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Research Article

# An Effective 'Off-On' Artificial Probe Based on Rhodamine B Derivative for Sensitive and Selective Recognition of $Cu^{2+}$ Ions

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## Abstract

This paper describes the synthesis and optical application of a novel, xanthane-based colorimetric and fluorescent `*on-off*' sensor RhCHN for the selective recognition and discrimination of $Cu^{2+}$ ions. Its structural characterization was achieved by NMR ($^1$H and $^{13}$C), FT-IR sand LC-MS/MS analyses. Among the metal ions tested, only addition of $Cu^{2+}$ ions to the probe in buffer solution, the maximum increases in both absorption and fluorescent emission spectra at 565 and 588 nm, respectively, were observed, followed by an immediate change in color from colorless to purple, visible to the naked eye. It was found that the RhCHN probe showed remarkable selectivity and sensitivity to copper ions only among the metal ions initially used. According to Job's plot method, it was estimated that RhCHN is bound to the $Cu^{2+}$ ion to form a complex within a 1:1 coordination stoichiometry (RhCHN:$Cu^{2+}$). To the best of our knowledge, a strong bond interaction between $Cu^{2+}$ and RhCHN has most likely gone through the spirolactam ring opening process. With an acceptable detection limit for copper ions, the analytical applications implied that the chromogenic and fluorogenic double response probe RhCHN can be effectively used for direct visualization and detection of $Cu^{2+}$ in aqueous medium.

**Keywords:** Colorimetric, fluorescent, xanthane, detection, $Cu^{2+}$

## $Cu^{2+}$ İyonlarının Hassas ve Seçici Tayini için Rhodamine B Türevli Etkili Bir 'Off-On' Yapay Prop

## Öz

Bu çalışma, $Cu^{2+}$ iyonlarını seçici bir şekilde tanıma ve ayırt etmek için yeni bir ksanten-temelli kolorimetrik ve floresan 'on-off' sensörün sentezini ve optik uygulamasını tanımlamaktadır. Bu sensörün karakterizasyonu, NMR ($^1$H & $^{13}$C), FT-IR ve LC-MS/MS analizleri ile yapılmıştır. Test edilen metal iyonları arasından, tampon çözeltisi içerisindeki proba sadece $Cu^{2+}$ iyonları eklendiğinde, çıplak gözle görülebilen renksiz durumdan mor renge bir dönüşümün takip ettiği absorpsiyon ve floresan emisyonlarında (565 ve 588 nm) maksimum artışlar gözlenmiştir. RhCHN probun başlangıçta kullanılan metal iyonları arasından sadece bakır iyonlarına karşı önemli bir seçicilik ve duyarlılık gösterdiği bulunmuştur. Job's plot metoduna göre, RhCHN'nin 1:1 koordinasyon stokiyometrisi ile $Cu^{2+}$ iyonuna bağlandığı tahmin edilmiştir. Daha önceki çalışmalarda vurgulandığı gibi, $Cu^{2+}$ ile RhCHN arasındaki güçlü bağ etkileşimi muhtemelen halka yapısının açılması üzerinden gerçekleşmektedir. Bakır iyonları için kabul edilebilir bir tayin alt sınırıyla, analitik uygulamalar, kromojenik ve florojenik çifte cevap veren prop RhCHN'nin, sulu ortamdaki $Cu^{2+}$ iyonlarını direkt görüntüleme ve tayini için etkili bir şekilde kullanılabileceğini göstermiştir.

**Anahtar Kelimeler:** Kolorimetrik, floresan, ksanten, tayin, $Cu^{2+}$

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## 1. Introduction

Over the last two decades, the design and development of chromogenic and fluorogenic probes has aroused the important interest of researchers in fields such as environmental science, biology and chemistry (Jiao et al., 2018; Puangploy et al., 2014; Zhao et al., 2009). Especially, due to their excellent photophysical performance, such as good photostability, large extinction coefficients, broad fluorescence in the visible range and exceptional detection capability, many types of the fluorescent / colorimetric sensors based on rhodamine derivatives were synthesized and applied with great effort. Up to now, very succesful artificial probes for the selective and sensitive detection of heavy and transition metal ions (HTMs) in different kinds of samples have been described (Hu et al., 2019; Dai et al., 2014; Saleem et al., 2014; Wang et al., 2012; Yu et al., 2011; Wang et al., 2013; Yang et al., 2016; Adhikari et al., 2019).

To the best of our knowledge, the metal-free chemosensors derived from the spirolactam ring-closed form of xanthenes and related derivatives do not have the absorption band in the range of 450-600 nm, the solution color and thus the fluorescence emission. However, when different metal ions are added to a sensor solution, the spirolactam-open forms of the molecular structures generated by miscellaneous cations not only show the strong absorption bands and/or fluorescence emissions. There exists also a drastic change in color from colorless to a colour - such as pink or purple - allowing the naked-eye detection of target metal ions (Wang et al., 2017; Sun et al., 2015; Tang et al., 2013; Sahana et al., 2017; Wang et al., 2014; Li et al., 2015; Chatterjee et al., 2016; Ozdemir, 2016; Wang et al., 2012).

