Talanta 119 (2014) 601–605

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Highly sensitive and selective determination of cupric ions by using N,N′-bis(salicylidene)-*o*-phenylenediamine as fluorescent chemosensor and related applications

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ARTICLE INFO

Article history:
Received 15 August 2013
Received in revised form
18 November 2013
Accepted 24 November 2013
Available online 1 December 2013

Keywords:
Fluorescence quenching
N,N′-bis(salicylidene)-*o*-phenylenediamine
Copper ions
Fluorescence switch

ABSTRACT

A sensitive and selective copper(II) fluorescence Schiff base chemical sensor receptor 1 (short for N,N′-bis (salicylidene)-*o*-phenylenediamine) has been prepared. The fluorescence of receptor 1 in pH 8.2 phosphate buffer solution can be dramatically quenched by Cu^{2+}, whereas it is nearly unaffected by other metal ions. Based on this, a sensitive and selective fluorescent quenching method for Cu^{2+} detection has been established. Under the optimum conditions, a good linear relation exists between the quenching efficiency (F_0/F) and the concentration of Cu^{2+} in the range of 1.0×10^{-7}–2.5×10^{-6} mol L^{-1}. The detection limit (3σ) for Cu^{2+} determination is 2.0×10^{-8} mol L^{-1}. The present method has been successfully used for quantification of Cu^{2+} in soybean milk powder. Furthermore, the fluorescence switch property of the system was explored, and the system might be applied for determination of glutathione and construction of molecular logic gate.

1. Introduction

Copper is one of the most important heavy metals and additives, and widely utilized in industrial and agricultural field. Although copper is an important trace mineral for metabolism of life, the excessive intake of copper could result in liver damage in infants and some functional handicaps [1]. Therefore, precise and accurate determination of trace amounts of copper in food products and drinking water is an important problem.

Numerous analytical methods have been applied for detection of copper. The common methods for determination of traces of copper are atomic spectroscopy, such as FAAS and ICP-OES [2,3]. The atomic spectroscopy methods offer low detection limits and accurate results but require expensive analytical instrumentation. Electrochemical methods, for instance, square wave anodic stripping voltammetry [4] and potentiometric analysis [5,6] have been used in copper determination. Spectrophotometric methods based on diphenylcarbazide [7] and 5-Br-PSAA [8] have been developed. Compared with these methods, spectrofluorimetry has merit in view that it is more sensitive, convenient, and simpler than other techniques. In fluorimetric methods, fluorescence enhancing techniques based on chemosensors triazolylpyrene [9], naphthalimide modified rhodamine B [10] and 8-aminoquinoline-5-azo derivatives [11] have been utilized for the determination of copper(II). Among quenching effect, sensors based on Tiron [12], Lucifer Yellow embedded in a hydrogel [13] have been used for sensing Cu^{2+}. Unconventional fluorescence probes such as lanthanide chelates [14–17] and quantum dots(QD) [18–20] have been presented for Cu^{2+} sensitive detection. Recently, a new carbon-based QDs system [21] with low toxicity compared with heavy-metal-based QDs has been designed for Cu^{2+} ions detection.

Among these fluorescence methods, Schiff bases are attractive fluorescent chemosensors, because they enable simple and inexpensive determinations of various metal ions [22]. Owing to the relatively simple preparation procedures of Schiff base, it is possible to obtain ligands of different design and characteristics by selecting appropriate reactants. The Cu^{2+} ion is known to have a particularly high thermodynamic affinity for typical N, O-chelators. Therefore, a high sensitive and selective Cu^{2+} Schiff base fluorescence chemosensor receptor 1 (short for N,N′-bis (salicylidene)-*o*-phenylenediamine) was designed in our work by the condensation reaction of salicylaldehyde with *o*-phenylenediamine. Also receptor 1 has been used to measure mercury by electrochemical method [23] and determine copper(II) by optical method [24]. Moreover, applications of [receptor 1]–metal complexes in biomedical field, such as antitumor activity [25], inhibition of proteasome [26] and overcoming multiple drug resistance in lymphoma and leukemia cells [27] have been previously reported. However, the use of receptor 1 for the determination of cupric ions in aqueous solutions by means of fluorescence has not been reported so far.

