2$ =2.7 Hz, 1H), 3.39 (q, J=6.8 Hz, 8H), 1.33(s, 1H), 1.18 (t, J=6.9 Hz, 12H). ESI-MS, m/z: 511.4  $[M+H]^+$ . Elemental analysis, calcd. for  $C_{31}H_{34}N_4O_3$ : C 79.92, H 6.71, N 10.97%; found, C 79.69, H 6.93, N 10.80%.

#### 2.4 General procedure for Cu(II) detection

Unless otherwise noted, all the measurements were performed according to the following procedure. In a 10 mL tube, 5 mL of 0.2 mol/L Palitzsch's buffer solution (pH 7.0), 1.0 mL of THF, and 0.2 mL of CARB stock solution were mixed, followed by addition of an appropriate volume of  $Cu^{2+}$  sample solution. The final volume was adjusted to 10 mL with water, and the reaction solution was thoroughly mixed. After 5 min at room temperature, a suitable volume of the reaction solution was transferred into a quartz cell with 1 cm optical length to measure absorbance at 567 nm. In the meantime, a blank solution containing no  $Cu^{2+}$  was prepared and measured under the same conditions for comparison.

## 3 Results and discussion

#### **3.1** Absorption properties

The color reaction of CARB with  $Cu^{2+}$  was initially examined in some pure aqueous buffer solutions such as phosphate, Tris-HCl, and Palitzsch's buffer. No or only weak color reaction was observed in the two former media, but in the latter (Palitzsch's buffer) a much stronger color reaction occurred immediately. However, the reaction in the Palitzsch's buffer was unstable and the absorption spectrum of CARB in the presence of  $Cu^{2+}$  was gradually red-shifted with the increase of time. This may result from the lower water solubility of the  $Cu^{2+}$ -CARB complexes. Then, we examined the effect of organic solvents (e.g., methanol, acetonitrile, and THF) on the color reaction. The results show that a stable pink color-on reaction could be produced in the Palitzsch's buffer (pH 7.0) containing 10% (v/v) THF or acetonitrile, and THF was used as a co-solvent for Cu<sup>2+</sup>-CARB complexes.

Figure 1 shows absorption spectra of CARB and its reaction solutions with  $Cu^{2+}$  at various concentrations. As can be seen, CARB itself has nearly no absorption in the visible region (curve 1), which may be attributed to its closed spirolactam form [1, 2, 25]. Upon gradual addition of  $Cu^{2+}$ (curves 2–18), however, a pink color appears, with a maximum absorption at 567 nm and a molar absorptivity of  $2 \times 10^4$  (mol/L)<sup>-1</sup> cm<sup>-1</sup>. This may be ascribed to the opening of the closed spirolactam structure promoted by  $Cu^{2+}$  complexation. Surprisingly, the reaction system of CARB with  $Cu^{2+}$  barely produces fluorescence (data not shown), which might result from the quenching effect of  $Cu^{2+}$  through a special binding mode (*vide infra*, Scheme 2, Section 3.6).

Most notably, the color-on reaction of CARB exhibits a high selectivity for  $Cu^{2+}$  over other common ions (Figure 2). This property enables  $Cu^{2+}$  to be distinguished easily from



**Figure 1** Absorption spectra of CARB (20  $\mu$ mol/L, curve 1) and its reaction solutions with Cu<sup>2+</sup> at various concentrations (2–50  $\mu$ mol/L, curves 2–18) in 0.1 mol/L Palitzsch's buffer media (pH 7.0) containing 10% ( $\nu/\nu$ ) THF. The inset depicts the absorbance at 567 nm in curves 2–18 as a function of Cu<sup>2+</sup> concentration.



Figure 2 Absorption spectra of CARB (10  $\mu$ mol/L) reacting with various ions (20  $\mu$ mol/L). Reactions were conducted at room temperature for 30 min in 0.1 mol/L Palitzsch's buffer media (pH 7.0) containing 10% (v/v) THF.

the other ions directly by the naked eye, and such a test is shown in Figure 3. As can be seen,  $Cu^{2+}$ , whether present alone (b) or in mixed ions (u), displays the expected pink color, but the other ions do not show any color.