Copper is an essential element for the most aerobic organisms and plants to continue their life, and it is the third most abundant trace metal (after $Fe^{3+}$ and $Zn^{2+}$) among the biologically relevant transition metals. Copper as a cofactor in many metalloenzymes with strong redox activity also plays an important role in maintaining nerve cells and the immune system. The consequence of excessive accumulation of toxic copper in the liver, fibrous and brain of the human body by copper-contaminated water has currently been linked to the symptoms of several diseases, including Alzheimer's, Wilson, Menkes, Prion and Parkinson. In contrast, a copper deficiency in humans can cause heart attack, fatigue, weakness and other problems (Li et al., 2012; Dong et al., 2010; Wu et al., 2012; Mergu et al., 2015; Li et al., 2013; Kempahanu makkagaari et al., 2014; Li et al., 2015; Jiang et al., 2013; Wang et al., 2014). Therefore the cost-effective detection and quantification of $Cu^{2+}$ ions in food, tap water, pharmaceuticals and industry is a major challenge in future projects.

Currently, there are various analytical methods, including atomic fluorescence, atomic absorption, inductively coupled plasma mass and inductively coupled plasma atomic emission spectrometries, and electrochemical methods, surface plasmon resonance techniques and quantum dots are available to quantify the copper content in different types of real samples (Xu et al., 2017; Lv et al., 2017; Xu et al., 2014; Jiao et al., 2018). Although they are high performance systems, these procedures generally require complex sample pretreatment, expensive and sophisticated instruments, operational management requirements and therefore a time-consuming process. In this regard, fluorescent receptor dyes capable of binding to toxic transition metal ions have recently become the focus of scientific interest due to their distinct advantages; ease of use, low cost, real-time analysis and rapid response, high reliability, and selectivity and sensitivity to metal ions in aqueous solutions (Ding et al., 2017; Udhayakumari et al., 2017; Xu et al., 2011; Tang et al., 2018; Lv et al., 2018; Zhou et al., 2009; Min et al., 2013; Yu et al., 2013).

In the present study, a new colorimetric and fluorescent dual-channel "*off-on*" sensor RhCHN based on a rhodamine B derivative was developed and synthesized for the selective visualization and quantification of $Cu^{2+}$ ions in real samples. The probe showed both chromogenic and fluorogenic reactions after treatment with $Cu^{2+}$ ions. This case was visible to the naked eye by a fast color change from colorless to violet. In contrast, the addition of the other competitive metal cations did not lead to significant changes in solution color and absorption or fluorescence emission spectra. The results obtained from optical investigations revealed that the RhCHN probe could have an application for fast and accurate determination of $Cu^{2+}$ ions in tap water.

## 2. Materials and Methods

### 2.1. Instruments

Molecular structure of probe RhCHN was confirmed by FT-IR, NMR ($^1H$ and $^{13}C$) spectroscopies, and LC-MS/MS spectrometry as well as fluorescence and UV/Vis absorption spectral methods. All the NMR experiments were conducted on a Bruker AVANCE III HD 600 MHz NMR spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. FT-IR measurements were performed over the range 400 to 4000 $cm^{-1}$ on a PerkinElmer Spectrum 100 spectrometer. LC-MS/MS analyses were carried out using a PerkinElmer’s Tandem mass spectrometry. The fluorescence spectra were measured with a Hitachi F-7000 fluorescence spectrophotometer. A Perkin Elmer UV / VIS spectrophotometer was employed for the absorption studies. All studies reported here were conducted at room temperature (298 K).

### 2.2. Reagents and methods

All chemicals were obtained from commercial suppliers in analytical quality and used as received without any further refining. Rhodamine B hydrazide and dibenzalacetone were synthesized by the method described in previous work (Ozdemir, 2017; Hu et al., 2016; Franco et al., 2012). As a chromatographic technique, TLC was used to monitor the progress of the reaction on silica gel plates. Silica gel column chromatography was applied for the purification and separation technique, with dichloromethane-methanol (95:5, v/v) serving as the mobile phase. Ethylenediaminetetraacetic acid (EDTA) was exploited as complexing agent in the reversibility test. To obtain the stock solution of RhCHN at 1 mM (1 $mmol.L^{1-}$), it was prepared by dissolving 10.1 mg probe in 15 ml ethanol. The nitrate or chloride salts — $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Cr^{3+}$, $Fe^{3+}$, $Hg^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cd^{2+}$, $Al^{3+}$, $Mn^{2+}$ and $Ag^+$—were dissolved in distilled water to afford 1 mM aqueous solutions. All stock solutions were stored in the fridge at 5-7 $^0$C.

### 2.3. UV/Vis absorption and fluorescence emission measurements

After preparing the standard solution of RhCHN at 1.0 x $10^{-3}$ M in ethanol, it was then diluted to micromolar (μM) concentrations using EtOH-$H_2O$ (2:1, v/v, 10 mM HEPES, pH 7.2) for all tests performed under identical conditions at room temperature (298 K). All spectroscopic titration studies were achieved by stepwise addition of a suitable concentration of metal cations to RhCHN in solutions. For the titration experiments in 450-650 nm range of absorption, a Perkin Elmer UV / VIS spectrophotometer was used with a quartz cuvette (path length 1 cm). A Hitachi F-7000 fluorescence spectrophotometer was performed with an excitation wavelength ($\lambda_{ex}$) of 530 nm in a quartz cell with a slit width of 5 nm for the fluorescence measurements. The emission spectra were recorded without delay, whenever probe RhCHN was treated with any metal ions in solution.