In this work, we firstly investigated a Schiff base receptor 1 as fluorescence probe for Cu^{2+} detection in aqueous system, including quenching mechanism, selectivity, sensing conditions optimization,

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http://dx.doi.org/10.1016/j.talanta.2013.11.066

measuring range and practical application. This sensing method has been proved to be a rapid, selective, sensitive way for trace copper detection. What is more, we further studied fluorescence switch property of receptor 1 based on the control of Cu^{2+} and glutathione.

2. Experimental section

2.1. Apparatus

Fourier transform infrared (FT-IR) spectrum was performed on an FT-IR spectrophotometer (IR-408PC, BRUKER). ^{1}H NMR spectra were acquired on a Varian Mercury VX-300 MHz spectrometer. UV/vis absorption spectra were obtained on a UV/vis spectrophotometer (UV-2550, SHIMADZU). Fluorescence spectra were recorded by fluorescence spectrophotometer (RF-5301PC, SHIMADZU). Atomic absorption spectrophotometer (AA-300, PERKINELMER) was used to determine the concentration of Cu^{2+} in sample.

2.2. Reagents and solutions

Salicylaldehyde (CP), *o*-phenylenediamine (CP) and ethanol (AR) all purchased from Sinopharm Chemical Reagent Co., Ltd. (China) were used to prepare receptor 1. Stock solution of receptor 1 (4.05×10^{-4} mol L^{-1}) was prepared by dissolving 0.0032 g receptor 1 with 2 mL DMF, 0.25 mL HCl (0.10 mol L^{-1}), then diluting to 25 mL volumetric flask with double-distilled water. The receptor 1 solution was stored in a refrigerator at 4 °C and could remain stable for 5 days. Standard working solutions of receptor 1 were obtained by suitable dilutions of the stock solution immediately prior to use. All other reagents were of analytical grade. Doubly distilled water was used throughout the experiments. Cu(II) standard solutions were prepared from $Cu(NO_3)_2 \cdot 3H_2O$ (AR, Sinopharm Chemical Reagent Co., Ltd., China). Phosphate buffer solutions (0.20 M) of different pH values were prepared by mixing Na_2HPO_4 solution (0.20 M) and NaH_2PO_4 solution (0.20 M) in right ratios.

2.3. Synthesis and characterization of receptor 1

Receptor 1 was prepared according to previously reported synthesis method of Schiff base [28] and well characterized by ^{1}H NMR and FT-IR spectroscopic techniques. Synthesis of receptor 1(as depicted in Fig. 1): A solution of *o*-phenylenediamine (1.08 g, 0.01 mol) and salicylaldehyde (2.44 g, 0.02 mol) in ethanol (15 mL) was refluxed for 4 h. An orange solid product was separated out and washed with ethanol for three times. After drying the product, 2.58 g orange solid was obtained with the yield of 81.7 %.

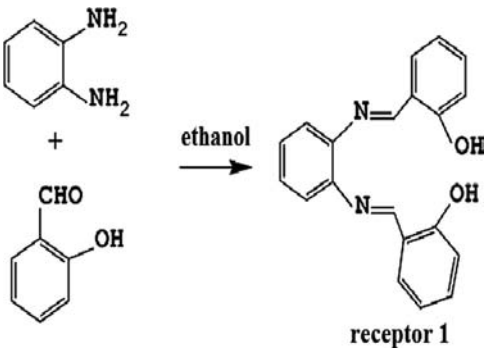

Fig. 1. Synthesis of receptor 1.

