J Fluoresc
DOI 10.1007/s10895-016-1922-0

ORIGINAL ARTICLE

A Readily-Synthesized Fluorescent Probe Based on N, N-Bis (Pyridin-2-Ylmethyl) Aniline for Copper(II) Detection in Aqueous Solution

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Received: 22 June 2016 / Accepted: 26 August 2016

Abstract In this paper, probe **1** based on N, N-bis (pyridin-2-ylmethyl) aniline is prepared as an effective fluorescent probe for Cu^{2+}. It exhibits good sensitivity and selectivity for Cu^{2+} over other metal ions in aqueous solution. The detection limit of probe **1** is 56 nM. Furthermore, probe **1** is pH-insensitive under near-neutral conditions and can work well in environmental samples.

Keywords Fluorescent probe · Copper ion · Aqueous solution

Introduction

Copper has received great attention in chemical, environmental, and biological systems in that it is a transition metal essential for plants, animals and humans [1, 2]. At higher level, however, it is very harmful to organisms such as bacteria, viruses, and humans [3, 4]. Variety of copper ions was investigated to be associated with some serious diseases such as prion diseases and Alzheimer's disease [5–7]. In addition, copper deficiency may cause Menkes disease, colon cancer, and so on [8, 9]. Therefore, the measurement and detection of copper ions has become more and more significant. So far, many analytical technologies have been developed to detect Cu^{2+}, including Cu^{2+}-specific-DNAzyme based fluorescence probes [10], atomic absorption spectrometry [11], quantum-dot based measurements [12] and electrochemical probes [13]. Although these technologies show advantages of good sensitivity and selectivity, their application is affected by the need for complicated equipment and difficult synthesis procedure. Different from other analytical techniques, fluorescent probes for the detection of Cu^{2+} have had great popularity due to specificity, high sensitivity, low cost, convenient, and real-time monitoring in biological and environmental samples [14]. In this terms, the design and synthesis of Cu^{2+} probes have attracted considerable attentions in recent years and demand is still high [15–17]. In the course of practical application, different environmental samples were measured, for example, tap, lake and river water or human hair, serum and urine, which have been widely reported [18–20]. It can be found that many factors can have an impact on measuring environmental samples, such as pH, coexisting ions interference, temperature, and so on. In view of the above questions, we designed a simple, water-soluble fluorescent probe, probe **1**, based on N, N-bis (pyridin-2-ylmethyl) aniline, applied to the detection Cu^{2+} in pure water system. It exhibits good sensitivity and selectivity for Cu^{2+} over other metal ions in aqueous solution. Furthermore, probe **1** is pH-insensitive under near-neutral conditions and can work well in environmental samples.

Experimental

Materials and Instruments

All materials were purchased from commercial suppliers and used without further purification. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) was used for column

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Electronic supplementary material The online version of this article (doi:10.1007/s10895-016-1922-0) contains supplementary material, which is available to authorized users.

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chromatography. ^{1}H NMR spectra were acquired on a Bruker AMX400 spectrometer. Chemical shifts (δ) were reported in ppm relative to a Me_4Si standard. Electrospray ionization (ESI) mass spectra were measured with an LC-MS 2010 A (Shimadzu) instrument. Fluorescence measurements were obtained on a Hitachi F-2500 fluorescence spectrometer with a 10 mm quartz cuvette. The pH measurements were made with a Sartorius basic pH-meter PB-10.

Synthesis of Compound 1

The $POCl_3$ (9 mL, 97 mmol) was added to DMF (30 mL), the mixture was cooled to 0 °C in an ice-water bath. After stirring for 0.5 h, a mixture of N, N-bis (pyridin-2-ylmethyl) aniline (5.4 g, 20 mmol) and DMF (10 ml) was added. The reaction solution was heated to 95 °C for 5 h, then the reaction was terminated. The reaction solution was adjusted to a neutral pH value by a saturated K_2CO_3 solution, extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, finally Compound **1** was isolated by silica gel column (Fig. 1) (VPE / VEA = 5/1). NMR of Compound **1** is shown in Supporting Information, Fig. S1.

Synthesis of Probe 1

(4-methyl-n-methyl-pyridinium hydriodide) (2.36 g, 10 mmol), compound **1** (3.03 g, 10 mmol) and 30 mL morpholine (morpholine) was sequentially added to 50 ml of n-butanol, and refluxed 2.5 h. Then, the reaction mixture was cooled to room temperature, orange solid was precipitated, suction filtration, and further separated by silica gel column (CH_2Cl_2 as eluent). ESI -MS calcd for $C_{26}H_{24}N_4$ $[M]^+$ 393.2074, found 393.2067. Probe **1** was finally collected (Fig. 1) (2.5 g, 49.3 %). HRMS of Probe **1** is shown in Supporting Information, Fig. S2.