### 2.4. Synthesis of probe RhCHN

Probe RhCHN was derived from Rhodamine B hydrazide and dibenzalacetone by condensation reaction in hot ethanol, as shown in the Figure 1. In short, dibenzalacetone (468.6 mg) in 20 ml ethanol was slowly poured into the stirred ethanolic solution of rhodamine B hydrazide (913.2 mg) in a 100 ml flask with a few drops of glacial acetic acid. This mixture was then heated to reflux (using an oil bath) for 8 hours under inert gas atmosphere (*$N_2$*). In the meantime, the TLC procedure was carefully performed to check the reaction progress. Next, the reaction medium was placed on a dark side to cool down to room temperature in the flask, followed by the process of vacuum evaporation at lower atmospheric pressure. Finally, the raw residue was subjected to column chromatography on silica gel ($CH_2Cl_2/CH_3OH$, 20: 1-5, vol./vol.) to afford a glassy dark orange solid RhCHN (502 mg, 55% yield).

FT-IR (ν/cm$^{-1}$): 2968.6 (w, aromatic, $\nu_{C\text{-}H}$), 2936.1 (w, alkenyl, $\nu_{C\text{-}H}$), 2870.4 (w, aliphatic, $\nu_{C\text{-}H}$), 1690.7 (s, amidic carbonyl, $\nu_{C=O}$); 1613.3 (vs, azomethine, $\nu_{C=N}$), 1547, 1512.5 (vs, aromatic, $\nu_{C=C}$), 1447, 1325, 1263, 1218, 1115.6 (vs, $\nu_{C\text{-}O}$), 1075, 971, 818, 784, 756, 696 (Figure S1). $^1$H NMR (600 MHz, $CDCl_3$), δ(ppm): 7.93 (d, 1H, Ar-$\underline{H}$), 7.73 (d, 1H, Ar-$\underline{H}$), 7.62 (t, 2H, Ar-$\underline{H}$), 7.39-7.47 (m, 5H, Ar-$\underline{H}$), 7.26-7.31 (m, 5H, Ar-$\underline{H}$), 6.56 (d, 2H, xanthene-$\underline{H}$), 6.45 (d, 2H, xanthene-$\underline{H}$), 6.41 (s, 1H, xanthene-$\underline{H}$), 6.37 (s, 1H, xanthene-$\underline{H}$), 6.29 (s, 1H, alkenyl=C-$\underline{H}$), 6.28 (s, 1H, alkenyl=C-$\underline{H}$), 6.22 (s, 1H, alkenyl=C-$\underline{H}$), 6.20 (s, 1H, alkenyl=C-$\underline{H}$), 3.34 (q, 8H, -$NC\underline{H}_2CH_3$), 1.15 (t, 12H, -$NCH_2C\underline{H}_3$), (Figure S2). $^{13}$C NMR (600 MHz, $CDCl_3$**)**, δ(pmm)**:** 169.03 (carbonyl carbon, $\underline{C}$=O), 166.12 (azomethine, -$\underline{C}$=N-), 161.08, 153.87, 153.7, 151.56, 148.89, 148.64, 143.35, 130.53, 128.99, 128.82, 128.74, 128.69, 128.59, 128.58, 128.42, 128.12, 127.46, 127.33, 125.43, 108.03, 107.7, 106.65, 104.57, 97.83, 76.82 (solvent, $CDCl_3$), 67.32 (quaternary carbon, $\underline{C}$), 44.38 (-$N\underline{C}H_2CH_3$), 12.66 (-$NCH_2\underline{C}H_3$) (Figure S3). Tandem-MS: The mass spectra of probe RhCHN was represented in Figure S4. Fragments at m/z = 673 and 674 were assigned to molecular ion peaks $[C_{45}H_{44}N_4O_2]^+$ and $[C_{45}H_{44}N_4O_2+H]^+$, respectively.

## 3. Result and Discussion

In the last two decades, rhodamine-based fluorophores have been used as a signal switcher in the development of colorimetric or fluorescent probes to qualitatively and quantitatively detect target analyst in different types of fields such as medicine, biochemistry, chemistry and environmental sciences due to their important photochemical properties. In general, these artificial molecular platforms existed in two forms: ring-closed spirolactam (fluorescence "*Off*") or ring-opened amide form (fluorescence "*On*"). During the interaction of probes with metal cations, the recognition process occurs by converting the molecular structure from the spirocyclic to the ring-opened amide forms of the xanthan units (Ozdemir, 2017; Hu et al., 2016; Li et al., 2014; Zhou et al., 2012).

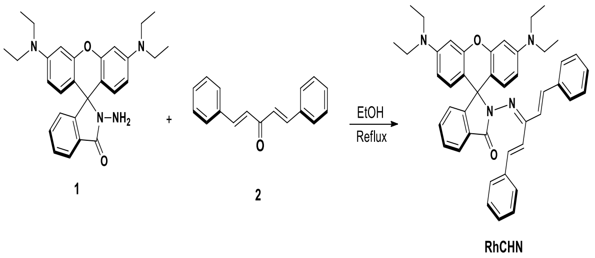

**Figure 1.** Synthesis of RhCHN

As shown in the Figure 1, probe RhCHN was produced as a member of the hydrazone Schiff-base ligand by condensation of rhodamine B-hydrazide and dibenzalacetone. Its structural confirmation was carried out using some instrumental methods. The spectral data evaluated are also included in the supplementary information.