2.4. Sample pretreatment

3.0 g soybean milk powder in crucible was heated on the electric furnace until it became ash. For further incineration, the sample was heated for another 2 h in a muffle furnace under 800 °C. After being completely incinerated, it was dissolved by approximately 3 mL of concentrated nitric acid and diluted to 20 mL. The sample solution was appropriately diluted and adjusted to the same acidity of standard copper(II) ions solution before being detected.

2.5. Analytical procedure

250 μL 1.0×10^{-4} mol L^{-1} receptor 1, 1 mL 0.20 M PBS (pH=8.2), certain volume of standard solution of Cu^{2+} (1.0×10^{-4} mol L^{-1}) were successively added into a 10 mL colorimetric tube and diluted to the mark with double-distilled water. 5 min for system stability, the fluorescence measurements were performed at $\lambda_{ex}/\lambda_{em}$ 324/430 nm.

3. Results and discussion

3.1. Fluorescence property and fluorescence response to Cu^{2+} of receptor 1

The fluorescence property of receptor 1 was firstly investigated. As it shown in Fig. 2, 2.5 μM receptor 1 solution exhibits strong fluorescence emission with maximum excitation wavelength at 324 nm. The strong emission of receptor 1 can be quenched obviously in the presence of 1.5 μM Cu^{2+} ions. Therefore, a sensing method for Cu^{2+} is possibly established based on quenching the fluorescence of chemosensor receptor 1.

3.2. Fluorescence quenching mechanism

The UV/vis absorption results (Fig. 3) show that the receptor 1 has two absorption bands centered at 256 nm and 324 nm. However, the addition of Cu^{2+} into the receptor 1 solution results in the increase of the absorption band centered at 324 nm and emerging a new absorption band range from 350 nm to 450 nm, and these two absorption bands enhance with the addition of increasing amount of Cu^{2+} ions. The variations of UV/vis absorption indicate that a nonluminous complex is formed through the

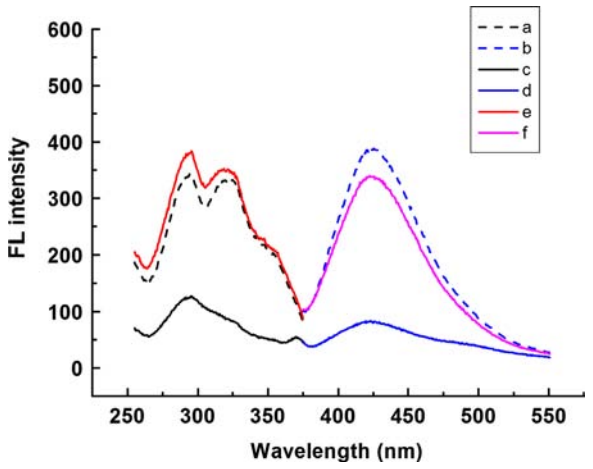

Fig. 2. Fluorescence excitation(a, c, e) and emission(b, d, f) spectra of 2.5 μM receptor 1 (a, b), 2.5 μM receptor 1 quenched by 1.5 μM Cu^{2+} ions (c, d), 2.5 μM receptor 1 quenched by 1.5 μM Cu^{2+} ions and recovered by 5 μM EDTA (e, f). Above solutions were regulated by PBS (0.2 M, pH 8.2).

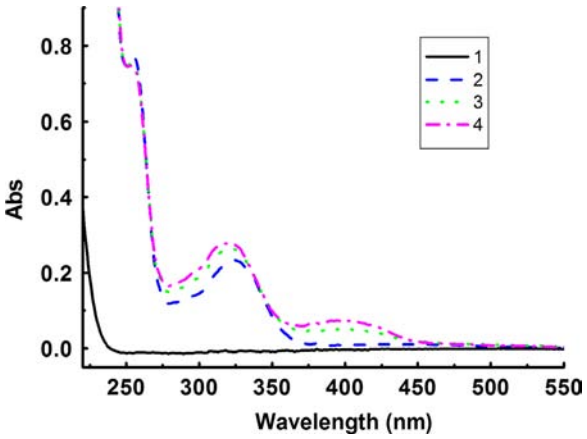

Fig. 3. UV/vis absorption spectra of (1) 5.0×10^{-5} mol L^{-1} Cu^{2+}, (2–4) 5.0×10^{-5} mol L^{-1} receptor 1 + Cu^{2+}, concentrations of Cu^{2+} added for spectrum (2)–(4) were 0, 1.0×10^{-5}, 5.0×10^{-5} mol L^{-1}. Above solutions were regulated by PBS (0.2 M, pH 8.2).

