

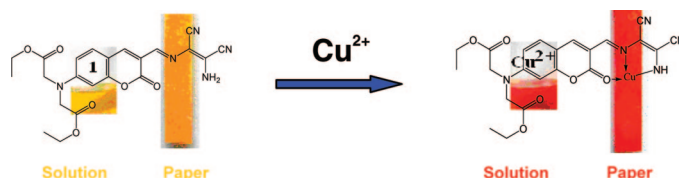
Colorimetric Test Kit for Cu^{2+} DetectionRuilong Sheng,[†] Pengfei Wang,^{*,†} Yunhua Gao,[†] Yan Wu,[†] Weimin Liu,[†]
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ABSTRACT



A coumarin-based colorimetric chemosensor **1** was designed and synthesized. It exhibits good sensitivity and selectivity for the copper cation over other cations such as Zn^{2+} , Cd^{2+} , Pb^{2+} , Co^{2+} , Fe^{2+} , Ni^{2+} , Ag^+ , and alkali and alkaline earth metal cations both in aqueous solution and on paper-made test kits. The change in color is very easily observed by the naked eye in the presence of Cu^{2+} cation, whereas other metal cations do not induce such a change. The quantitative detection of Cu^{2+} was preliminarily examined.

The development of sensitive chromogenic chemosensors has been receiving much attention in recent years because of the potential application in clinical biochemistry and the environment. A number of chemosensors have been developed for selective recognition of different species on the basis of different host–guest interactions, such as hydrogen-bonding, electrostatic force, metal–ligand coordination, hydrophobic and van de Waals force interactions, etc.¹ Most of these chemosensors have been employed in solution by means of spectroscopic instrumentation.² This will significantly restrict the practical applications of these chemosensors. For simplicity, convenience and low cost, the fabrication of small molecular chemosensors into colorimetric test kits is highly demanding. For example, the current concern regarding

children's toys requires the development of test kits for toxic metals such as lead. On the other hand, copper is a widely used industrial metal, and its cation is toxic at high concentration³ and is involved in brain diseases such as Alzheimer's, Parkinson's, and Prion at a trace amount.⁴ Even though some colorimetric/fluorescent chemosensors have been developed for the detection of Cu^{2+} so far,⁵ the sensing methods for fast detection of Cu^{2+} in aqueous solution, especially using colorimetric sensors without resorting to instruments, are relatively rare.^{5a,b,f} In this Letter, a simple colorimetric test kit based on a coumarin Schiff base derivative, chemosensor **1**, was fabricated for the qualitative and quantitative detection of Cu^{2+} in aqueous solution.

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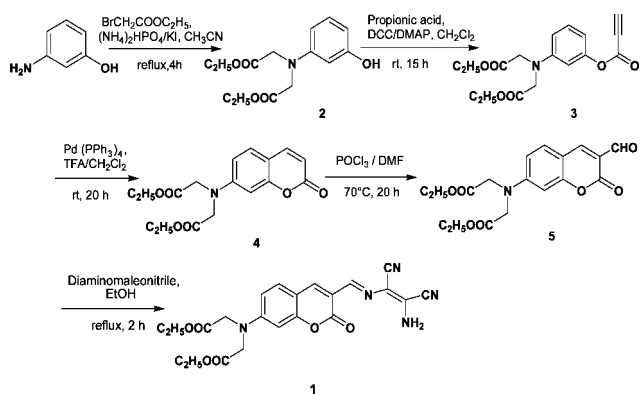
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Coumarin and its derivatives are excellent chromogenic and fluorogenic dyes that are widely utilized as reporters in chemosensors.⁶ In this work, we designed and synthesized a coumarin derivative as a new colorimetric chemosensor **1** (Scheme 1), in which 7-di(ethoxy-carbonylmethyl)amino coumarin serves as chromophore core. Diaminomaleonitrile was coupled with coumarin at the 3-position carbonyl to form Schiff base derivative. An intramolecular charge transfer (ICT) is thus enhanced as a result of the extended π -conjugation and the stronger electron-withdrawing ability of the nitrile group. The extended ICT usually exhibits high sensitivity to external perturbations such as the polarity of solution and the electric field in its vicinity, often showing as remarkable color changes.⁷ The formed imine and the rest of the amine groups in chemosensor **1** act as chelating sites of metallic cations, in particular, transition and post-transition metal cations.

Scheme 1. Synthesis of Chemosensor **1**^a



^a For synthetic protocols and compound characterization see Supporting Information.