### 3.1. UV-vis absorption and fluorescence spectral responses of probe to $Cu^{2+}$

To investigate the remarkable properties such as the photophysical and the chelation behaviors of free RhCHN towards $Cu^{2+}$, UV/Vis titration experiments were carried out in buffer solution (EtOH-$H_2O$, 2:1, v/v, 10 mM HEPES, pH 7.2) (Zhang et al., 2007; Guo et al., 2014; Xiang et al., 2006; Ding et al., 2012). Probe RhCHN, like most rhodamine-based chemosensors, remained colorless without the coexistent metal ions and did not show any significant absorption band above 450 nm in the UV/Vis spectra. This fact demonstrates that the RhCHN probe consists mainly of the spiro form (ring-closed). Based on the coordination interaction with metal cations, RhCHN is expected to behave like a signal switch, resulting in a color change with excellent photochemical characteristics such as photon absorption and emission via a large π conjugated system (Hu et al., 2011; Tang et al., 2011; Wang et al., 2017; Huo et al., 2013; Lou et al., 2009; Yoon et al., 2017). After addition of $Cu^{2+}$ to RhCHN in solution, as expected, a significant change from colorless to violet was immediately observed, leading to strong absorption maxima at 565 nm. Under natural light, this change was detectable by the naked eye. As can be seen from the Figures 2, 3, and S5, the presence of each tested metal ion, such as $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Cr^{3+}$, $Fe^{3+}$, $Al^{3+}$, $Mn^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Fe^{2+}$, $Hg^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cd^{+2}$ and $Ag^+$, did not cause any important changes in the color and absorption spectra under the same circumstances. Further, the RhCHN probe solution showed an amplification of the broad absorption bands with maxima centered at 565 nm when exposed to different $Cu^{2+}$-doping concentrations, as depicted in the Figure 8. Actually, clear evidence for the conversion of the RhCHN receptor from the spirolactam form to the ring-opened amide form was obtained from the changes in the color and optical spectral bands, suggesting that RhCHN separates strongly and coordinates to the $Cu^{2+}$ ion to build a stable chelate complex. The results of UV/Vis absorption measurements confirmed that the RhCHN chemosensor can detect copper ions in solutions with high selectivity and sensitivity.

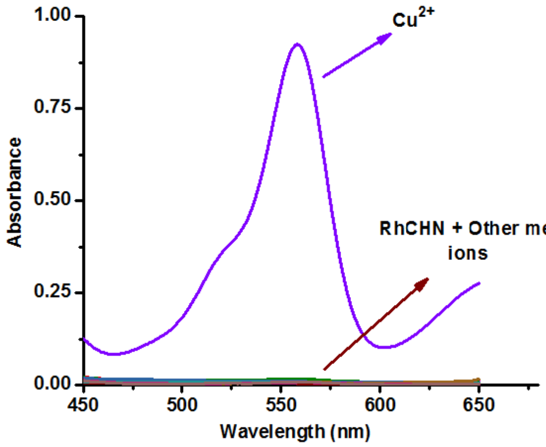

**Figure 2.** UV-vis absorption spectra of RhCHN (50 μM) in the absence/presence of 2.0 equiv. of competitive metal ions in buffer solution (EtOH-$H_2O$, 2:1, v/v, 10 mM HEPES, pH 7.2).

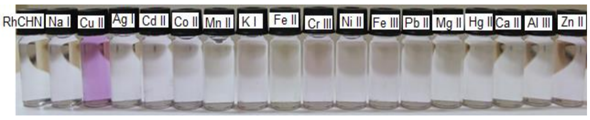

**Figure 3.** The color change of RhCHN after addition of each competitive metal ions (2.0 equiv) in buffer solution.

In order to gain a better insight into the $Cu^{2+}$ interaction of the RhCHN probe, fluorescence titrations were carried out in the presence of the commonly used metal species under the same test conditions. The excitation wavelength was set at 530 nm (Slit width: 5 nm), and then the fluorescence emission were gathered in the range from 560 to 660 nm. Due to the dominance of the spirolactam structure in the rhodamine fluorophore, the free probe RhCHN showed almost no significant change in the emission spectra after addition of sixteen competitive metal ions. Interestingly, however, a color change was observed when RhCHN was treated with $Cu^{2+}$, which was accompanied by an important increase in emission intensity, as indicated in the Figures 4 and S6.

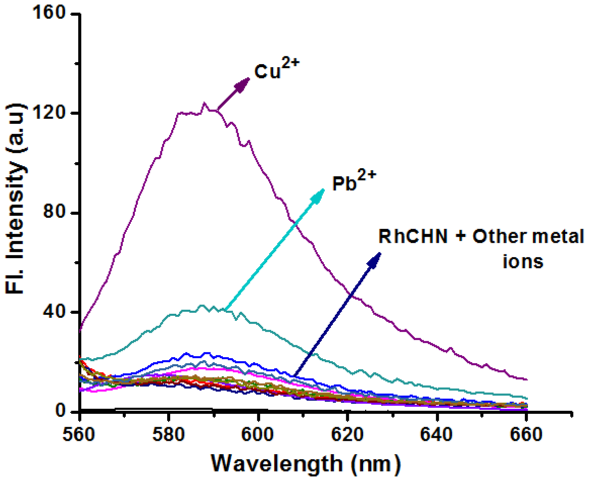

**Figure 4.** Fluorescence emission spectra of RhCHN (50 μM) after addition of each interfering metal ions (2.0 equiv.) in buffer solution (EtOH-$H_2O$, 2:1, v/v, 10 mM HEPES, pH 7.2).

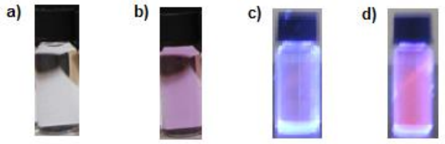

**Figure 5.** The picture shows the color change of free RhCHN (a); and after addition of 2.0 equiv. of $Cu^{2+}$ (b); under natural light; (c) and (d) the fluorescence image of the same solutions (a & b) excited by UV lamp ($\lambda_{ex}$= 365 nm) in buffer solution.

