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A novel jointly colorimetric and fluorescent sensor for Cu^{2+} recognition and its complex for sensing S^{2-} by a Cu^{2+} displacement approach in aqueous media

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Abstract: In this work, a simple and easily synthesized Schiff-based derivative colorimetric and fluorescent sensor (**1**), 4-dimethylamino-benzoic acid (2-imidazole formaldehyde)-hydrazide, was obtained for the detection of Cu^{2+} and S^{2-}. The compound **1** exhibited dual spectral responses to Cu^{2+}, that is, vivid color change and fluorescence enhancement in the presence of Cu^{2+}. The detection limits were valued as 0.46 μM and 15 nM according to absorption and fluorescent response, respectively. Both of them are below the World Health Organization (WHO) guidelines for drinking water (31.5 μM). In addition, the ensemble (**1-Cu^{2+}**) selectively and sensitively detected a low concentration of S^{2-}. As the addition of S^{2-} instantly removed Cu^{2+} from the ensemble (**1-Cu^{2+}**) resulting in a color change from yellow to colorless and a "turn-off" fluorescent response. The detection limit for S^{2-} was estimated as 0.12 μM (from fluorescent method) and 0.68 μM (from absorption method), respectively, each of which was also lower than the maximum allowable level of S^{2-} (15 μM) in drinking water defined by the WHO. The binding process was confirmed via UV-vis absorption, fluorescence measurements, ^{1}H NMR, mass spectroscopy and density functional theory calculation. What's more, successful practical application of test paper is used to inspect the S^{2-} which means the convenient and rapid assay in real samples can be achieved.

Keywords: 4-Dimethylamino-benzoic acid hydrazide derivative, colorimetry, fluorescence, Cu^{2+}, S^{2-}

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1. Introduction

Biological important ions, such as metal ions and anions, play indispensable roles in cell biology. Keeping the integrity and homeostasis of these species would be very significant for physiological and pathological processes [1]. Among various metal ions, copper is an essential trace element and plays important roles in many biological systems [2]. As the third plentiful essential trace element found in the human physiology, copper is indispensable for carrying out several necessary processes and plays diverse parts in human physiopathology [3]. However, excess of copper causes oxidative stress and related symptoms, which may lead to diabetes and many neurodegenerative disorders such as Parkinson's [4], Alzheimer's [5], Wilson's [6], and Menke's diseases [7]. Therefore, the development of ideal chemosensors for the monitoring of Cu^{2+} with high selectivity, low detection limit, and rapid response is highly needed [8, 9].

As a toxic traditional pollutant, sulfide can be released during industrial processes and also formed in biological systems due to the formation of the sulfur-containing amino acids in meat proteins or microbial reduction of sulfate by anaerobic bacteria [10, 11]. The protonated forms, HS^- and H_2S, are even more toxic than sulfide itself [12]. Sulfide can damage the human respiratory and nerve systems, causing people to lose consciousness or even die at a very low concentration (ppm level) [13]. On the other hand, some studies have shown that sulfide participates in many physiological processes, such as vasodilation, angiogenesis, neuromodulation regulation of inflammation and apoptosis [14]. Nevertheless, abnormal sulfide production is linked to human diseases such as hypertension, Down's syndrome, Alzheimer's disease, as well as liver cirrhosis [15]. So, the quantitative detection of S^{2-} is of great importance for both environmental and biological systems.

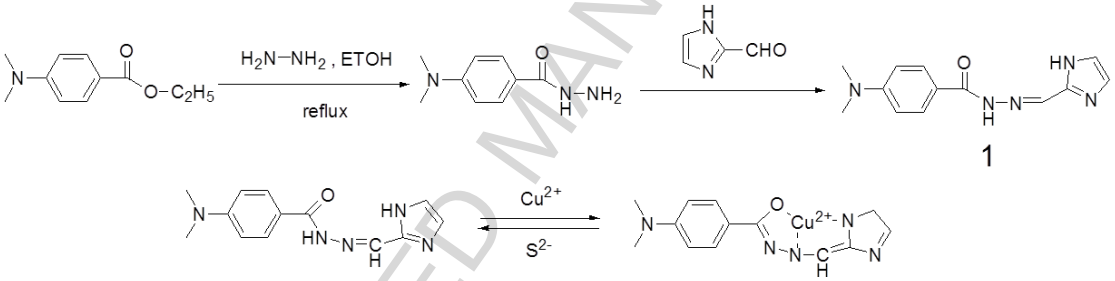
Owing to distinct advantages of simplicity, sensitivity, selectivity, and low cost [16-18], as well as the applicability in rapid tracking of targets in toxicological, environmental and biological samples [19-21], optical chemosensors like fluorescent and colorimetric ones, have received considerable attention for the detection of Cu^{2+} or S^{2-}. Nevertheless, the development of the chemosensors capable of sequential detection both of Cu^{2+} and S^{2-} is still in high demand. More importantly, probes with colorimetric and fluorescent feature to accurately monitor the two targets both by the naked eye and fluorescent microscopy are rarely reported.

In recent years, the displacement methods using the Cu^{2+}-based chemosensing ensembles have been widely used for highly selective detection of anions just like S^{2-} [22, 23]. In these displacement methods, the sensor itself displayed a specific recognition toward Cu^{2+} with a change in the color or in the fluorescent intensity by forming a stable ensemble with Cu^{2+}. And then Cu^{2+} will be removed from the ensemble due to the formation of more stable complex $[Cu(S)_x]^{n-}$ with the addition of S^{2-}. However, most of the reported displacement methods using the Cu^{2+}-based ensembles showed limitations to some aspects, including time-consuming synthetic procedures [24], and the interference from the other sulfur-containing anions like HSO_3^- [25].

Thus, it is still challenging to develop appropriate jointly colorimetric and fluorescent chemosensors for Cu^{2+} recognition and S^{2-} sensing with Cu^{2+}-based ensemble.

