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A New Heptamethine Cyanine-Based Near-Infrared Fluorescent Probe for Divalent Copper Ions with High Selectivity

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ABSTRACT

A new near-infrared fluorophore 2-(2-Aminoethyl) pyridine-tricarbocyanine (**1**) was rationally designed and synthesized as a fluorescent probe for detection of Cu^{2+} with high selectivity. The response of Probe **1** is based on the fluorescence quenching upon binding to Cu^{2+}. The sensing performance of the proposed Cu^{2+}-sensitive Probe **1** was then investigated. The probe can be applied to the quantification detection of Cu^{2+} with a linear concentration range covering from 4.8×10^{-7} to 1.6×10^{-4} mol/L and a detection limit of 9.3×10^{-8} mol/L. The experimental results showed that the response of **1** to Cu^{2+} was independent of pH in medium condition (pH 6.0 - 8.0), and exhibited excellent selectivity towards Cu^{2+} over other common metal cations.

Keywords: Near-Infrared Dye; Fluorescent Probe; Cu^{2+} Ions

1. Introduction

The design and synthesis of fluorescent probes for selective and sensitive detection of metal ions have attracted wide-spread interests of chemists, biologists, clinical biochemists and environmentalists in recent years [1]. Copper is an essential trace element in both plants and animals, including humans. Among the essential heavy metals, the abundance of copper ranks the third in human body. It participates in many biological processes, such as haemoglobin synthesis (in utilization of Fe and regeneration of Hb), development of connective tissue, normal functions of the central nervous system, and oxidative phosphorylation [2-4]. Nevertheless, copper of high concentration is highly toxic to some organisms such as many bacteria and viruses [5]. Owing to its toxicity for bacteria, elevated concentrations of copper would ham- per the self-purification capability of the sea or rivers, and destroy the biological reprocessing systems in water. Copper is also found to be harmful to human at high concentration and has been suspected to cause the damage of infant liver in recent years. Accordingly, searching for efficient and reproducible analytical methods for the copper assay is of great importance for environment and human health.

Many analytical methods for detection of copper, including atomic absorption spectrometry (AAS) [6], inductively coupled plasma-mass spectroscopy (ICP-MS) [7], inductively coupled plasma-atomic emission spectrometry (ICP-OES) [8], spectrophotometry [9], voltammetry [10] and fluorescence spectroscopy [11], have been developed so far. Among these methods, fluorescence spectroscopy offers significant advantages due to its nondestructive character, high sensitivity and specificity, and the availability of a wide range of indicative dyes. Several fluorophores have been used to design fluorescent probes for divalent copper ions including calcein [12], rhodamine [13], naphthalimide [14,15], pyrene [16,17], tris(2,2'-bipyridine)-ruthenium(II) [18], ben-

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zoxazole [19], porphyrin [20,21], spiropyran [22,23], BODIPY [24], and so on. Unfortunately, limitations of the currently available probes for Cu^{2+} include low sensitivity, and/or excitation profiles in the ultraviolet or visible region, which can damage living samples and cause interfering autofluorescence from native cells.

The light in the near-infrared region (NIR) around 650 - 900 nm can penetrate more deeply into tissues, which is of importance to study on living organism imaging. Moreover, it has a further advantage that autofluorescence is not observed upon NIR excitation. Heptamethine cyanine dyes [25], one of the important kinds of NIR dyes, has been widely used in various fields, and been employed as fluorescent labels in the studies of fluorescence imaging with biological mechanisms. And a few probes based on heptamethine cyanine dyes have been employed to detect metal ions or small molecules [26]. However, to the best of our knowledge, only few NIR fluorescent probes based on cyanine dyes have been reported for divalent copper ion assay detection [27]. Searching for new NIR probe for copper detection with high selectivity is still an active field as well as a challenge for the analytical chemistry research.

Herein, we report the synthesis and properties of a novel NIR fluorescent probe 2-(2-Aminoethyl) pyridine-tricarbocyanine (**1**) for the detection of Cu^{2+} with good selectivity and high sensitivity. Tricarbocyanine and 2-(2-aminoethyl) pyridine were selected as the reporter and chelator, respectively. The probe exhibited stable response towards Cu^{2+} over the concentration range from 4.8×10^{-7} to 1.6×10^{-4} mol/L with a working pH range from pH 6.0 to 8.0.