Results and Discussion

Spectral Response of Probe 1 to Cu^{2+}

The spectral response of probe **1** to Cu^{2+} was studied in PBS buffer (5 mM, pH 7). In the absence of Cu^{2+}, probe **1** displayed one major absorption band centered at 426 nm with a corresponding orange-colored fluorescence maximum at 575 nm (Figs. 2a, b). After Cu^{2+} addition, the maximum absorption peak underwent a 81 nm blue shift from 426 to 345 nm, coupled with the fluorescence intensity decreased to original 8 % (Fig. 2b).

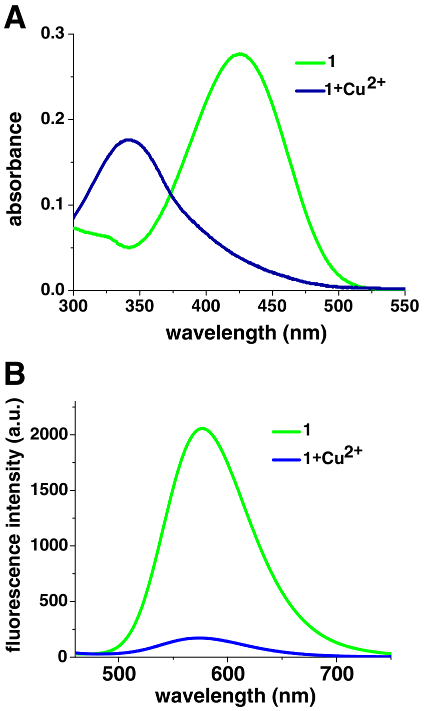


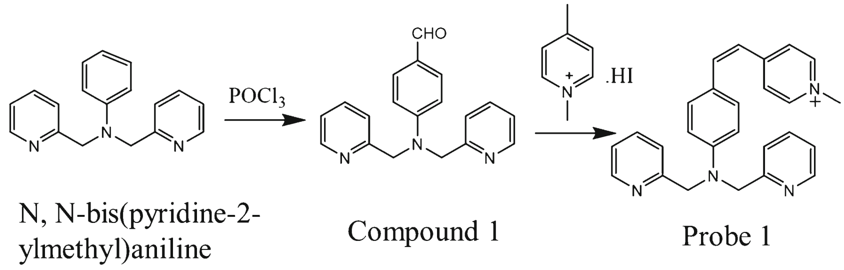
Fig. 2 **a** Absorption spectra and **b** fluorescence spectra of probe **1** (5 μM) before and after addition of Cu^{2+} (15 μM), measured in PBS buffer (5 mM, pH = 7). The spectrum was acquired after Cu^{2+} addition for 0.5 h at room temperature. Λ_{ex} = 385 nm

The fluorescence quenching phenomenon was attributed to the strong chelation of Cu^{2+} with probe **1**, which decreases the electron-donating ability of the central nitrogen atom of probe **1** and thereby results in a decrease in intramolecular charge transfer (ICT) efficiency [21]. In addition, the paramagnetic nature of Cu^{2+} can also be a factor for the quenching. According to Job plot (Fig. S3), probe **1** and Cu^{2+} formed a 1:1 stoichiometry complex [22].

Sensitivity of Probe 1 toward Cu^{2+}

Probe **1** showed high sensitivity for Cu^{2+} detection in PBS solution (5 mM, pH =7). As seen in Fig. 3a, upon an increasing

Fig. 1 The schematic synthesis route of probe **1**



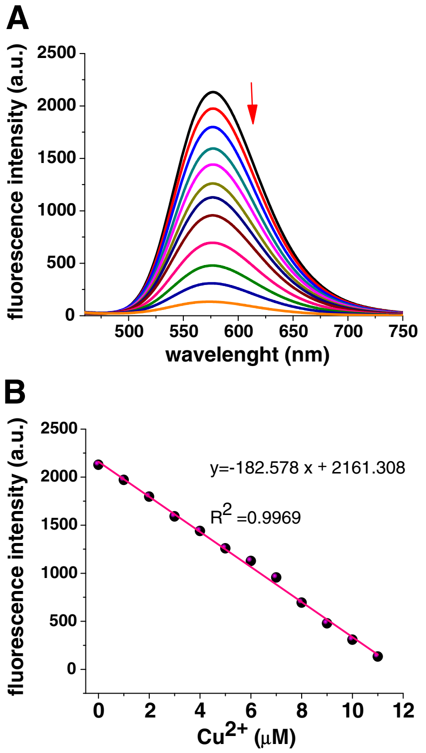


Fig. 3 **a** Fluorescence responses of probe **1** (5 μM) toward Cu^{2+} (final concentration: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 μM). **b** the linear relationship between fluorescence intensity and Cu^{2+} concentrations. All experiments were performed after reacting with metal ions for 0.5 h in PBS solution (5 mM, pH = 7). λ_{ex} = 385 nm