Chemosensor **1** was synthesized with 3-aminophenol as a starting material (shown in Scheme 1). N-Alkylation of 3-aminophenol with ethyl bromoacetate or ethyl chloroacetate was catalyzed by ammonium dihydrogen phosphate in acetonitrile to produce **2** (yield 70%). Compound **2** was esterified with propionic acid to form ester **3** by DCC/DMAP coupling reaction (yield 30%). The intramolecular ring closure of ester **3** was catalyzed by 0.1 equiv of tetrakis(triphenyl phosphine)palladium(0) (Pd(PPh₃)₄) in TFA at room temperature to yield coumarin **4** with a yield of 40%.⁸ Coumarin aldehyde **5** was obtained from the classic Vilsmeier reaction⁹ of coumarin **4**

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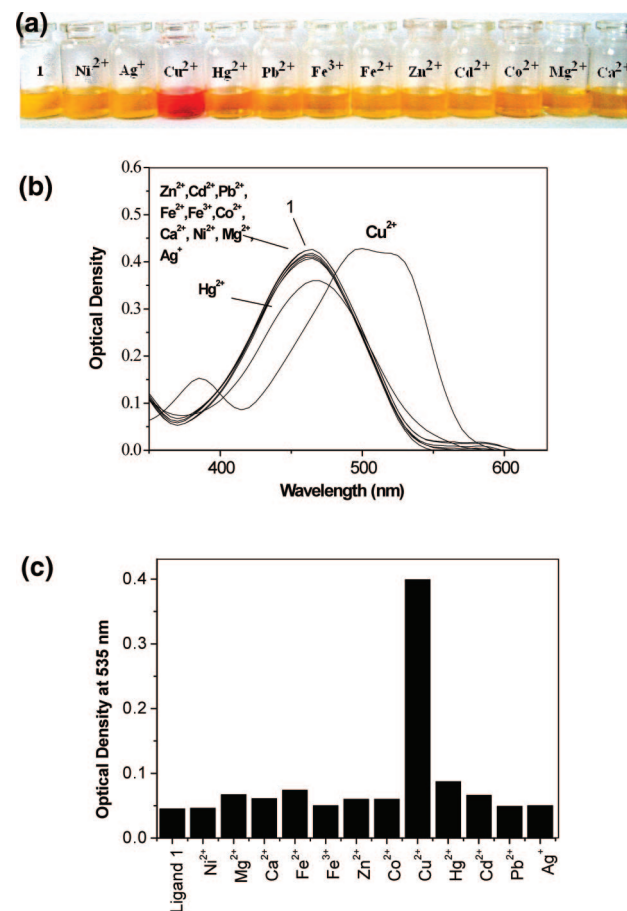


Figure 1. (a) Solutions of **1** upon addition of different metal ions. (b) Absorption changes of **1** in H₂O/DMSO (v/v = 60/40) (1.0 × 10^{−5} M) upon addition of 1 equiv of different perchlorate salts (1.0 × 10^{−5} M). (c) Optical density of **1** at 535 nm upon the addition of different cations.

and POCl₃ in DMF (yield 40%). The final condensation of coumarin aldehyde **5** and diamino maleonitrile in ethanol gave rise to chemosensor **1** (yield 70%). Detailed procedures and characterizations are reported in Supporting Information.

Chemosensor **1** is soluble in a mixture solvent of H₂O and DMSO, further diluted for analyte detection in aqueous solution. The yellow solution of **1** is stable within a pH range of 7–10 (Figure S1, Supporting Information) in H₂O/DMSO (v/v = 60/40). A strong and broad absorption band from 380 to 530 nm peaked at 460 nm ($\epsilon = 4.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) was observed. By comparison to that of coumarin aldehyde **5**, the absorbance of **1** shifts to the red with a similar molar absorption coefficient, reflecting the enhanced intramolecular charge transfer due to the extended π -conjugation and strong electron-withdrawing ability of nitriles after the formation of Schiff base between coumarin aldehyde **5** and diaminomaleonitrile. The responses of **1** in H₂O/DMSO (v/v = 60/40) to a series of individual metallic cations were investigated. As shown in Figure 1a,

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solutions of **1** upon the addition of 1 equiv of Ag^+ , Fe^{3+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+} , Cu^{2+} , Hg^{2+} , Pb^{2+} , Ca^{2+} , and Mg^{2+} display distinguishing red color with Cu^{2+} compared with those with other cations, indicating the sensitivity and selectivity of **1** to Cu^{2+} over other cations. Their corresponding UV–vis absorption spectra are depicted in Figure 1b.