The reversibility of the reaction was also investigated. As shown in Figure 4, addition of 1 equiv of EDTA to the reaction solution containing 1 equiv of  $Cu^{2+}$  causes the disappearance of the absorption band at 567 nm (curve c) due to the stronger binding ability of EDTA. Further introduction of 1 equiv. of  $Cu^{2+}$  into the reaction system re-produces the pink color (curve d; the incomplete recovery is due to the presence of EDTA). These results indicate that the complexation of CARB with  $Cu^{2+}$  is reversible, which is important for monitoring dynamic changes of  $Cu^{2+}$  concentration.



Figure 3 A photograph of color reactions of CARB (10  $\mu$ mol/L) with various ions. Reactions were performed at room temperature for 30 min in 0.1 mol/L Palitzsch's buffer solution (pH 7.0) containing 10% ( $\nu/\nu$ ) THF. (a) CARB only (reagent blank); (b) CARB + 10  $\mu$ mol/L Cu<sup>2+</sup>; (c–t) CARB + 20  $\mu$ mol/L of Al<sup>3+</sup>, Ag<sup>+</sup>, Ba<sup>2+</sup>, Ca<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, La<sup>3+</sup>, Fe<sup>3+</sup>, Fe<sup>2+</sup>, Hg<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Sn<sup>2+</sup>, Zn<sup>2+</sup> and ClO<sup>-</sup>, respectively; (u) the sample b + other mixed ions (20  $\mu$ mol/L of Al<sup>3+</sup>, Ag<sup>+</sup>, Ba<sup>2+</sup>, Ca<sup>2+</sup>, Ca<sup>2+</sup>, Ca<sup>2+</sup>, Ca<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, La<sup>3+</sup>, Fe<sup>3+</sup>, Fe<sup>3+</sup>, Fe<sup>3+</sup>, Fe<sup>2+</sup>, Hg<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Sn<sup>2+</sup>, Zn<sup>2+</sup> and ClO<sup>-</sup>).

#### 3.2 Optimization of experimental conditions

Different experimental conditions, such as THF concentration, pH, reaction temperature, and time, were examined for optimization. To stabilize absorption spectra, at least 6% (v/v) of THF is required, and 10% (v/v) of THF may be used in this work. The effect of pH, reaction temperature, and time on the absorbance is shown in Figure 5. As can be seen from Figure 5(a), CARB does not react with  $Cu^{2+}$  in the alkaline media with pH > 7.8, consistent with the behavior of N-benzoyl rhodamine B hydrazide [5] that possesses the same reaction group. Because CARB shows the best performance at about pH 7, the combination of pH 7.0 Palitzsch's buffer and 10% (v/v) THF is selected for the present reaction system. Change in temperature from 15 to 45 °C hardly affects the absorbance (Figure 5(b)), and thus room temperature may be employed in this experiment. Time course studies reveal that the color reaction is fast, and is almost completed in 1 min (Figure 5(c)). Moreover, the formed pink color is stable and could remain constant for at least 2 days. Based on the above observations, the optimized reaction conditions for Cu<sup>2+</sup> analysis include a reaction time of 5 min at room temperature in 0.1 mol/L Palitzsch's buffer



**Figure 4** Effect of EDTA on absorption spectra of CARB reacting with  $Cu^{2+}$  in 0.1 mol/L Palitzsch's buffer solution (pH 7.0) containing 10% ( $\nu/\nu$ ) THF. (a) CARB (20 µmol/L) alone; (b) CARB (20 µmol/L) +  $Cu^{2+}$  (40 µmol/L); (c) the sample b + EDTA (40 µmol/L); (d) the sample c +  $Cu^{2+}$  (40 µmol/L).



**Figure 5** Effect of pH (a), reaction temperature (b) and time (c) on the absorbance at 567 nm of CARB ( $10 \mu mol/L$ ) reacting with Cu<sup>2+</sup> ( $20 \mu mol/L$ ). Conditions: (a) the reaction was carried out at room temperature for 5 min in 0.1 mol/L Palitzsch's buffer solution containing 10% ( $\nu/\nu$ ) THF with different pH values; (b) the reaction was performed in 0.1 mol/L Palitzsch's buffer solution (pH 7.0) containing 10% ( $\nu/\nu$ ) THF at different temperatures for 5 min; (c) the reaction was conducted at room temperature for different time in 0.1 mol/L Palitzsch's buffer solution (pH 7.0) containing 10% ( $\nu/\nu$ ) THF.

solution (pH 7.0) containing 10% (v/v) THF.

