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A fluorescence "turn-on" chemodosimeter for Cu^{2+} in aqueous solution based on the ion promoted oxidation†

Junbo Li,* Yang Zeng, Qihui Hu, Xianglin Yu, Jia Guo and Zhiquan Pan*

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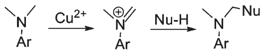
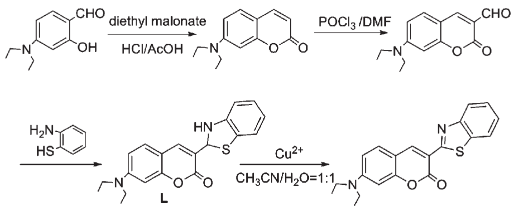
We developed a novel method for Cu^{2+} detection based on the ion promoted oxidation reaction. Chemodosimeter L (weak fluorescence) can be oxidized into 3-benzothiazoly-7-*N*,*N*-diethylaminocoumarin (strong green fluorescence, coumarin 6) by Cu^{2+} with high selectivity and sensitivity in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN solution.

Nowadays, much attention has been paid to the development of new fluorescent chemosensors for metal ions because of their potential applications in the fields of clinical biochemistry, analytical chemistry and environmental chemistry.[1–6] Among these metal ions, Cu^{2+} is a widely used ion in industry and also the third-most abundant transition metal ion in the body, which plays a critical role as a catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome *c* oxidase, and tyrosinase.[7,8] Despite its important role in organisms, the accumulation of excess amounts of copper ions or their misregulation can cause a series of severe diseases such as Alzheimer's and Parkinson's diseases.[9] Therefore, the rapid detection of Cu^{2+} is very important in environmental and biological systems. So far, many Cu^{2+} fluorescent probes have been reported, however, most of them, especially the early reported ones,[10–12] were based on the fluorescence quenching response upon binding with Cu^{2+}, which is not as sensitive as fluorescence enhancement response. Currently, many signal processes for fluorescence enhancement response for Cu^{2+} have been proposed such as the ring opening of rhodamine derivatives,[13,14] the prohibition of the isomerization of C=N,[15,16] the coordination of N or O atoms,[17,18] the hydrolysis of ligand,[19,20] and the click cycloaddition reaction.[21] However, it should be noted that the Cu^{2+} promoted oxidation reaction has not been used as a signal process for chemosensors, although it has been used widely in organic synthesis.[22–24]

In this manuscript, we demonstrated a new method for Cu^{2+} detection by metal ions induced oxidation, which was based on the easy oxidation of benzothiazoline.[25–27] Wang *et al.* reported the synthesis of 3-benzothiazoliny-7-*N*,*N*-diethylaminocoumarin (**L**), which showed weak fluorescence due to the effective photo-induced electron transfer (PET) process from N atom to coumarin. They demonstrated the enzyme sensing properties of **L** based on the controllable oxidation of benzothiazoline.[28] Our group focuses our interests on the transition metal ions detection using functional dyes such as rhodamine and coumarin.[29,30] Cu-catalyzed oxidative coupling reactions have been reported widely (Scheme 1).[22–24] The structure of benzothiazoline is similar to the amines used in these catalyzed oxidative coupling reactions. With this idea in mind, we proposed that the oxidation of 3-benzothiazoliny-7-*N*,*N*-diethylaminocoumarin (**L**) (weak fluorescence) to 3-benzothiazoly-7-*N*,*N*-diethylaminocoumarin (strong green fluorescence) can be promoted by metal ions such as Cu^{2+}. Fortunately, the fluorescence of 3-benzothiazoliny-7-*N*,*N*-diethylaminocoumarin (**L**) in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN solution is weak ("off" state). However, when 10 equiv. of Cu^{2+} was added into the solution, a strong green fluorescence ("on" state) was observed. Meanwhile, other common metal ions such as Fe^{3+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+}, Ag^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Na^{+} and K^{+} induced little fluorescence intensity enhancement.