2. Experimental

2.1. Reagents

Before being used, N, N'-dimethylformamide (DMF) was subjected to simple distillation from K_2CO_3. IR-780 iodide was purchased from Sigma-Aldrich. 2-(2-Aminoethyl) pyridine was purchased from Alfa Aesar. All other chemicals were of analytical reagent grade, purchased from Shanghai chemical Reagent Corporation (Shanghai, China), and used without further purification. Twice distilled water was used throughout all experiments. Thin layer chromatography (TLC) was carried out using silica gel 60 F254, and column chromatography was conducted over silica gel (100 - 200 mesh), both of which were obtained from the Qingdao Ocean Chemicals (Qingdao, China).

2.2. Synthesis of Compound 1

Synthetic route for Compound **1** was depicted in **Scheme 1**. Briefly, IR-780 iodide (11.3 mg, 0.0167 mmol) and 2-(2-Amino-ethyl) pyridine (20.4 mg, 0.167 mmol) were
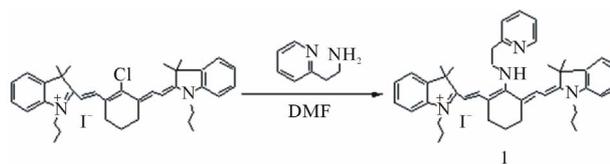
Scheme 1. Synthetic pathway of Compound 1.

dissolved in anhydrous DMF (3 mL) in a 25 mL round bottom flask. The mixture was stirred at 80°C for 4 h under an argon atmosphere. The solvent was removed under reduced pressure, then purified on silica gel chromatography eluted with CH_2Cl_2/ethanol (100:1, V/V) to afford the desired product as a blue solid (7.1 mg, yield 56%). ^1H NMR (400 MHz, CDCl₃): δ 0.93 (t, 6H, J = 7.4 Hz), 1.55(s, 12H), 1.63 - 1.73(m, 6H), 2.45 (t, 4H, J = 6.0 Hz), 3.21(t, 2H, J = 6.4 Hz), 3.92 (t, 4H, J = 6.8 Hz), 4.14(d, 2H, J = 6.4 Hz), 5.76 (m, 2H), 7.05(t, 2H, J = 6.8 Hz), 7.15(d, 2H, J = 8.0 Hz), 7.27 - 7.33(m, 4H), 7.43(m, 2H), 7.60(d, 2H, J = 12.8 Hz), 7.75(m, 1H), 8.50(d, 1H, J = 5.2 Hz), 8.68(br, 1H). ESI-MS: [M-I⁻] = 625.3, calculated: [M]⁺ = 625.9.

2.3. Apparatus

^1H NMR spectra were recorded on a INOVE-400 (Varian) spectrometer operating at 400 MHz. All chemical shifts are reported in the standard δ notation of parts per million. LC-MS analyses were performed using an Agilent 1100 HPLC/MSD spectrometer; UV-Vis absorption spectra were recorded with a Shimadzu MultiSpec-1501 spectrophotometer. All fluorescence measurements were carried out on a HITACHI F4500 (Japan) with excitation slit set at 10.0 nm and emission at 20.0 nm. The pH measurements were carried out on Mettler-Toledo Delta 320 pH meter (Shanghai, China).

2.4. Measurement Procedures

4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) buffer {0.1 mol/L, pH = 7.4, I = 0.1 (NaNO₃)} were prepared by dissolving appropriate HEPES and NaNO₃ in water, adjusting to pH = 7.4 by 1.0 mol/L NaOH with the volume to 1000 ml in a volumetric flask.

A 2.0×10^{-5} mol/L stock solution of **1** was prepared by dissolving **1** in CH₃CN. A stock standard solution of Cu^{2+} (0.01 mol/L) was prepared by dissolving an appropriate amount copper in water and adjusting the volume to 100 ml, then further diluted to 1×10^{-3} - 1×10^{-7} mol/L stepwise. The buffered solutions of wide pH range were obtained by adjustment of 0.1 mol/L HEPES solution with HCl or NaOH solution. The complex solution of Cu^{2+}/**1** was prepared by adding 5.0 mL the stock solution of **1** and 1.0 mL mentioned above solution of Cu^{2+} in a 10 mL volumetric flask. Then the mixture was diluted to 10mL with pH 7.4 HEPES buffer solution. In the ob-

tained solution, the concentrations were 1×10^{-5} mol/L of **1** and 1×10^{-3} - 1×10^{-8} mol/L of Cu^{2+}. Blank solution of **1** was prepared under the same conditions without Cu^{2+}. All the solution above were protected from light and kept at 4˚C for further use.