# 3.3 Linearity

Under the optimized condition, a linear relationship was determined by plotting the absorbance of CARB against  $Cu^{2+}$  concentration. A linear equation of  $A_{567nm} = -0.0675 + 0.0248 \times [Cu^{2+}]$  (n = 16,  $R^2 = 0.990$ ) was obtained in the concentration range of 4–40 µmol/L Cu<sup>2+</sup> (see also the inset of Figure 1). The detection limit for Cu<sup>2+</sup> was  $4.5 \times 10^{-7}$  mol/L based on 11 blank determinations (*S/N*=3).

## 3.4 Binding stoichiometry of CARB with Cu(II)

To determine the binding stoichiometry, the continuous variation method (Job's plot) [29] was used by measuring the absorbance of the reaction system at 567 nm. As shown in Figure 6(a), a maximum absorbance is produced when the molecular fraction of  $Cu^{2+}$  is close to 67%, indicating that the binding ratio of CARB to  $Cu^{2+}$  is 1:2. This result is also supported by the molar ratio method (Figure 6(b)) [30–32]. On the basis of 1:2 stoichiometery, the stability constant of the CARB-Cu<sup>2+</sup> complex is estimated to be  $4.9 \times 10^{15} (\text{mol/L})^{-2}$ .

# 3.5 Interference studies

The effects of various ions on the absorbance were investigated by analyzing the CARB solutions containing 20  $\mu$ mol/L Cu<sup>2+</sup>. The tolerable concentration was estimated by the criterion at which a species gave a relative error of no more than 10% in recovery for 20  $\mu$ mol/L Cu<sup>2+</sup>. As shown in Table 1, high-valent cations such as Al<sup>3+</sup> and Fe<sup>3+</sup> seem to have lower tolerable concentrations. However, higher levels of these ions in the media of pH 7.0 would lead to precipitation of metal hydroxides, which can be removed through filtration. In addition, all the other common ions produce little influence on the determination of Cu<sup>2+</sup>, indicating a highly selective feature of the present system.

#### 3.6 Reaction mechanism

As described above, the color reaction of CARB with  $Cu^{2+}$ is attributed to the ring-opening of the spirolactam structure promoted by  $Cu^{2+}$  complexation. However, the reaction system hardly shows fluorescence, which is rather different from that of the common rhodamine spirolactam derivatives [1, 2, 33]. To explore the reaction mechanism of CARB with  $Cu^{2+}$ , the model compound ARB, which only lacks the extra carboxyl group (Scheme 1), was synthesized for comparison. Interestingly, ARB does not show any color response to  $Cu^{2+}$  under the same conditions. This strongly indicates that the extra carboxyl group in CARB plays a crucial role in the color-on reaction, and without it the reaction can not occur. Considering the above result of 1:2



**Figure 6** Binding stoichiometry of CARB and  $Cu^{2+}$ . (a) Job's plot for the system of CARB with  $Cu^{2+}$  (the total concentration of  $Cu^{2+}$  and CARB was kept constant at 30 µmol/L); (b) molar ratio method (the concentration of CARB was constant at 20 µmol/L). The absorbance was measured at 567 nm.

Table 1 Recovery of 20  $\mu mol/L~Cu^{2+}$  in the presence of various coexisting ions

Ions	Concentration (mmol/L)	Molar ratio of the added ion to Cu <sup>2+</sup>	Recovery (%)	
K <sup>+</sup>	4	200	108	
Ca <sup>2+</sup>	12	600	106	
Mg <sup>2+</sup>	20	1000	101	
Al <sup>3+</sup>	0.4	20	93	
$Ag^+$	0.2	10	99	
Ba <sup>2+</sup>	2	100	98	
$Cd^{2+}$	2	100	101	
Co <sup>2+</sup>	2	100	102	
Fe <sup>3+</sup>	0.1	5	94	
Fe <sup>2+</sup>	0.4	20	97	
Hg <sup>2+</sup>	1	50	98	
La <sup>3+</sup>	1	50	102	
Mn <sup>2+</sup>	4	200	102	
Ni <sup>2+</sup>	4	200	95	
Pb <sup>2+</sup>	3	150	104	
Sn <sup>2+</sup>	0.3	15	95	
Zn <sup>2+</sup>	2	100	103	
ClO <sup>-</sup>	0.4	20	108	
$CO_{3}^{2-}$	2	100	103	
$HPO_4^{2-}$	0.8	40	95	
$NO_3^-$	6	300	104	
$SO_4^{2-}$	4	200	102	
SiO <sub>3</sub> <sup>2-</sup>	10	500	106	