In the present work, as shown in Scheme 1, a *p*-dimethylaminobenzamide derivative **1** was used for colorimetric and fluorescent detection of Cu^{2+} and subsequently, its Cu^{2+}-based ensemble can selectively detection S^{2-}. Generally, *p*-dimethylaminobenzamide and its derivatives have a wide range of applications including medicine [26], anticorrosion [27] and sensing [28]. In order to meet the demand of the recognition of different ions and testing, we can use various kinds of aldehydes and ketones as raw materials and synthesize different types of Schiff-base compound.

The chemosensing ensemble we prepared showed several promising sensing properties: (1) The sensor for detection of Cu^{2+} was based on fluorescence enhancement with the almost zero background, which is very different existing reports based on paramagnetic nature of Cu^{2+}; (2) Highly selective and sensitive detection of Cu^{2+} and S^{2-} over the other anions in aqueous solutions were achieved by colorimetric and fluorescence detection methods; (3) The Schiff-based derivative as the probe was synthesized easily with a high yield. To the best of our knowledge, this is an appropriate example of a Schiff-based system for sequential detection both of Cu^{2+} and S^{2-} in aqueous solutions.



Scheme 1 The synthesis process of compound **1** and binding model among **1**, Cu^{2+} and S^{2-}.

2. Experimental

2.1. Materials and instrumentation

All reagents and solvents were used without purification. Imidazole-2-carboxaldehyde, 4-dimethylamino-benzoic acid ethyl ester and hydrazine hydrate were bought from Aladdin Reagent Holding Limited Liability Company (Shanghai, China). Inorganic salts including $AgNO_3$, $AlCl_3$, $Ba(NO_3)_2$, $Cu(NO_3)_2$, $Ca(NO_3)_2$ $CdCl_2$, $CrCl_3$, $CoCl_2$, $FeCl_3$, $HgCl_2$, KNO_3, $Mg(NO_3)_2$, NaCl , $NiCl_2$, $Pb(NO_3)_2$ and $ZnCl_2$ were purchased from Qing Xi Technology Limited Liability Company (Shanghai, China). Other anionic (Ac^-, Br^-, Cl^-, F^-, HCO_3^-, HPO_4^{2-}, I^-, $S_2O_3^{2-}$, SCN^-) and some compounds (EDTA, CH_4N_2S, GSH, Hcy, Cys) were purchased from Elaboration Institute of Technology (Shanghai, China). All aqueous solutions were prepared using ultrapure water from a Milli-Q system.

Absorption measurements were carried out on Shimadzu-2550 UV-vis spectrophotometer (Shimadzu, Japan) using a 1.0 cm quartz cell. Fluorescence spectra were recorded on F-4600 spectrofluorimeter (Hitachi, Japan) equipped with a xenon lamp source and a 1.0 cm quartz cell, the scan speed was 12000 nm min^{-1} and the band pass of excitation and emission was set as 5 and 2.5 nm, respectively, and voltage was 700 V. ESI-MS data were recorded on a Waters ZQ4000/2695 mass spectrometer (Waters, America). NMR spectra were recorded on an Agilent Technologies 400/54 Annual Refill (Bruker BioSpin, America) with DMSO-d_6 as the solvent and tetramethylsilane (TMS) as internal standard. Elemental analyses (C, H and N) were performed on an Elementary Vario EL analyzer (Elementar, Germany).

*2.2 Synthesis of compound **1***

As shown in Scheme 1, the reflux of 4-dimethylamino-benzoic acid ethyl ester (0.3584 g, 2 mmol) and hydrazine hydrate (85%, 0.5 ml) in 25 mL of ethanol solution were removed until the complete consumption of imidazole-2-formaldehyde, which was monitored by TLC (about 16 h). 4-Dimethylamino-benzoic acid hydrazide (0.1791 g, 1.0 mmol) and imidazole-2-formaldehyde (0.096 g, 1.0 mmol) were solved in 25 mL ethanol and kept refluxing until complete consumption of imidazole-2-formaldehyde, which was monitored by TLC (about 12 h). The reaction mixture was cooled to room temperature and a white precipitate was obtained through recrystallization. The white precipitate was collected by filtration and washed three times with methanol to get purified precipitate, and the total yield was up to 73%. Ultimately, a white solid of compound **1** was obtained by the vacuum dryer and was characterized by NMR, Elemental analyses and ESI mass data, which were consistent with the proposed formulation.

Compound **1**: ^{1}H NMR (400 MHz, DMSO-d_6): δ (*ppm*) 12.73 (s, 1H), 8.30 (s, 1H), 7.78(d, J=12Hz, 2H), 7.10(s, 1H), 6.96(d, J=8Hz, 2H), 6.73(d, J=8Hz, 2H), 2.98(s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 167.94, 152.59, 143.49, 132.93, 129.55, 123.84, 119.60, 111.30, 39.91; ESI-MS m/z $[M-H]^+$: calcd, 258.13 found, 258.14; Element Anal.: calcd. for $C_{13}H_{15}N_5O$: C, 60.69; H, 5.88; N, 27.22%, found: C, 61.28; H, 5.62; N, 26.65%.

2.3 UV-vis and fluorescence titration measurement

A stock solution of compound **1** at a concentration of 1.0 mM was prepared in acetonitrile and stored in a cold and dark place. Absorption and fluorescence experiments were carried after appropriate dilution. All absorbance and fluorescence measurements were carried out in CH_3CN/H_2O (v/v = 3:2) pH 7.4 Tris-HCl solution.

2.4 Calculation methods

The density functional theory using Becke's three parameterized Lee-Yang-Parr (B3LYP) exchange functional with 6-311G basis sets, in Gaussian-09 programs has been employed to obtain optimized structure of **1** and **1**-Cu^{2+} complex in gaseous state.