3. Results and Discussion

3.1. Spectral Characteristics

Figure 1 showed the fluorescence spectra of **1** in HEPES buffer solutions with different concentrations of Cu^{2+}, which recorded at excitation wavelength of 640 nm and emission wavelength of 670 - 800 nm. The spectrum of free **1** exhibited very strong fluorescence emission in a buffer solution. Addition of Cu^{2+} to a solution of **1**, fluorescence signal exhibited a remarkable quenching. The fluorescence intensity of **1** was gradually decreased with increasing Cu^{2+} concentration. These results provided a proof for the formation of an inclusion complex of **1** with Cu^{2+}, which constituted the basis for the determination of Cu^{2+} concentration with **1**. It is worthy to note that the fluorescence intensity of Probe **1** can be recovered upon addition of the coordinating reagents ethylenediaminetetraacetic acid (EDTA).

In order to better understand the variation of fluorescence intensity with the concentrations of Cu^{2+}, the absorption spectra of **1** in the absence and presence of Cu^{2+} were recorded (**Figure 2**). In the absorption spectrum of **1,** the results showed a strong absorption band at 643nm in the absence of Cu^{2+}, while the addition of Cu^{2+} ions decreased with no obvious shift in absorbance at 643 nm. From the fluorescence spectra and UV-is spectra, it is indicated that the fluorescence changes of **1** were more likely to be caused by the change of quantum yield rather than spectral shifts. Similar results were reported by Tang *et al.* [28].

In addition, Probe **1** works well and no detectable change in the linear range, detection limit or other analytical performance is found after it has been stored for several weeks in the dark at 4˚C, which implies that the NIR fluorescent probe used is stable.

3.2. Principle of Operation

The complexation equilibrium of **1** (A) with Cu^{2+} (B) with an association constant K can be expressed by the following equation:

$$mB + nA \xleftrightarrow{K} A_nB_m \tag{1}$$
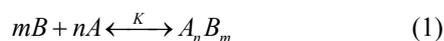
where Cu^{2+} (B) and **1** (A) is established by formation of a complex with a complexing ratio of m:n. According to the modified Stern–Volmer equation [29], the relationships for the changes of fluorescence intensities, the concentration of Cu^{2+} [B] in solution and the concentration of **1** [A] in solution can be expressed as follows:
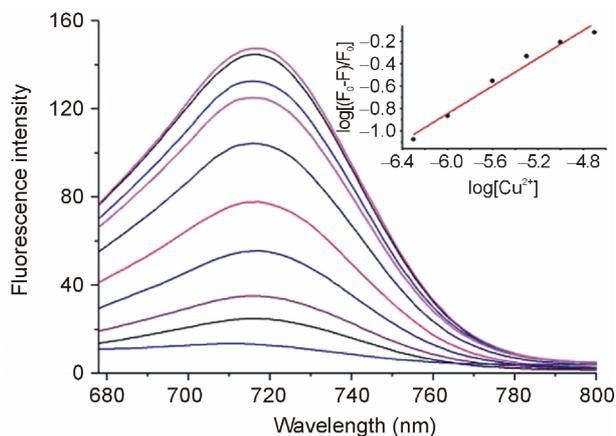

Figure 1. Fluorescence emission spectrum of 1 (10 µmol/L) with different concentration of Cu^{2+} (From top to bottom: 0, 0.5, 1.0, 2.5, 5.0, 10, 20, 50, 100, 200 µmol/L) in CH_3CN/H_2O (1:1, v/v) solution. Inset shows the linear responses with divalent copper ions concentrations.

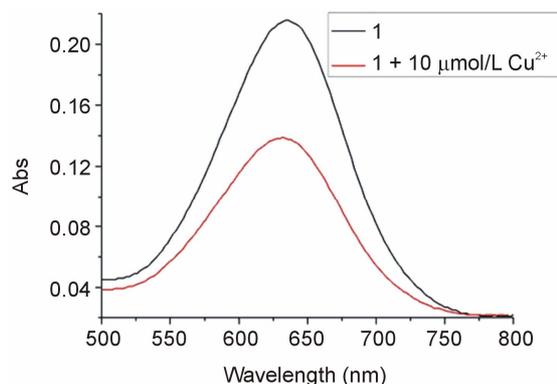**Figure 2. Chages in the UV-vis spectra of 1 (10 µmol/L) (black line) upon the addition of Cu^{2+} (10 µmol/L) (red line).**

$$\frac{F_0 - F}{F} = K_q [A]^{n-1} [B]^m \tag{2}$$

Assuming $\Delta F = F_0 - F$, one can obtain:

$$\log\left(\frac{\Delta F}{F}\right) = \log K_q + (n-1)\log[A] + m\log[B] \tag{3}$$