From the instrumental read-outs, the spectral responses of **1** to different cations can be classified into three types. For type 1 cations such as Zn^{2+} , Cd^{2+} , Pb^{2+} , Ag^+ , Fe^{3+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Ca^{2+} , and Mg^{2+} , the spectral changes of aqueous solutions of **1** were negligible before and after these cations were added. Hg^{2+} is the type 2 cation, which induced the slightly red-shifted absorbance of **1** solution with decreased molar absorption coefficient. The UV–vis absorption spectrum of **1** solution shifted to the red about 70 nm when combined with Cu^{2+} , the type 3 cation. For comparison, their optical densities at 535 nm upon the addition of these cations to **1** solution are charted in Figure 1c. The differential optical responses of a solution of **1** to these cations indicate that the interactions between **1** and these cations increase from type 1 to type 2 and, in turn, to type 3 (Figure 1b).

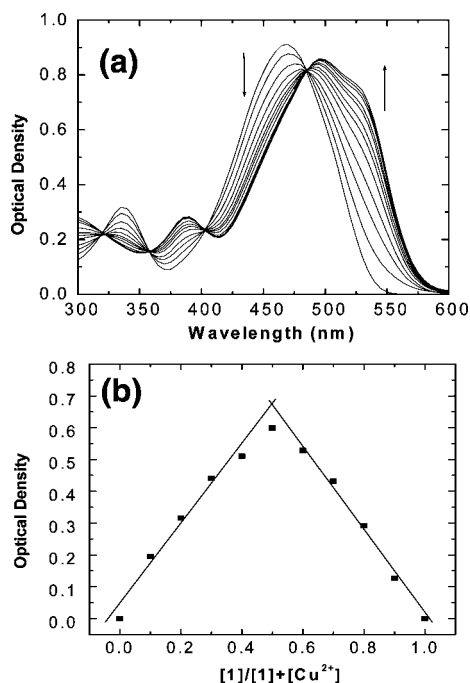


Figure 2. (a) Titration curves of chemosensor **1** in $\text{H}_2\text{O}/\text{DMSO}$ ($v/v = 60/40$) (2.0×10^{-5} M) upon addition of Cu^{2+} at 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4, and 4.8×10^{-5} M. (b) Job's plot of the complex formed by **1** and Cu^{2+} .

To elicit the interactions between **1** and Cu^{2+} , UV–vis absorption spectral variation of **1** (2.0×10^{-5} M) in $\text{H}_2\text{O}/\text{DMSO}$ solution was titrated with Cu^{2+} from 0 to 4.8×10^{-5} M. As shown in Figure 2a, the maximum absorbance at 460 nm gradually decreased, and concomitantly a rising new absorbance that peaked at 535 nm appeared. An isosbestic point is clearly observed at 480 nm, indicating the formation of a new complex between **1** and Cu^{2+} . The Job's plot in

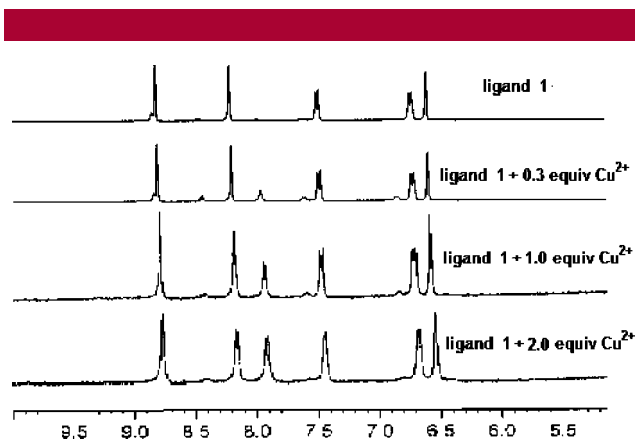


Figure 3. ^1H NMR spectra of chemosensor **1** upon addition of 0.3, 1.0, and 2.0 equiv of Cu^{2+} in $\text{D}_2\text{O}/d_6\text{-DMSO}$ ($v/v = 60/40$).

Figure 2b gives a 1:1 stoichiometric ratio between **1** and Cu^{2+} for the newly formed species, corroborated by MALDI-TOF mass spectroscopy in which a maximum peak is found at $m/z = 513.8$ for $[\text{1} + \text{Cu}^{2+} - \text{H}^+]^+$ (calcd 513.9) (Figure S2, Supporting Information). On the basis of 1:1 stoichiometry, the stability constant of the complex between **1** and Cu^{2+} was estimated to be $6.6 \times 10^5 \text{ M}^{-1}$ ($r^2 = 0.998$) by using nonlinear curve fitting (Figure S3, Supporting Information).¹⁰ The plot of optical density change (A/A_0) at 535 nm against added concentrations of Cu^{2+} shows that the limit of detection is about 1.2×10^{-6} M (Figure S4, Supporting Information).