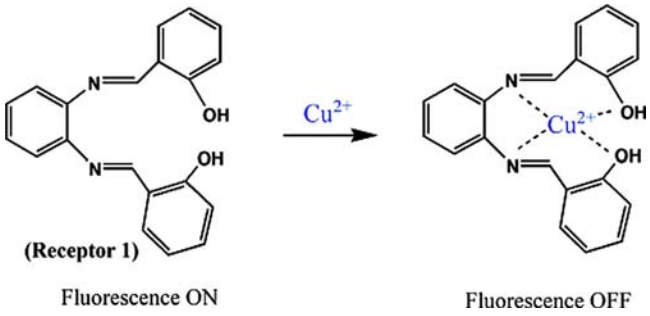

Fig. 4. Reaction scheme of the "On–Off" fluorescence procedure.

combination of Cu^{2+} with N, O of receptor 1, which proves that the fluorescence of receptor 1 can be quenched by the static fluorescence quenching effect. On the another hand, the absorption band centered at 324 nm partially overlap with the excitation spectrum of receptor 1, and the absorption (350–450 nm) overlaps a little with emission spectrum of receptor 1. Hence, this inner filter effect (the complex formed by Cu^{2+} and receptor 1 absorbs excitation and emission light of receptor 1) also plays a partial role in quenching mechanism [21,29].

The quenching is analyzed by the equation [30]: $\lg(F_0/F-1)=\lg K+n\lg[Q]$, where F_0 and F are the fluorescent intensity at 430 nm in the absence and presence of Cu^{2+} ions, respectively, K is the binding constant and n is the number of binding sites per receptor 1. According to Fig. S2, the binding constant and the number of binding sites n are obtained as $K=3.94$ L mol^{-1} and $n=1.05$, respectively. This indicates that the complex has a 1:1 (metal-to-ligand) stoichiometry. This reaction mechanism between receptor 1 and Cu^{2+} also has been testified by previous work [24]. Therefore, the reaction scheme between receptor 1 and Cu^{2+} can be described by Fig. 4.

The quenched florescence of receptor 1 by Cu^{2+} can be recovered by adding a strong metal ion chelator such as ethylenediaminetetraacetate (EDTA) (see line e and f in Fig. 2), illustrating that the quenching of receptor 1 by Cu^{2+} might be recovered by a competitive complexation reaction.

3.3. Effect of pH

The quenching behaviors of receptor 1 by Cu^{2+} in different buffer solution mediums such as HBO_3–$Na_2B_4O_7$, B–R and Na_2HPO_4–NaH_2PO_4 have been studied. The fluorescence of receptor 1 in Na_2HPO_4–NaH_2PO_4 solution reveals the biggest Stokes shift, so PBS is chosen as optimum sensing medium.

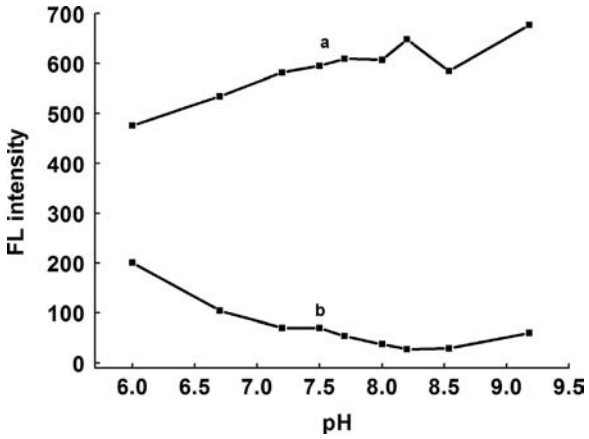

Fig. 5. Fluorescence responses of 2.5 μM receptor 1 in the absence (a) and presence (b) of 2.5 μM Cu^{2+} at different pH values.