Here F_0 and F denote the fluorescence intensities of **1** in the absence and presence of Cu^{2+}, respectively. K_q is fluorescence quenching constant. The calibration curve was constructed by recording the fluorescence intensity values of **1** in the presence of different Cu^{2+} concentration. In the range between 1.0×10^{-7} and 5.0×10^{-5} mol/L Cu^{2+}, the fluorescence intensity is linearly dependent on the Cu^{2+} concentration. The dependency can be described by the following equation:

$$\log\left(\frac{\Delta F}{F}\right) = 0.9343\log\left[Cu^{2+}\right] + 4.80 \tag{4}$$

It is obvious from Equation (4) that m is the slope of

log(ΔF/F) versus log[Cu^{2+}], which was calculated to be 1 approximately. Quenching constant (Kq) is 6.3×10^4. The relative fluorescence intensity α is defined as the ratio of free A [A]$_f$, to the total amount of A [A]$_t$, in the solution. It can be experimentally determined by measuring the fluorescence intensity of **1** in the solution:

$$\frac{[A]_f}{[A]_t} = \frac{F - F_t}{F_b - F_t} \quad (5)$$

Here F_b is the fluorescence intensity of **1** in the blank buffer solution and F_t represents the fluorescence intensity of **1** in the solution when **1** is completely complexed with Cu^{2+}. F is the fluorescence intensity of **1** actually measured when in contact with Cu^{2+} solutions of a given concentration. The relationship between the α and Cu^{2+} concentration [B] can be represented as:

$$\frac{\alpha^n}{1-\alpha} = \frac{1}{nK[A]_t^{n-1}[B]^m} \quad (6)$$

The response of **1** for different concentrations of Cu^{2+} was shown in **Figure 3**. Three curves are calculated using Equation (6) with different K and ratios of Cu^{2+} and **1**. It can be seen that the best curve was 1:1 complex ratio and an appropriate K of 1.09×10^5 fits form the experimental data. The curve can serve as the calibration curve for the detection of Cu^{2+} concentration. A practically usable range for quantitative determination covered from 4.8×10^{-7} to 1.6×10^{-4} mol/L ($0.05 \leq \alpha \leq 0.95$) [29]. The detection limit was 9.3×10^{-8} mol/L (defined as three times standard deviation of blank solution).

3.3. Effect or pH

The effects of pH on the fluorescence intensity of **1** in the presence of Cu^{2+} were carried out at a pH range from 5.0 to 9.0 with fixed the Cu^{2+} concentration at 5 μmol/L (**Figure 4**). In lower pH value, the fluorescence intensity of **1** decreased with decreasing pH value, which might be caused by the protonation of Compound **1** without binding with the metal ion. On the other hand, too high pH would lead to form the precipitation of Cu(OH)$_2$, and reduce its complexation with **1**. In a wide range of pH from 6.0 to 8.0, acidity did not affect the determination of Cu^{2+} with Compound **1**. In other words, the response behavior of Compound **1** is independent of pH in medium condition, which is convenient for practical applications of the proposed probe in determination of Cu^{2+}.

3.4. Selectivity

Under the same conditions, the ability of **1** to recognize Cu^{2+} was further investigated by mixture 100 μmol/L Cu^{2+} with the other background anions and metal ions. The experiments were carried out by recording the

changes of the fluorescence intensity before and after adding the interferants into the pH 7.4 HEPES buffer solution. As shown from **Figure 5**, one can see that the proposed probe exhibited a relatively high selectivity for Cu^{2+} ions over a large number of mono-, bi-, and trivalent cations. Fortunately, normal interferents like Hg^{2+} do not interfere, which is better than that of the probe reported in literatures.

3.5. Preliminary Analytical Application

The proposed probe was applied to the determination of copper ions in water samples of Xiang River. The river water samples were simply filtrated and showed that no Cu^{2+} was present in them. All the water samples were spiked with standard Cu^{2+} solutions at different concentration levels, and then analyzed their concentrations with proposed Probe **1**. Results are shown in **Table 1**. One can see that the recovery study of spiked Cu^{2+}
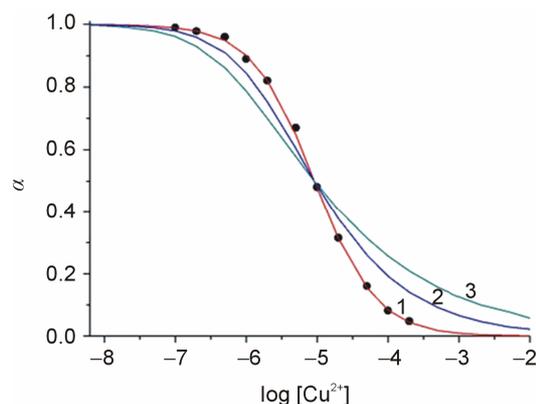