3. Results and discussion

*3.1 Colorimetric and fluorescent responses of compound **1** to Cu^{2+} in aqueous buffered solutions*

As the free amino active hydrogen plays an important role in the tight binding with Cu^{2+} [29, 30], we expect compound **1** could combine with copper ions directly and would induce the change of the absorption and/or the fluorescence spectra. The spectroscopic experiments were carried out in CH_3CN/H_2O (v/v = 3:2) pH 7.4 Tris-HCl solution. The optimal condition was obtained from experimental results shown in Fig.S1 and Fig.S2. The absorption spectrum of compound **1** was measured in the presence of an increasing concentration of Cu^{2+}. As shown in Fig.1a, compound **1** showed a strong absorption band at 342 nm. Upon gradual addition of Cu^{2+}, the absorbance peaked at 342 nm gradually decreased and a new absorption band peaked at 406 nm appeared along with color change from colorless to yellow. Moreover, two isosbestic points at 286 and 365 nm were observed, demonstrating that only one compound between compound **1** and Cu^{2+} was formed.

Similarly, fluorescent response to Cu^{2+} was investigated in detail. As shown in Fig.1b, compound **1** emitted very weak fluorescence. The fluorescence intensity at 470 nm enhanced as the concentration of Cu^{2+} increased. As shown in Fig.S3, the linear equations for UV and fluorescence followed as y=0.040x+0.0035 (R=0.9988) and y=106.8x-13.08 (R=0.9997), respectively. The detect limit for Cu^{2+} were estimated as 0.46 μM and 15 nM according to absorption and fluorescent response using the basis of 3σ/k, where σ is the standard deviation of the blank measurements and k is the slope of the intensity as a function of the concentration of Cu^{2+}. The binding stoichiometry between compound **1** and Cu^{2+} was investigated by Job's plot analysis (Fig.S4). When the mole fraction of Cu^{2+} was increased to 0.5, the fluorescence intensity variation at 470 nm reached the maximum. This result show that a 1:1 complex formation between compound **1** and Cu^{2+}. The binding constant of **1**-Cu^{2+} complex was calculated to be 4.3×10^7 M^{-1} and 5.4×10^7 M^{-1} (Fig.S5). That result indicated that compound **1** had a steady binding affinity for Cu^{2+}.

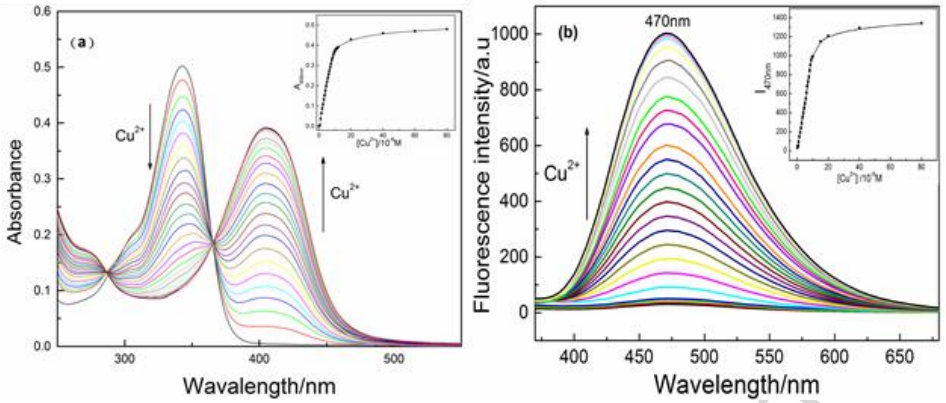


Fig.1 (a) Absorption spectral change of **1** (10 μM). Inset: the absorbance at 406 nm versus the concentration of Cu^{2+} added. (b) Fluorescence spectral change of **1** (1 μM) with increasing concentration of Cu^{2+}. Inset: fluorescence intensity at 470 nm versus the concentration of Cu^{2-} added.

The colorimetric and fluorescent responses of compound **1** to various metal ions (Ag^{+}, Al^{3+}, Ba^{2+}, Cu^{2+}, Cr^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Ni^{2+}, Na^{+}, Pb^{2+} and Zn^{2+}) were investigated in aqueous solutions (Fig.2). Among the tested metal ions, only Cu^{2+} induced change of the absorption and the huge increase of fluorescence emission sensitively. In addition, the color and emission responses to Cu^{2+} were not considerably interfered by other metal ions like 1000 times the concentration of Na^{+}, K^{+} Al^{3+}, Cd^{2+} and Co^{2+}; 500 times of Ca^{2+}, Ag^{+} and Pb^{2+}, 100 times of Cr^{3+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+} and Hg^{2+} (Fig. 3). The overall results indicate that **1** possesses a more steady binding affinity for Cu^{2+} than any other metal ions. To verify the feasibility of this method, using the standard addition method of copper ion in tap water, lake water and spring water were tested and obtained satisfactory results (Table S1). Recovery and the relative standard deviation are 99%-103% and 0.4%-2.1%, respectively.