addition of Cu^{2+} (final concentration: 0 ~ 11 μM), the solution containing probe **1** (5 μM) presented a gradual decrease in fluorescent intensity at 575 nm. There was a good linearity (R^2 = 0.9969) between the fluorescence intensity and Cu^{2+} concentrations in the range of 0 ~ 11 μM (Fig. 3b). The detection limit of probe **1** is 56 nM (Detection limit =3σ/k [26], Where σ is the standard deviation of blank measurement, k is the slope of the plot of fluorescence intensity versus Cu^{2+} concentration.). As shown in Table 1, the detection limit of several techniques was listed and compared [23–25].

The Selectivity of Probe 1 to Cu^{2+}

To evaluate the selectivity of probe **1**, it was tested with other environmentally relevant metal ions, including K^+, Na^+, Ag^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Al^{3+} and Fe^{3+}. Under the same conditions (5 mM PBS, pH = 7), probe **1** exhibited no apparent fluorescence change in the presence of other metal ions (Fig. 4). Moreover, the interference of the various metal ions on monitoring Cu^{2+} was also studied. These results showed probe **1** possesses high selectivity toward Cu^{2+} even in the presence of other metal ions.

Table 1 The comparison of detection limit of several techniques

Technique	Detection limit	Reference
Electrochemical sensor	0.03 μM	23
Cu^{2+}-dependent DNA ligation DNAzyme	5 μM	24
Colorimetric methods	0.9 μM	25
Fluorescent probe	0.056 μM	This work

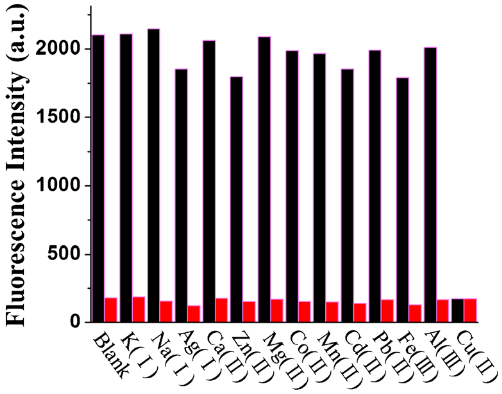


Fig. 4 The black bar: fluorescence responses of probe **1** (5 μM) toward various metal ions (15 μM for all ions); The red bar**:** fluorescence responses of probe **1** (5 μM) toward Cu^{2+} in the presence of various metal ions (15 μM for all ions). All experiments were performed after reacting with metal ions for 0.5 h in PBS solution (10 mM, pH = 7). λ_{ex} = 385 nm

pH-Dependent Fluorescence of Probe 1

Finally, the effect of pH changes on the fluorescence detection of Cu^{2+} was tested. As shown in Fig. 5, probe **1** presented limited changes toward pH in the range of 3–11, which proves that probe **1** is pH-insensitive under near-neutral conditions. The same result was obtained when Cu^{2+} was added in the presence of the probe. Therefore, probe **1** could work well in environmental samples.

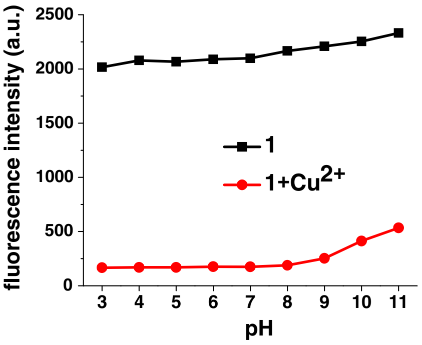


Fig. 5 The fluorescence intensity at 575 nm responds to various PBS solution with different pH values = 3, 4, 5, 6, 7, 8, 9, 10, 11; the black line: probe **1** (5); the red line: probe **1**(5 μM) + Cu^{2+} (15 μM); λ_{ex} = 385 nm

Conclusions

In summary, We have successfully developed a readily-synthesized fluorescent probe for copper ions (Cu^{2+}) in aqueous solution. It displayed several adventages because of high sensitivity, selectivity, low-cost and operational simplicity. The detection limit of probe **1** is 0.056 μM, which was far below the WHO acceptable limit (31.5 μM) in drinking water. In our further study, practical applications using real environmental samples will be carried out, and the proposed method may have potential use in many application areas such as biological and ecological samples.

Acknowledgments We gratefully acknowledge assistance from Dr. Hailiang Nie.

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