The fluorescence intensity variations of receptor 1 in the absence and presence of Cu^{2+} at different pH PBS are shown in Fig. 5. The initial fluorescence intensity (in the absence of Cu^{2+}) and the quenched fluorescence intensity (in the presence of Cu^{2+}) are both pH-dependent in the range of pH 6.0–9.2. The receptor 1 (line a in Fig. 5) has higher fluorescence activities in the range of pH 7.0–9.2 than in pH 6.0–7.0. On the other hand, in pH 6.0–7.0, the fluorescence quenching of the receptor 1 by Cu^{2+} is poor (line b in Fig. 5), which may be attributed to the fact that the amino groups of receptor 1 are protonated and thus unable to complex with Cu^{2+}. In contrast, in weakly alkaline solutions (pH 7.0–9.2) the quenching efficiencies are quite satisfactory and reach a maximum at pH 8.2, but are slightly weakened when pH is higher than 8.2 as a result of the partial hydrolysis of Cu^{2+} ion in the alkaline media.

3.4. Selectivity of fluorescence probe towards Cu^{2+}

Specific recognition of the target is particularly important for practical applications of a chemosensor. According previous reports [31,32], some chemosensors in the detection of Cu^{2+} are usually interfered with other paramagnetic ions such as Fe^{3+}, Co^{2+}, and Hg^{2+}. The fluorescence responses of receptor 1 to Cu^{2+} and various possible interfering metal ions were tested. As shown in Fig. S3, fluorescence of receptor 1 is dramatically quenched by Cu^{2+} ions, while little influence is observed from other alkali-earth and transition metal ions. Furthermore, in the competition experiments, the influences of interfering ions on fluorescence response of receptor 1 to Cu^{2+} were measured. As we can see in Table 1, among the foreign metal ions in their tested concentrations, only Hg^{2+} has partial influence on the sensing of Cu^{2+}, while other interfering metal ions have no obvious influence on the function of receptor 1. These findings indicate that receptor 1 is a selective fluorescent chemosensor for Cu^{2+} in aqueous buffer solution.

3.5. Effects of time on receptor 1 and receptor 1 with Cu^{2+}

The effects of time on the fluorescence of receptor 1 with and without Cu^{2+} were investigated. As shown in Fig. S4, the fluorescence signal of 2.5 μM receptor 1 working solution (line a in Fig. S4) remains stable for 1 h, indicating a good fluorescence stability of the receptor 1 working solution. In addition, the fluorescence of 2.5 μM receptor 1 is quenched immediately with the addition of 0.5 μM Cu^{2+} (line b in Fig. S4) and remains stable in the following 1 h. The result proves that this quenching method is a rapid and stable one for the detection of Cu^{2+} ions.

3.6. Influence of receptor 1 concentrations

For the fluorescence quenching method, the concentration of fluorophore affects the sensitivity and linear range. Generally, higher sensitivity but narrow linear range can be found when using low concentration of fluorophore. On the contrary, high concentration of fluorophore leads to low sensitivity and wide linear range.

A good linear relationship between fluorescence intensity and concentration of receptor 1 in the range of 0–1.4×10^{-5} mol L^{-1} was observed. Therefore, concentrations of receptor 1 at 2.5×10^{-6} mol L^{-1} and 7.5×10^{-6} mol L^{-1} were studied. At these two concentrations, the linear ranges are 1.0×10^{-7}–2.5×10^{-6} mol L^{-1} (R=0.995) and 5.0×10^{-7}–6.0×10^{-6} mol L^{-1} (R=0.996). Taking sensitivity into consideration, 2.5×10^{-6} mol L^{-1} was finally chosen.