When the violet color of the RhCHN probe in solution with $Cu^{2+}$ ions was excited by ultraviolet radiation ($\lambda_{ex}$ = 365 nm) under the identical circumstances, a slight color change from violet to light orange was observed, as can be seen in the Figure 5. The ring opening reaction mechanism leading to a large $\pi$ conjugated molecule was also confirmed by this color change according to previous studies (Kempahanumakkagaari et al., 2014; Xiang et al., 2006).

## 3.2. Determination of stoichiometry and association constant

For the purpose of identification of the bonding mode between RhCHN and $Cu^{2+}$, the job's plot assay was performed. The buffer solution of RhCHN in EtOH-H 2 O (2: 1, vol./vol., 10 mM HEPES, pH 7.2) was titrated using different concentrations of $Cu^{2+}$ ions, while the total concentration was continuously maintained at 25 uM (Puangploy et al., 2014; Wang et al., 2014). When the mole fraction is about 0.5, the absorption value passes through a maximum, indicating a 1:1 complexation stoichiometry (RhCHN:$Cu^{2+}$), as shown in the Figures 6 and S7.

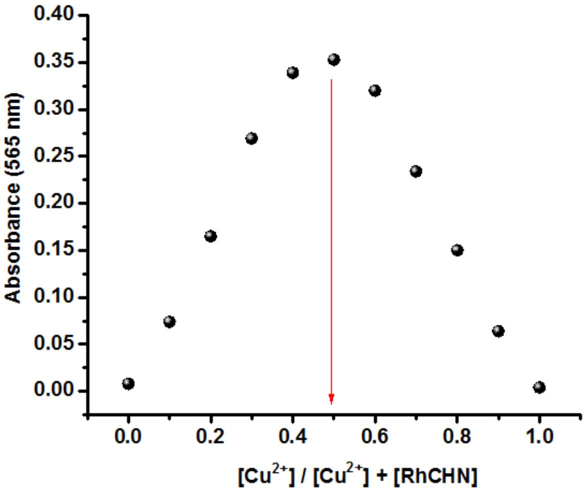

**Figure 6.** Change in absorbance of RhCHN at 565 nm in the presence of $Cu^{2+}$ with a total concentration of 25 μM ([RhCHN]+[ $Cu^{2+}$]) in buffer solution, indicating a 1:1 binding stoichiometry for the complex formation.

The association constant value of $Cu^{2+}$ with probe RhCHN was calculated from the absorbance values at 565 nm. According to Benesi-Hildebrand the equation used is as follows (Xu et al., 2011; Hu et al., 2016):

$$\frac{1}{(A-A_0)} = \frac{1}{K_a(A_{max}-A_0)[Cu^{2+}]^n} + \frac{1}{(A_{max}-A_0)}$$

In the absence of $Cu^{2+}$, the absorbance of RhCHN is $A_0$. At each addition of $Cu^{2+}$, the absorbance is $A$. At saturation point, the absorbance value is $A_{max}$, and $Cu^{2+}$ concentration added to RhCHN solutions (μM) is $[Cu^{2+}]$. $n$ means the binding ratio of the complex between RhCHN and copper ion, and $K_a$ represents the association constant. From the application of 1 / ($A$-$A_0$) against 1 / [$Cu^{2+}$], a linear relationship between absorption signals and the added $Cu^{2+}$ concentration (0 to 65 μM) was determined, as shown in the Figure 7. According to equation 1 / ($A$-$A_0$) = 145.0588 x 1 / [$Cu^{2+}$] + 0.1827, $K_a$ and $n$ were evaluated to be $5.7\text{x}10^5$ $M^{-1}$ and 1, respectively, with a coefficient of correlation ($R^2$: 0.99274).

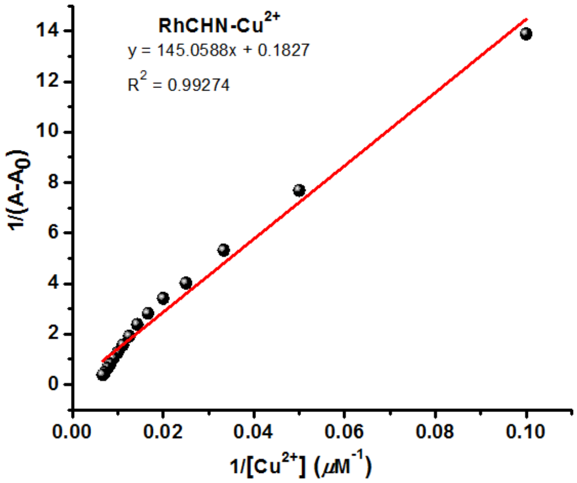

**Figure 7.** Benesi-Hildebrand application from UV-vis absorption titration experiments of RhCHN (absorbance at 565 nm) with $Cu^{2+}$

ions, showing a 1:1 binding mode between probe and copper.

