A “turn-on” fluorescence probe for Cu$^{2+}$ detection has been reported according to a Cu$^{2+}$ triggered spirolactam ring-opening reaction. The probe is a double-responsive fluorescent and colorimetric Cu$^{2+}$-specific sensor in aqueous solution containing 20% of acetonitrile with high selectivity and excellent sensitivity (limit of detection is 12 μg L$^{-1}$). Furthermore, the significant color changes visible to the naked eye at the concentration of 3 μM (ca. 0.20 mg L$^{-1}$) are about ten times lower than the WHO (World Health Organization) recommended level (2.0 mg L$^{-1}$) for Cu$^{2+}$ ions in drinking water.

1. Introduction

Copper is the third-most abundant transition metal in the human body, and plays a critical role as a catalytic cofactor for a variety of metalloenzymes and transcriptional events. However, it was reported that overloaded Cu$^{2+}$ might stimulate the production of reactive oxygen species and thus exhibits toxicity associated with Alzheimer’s and Wilson’s diseases. In recent years, Cu$^{2+}$ has also been suspected to cause infant liver damage. Accordingly, the World Health Organization (WHO) has set the maximum allowable level of copper in drinking water at 2.0 ppm (~30 μM). Nevertheless, copper contamination and its potential toxic effects on human beings continue to be challenging problems throughout the world due to the widespread use of Cu$^{2+}$ in agriculture and industry. Therefore, a fast, convenient, and reliable method for Cu$^{2+}$ detection is required, particularly in drinking water monitoring. Fluorescent chemosensors with high specificity and sensitivity, ease and safety of handling have received considerable attentions.

A multifunctional fluorescent chemosensor by modulating the selectivity to various metal ions with the change of the tested media appears to be particularly attractive because of its convenience in real application. Sensitive probes have been designed to selectively differentiate Pb$^{2+}$ and Hg$^{2+}$, Au$^{3+}$ and Hg$^{2+}$, Zn$^{2+}$ and Cu$^{2+}$, and Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ via simple changes of different testing media. These studies demonstrated that solvent effect has a significant influence on detecting selectivity of chemosensors.

Recently, Duan reported a sugar–rhodamine fluorescence probe 1 that could selectively detect Hg$^{2+}$ in natural water and living cells. The introduction of a sugar residue in probe 1 can improve both water solubility and bio-compatibility, thus increasing colorimetric and fluorescent “turn-on” selectivity, and sensitivity toward Hg$^{2+}$ in 100% water and in cellular systems as well. Carbohydrate moieties, containing multi-OH groups, are believed to be good receptors for metal ions, and thus it is reasonable to expect that the probe 1 provides different selectivity through changing the solvent. Herein, we disclose that the sugar–rhodamine fluorescence probe 1 (Scheme 1) would exhibit a highly selective and sensitive fluorescence enhancement responding to Cu$^{2+}$ ion.

2. Results and discussion

As mentioned in a previous report the synthesized probe 1 has high aqueous solubility and can completely dissolve in 100% pure water without adding any toxic organic co-solvent, which was used to detect Hg$^{2+}$ ion in pure water with the fluorescent method. However, a very interesting phenomenon was found in our experiment, that is, the fluorescence signal of probe 1 to Hg$^{2+}$ was significantly quenched by adding acetonitrile (CH$_3$CN) into aqueous media (Fig. 1a). Instead, the probe 1 exhibited a strong fluorescent signal to Cu$^{2+}$, and the fluorescence intensity reached maximum when the ratio of acetonitrile to water was 20/80 (v/v) (Fig. 1b). As shown in three-dimensional excitation emission matrix fluorescence spectroscopy (3D-EEM) (Fig. S1†), the excitation and emission wavelengths of probe 1-Cu$^{2+}$ were at 520 nm/560 nm. A variation of pH from 5.8 to 9.8 does not cause significant changes in fluorescence intensity (Fig. 2), which

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Scheme 1 Possible reaction mechanism of probe 1 with Cu$^{2+}$.
The reversible cation-catalyzed reaction (Scheme 1). Importantly, the

titration of probe 1 with Cu$^{2+}$ (from 1 to 7 equiv.) showed saturation behavior at 2 equiv. of Cu$^{2+}$ (Fig. 4). In order to
explore the reaction mechanism of the present system, detailed
MALDI-TOF mass spectral analyses were conducted (Fig. S3 and S4†). In a short 5 min reaction time, three major peaks at m/z 591.3, 429.2 and 415.2 were observed, which were identical to the
remaining probe 1 (calcd m/z 591.3 [M + H]$^+$), compound 2 (calcd m/z 429.2 [M + H]$^+$) and compound 3 (calcd m/z 415.2
[M + H]$^+$). By elongating the reaction time to 30 min only the
peak at m/z 415.2 corresponding to compound 3 was observed.