Table 1
Effect of different interferents on fluorescence of receptor 1–Cu^{2+} system.

Interferent	Concentration[a] (mol L^{-1})	Relative error[b] (%)
K^{+}	5.0×10^{-5}	−4.9
Ca^{2+}	1.0×10^{-5}	0.2
Al^{3+}	5.0×10^{-7}	−5.1
Ni^{2+}	5.0×10^{-7}	−4.8
Pb^{2+}	5.0×10^{-7}	−3.2
Co^{2+}	2.5×10^{-6}	0.2
Mn^{2+}	5.0×10^{-7}	−0.53
Fe^{3+}	5.0×10^{-7}	−4.9
Fe^{2+}	5.0×10^{-7}	−2.7
Zn^{2+}	5.0×10^{-6}	−1.9
Hg^{2+}	5.0×10^{-7}	16.2
Cd^{2+}	5.0×10^{-7}	5.0
Sn^{2+}	5.0×10^{-7}	5.6

[a] The experiment was carried out with a fixed concentration of Cu^{2+} at 5.0×10^{-7} mol L^{-1}.
[b] Relative error is defined as %RE=$[(F_0-F)/F_0]\times100$.

Table 2
Results of copper(II) detection in soybean milk powder sample.

Sample	Contents in sample (μg g^{-1})		Er (%)
	The proposed method	AAS	
1	163.0	208.0	−21.6
2	298.0	296.7	0.4

3.7. Calibration curve and detection limit

As Fig. S5 shown, the fluorescence intensity of receptor 1 decreases as increasing concentration of Cu^{2+} is added. A good linear relationship between the quenching efficiency (F_0/F) and concentration of Cu^{2+} in the range from 0.1 to 2.5 μM exists, as shown in the inset of Fig. S5. The linear regression equation is expressed as $F_0/F=1+6.19c$ (c: 10^{-6} mol L^{-1}, n=8) with a correlation efficiency of 0.997. The detection limit of Cu^{2+} (3S/N) is 2.0×10^{-8} mol L^{-1}, and the relative standard deviation is 4.6% (n=6) for detection of 8.0×10^{-7} mol L^{-1} standard copper(II) ions.

3.8. Quantification of content of copper(II) in soybean milk powder

The applicability of this sensing method was evaluated through detecting the content of Cu^{2+} in soybean milk powder. The results are shown in Table 2, the concentrations of Cu^{2+} in two parallel samples determined using the proposed method are basically consistent with the results measured by atomic absorption spectrometry (AAS).

3.9. Comparison of methods

The comparison of receptor 1 with other fluorescence reagents for copper is shown in Table 3. It can be seen in Table 3, the procedure for determination of Cu^{2+} based on receptor 1 is simpler than on 8-aminoquinoline-5-azo derivatives [11], Tiron [12], Lucifer Yellow [13], PMAQ [33] and Naphthol derivative [34]. Moreover, receptor 1 displays a higher sensitivity for Cu^{2+} detection than SAPYA [22]. In the displayed analytical features, only NRC [10] presents higher superiority than receptor 1 for sensing Cu^{2+}. To sum up, the proposed method is relatively simple, rapid, selective and sensitive for determination of copper.

3.10. Construction of fluorescence switch

The quenched fluorescence of receptor 1 by Cu^{2+} might be recovered when adding strong metals chelator (such as EDTA) or strong reducer (such as glutathione and L-cysteine [35]). Therefore, the fluorescence switch property of receptor 1 based on the control of Cu^{2+} and glutathione was investigated. The fluorescence intensity of 2.5 μM receptor 1 in pH 8.2 PBS solution is quenched by adding 1.5 μM Cu^{2+} on account of a complex that is formed between Cu^{2+} and receptor 1. However, fluorescence of the above quenched system is recovered with the addition of 2.0 μM glutathione since glutathione reduces Cu^{2+} ions to Cu^{+}

Table 3
Comparison of the main characteristics for fluorimetric determination of copper with several organic reagents.