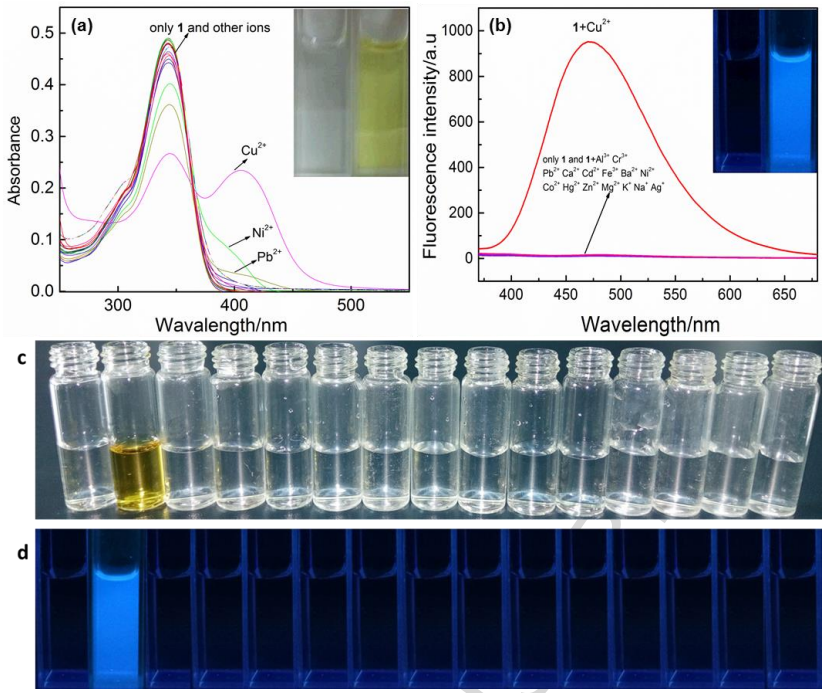


Fig.2 (a) Absorption and (b) fluorescence spectra (λ_{ex} = 350nm, slit 10 nm / 2.5 nm), visible color change under ambient light (c), and emission color change under UV light (λ_{ex} = 365 nm) (d) of compound **1** in the presence of various metal ions (50 equiv). (left to right: only **1**, Cu^{2+}, Ag^{+}, Al^{3+}, Ba^{2+}, Cr^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Ni^{2+}, Na^{+}, Pb^{2+} and Zn^{2+}).

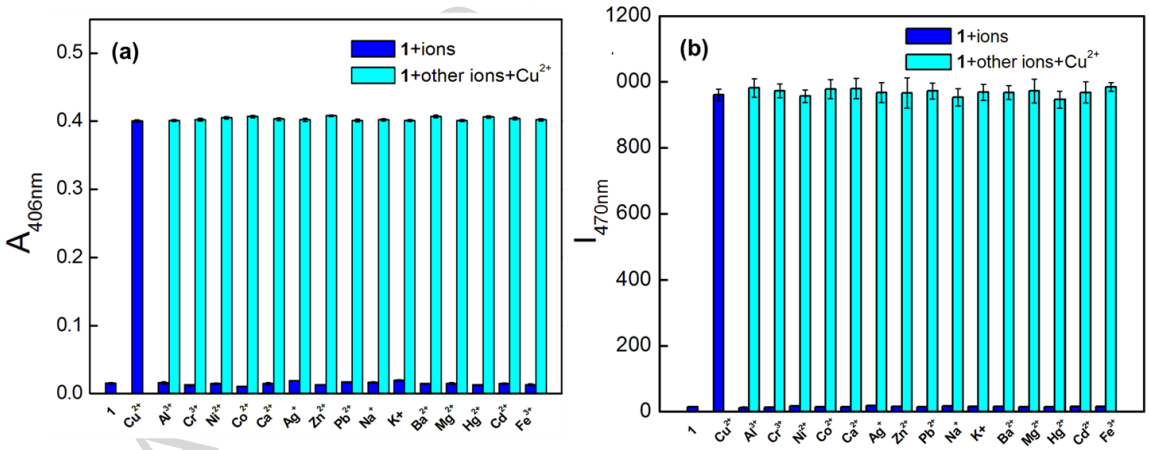


Fig.3 (a) Absorption and (b) fluorescence responses of **1** towards Cu^{2+} and other competitive ions and compounds in CH_3CN/H_2O (v/v = 3:2, pH 7.4) Tris-HCl solution.

3.2 Colorimetric and fluorescent responses of the ensemble (**1-Cu^{2+}**) *to* S^{2-} *in aqueous buffered solutions*

We prepared the ensemble (**1-Cu^{2+}**) by adding 1 equiv. of Cu^{2+} to the aqueous solutions containing compound **1** (10 μM) and investigated the responses to S^{2-} in CH_3CN/H_2O (v/v = 3:2) pH 7.4 Tris-HCl solution. As shown in Fig. 4a, a gradual decrease of absorbance (406 nm) was observed by increasing the concentration of S^{2-}. Meanwhile, the fluorescence intensity at 470 nm

decreased gradually upon the addition of S^{2-} and reached the saturation point at about 10 equiv. of S^{2-}, as shown in Fig. 4b. The absorption and fluorescence spectra of the ensemble in the presence of S^{2-} returned to the original spectra of compound **1**. The change of the fluorescent intensity at 470 nm induced by S^{2-} as a function of time was measured and the response time of the ensemble to S^{2-} needed 2 minutes. This result indicated that the ensemble showed rapid response which is good for application in real samples.

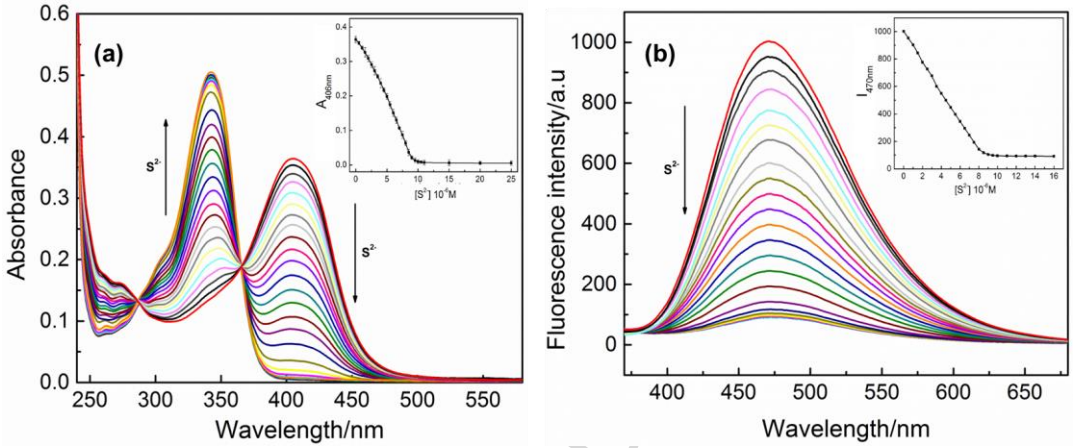


Fig.4 (a) Absorption spectra of **1-Cu^{2+}** (10 μM) (λ = 406 nm). Inset: absorbance at 406 nm versus the concentration of S^{2-} added. (b) Fluorescence spectra of **1-Cu^{2+}** (1.0 μM) with increasing concentration of S^{2-} (λ_{ex} = 350 nm). Inset: fluorescence intensity at 470 nm versus the concentration of S^{2-} added.