To evaluate the selectivity of probe 1, eleven of various cations
(including heavy metal ions that not only coexist but also often
have a similar reactivity) were examined in parallel under the
same working conditions. As shown in Fig. 5, the reaction of
probe 1 (10 μM) with Cu$^{2+}$ (10 μM) produced strong fluorescence
response at λem = 560 nm, while the other cations did not exhibit
the similar behavior even when their concentrations were set up
at hundred times of Cu$^{2+}$ (1.0 mM). This result indicates that the
probe 1 has high specificity toward Cu$^{2+}$ under the working
conditions.

Fig. 4 Fluorescence intensity change (λex/λem = 520/560 nm, slit widths
1.0 nm) of probe 1 (10 μM in water containing 20% of acetonitrile) as
a function of equivalent of Cu$^{2+}$ after 30 min for each addition at room
temperature.
Similarly, the UV absorbance at 520 nm enhanced from 0.001 to 0.17 when increasing the concentration of Cu²⁺ from 0 to 10 μM (Fig. S5†). A satisfactory linear relationship between UV absorbance and Cu²⁺ concentration was observed with the correlation coefficient as high as 0.9940.

Furthermore, the detection limit of Cu²⁺ by the fluorometric method with the probe 1 was determined using both the S/N ratio test method and serial dilution method. It was found that the detection limit was as low as 0.20 μM (12 μg L⁻¹), the relative standard deviation of eleven runs was 5.1%. The detection limit (12 μg L⁻¹) of probe 1 to Cu²⁺ is ca. 160 times lower than the recommended water quality standard of Cu²⁺ (2.0 mg L⁻¹) for drinking water by WHO, EU, and Australia. The present result implies that the probe 1 can be simply, rapidly, and satisfactorily used to detect Cu²⁺ concentration. Apparently, under naked-eye conditions, the pink color of the solution is of correlation with the concentrations of probe 1 and Cu²⁺. Fig. 7 shows the color variations of probe 1 solution in terms of Cu²⁺ concentration. It can be seen that the color changes could be exclusively confirmed with the naked-eye when the Cu²⁺ concentration increased to 3 μM (ca. 0.20 mg L⁻¹), ten times lower than the recommended standard value for Cu²⁺ in drinking water by WHO, EU and Australia. This observation suggested that probe 1 could be used conveniently to screen the Cu²⁺ concentration in the sample with the naked-eye. Based on the optical variation of probe 1, a color chart was prepared according to the correlation of color change and Cu²⁺ concentration. As shown in Fig. 8, the Cu²⁺ concentration in water sample could be identified through comparing the color in the test vial and chart with the naked eye.

3. Conclusions

We have investigated the properties of the fluorescent probe 1 for Cu²⁺ detection based on a Cu²⁺ triggered ring-opening reaction of spirolactam in aqueous medium. The probe 1 is a dual-responsive fluorescent and colorimetric Cu²⁺-specific sensor in aqueous media. Fluorescent probe 1 exhibited an excellent Cu²⁺ ions selectivity over other cations, as well as a satisfactory detection limit at 12 μg L⁻¹. More importantly, the probe displays significant naked-eye color changes as the Cu²⁺ concentration increases, the formed pink color can be obviously recognized by naked eye even at 3 μM (ca. 0.20 mg L⁻¹) of Cu²⁺, which is about ten times lower than the WHO recommended value for Cu²⁺ (2.0 mg L⁻¹) in drinking water. A color chart was prepared according to the correlation of the color appeared and the concentration of Cu²⁺, providing a potential method for visible detection of Cu²⁺ ions. We anticipate that the rapid and accurate naked-eye identification of Cu²⁺, as well as the satisfactory detection limit, would make this approach a very promising choice for Cu²⁺ detection in water. The most interesting is that the same chemosensor can be successfully applied to detect Hg²⁺ and Cu²⁺ by controlling test media. The theme of the molecular design presented here may help the development of more efficient rhodamine-based chemosensor platforms.

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Notes and references


Fig. 6 (a) Fluorescence titration spectra (λex = 520 nm, with slit widths 1.0 nm) of probe 1 (10 μM in water containing 20% of CH₃CN) with Cu²⁺ from 0 to 10 μM at room temperature. (b) The plot of fluorescence intensity change (I/I₀ = 520/560 nm, slit widths 1.0 nm) of probe 1 (10 μM in water containing 20% of CH₃CN) against varied concentrations of Cu²⁺ from 0 to 10 μM at room temperature. (Inset) Dependence of fluorescence intensity at 560 nm with respect to concentrations of Cu²⁺ ions ranging from 0 to 1.0 μM.

Fig. 7 “Naked-eye” color changes of probe 1 (0.5 equiv. to Cu²⁺) in water containing 20% of CH₃CN under natural light upon addition of different concentrations of Cu²⁺ (from 0 to 50 μM) at room temperature.

Fig. 8 The prepared color chart according to the solution color with different concentrations of Cu²⁺ ions.