### 3.3. Tolerance of RhCHN to $Cu^{2+}$ over other coexistent metal ions

Selectivity is an important aspect of colorimetric and fluorescent artificial probes for the identification of target metal cations that coexist with other interference ions in real samples (Wang et al., 2012; Sun et al., 2015). To study the ability of $Cu^{2+}$ selective detection of RhCHN in buffer solution (EtOH-$H_2O$, 2:1, v/v, 10 mM HEPES, pH 7.2), the change in emission intensity was investigated by adding 2.0 equiv. of $Cu^{2+}$ to the solutions of the probe, individually incubated with the excess amounts of competitive metal ions, including $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Ag^+$, $Cr^{3+}$, $Fe^{3+}$, $Al^{3+}$, $Mn^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Hg^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Fe^{2+}$, and $Cd^{+2}$. When 2.0 equiv. of $Cu^{2+}$ was added into the solution of RhCHN in the absence or presence of the metal ions tested, as expected, remarkable increases in emission intensity at 588 nm were observed in the fluorescence spectra, as shown in the Figure S8. However, the probe solution incubated with $Fe^{2+}$ showed no reflection for the addition of $Cu^{2+}$, which was also explained in detail in our previous work (Ozdemir, 2019). Based on the above results it could be negotiated that the $Cu^{2+}$-selective property of RhCHN is hardly disturbed by the other competitive metal ions.

### 3.4. The reversibility study

For practical application, reversibility, which indicates the capability of restoring the metal-free sensor from the complex form, is a desired property of molecular sensing platforms (Wang et al., 2014; Lv et al., 2018). An effective complexing reagent for metal cations, EDTA, was operated to investigate the reversibility and reproducibility of receptor RhCHN for $Cu^{2+}$. When the different concentrations of EDTA were added into RhCHN in solution with 2.0 equiv. of $Cu^{2+}$, the UV/Vis spectral band with a maximum at 565 nm gradually decreased, accompanied by a marked change in color from violet to colorless, as exhibited in the Figure 8. This case shows that, compared to the RhCHN-$Cu^{2+}$ complex, the strong coordination between EDTA and $Cu^{2+}$ occurs, leading to the regeneration of free RhCHN (Hu et al., 2016; Nair et al., 2015; Reddy et al., 2014).

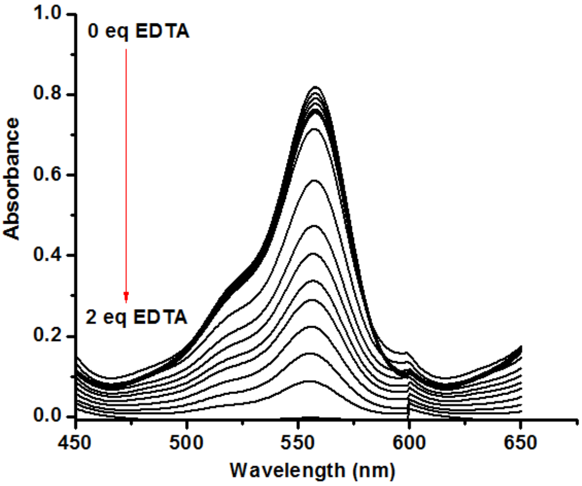

**Figure 8.** UV-vis absorption titration profile of RhCHN (50 μM) in the presence of $Cu^{2+}$ (2.0 equiv.) with increasing concentration of EDTA in buffer solution.

When $Cu^{2+}$ was added again to the solution containing the metal-free RhCHN and EDTA-$Cu^{2+}$ complex, the absorbance value at 565 nm was almost restored. Simultaneously, the colorless solution quickly changed from colorless to violet again. The recycle test determinations with the regular addition of $Cu^{2+}$ or EDTA were quoted four times, as demonstrated in the Figure 9. The results obtained thus unraveled that the complexation of $Cu^{2+}$ with probe

RhCHN is reversible, as depicted in the Figure 10.

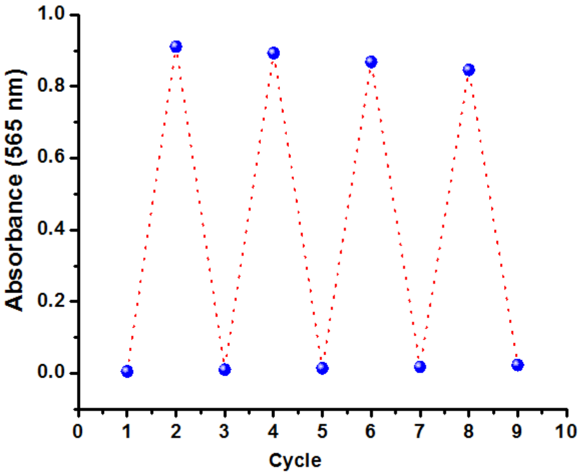

**Figure 9.** Change in absorbance of RhCHN (50 μM) at 565 nm after the sequential addition of 2.0 equiv. $Cu^{2+}$ and EDTA in buffer solution.

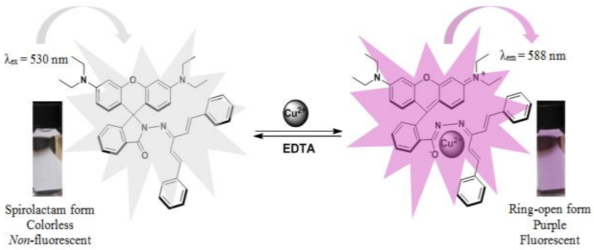

**Figure 10**. The reversible mechanism of $Cu^{2+}$-coordination to RhCHN by EDTA in buffer solution. Inset photograph shows changes in solution color of RhCHN in the absence (*Off*-form, left) and presence of $Cu^{2+}$ ions (*On*-form, right), respectively.