Scheme 1 Mechanism for Cu^{2+} catalyzed oxidative coupling reaction.



Scheme 2 Synthesis and Cu^{2+} promoted oxidation of chemodosimeter **L**.

Chemodosimeter **L** was readily synthesized in three steps (Scheme 2). The 7-diethylaminocoumarin was prepared according to the literature[31] and the 7-diethylaminocoumarin-3-aldehyde was obtained using the classical Vilsmeier reaction.[32] Chemodosimeter **L** was obtained by condensation of 7-

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Hubei Novel Reactor & Green Chemical Technology Key Laboratory, Key Laboratory for Green Chemical Process of Ministry of Education, Wuhan Institute of Technology, 693 Xiongchu Avenue, Wuhan, Hubei Province, 430074, PR China. E-mail: jbli@mail.wit.edu.cn, zhiqpan@163.com

† Electronic supplementary information (ESI) available: Synthesis of compound **L**, experimental details, NMR spectra, MS spectra, and additional spectra. See DOI: 10.1039/c2dt12497a

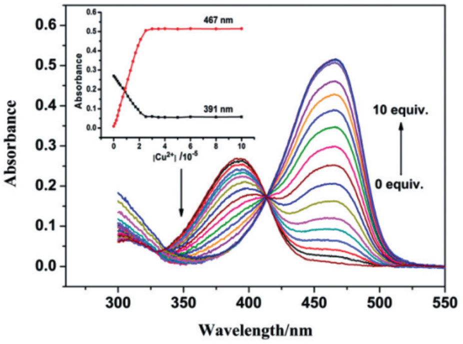
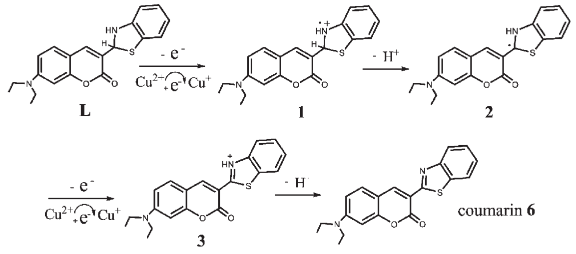


Fig. 1 The UV-vis absorption spectra of **L** (10 μM) in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN in the presence of increasing concentrations of Cu^{2+} (0–10 equiv.). The inset plots the absorbance at 391 nm and 467 nm *versus* the Cu^{2+} concentration.



Scheme 3 Possible mechanism for Cu^{2+} promoted oxidation of **L**.

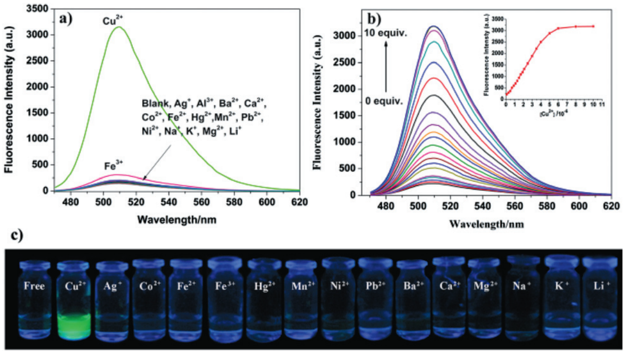


Fig. 2 (a) Fluorescence response of **L** (1 μM) upon addition of different metal ions (10 equiv.) in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN. (b) Fluorescence response of **L** (1 μM) upon addition of different concentration of Cu^{2+} (0–10 equiv.). The inset plots the fluorescence intensity at 509 nm *versus* Cu^{2+} concentration. (c) Photograph of **L** (1 μM) with different metal ions (10 equiv.).

diethylaminocoumarin-3-aldehyde with 2-aminobenzenethiol with a yield of 60%.[28]