Reagent[a]	$\lambda_{ex}/\lambda_{em}$ (nm)	Linear range (mol L^{-1})	LOD (mol L^{-1})	Experimental condition	Interfering ions[b]	Ref.
NRC	360/580	5.0×10^{-8}–4.5×10^{-6}	1.8×10^{-8}	Fluorescence increase, pH 7.0, in ethanol/HEPES (1:9, v:v, 50 mM)	No	[10]
FCFPAQ	328/368	6.2×10^{-8}–2.2×10^{-6}	1.2×10^{-8}	Fluorescence increase, pH 5.4, boiled for 15 min	No	[11]
FCPBSQ	326/362	1.6×10^{-8}–3.1×10^{-6}	3.1×10^{-9}	Fluorescence increase, pH 6.4, boiled for 5 min	No	[11]
BAQABP	296/382	4.7×10^{-8}–2.3×10^{-6}	7.8×10^{-9}	Fluorescence increase, pH 8.4, boiled for 20 min	No	[11]
Tiron	294/350	5.0×10^{-7}–1.0×10^{-5}	3.8×10^{-7}	Fluorescence quenching, pH 8.0, heated up to 80 °C for 90 min	Co^{2+}	[12]
Lucifer Yellow	430/535	1.0×10^{-8}–1.0×10^{-4}	1.0×10^{-8}	Fluorescence quenching, pH 4.0–6.8, LY embedded in a hydrogel	Hg^{2+}	[13]
SAPYA	340/385	4.7×10^{-7}–5.5×10^{-6}	No data	Fluorescence quenching, pH 8.9, in 50% dioxan in water	No	[22]
PMAQ	310/434	3.9×10^{-7}–6.89×10^{-6}	2.8×10^{-7}	Fluorescence quenching, pH 4.5, kept for 20 min	Co^{2+}, Fe^{3+}	[33]
Naphthol derivative	420/508	2.0×10^{-7}–2.0×10^{-6}	No data	Fluorescence quenching, in acetonitrile	Al^{3+}	[34]
Receptor 1	324/430	1.0×10^{-7}–2.5×10^{-6}	2.0×10^{-8}	Fluorescence quenching, pH 8.2, kept for 5 min	Hg^{2+}	This work

[a] The abbreviation of the reagents represented as follows: NRC: naphthalimide modified rhodamine B; FCPAQ: 5-(3-fluo-4-chlorophenylazo)-8-aminoquinoline; FCPBSQ: 5-(3-fuo-4-chlorophenylazo)-8-benzenesulfonamidoqu inoline; BAQABP: 4,4′-bis(8-aminoquinoline-5-azo)-biphenyl; SAPYA: N,N′-bis(salicylidene)-2,3- pyridinediamine; PMAQ: 8-[(2-pyridine) methylideneamino] quinoline; Naphthol derivative: coupling of 1-hydroxynaphthalene-2-carbaldehyde and 1-amino-2-naphthol hydrochloride.
[b] Could produce interference at the same concentration of copper(II).

ions, and Cu^{+} ions are stabilized through the formation of a Cu(I)–glutathione complex [36]. As shown in Fig. S6, the fluorescence intensity of receptor 1 is reversibly controlled by alternate addition of Cu^{2+} and glutathione for four times this way, implying the relative excellent fluorescence switch character of receptor 1.

4. Conclusions

A new method for determination of Cu^{2+} ions has been developed based on quenching the fluorescence of an easily synthesized Schiff base in aqueous solution. The proposed method shows excellent sensitivity and good selectivity and has been successfully applied to the detection of Cu^{2+} in soybean milk powder. Furthermore, the fluorescence switch property of receptor 1 based on the control of Cu^{2+} and glutathione has been demonstrated. Because strong reducers such as glutathione and L-cysteine could recover the quenched fluorescence of receptor 1 by Cu^{2+}, the system has potential for determination of total content of glutathione and L-cysteine in human plasma.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.066.

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