In order to evaluate the selectivity of the ensemble sensing for S^{2-} in aqueous solutions, the absorption and emission spectra of the ensemble (**1-Cu^{2+}**) were measured in the presence of various anions (Ac^-, Br^-, Cl^-, F^-, HCO_3^-, HPO_4^{2-}, I^-, $S_2O_3^{2-}$, SCN^-) and some compounds (EDTA, CH_4N_2S, Cys, Hcy, GSH). As shown in Fig.5, only S^{2-} induced the change of the absorption spectrum and the turn-off response of the fluorescence, whereas the other anions (including sulfur-containing ones) did not induce any significant change in the absorption and emission spectra. Fig.5c presents a visible color change of the ensemble under ambient light, and Fig.5d presents a visible emission color change of the ensemble under UV light. The ensemble solution containing S^{2-} displayed colorless and emitted weak fluorescence. In contrast, the ensemble solution displayed a yellow color as well as emitted bright blue fluorescence in the absence or presence of other competing anions.

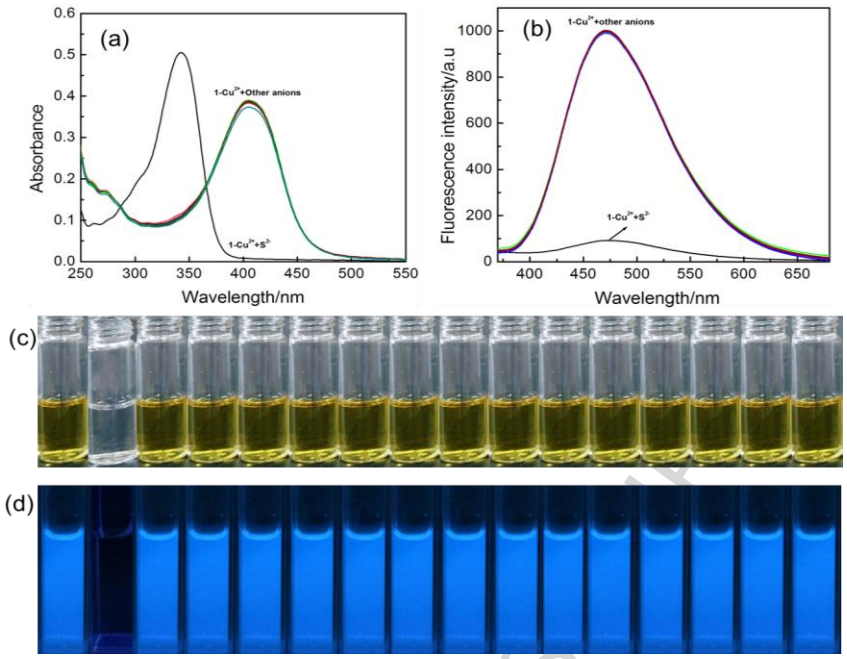


Fig.5 (a) Absorption and (b) fluorescence spectra (λ_{ex} = 350nm, slit 10 nm/2.5 nm), (c) visible color change under ambient light, and (d) emission color change under UV light (λ_{ex} = 365 nm) of **1-Cu^{2+}**(1 μM) in the presence of various anions (10 equiv). From left to right was None, S^{2-}, Ac^{-}, Br^{-}, Cl^{-}, F^{-}, HCO_3^{-}, HPO_4^{2-}, I^{-}, $S_2O_3^{2-}$, SCN^{-}, EDTA, CH_4N_2S, Cys, Hcy, GSH)

Further, the effect of coexisting substance on the detection of S^{2-} was investigated. The presence of the following amounts of foreign species compared with the concentration of S^{2-} resulted in less than ±10% error: 1000 equiv. of F^{-}, Cl^{-}, Br^{-}, Ac^{-}, HPO_4^{2-}, 500 equiv. of I^{-}, HCO_3^{-}, $S_2O_3^{2-}$, Cys, Hcy, GSH, EDTA, 200 equiv of CH_4N_2S, SCN^{-} (Fig. 6). In addition, the fluorescent response to S^{2-} was not affected by the presence of the other anions. The results indicated that the method possessed high selectivity for detection S^{2-} via colorimetric as well as fluorescent responses. The LOD of the ensemble (**1-Cu^{2+}**) for S^{2-} in aqueous solutions was investigated by a linear response of **1-Cu^{2+}** as a function of S^{2-} concentration (Fig. S6). The linear equations for UV and fluorescence are y=0.384-0.039x (R = 0.9968) and y=998.8-108.58x (R = 0.9942), respectively. The LOD of the ensemble (**1-Cu^{2+}**) for S^{2-} were calculated to be 0.68 μM (from absorption index) and 0.12 μM (from fluorescence index). The detection limit for S^{2-} was lower than the maximum allowable level of S^{2-} (15 μM) in drinking water set by the WHO. This result demonstrated that the assay was suitable to monitoring S^{2-} concentration in environment water.