Fig. 1 shows the UV-vis absorption spectra of **L** in the presence of different concentrations of Cu^{2+} ions. When increasing concentrations of Cu^{2+} were introduced in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN solution, the maximum absorption wavelength at 391 nm was decreased accomplished with the increase of the absorption at 467 nm. Furthermore, an isosbestic point at 413 nm was observed, which indicated the formation of a new compound. Meanwhile, **L** showed weak fluorescence in the absence of Cu^{2+} in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN. By contrast, upon addition of 10 equiv. of Cu^{2+}, a remarkable fluorescence intensity enhancement at 509 nm was observed. The fluorescence intensity reached maximum within 1 min (Fig. S1†). Furthermore, the addition of the chelating reagent EDTA or diethylenetriamine to the solution did not reduce the fluorescence intensity (Fig. S2†), which demonstrated that the oxidation process is irreversible.[33,34] Moreover, the absorption and fluorescence of **L** in the presence of 10 equiv. of Cu^{2+} were the same as those of the authentic sample of 3-benzothiazoly-7-*N*,*N*-diethylaminocoumarin (coumarin 6), indicating the Cu^{2+} induced formation of it (Fig. S3, S4†). Besides, for a practical application, the stability of **L** solution in different pH was also investigated. As demonstrated in Fig. S5,† **L** solution (50% (v/v) water–CH_3CN) was stable at a wide pH span (4–11). Meanwhile, **L** could also detect Cu^{2+} smoothly at the pH span from 4 to 11.

In order to investigate the necessity of the oxygen in the oxidation of **L**, the solution of **L** was firstly purged with nitrogen for 20 min to remove the oxygen, then the 10 equiv. of Cu^{2+} was added and the fluorescence spectra were recorded (Fig. S6†). The results showed that the fluorescence spectra were similar in the absence and presence of oxygen, indicating that the benzothiazoline was oxidized by Cu^{2+}. It is well known that the oxidizability of Fe^{3+} is stronger than Cu^{2+}. However, when 10 equiv. Fe^{3+} was added into the solution of **L**, little fluorescence intensity enhancement had been observed even at elevated temperatures (Fig. S7†), which indicated that Fe^{3+} cannot oxidize benzothiazoline to benzothiazole in the used solvent system. Furthermore, the common oxidants such as H_2O_2, TBHP, NaClO (10 equiv.) could lead to a weak fluorescence intensity enhancement (Fig. S8†). We proposed that the oxidation of benzothiazoline to benzothiazone by Cu^{2+} was the synergistic effect of coordination and oxidization, and some substances with stronger oxidizability such as Fe^{3+}, H_2O_2, TBHP, NaClO showed little oxidation. Moreover, as the ratio of the solvent (V_{CH3CN}/V_{HEPES}) changed from 9 : 1 to 1 : 9, the fluorescence intensity of the solution in the presence of 10 equiv. Cu^{2+} was decreased and the maximum emission wavelength was also red shifted from 505 nm to 511 nm (Fig. S9†), the reason for which might be that the water weakened the coordination between Cu^{2+} and ligand **L**. The possible oxidative mechanism was shown in Scheme 3. The oxidation of **L** using Cu^{2+} proceeds *via* initial one-electron oxidation to give an aminium cation radical (**1**), and then the radical (**2**) may also be formed by the proton loss from **1** followed by a further one electron oxidation to give an iminium cation (**3**).[35,36] Finally, the one proton loss gives the oxidation product coumarin **6**. Moreover, the formed oxidation product was separated and confirmed by the characterization such as ^1H NMR, ^{13}C NMR and MS.