## 3.5. Effect of pH level

To verify the tolerance of acidic and basic conditions in the emission spectra of free RhCHN and its copper complex, the fluorescence titration experiments were performed in buffer solution in mixed ethanol-water solvents (2:1, v/v, 10 mM HEPES, pH 7.2) over a range of pH values (4.0 to 12.0), and the results are given in the Figure 11 (Guo et al., 2014; Xiang et al., 2006). In the absence of $Cu^{2+}$, probe RhCHN exhibited no characteristic changes in color and emission intensity between pH 4.0 and 12.0, representing that the spirolactam ring of RhCHN is hardly affected by pH changes. In contrast, after the addition of $Cu^{2+}$ to the solution of RhCHN, a maximum increase in emission intensity at 588 nm in the fluorescence spectra was detected at about pH 7.0. Under acidic (pH< 6.5) or basic conditions (pH >8.5), the emission values gradually decreased, which means that the complex form of RhCHN with $Cu^{2+}$ ions has no further stability. From the experimental approaches, it was proposed that the $Cu^{2+}$ detection property of RhCHN strongly depends on the pH level. Nevertheless, probe RhCHN can be performed to selectively detect the $Cu^{2+}$ ions under physiological pH conditions.

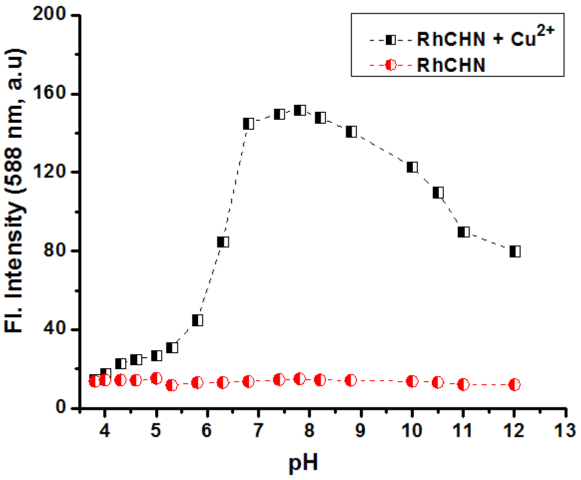

**Figure 11.** Variation of fluorescence intensity of RhCHN (50 μM) at 588 nm in the absence and presence of 2.0 equiv. of $Cu^{2+}$ in buffer solution at different pH values. To adjust the pH of the solutions, 0.1 M HCl or NaOH stock solution was used.

### 3.6. Detection limit (DL)

The fluorescence titrations to determine the detection limit (DL) of RhCHN for $Cu^{2+}$ ions were performed by adding different copper concentrations into the solution of RhCHN in buffer solution in mixed ethanol-water solvents (2:1, v/v, 10 mM HEPES, pH 7.2). Officially, the World Health Organization (WHO) and the US Environmental Protection Agency (EPA) have set a maximum level of $Cu^{2+}$ pollutants in drinking water of 31 µM and 20 µM, respectively (Muthuraj et al., 2014; Sun et al., 2018). As seen in from the Figure 12, plotting the emission intensity at 588 nm ($F$-$F_0$) of the solutions against the copper concentration ($[Cu^{2+}]$) showed a linear relationship. According to the IUPAC definition, DL was then calculated using the data obtained from the titration curve of $Cu^{2+}$, the equation being as follows (Wang et al., 2017):

$$DL = 3\sigma/m$$

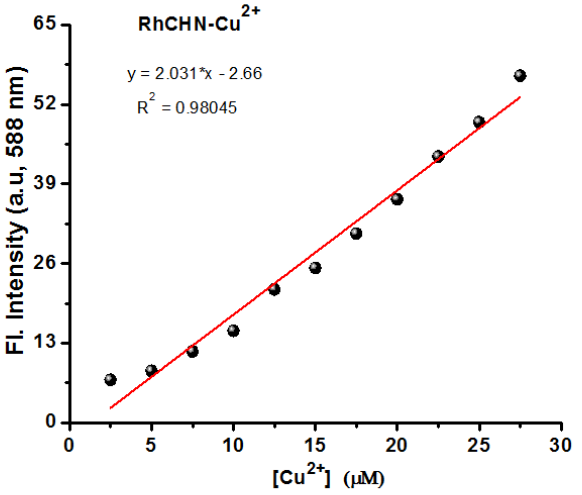

**Figure 12.** Change in emission intensity of RhCHN (25 µM) at 588 nm in buffer solution upon gradual addition of $Cu^{2+}$ (0 to 27.5 µM).

The standard deviation of the blank measurement is $\sigma$, and $m$ is the slope of the linear equation between the intensity changes of the fluorescence ($F$-$F_0$) and the concentrations of $Cu^{2+}$. With the fluorescence detection limit for $Cu^{2+}$ ions far below the permissible limits for drinking water quality, $66\times10^{-8}$ M was determined.

### 3.7. Performance comparison

So far, a series of chromogenic and fluorescent artificial chemosensors based on rhodamine derivatives has been developed and described for the selective detection of $Cu^{2+}$ ions in various samples. Compared to previous reports in this context, the newly developed sensor RhCHN shows remarkable optical properties towards $Cu^{2+}$ ions against other different metal ions tested in aqueous medium, as exhibited in Table 1.

## 4. Conclusion

To sum up, we report a new colorimetric and fluorescent "off-on" sensor RhCHN for the selective detection of $Cu^{2+}$ ions in aqueous environments. The double-response probe RhCHN showed high sensitivity and selectivity only for $Cu^{2+}$ ions, even when exposed to other selected metal cations, except for $Fe^{2+}$. When $Cu^{2+}$ ions were added to RhCHN in solution, both a strong absorption band with a maximum at 565 nm and a visual change from colorless to violet was observed. Besides that, under the UV lamp, this solution showed a slight color change from violet to orange, which was visible to the naked eye. Further, RhCHN showed reversibility to $Cu^{2+}$ ions via a 1:1 bond stoichiometry and the ability to restore the free sensor from its complex form. From the titration experiments it was also observed that RhCHN could be used for $Cu^{2+}$ quantification with the detection limit of 66 ×

$10^{-8}$ M in aqueous media. Finally, the RhCHN sensor, which offers high selectivity, ease of use, reversibility, and low cost, can be used to quickly detect $Cu^{2+}$ ions in real water samples.