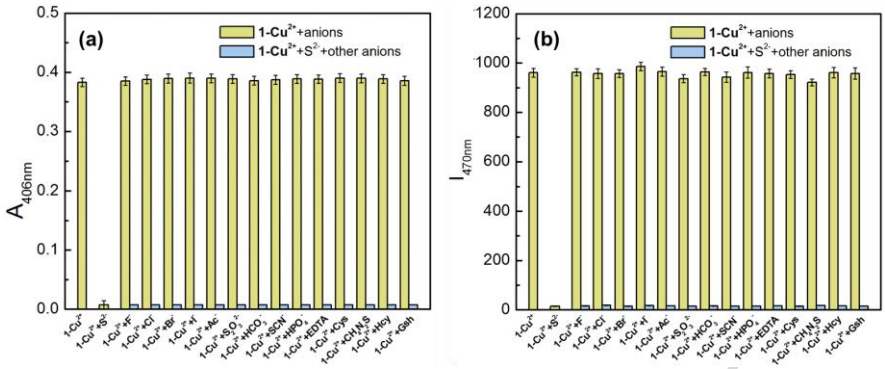


Fig.6 (a) Absorbance and (b) fluorescence responses of **1-Cu^{2+}** toward S^{2-} and various competitive anions and compounds.

To further investigate practical application of the ensemble (**1-Cu^{2+}**), the test papers were immersed into the **1-Cu^{2+}** aqueous solution for 2 days and then air-dried to detect S^{2-} in real samples. As depicted in Fig.7, the yellow color of the test strips was faded gradually with the increasing of Cu^{2+} concentration. Also, it can be seen that the blue emission color weakened gradually. This observation suggested that the test paper immersed **1-Cu^{2+}** can be used conveniently to sense S^{2-} by the naked-eyes.

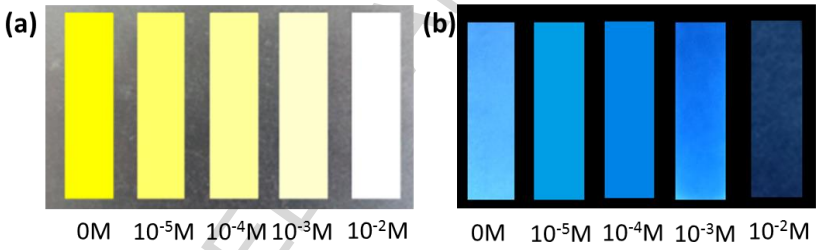


Fig.7 (a) Visible color change (from yellow to white) of the test strips after the addition of S^{2-}. (b) The corresponding emission color change (from light blue to colorless) of the test strips under a hand-held UV lamp.

For analysis of S^{2-} in real sample, the samples (tap water, lake water and blood samples) were spiked with 2.0, 4.0 and 6.0 μM of S^{2-}, and analyzed by proposed fluorescent method. A good agreement was obtained between the spiked and measured S^{2-} amounts. The recovery percentage was found in the range of 97.69-102.7%. All the measurements were performed three times. The results are listed in Table 1, which showed satisfactory recovery and RSD values for the sample. The recovery value for S^{2-} was greater than 97.69 %, and the relative error was lower than 2.27% for S^{2-}. These values were quantitative and it suggests that the presented procedure could be applied for the detection of S^{2-} in real samples.

Table 1 Determination of S^{2-} in samples*

Sample	Added (μM)	Found (μM)			Average (μM)	RSD (%) (n=3)	Recovery (%)
Tap water	2.0	2.037	2.082	2.036	2.052±0.021	1.045	102.6
	4.0	4.041	4.048	4.039	4.043±0.005	0.117	101.1
	6.0	6.015	6.017	6.036	6.023±0.012	0.192	101.0
Lake	2.0	2.065	2.002	2.093	2.053±0.047	2.270	102.7
water	4.0	4.095	4.011	4.046	4.050±0.042	1.042	101.3
	6.0	6.099	6.072	6.015	6.062±0.043	0.707	101.0
Blood	2.0	1.883	1.892	1.899	1.991±0.008	0.403	99.57
	4.0	3.904	3.910	3.909	3.908±0.003	0.082	97.69
	6.0	5.879	5.899	5.863	5.880±0.018	0.302	98.00

*****No measured ions were detected in the tested samples

3.3 Reversible detection

As the presence of S^{2-} alone has no influence on the emission of probe **1**, the decrease in the fluorescence intensity is due to the interaction between Cu^{2+} and S^{2-} apparently. The fluorescence is recovered following the addition of further Cu^{2+} (1.0 equiv.). The reversible cycles, as shown in Fig. 8, fluorescence on and off, could be repeated for 5 times under the same condition. This alteration showed the capability of sensor **1** as a good reversible and reusable sensor.

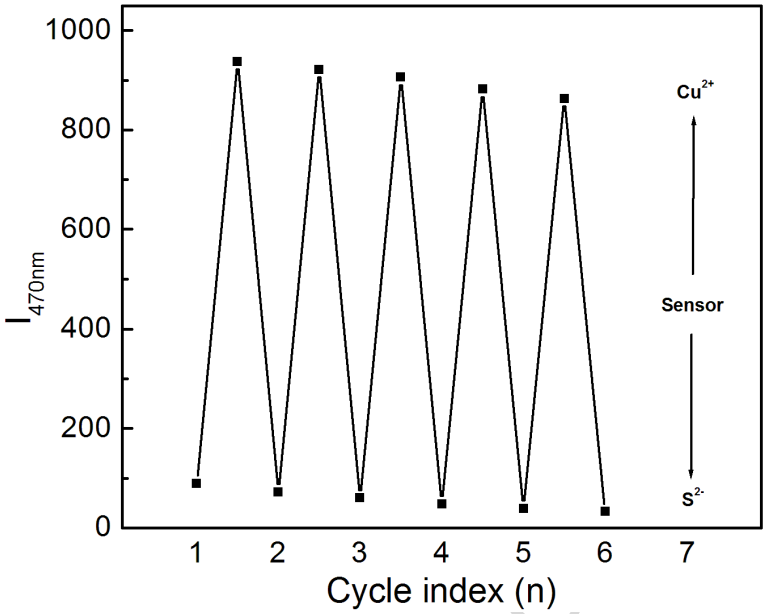


Fig.8 Reversible changes in the emission intensity of **1** at 470 nm (ex = 350 nm) upon sequential addition of Cu^{2+} and S^{2-} solution for 5 cycles.