To investigate the selectivity, representative ions such as Cu^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+}, Ag^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Na^+ and K^+ were added into the solution of chemodosimeter **L** in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN. Changes of the fluorescence spectra of **L** upon addition of different metal ions were shown in Fig. 2a. Chemodosimeter **L** only showed very weak fluorescence due to

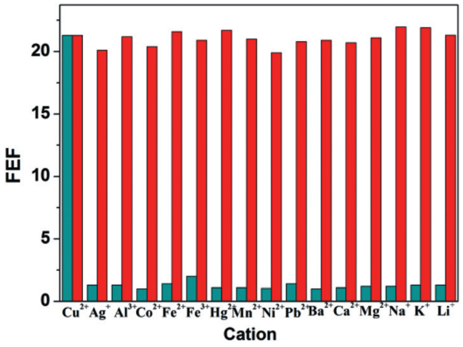


Fig. 3 Fluorescence enhancement factors (FEF) of **L** (1 μM) in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN. Blue bars represent addition of different metal ions (10 equiv.) to the solution. Red bars represent addition of Cu^{2+} (10 equiv.) and different metal ions (10 equiv.) to the solution. Excitation and emission were at 457 nm and 509 nm, respectively.

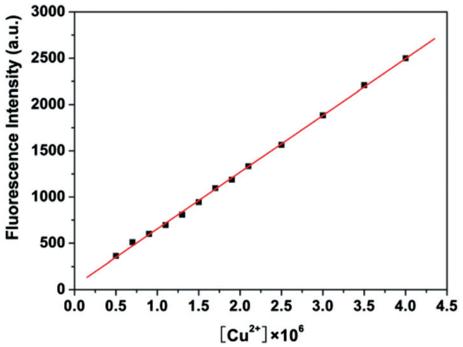


Fig. 4 Plot of fluorescence intensity *versus* the concentration of Cu^{2+}.

the effective PET process from the N atom to coumarin. Upon addition of Cu^{2+} (10 equiv.), the maximum emission peak at 509 nm was enhanced by nearly 22-fold. While other metal ions showed no fluorescence enhancement except Fe^{3+} showed a 2-fold enhancement. Therefore, compound **L** could detect Cu^{2+} with high selectivity. Meanwhile, titration of **L** with Cu^{2+} was also performed in HEPES (10 mM, pH = 7.4) buffer containing 50% (v/v) water–CH_3CN. With increasing Cu^{2+} concentration, the emission of **L** was drastically increased with a fluorescence enhancement factor (FEF) up to 22-fold (Fig. 3). The interference of other metal ions to the detection of Cu^{2+} was also investigated. Cu^{2+} (10 equiv.) were added into the solution of **L** in the presence of other metal ions (10 equiv.). As shown in Fig. 3, apparently, Ag^+, Co^{2+} and Ni^{2+} show little interference with relative errors of 5.7%, 4.3% and 6.7%, respectively. Other metal ions had no interference on the detection of Cu^{2+} with relative errors of no more than 3.0%. The results showed that common metal ions had no interference on the detection of Cu^{2+}, which made it feasible for practical application. Meanwhile, the fluorescence intensity was linear with Cu^{2+} concentration in the range of 5×10^{-7}–4×10^{-6} M. The linear regression equation was determined to be $F = 613.50 \times 10^6 \times [Cu^{2+}] + 41.69$ ($n = 13$, $R = 0.99958$) (Fig. 4). The detection limit was calculated to be 58 nM with the equation: detection limit = $3S_d/\rho$, where S_d is the standard deviation of blank measurement; ρ is the slope between the fluorescence intensity *versus* Cu^{2+} concentration.[29]

In summary, we described a fluorescence turn-on chemodosimeter for Cu^{2+} based on the ion promoted oxidation of benzothiazoline to benzothiazole. Chemodosimeter **L** could detect Cu^{2+} with high selectivity and sensitivity in aqueous solution. Meanwhile, other common metal ions had little interference on the detection of Cu^{2+}. This method opens up a new way for the detection of metal ions. Further efforts in our laboratory will focus on the detection of other possible substances with this easy oxidation process from benzothiazoline to benzothiazole.

Acknowledgements

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