## 5. Aknowledgment

A short summary of this study as an oral presentation was discussed at the International Summit for Innovation in Medicine-4 of SANKO University in Gaziantep, Turkey.

**Table 1.** Performance comparison of some reported rhodamine-based receptors for the detection of $Cu^{2+}$ ions.

| **Ref.** | Fluorophores | Testing media | Detect ions | Responses | Reproducibility | Binding constants | LOD for $Cu^{2+}$ | Applications |
|---|---|---|---|---|---|---|---|---|
| Puangploy et al. (2014) | **Rh.B | $CH_3CN$/HEPES buffer pH 7.0 | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 1.18x10$^4$ M$^{-1}$ | 0.28x10$^{-6}$ M | Hela cells |
| Zhao et al. (2009) | Rh.B | $CH_3CN/H_2O$ (3:7, v/v, pH 7.0) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 2.67x10$^5$ M$^{-1}$ | 78x10$^{-9}$ M | Tap water |
| Dai et al. (2014) | Rh.B | Aqueous acetate/$CH_3CN$(2/3, pH 4.8 | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 4.23x10$^4$ M$^{-1}$ | 14.98x10$^{-6}$ M | Aqueous media |
| Wang et al. (2012) | Rh.B | $H_2O/CH_3CN$ (v/v, 7:3) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Irreversible | NA | 1.8x10$^{-6}$ M | MG63 cells |
| Sun et al. (2015) | Rh.B | Ethanol | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | NA | 4.8x10$^{-7}$ M | Logic function |
| Li et al. (2013) | Rh.B | $CH_3CN$/HEPES buffer (1:1) pH 7.2 | $Cu^{2+}$ | Colorimetric/ Fluorescence | Irreversible | 5.01x10$^4$ M$^{-1}$ | 12x10$^{-9}$ M | Hela cells |
| Xu et al. (2014) | Rh.B | EtOH-water(1:1,v/v,Tris-HCl pH 7.1) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | NA | 0.3x10$^{-6}$ M | H292 Lung cancer cells |
| Jiao et al. (2018) | Rh.B | $CH_3CN/H_2O$ (8:2) buffer pH 7.12 | $Cu^{2+}$ | Colorimetric/ Fluorescence | NA | 1.36x10$^5$ M$^{-1}$ | 0.47x10$^{-9}$ M | A549 cells |
| Xu et al. (2011) | Rh.B | DMSO/Tris-HCl buffer(1:9 , pH 7.0) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 2.83x10$^4$ M$^{-1}$ | 3.42x10$^{-6}$ M | Water samples |
| Lv et al. (2018) | Rh.B | $CH_3CN/H_2O$ (1:1) PBS buffer pH 7.0 | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 2.26x10$^6$ M$^{-1}$ | 28x10$^{-9}$ M | The maize roots |
| Zhou et al. (2009) | Rh.B | $CH_3CN$/HEPES buffer pH 7.4 | $Cu^{2+}$ | Colorimetric | Reversible | 2.5x10$^4$ M$^{-1}$ | NA | Aqueous media |
| Hu et al. (2016) | Rh.B | EtOH/water(9:1,v/v) HEPES (pH 7.0) | $Cu^{2+}$ | Colorimetric | Reversible | 6.06x10$^4$ M$^{-1}$ | 2.0x10$^{-6}$ M | Aqueous media |
| Zhou et al. (2012) | Rh.B | Aqueous solution | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | NA | 0.49x10$^{-6}$ M | Aqueous media |
| Guo et al. (2014) | Rh.B | $CH_3CN$/Tris-HCl(1:1, pH 7.2) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 6.47x10$^4$ M$^{-1}$ | 24.3x10$^{-9}$ M | Aqueous media |
| Xiang et al. (2006) | Rh.B | $CH_3CN/H_2O$Tris-HCl(1:1, pH 7.0) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 6.9x10$^4$ M$^{-1}$ | >25x10$^{-9}$ M | Aqueous media |
| Ding et al. (2012) | Rh.B | Acetate/buffer DMF(3/2, pH 3,6) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 5.72x10$^4$ M$^{-1}$ | 6.47x10$^{-6}$ M | Acidic aqueous solution |
| Wang et al. (2017) | Rh.B | EtOH-water(3:1, v:v, $Na_2HPO_4$ buffer, pH 7.0) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Irreversible | 1.06x10$^3$ M$^{-1}$ | 26x10$^{-9}$ M | Aqueous media |
| Yoon et al. (2017) | Rh.B | $CH_3CN$/HEPES buffer(6:5, pH 7.4) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 1.45x10$^3$ M$^{-1}$ | 0.95x10$^{-6}$ M | Aqueous media |
| **This work** | Rh.B | EtOH-$H_2O$(v/v, 2:1, HEPES, pH 7.2) | $Cu^{2+}$ | Colorimetric/ Fluorescence | Reversible | 5.7x10$^5$ M$^{-1}$ | 66x10$^{-8}$ M | Aqueous media |

*NA: not avaible; **Rh.B: Rhodamine B

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