3.4 The proposed reaction mechanism

The binding mode between **1** and **Cu^{2+}** was analyzed by mass spectrometry (MS). As shown in Fig. S7, peaks at m/z 258.1358 and 320.0673 belonged to [**1**+H^+] and [**1**+Cu^{2+}] respectively, which further proved the 1:1 stoichiometry ratio of between **1** and Cu^{2+}.

In order to verify the mechanism between Cu^{2+} and sensor **1** for the changes of fluorescence intensity and the proposed interaction, electronic properties of ground state and excited state of **1** and **1**-Cu^{2+} complex were applied by molecular orbital calculation. The calculate results showed that the lowest singlet/doublet electronic transition for sensor **1** and **1**-Cu^{2+} was HOMO–LUMO transition. As shown in Fig. 9, it was noticed that the fluorescence enhancement by Cu^{2+} could be explained in terms of the occupancy of the frontier orbitals. And the molecular orbitals were relevant to the excitations and the contributions of orbital transitions for **1** and **1**-Cu^{2+} complex. In sensor **1**, the electron densities of HOMO were only distributed around the imidazole moiety, while those of LUMO were distributed in the 4-dimethylamino-benzoyl moiety. Upon excitation of the sensor **1**, an electron cloud would be transferred from the imidazole to the 4-dimethylamino-benzoyl, resulting in the quenching of sensor **1**. Thus, a PET mechanism was confirmed. While for **1**-Cu^{2+} complex, due to the absorption of copper ion electronic effect, the p

electrons cloud in the HOMO of the **1**-Cu^{2+} complex were located around the Cu^{2+} center. The results show that, HOMO localized on the imidazole group. So the PET process was inhibited, and there was no electron transfer upon excitation. So the fluorescence was enhanced compared with that of sensor **1**.

On the other hand, the orbital energies and spatial distributions of the HOMO and LUMO of **1** and the **1**-Cu^{2+} complex were also generated. The calculated energy gap between the HOMO and LUMO of the **1**-Cu^{2+} complex was 1.78 eV, which is significantly lower than that of **1** (3.46 eV). The results showed that the HOMO and LUMO of the 1-Cu^{2+} complex were more stable than those of sensor **1**, also proving that the binding of Cu^{2+} to the sensor **1** stabilizes the system. These were in full agreement with experimental observations.

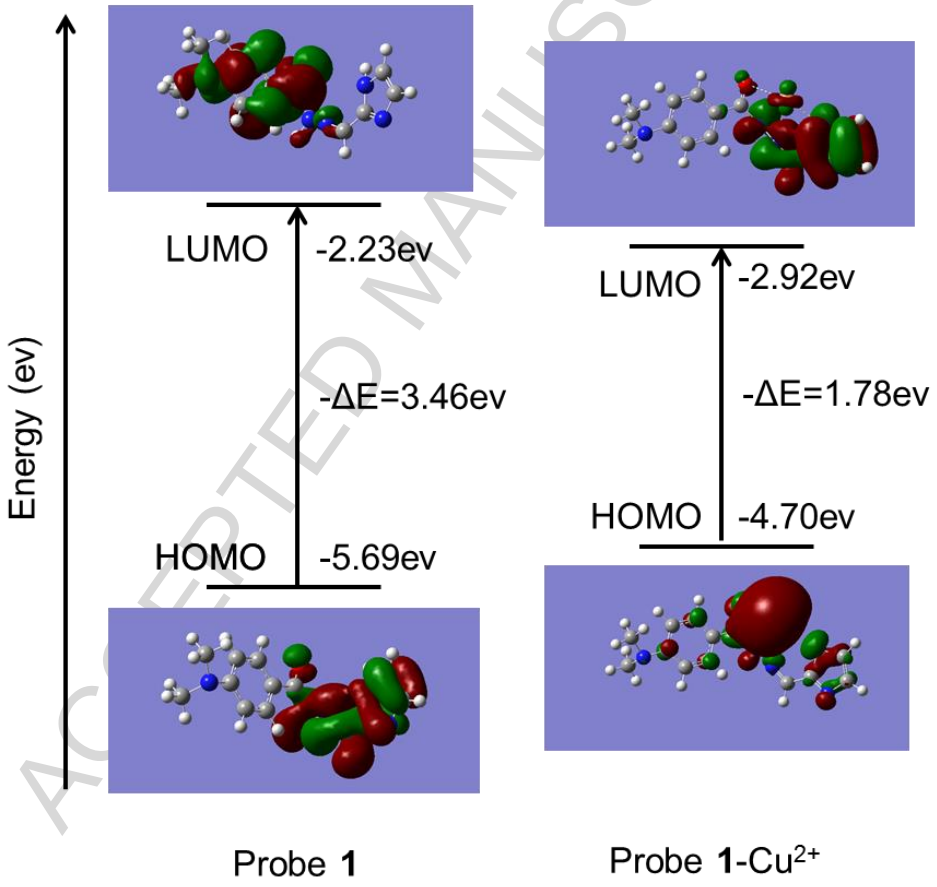


Fig.9 HOMO/LUMO and band gap energies ($-\Delta E = E_{HOMO}-E_{LUMO}$) of **1** and **1**-$Cu^{2+}$ complex by DFT calculation.