The ^1H NMR titration spectra of **1** solution with Cu^{2+} in D_2O solution illustrate its characteristically structural changes upon interactions with Cu^{2+} . As shown in Figure 3, the proton chemical shifts of the coumarin ring are shifted slightly upfield; more obviously, the integral of a new peak at 7.9 ppm assigned to aminyl increased from 0.31 to 0.96 in reference to those of coumarin protons when the added Cu^{2+} was increased from 0.3 to 2 equiv. These NMR results suggest that Cu^{2+} deprotonated the amine to form $\text{Cu}-\text{N}$ bonding, and coordinated with the imino of the Schiff base and 2-carbonyl of the coumarin core. This picture was confirmed by X-ray single crystal structures of metal complexes involving analogous ligands in the literature.¹¹ These results also elicit the complex stability constant in a 1:1 binding model between **1** and Cu^{2+} , which is different from those Cu^{2+} complexes with bidentate ligands in an 1:2 stoichiometry.¹² The large red-shifted UV–vis absorbance of **1** solution upon the addition of Cu^{2+} is thus rationalized to result from the extended ICT in such a push–pull $\text{Cu}(\text{II})$ Schiff base complex.

The selectivity of **1** (1.0×10^{-5} M) to Cu^{2+} (1.0×10^{-5} M) was further demonstrated in the presence of 3 equiv of

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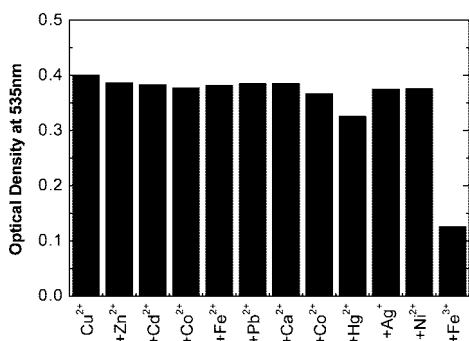


Figure 4. Optical density of **1** (1.0×10^{-5} M) at 535 nm with addition of Cu²⁺ (1.0×10^{-5} M) in the presence of 3 equiv of other metal cations.

other cations. As shown in Figure 4, the optical density of **1** and Cu²⁺ at 535 nm was not affected in the presence of other cations such as Ca²⁺, Zn²⁺, Cd²⁺, Pb²⁺, Co²⁺, Fe²⁺, Ni²⁺, and Ag⁺. These results are significantly different from other Cu(II) sensors⁵ that are usually affected by Co²⁺, Zn²⁺, Cd²⁺, and Ni²⁺. However, the optical density of **1** decreased about 10% when Hg²⁺ was added. In the presence of Fe³⁺, the optical intensity of **1** decreased about 70%. The interference of Hg²⁺ and Fe³⁺ on the optical response of **1** to Cu²⁺ might be due to the competitive coordination¹³ of these metals with aminomaleonitrile moiety.

To investigate the practical application of chemosensor **1**, test strips were prepared by immersing filter papers into a THF solution of **1** (0.1 M) and then drying in air. The test strips containing **1** were utilized to sense different cations. To these cation solutions, different test kits were immersed for 10 s. After several drops of ethanol (to dissolve **1**) were added, the obvious color change was observed only with Cu²⁺ solution. Also, these test strips were applied for sensing different Cu²⁺ concentrations, exhibiting colorimetric changes differentiable by naked eyes. As depicted in Figure 5, the red color of the test strips intensified from 0, to 1.0×10^{-4}

M, 5.0×10^{-4} M, 1.0×10^{-3} M and show that the discernible concentration of Cu²⁺ can be as low as 1.0×10^{-4} M.

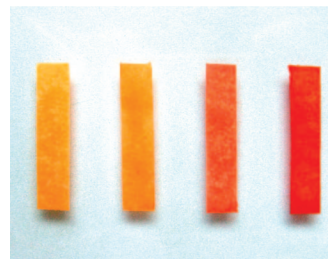


Figure 5. Photographs of the test kits with **1** for detecting Cu²⁺ ion in aqueous solution with different concentrations. Left to right: 0, 1.0×10^{-4} M, 5.0×10^{-4} M, 5.0×10^{-3} M.

In summary, a new colorimetric chemosensor **1** was designed and synthesized by coupling coumarin aldehyde **5** with diaminomaleonitrile to form Schiff base structure with the enhanced intramolecular charge transfer. The sensitivity and selectivity of **1** to Cu²⁺ over other metal cations in aqueous solution were demonstrated via its optical response, ascribed to the formation of the push–pull Cu(II) Schiff base complex. Based on the colorimetric response of **1** to Cu²⁺, test strips containing **1** were fabricated, which also exhibits a good sensitivity and selectivity to Cu²⁺ as in solution. The quantitative detection of Cu²⁺ using these test strips was attempted.

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Supporting Information Available: Synthesis of chemosensor **1** and Figures S1–S4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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