Figure 3. Relative fluorescence intensity α of 1 as a function of log[Cu^{2+}] in CH$_3$CN/H$_2$O (1:1, v/v) solution. The curves fitting the experimental data were calculated from Equation (6). (1) m:n = 1:1, K= 1.09×10^5; (2) m:n = 1:2, K= 1.09×10^{10}; (3) m:n = 1:3, K= 1.46×10^{15}. (●) data points experimentally obtained.
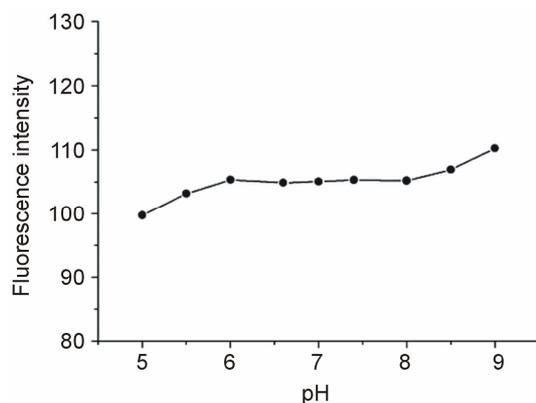

Figure 4. Effect of pH on the emission of 1 (10 μmol/L) with 5 μmol/L Cu^{2+} at 715 nm in CH$_3$CN/H$_2$O (1:1, v/v) solution. Excitation was provided at 640 nm.

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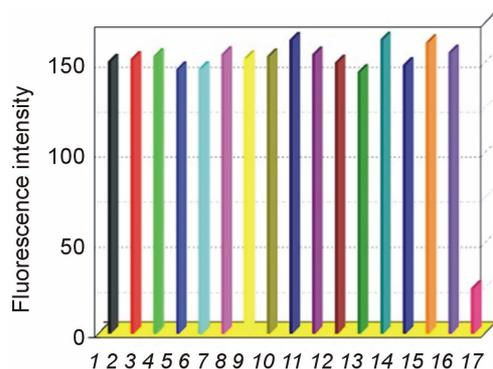**Figure 5. Emission change of 1 at 715 nm upon addition of each cation in CH_3CN/H_2O (1:1, v/v) solution at pH 7.40 {0.1 mol/L HEPES, I = 0.1 ($NaNO_3$)}: 1, none; 2, Zn^{2+}; 3, Cd^{2+}; 4, Mn^{2+}; 5, Ca^{2+}; 6, Mg^{2+}; 7, K^+; 8, Na^+; 9, Li^+; 10, Al^{3+}; 11, Co^{2+}; 12, Ni^{2+}; 13, Pb^{2+}; 14, Hg^{2+}; 15, Fe^{3+}; 16, Ag^+ 17, Cu^{2+}. With the exception of Cu^{2+}, Hg^{2+} and Al^{3+} where 10^{-4} mol/L of cation were added, each solution contained 10^{-3} mol/L of interest. The concentration of 1 was 10 µmol/L and excitation was provided at 640 nm.**

Table 1. Recovery study of spiked determination of copper in Xiang River water with proposed Probe 1.

Sample	Cu^{2+} spiked (mol/L)	Cu^{2+} recovered (mol/L)	Recovery (%)
1	0	0	–
2	1.0×10^{-5}	$(1.02^a \pm 0.03^b) \times 10^{-5}$	102.0
3	5.0×10^{-6}	$(4.93^a \pm 0.04^b) \times 10^{-6}$	98.6

[a]Average were calculated with n = 3, [b]Standard deviations.

determined by the **1**-based probe showed satisfactory results. The present probe is useful for the determination of Cu^{2+} in real samples.

4. Conclusion

In summary, a new near-infrared fluorescent probe was designed and synthesized for the detection of Cu^{2+} based on quenching the fluorescence of tricarbocyanine chromophore with **1**-Cu^{2+} complexation. Compared to reported fluorescent probes, **1**-based probe showed high selectivity and large stokes shift over existing reagents and methods for the fluorescence determination of Cu^{2+} in neutral medium. And the proposed method can be used for the determination of Cu^{2+} in real samples.

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