To further explore the working mechanism and binding mode of the sensor **1** to Cu^{2+} and (**1**-Cu^{2+}) to S^{2-}, the nuclear magnetic titration experiments were performed. As shown in Fig. 10,

when Cu^{2+} was added to sensor **1**, the H near the benzene ring has a certain movement and the signal of H_a and H_b disappeared. While adding S^{2-} sequentially, signals of H_a and H_b reappeared again. So, we guess the complex formation occurred between Cu^{2+} and **1**, and then the complex **1-Cu^{2+}** disassembled upon addition of S^{2-} as shown in Scheme 2. The N and O complexation between compound **1** and Cu^{2+} is formed through two five membered rings, which enhanced the planar rigid structure of compound **1** resulting in fluorescent enhancement. These results supported the proposed mechanism.

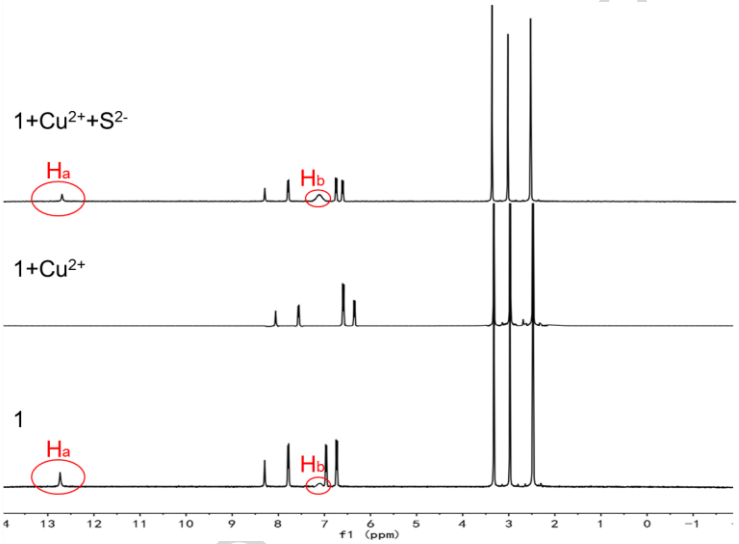
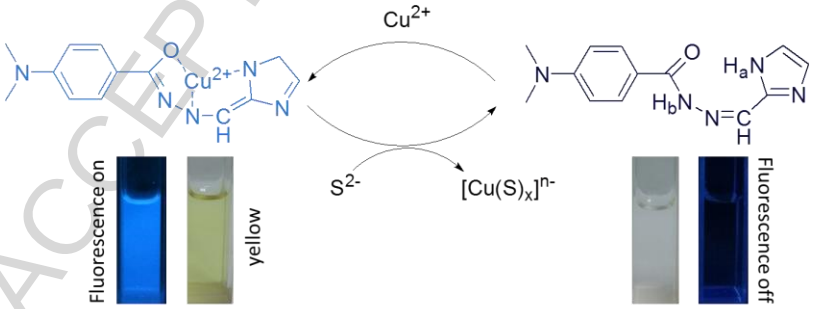


Fig.10 ^{1}H NMR spectral changes of **1**、**1-Cu^{2+}** and **1-Cu^{2+}** upon addition of S^{2-} in $(CD_3)_2SO$.



Scheme 2 Illustration of the proposed mechanism of **1** for the sequential detection of Cu^{2+} and S^{2-}.

4. Conclusion

In summary, a new colorimetric and fluorescent sensor **1** has been developed, which could selectively detect Cu^{2+} over other metal ions; what is more, the ensemble (**1-Cu^{2+}**) could detect a

low concentration of S^{2-} by a colorimetric change as well as a fluorescent change. Its application in quantitative detection of S^{2-} using simple paper-made test kits was performed. Sensor **1** demonstrated a highly selective ''off-on-off'' type fluorescent switching property. The combining response mechanism was confirmed via UV-vis absorption, fluorescence measurements, ^{1}H NMR, mass spectroscopy and density functional theory calculation.

Author contributions

The first two authors contributed equally to this work.

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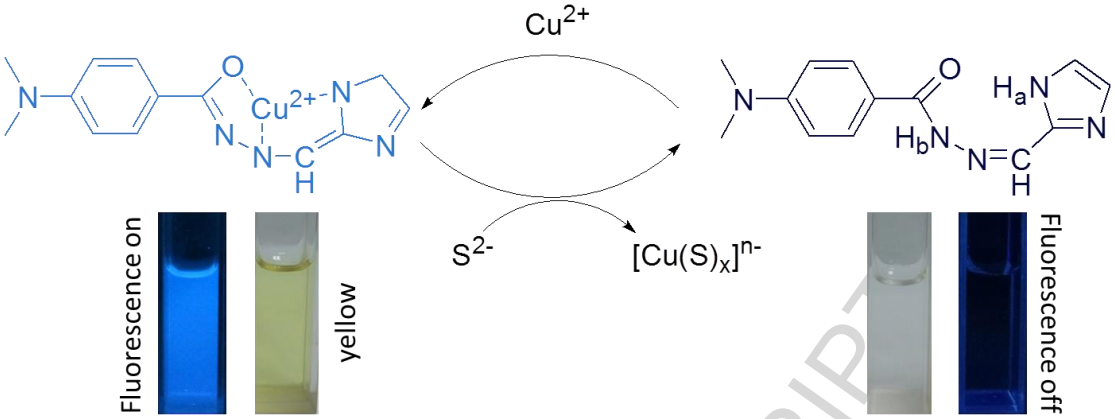
Graphical Abstract (for review)



Highlights

1. A colorimetric and fluorescent assay for Cu^{2+} and S^{2-} in aqueous solution based on 4-Dimethylamino-benzoic acid hydrazide was presented.
2. The sensor for detection of Cu^{2+} was based on fluorescence enhancement and the background was almost zero.
3. The colorimetric and fluorescent assay shows excellent selectivity over other ions by the naked eye and spectra.
4. The assay was applied to detect S^{2-} on the test strips.
5. The experiment has a low detection limit for Cu^{2+} and S^{2-}