Scheme 2 The possible reaction mechanism of CARB with Cu<sup>2+</sup>.

stoichiometery, the color-on reaction of CARB with  $Cu^{2+}$  may be explained by the route depicted in Scheme 2: the carboxyl and hydrazide groups in CARB produce a cooperation effect, leading to the formation of a 1:2 complex. The two  $Cu^{2+}$  ions in the complex may play different roles: one induces the opening of the spirocyclic structure and the other quenches the fluorescence of the xanthene moiety [34–36]. It is noted that the curve in Figure 6(b) appears somewhat in an S-shape, suggesting that the two  $Cu^{2+}$  ions might also have a cooperation effect in the reaction. Moreover, density functional theory calculations provide further structural evidence for the CARB- $Cu^{2+}$  1:2 complex. As depicted in Figure 7, the geometry of the complex, opti-

mized at the B3LYP/6-31G level by the Gaussian 03 program [37], shows that one  $Cu^{2+}$  is located in a caged structure by coordinating with two oxygen atoms and two nitrogen atoms. The other  $Cu^{2+}$  is coordinated with the extra carboxyl group and interacts with the  $\pi$ -conjugated benzene ring of xanthene.

# 3.7 Comparison with other rhodamine probes

A summary of the rhodamine-based probes for the detection of  $Cu^{2+}$  is given in Table 2. As can be seen, each probe has its own charactersistic. Compared with most of the probes,



**Figure 7** The calculated structure of the 1:2 CARB-Cu<sup>2+</sup> complex. Gray, red, blue and green atoms denote C, O, N and Cu atoms, respectively, where H atoms are omitted for clarity.

Table 2Comparison of different rhodamine probes for the determination of  $Cu^{2+}$ 

Probes	Reaction medium	Reaction time (min)	Interfering ion	Reversibility	Ref.
СООН	CH3CN	60	Hg <sup>2+</sup>	Yes	[11]
Rhodamine B hydroxylamide	pH 6.0 Tris-HCl with 60% (v/v) CH <sub>3</sub> CN	120	No	No	[25]
Rhodamine B hydrazide	pH 7.0 HEPES <sup>a</sup> with 80% (v/v) CH <sub>3</sub> CN	2	Hg <sup>2+</sup>	No	[36]
	pH 7.0 Tris-HCl with 90% (v/v) CH <sub>3</sub> CN	40	Zn <sup>2+</sup>	Not available	[38]

(Continued)
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Probes	Reaction medium	Reaction time (min)	Interfering ion	Reversibility	Ref.
Rhodamine B boronic acid	pH 7.4 HEPES	30	No	Yes	[39]
N N N N N N N N N N N N N N N N N N N	pH 7.2 HEPES with 30% (v/v) CH <sub>3</sub> CN	1	Hg <sup>2+</sup>	No	[40]
Platinum film-immobilized rhodamine	methanol	30	No	Yes	[41]
Salicylaldehyde rhodamine B hydrazone	pH 7.0 Tris-HCl with 50% (v/v) CH <sub>3</sub> CN	20	Fe <sup>2+</sup>	Yes	[42]
N-(3-Carboxy)acryloyl rhodamine B hydrazide	pH 7.0 Palitzsch's buffer with 10% (v/v) THF	5	No	Yes	This work

a) HEPES: 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid.

however, CARB exhibits an improved overall performance in terms of fast reaction time and good selectivity.

# 4 Conclusions

The above results clearly show that CARB displays a highly selective absorption response to  $Cu^{2+}$  with little response to other commonly coexisting ions. This property of CARB may allow  $Cu^{2+}$  to be determined by the naked eye. Studies on the reaction mechanism reveal that one CARB molecule combines two  $Cu^{2+}$  ions, and the two  $Cu^{2+}$  ions in the complex play different roles. This action behavior is also supported by a comparative study on the model compound *N*-acryloyl rhodamine B hydrazide as well as the density functional theory calculations. Moreover, CARB might also be used to detect hypochlorite anion in the alkaline media because it has the same reaction unit as N-benzoyl rhodamine B hydrazide [5], which is now under study in our laboratory.

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