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Ultrasensitive and highly selective detection of Cu²⁺ ions based on a new carbazole-Schiff

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Abstract: A new chemosensor for Cu^{2+} based on Schiff base with high sensitivity and selectivity was designed and synthesized. The fluorescence intensity of the chemosensor in CH₃CN solution was enhanced 160-fold after the addition of 10 equiv. Cu^{2+} over other metal ions. In addition, it also facilitates colorimetric detection for Cu^{2+} in CH₃CN solution. The chemosensor displayed low detection limit and fast response time to Cu^{2+} .

Keywords: Fluorescence; Chemosensor; Copper ion; Schiff base; Carbazole.

1. Introduction

Heavy metal ions are hazardous to human health and environment, of which, Cu²⁺ ions perform an important role in physiology of human beings [1-3]. Cu²⁺ ion is one kind of the essential trace metal ion to sustain normal human health and sever as a cofactor for a wide variety of enzymes in all living organisms. For example, Cu²⁺ ions play a cofactor role in various redox processes, enzyme functions and pigments [4]. Although Cu²⁺ are the third most abundant essential trace element after iron and zinc in the human body, they are harmful to humans and organism at high concentration [5]. Excessive loading copper ions can cause extremely negative health effects such as prion disease, several neurodegenerative diseases including Menkes, Wilson, Alzheimer and Parkinson [6-8] and could turn into a biologically hazard because of its ability to generate reactive oxygen species, which causes irritation of nose and throat resulting in nausea, vomiting, and diarrhea [9]. In our daily lives, Cu^{2+} ion is generated and accumulated in food chain and the environment with the development of various industries such as electroplating, painting, wood and paper industries. The limit of copper in drinking water is 1.3 ppm (~20 μM) set by US Environmental Protection Agency (EPA) [10]. The traditional detection methods for Cu²⁺ such as inductively coupled plasma atomic emission spectrometry (ICP-AES) [11-14], atomic absorption spectroscopy (AAS) [15-16], fluorescence techniques [17-19] and electrochemical methods (EM) [20-22] require sophisticated equipment, tedious sample preparation procedures and trained operators [23].

Fluorescent chemosensor is a highly sensitive and selective approach for detection of metal ions with speed, precise accurateness and low-cost [24-25]. Up to now, a variety of fluorescent and colorimetric Cu²⁺ chemosensor based on small organic molecules, conjugated polymers, nanoparticles

and biomolecules were reported [2, 26-31]. Even so, exploring of fluorescence emission probes for Cu²⁺ sensing with high selectivity and sensitivity is still necessary.

Schiff bases, condensation of reactive aldehydes and amines, are important compounds with various potential applications. Schiff bases attract much interest both from a synthetic and biological point of view. Some of Schiff base derivatives had been used as coordination unit in fluorescent chemosensors [32-35]. The bridged C=N structure of Schiff bases easily isomerize in the excited state and tend to exhibit very weak fluorescence. But when they coordinate with some special metal ions, the C=N isomerization is inhibited, and then Schiff bases show strong fluorescence [36-37].

Carbazole is a conjugated unit with interesting optical and electronic properties. At present, the most carbazole derivatives are usually used as photoelectric functional materials. There are a few reports on carbazole-based fluorescent chemosensor for selective detection of metal ions [10, 38-39]. Diaminomaleonitrile has special steric structure and containing four nitrogen atoms and C=C double bond, it can provide high selectivity in coordination process with metal ions.

In this paper, a novel Schiff base (Scheme 1) composed of carbazole and diaminomaleonitrile has been developed to behave as a highly selective and sensitive fluorescent chemsensor for Cu^{2+} in CH_3CN solution. Moreover, this Schiff base presented the application potential as fluorescent for the detection of metal ions.



Scheme 1. The synthetic route of sensor 1

2. Materials and Methods

2.1. Instruments and materials

Unless otherwise stated, solvents and reagents were of analytical grade from commercial suppliers and were used without further purification. All chromatographic examinations were performed using silica gel, and TLC was performed on silica plates (made in China). ¹H NMR

spectra and ¹³C NMR spectra were recorded on a Varian Unity Inova Spectrometer (500 MHz) at room temperature using d-chloroform as solvent. The IR spectra were recorded on a Nicolet 6700 Fourier Transform Infrared Spectrometer (FT-IR). The absorbance spectra were examined using a Varian Cary 500 UV-vis spectrophotometer. Deionized water was used throughout all experiments. The salts used in stock solutions of metal ions are Cu(NO₃)₂·3H₂O, Ni(NO₃)₂·6H₂O, Al(NO₃)₃·9H₂O, Zn(NO₃)₂·6H₂O, Cr(NO₃)₃·9H₂O, Cd(NO₃)₂·4H₂O, Fe(NO₃)₃·9H₂O, Co(NO₃)₂·6H₂O, Mg(NO₃)₂·6H₂O, KNO₃, AgNO₃, BaCl₂·2H₂O, MnCl₂, HgCl₂, PbCl₂, CaCl₂, SrSO₄ and FeSO₄·7H₂O.

2.2. Synthesis

2.2.1. Synthesis of N-hexyl carbazole 3

After the mixture of 85.0 ml DMF and KOH (13.0 g, 232.0 mmol) in a 250 ml flask was stirred for 20 min at room temperature, Carbazole (6.20 g, 37.0 mmol) was added into the mixture and continued to be stirred. After stirring for 40 min, bromohexane (5.27 ml, 37.0 mmol) was added dropwise into the mixture. Then, the mixture was stirred for another 9 h. The mixture was poured into cool water and the white precipitate was obtained. Then the filtered residue was chromatographed over silica gel using petroleum ether/dichloromethane (3/1) as an eluent to give N-hexyl carbazole, 9.10g. Yield: 98.0%. ¹H NMR (500 MHz, CDCl₃, ppm) : δ 8.10 (d, J = 4.0 Hz, 2H), 7.48-7.45 (m, 2H), 7.40 (d, J = 4.0 Hz, 2H), 7.25-7.21 (m, 2H), 4.30 (t, J = 7.5 Hz, 2H), 1.90-1.84 (m, 2H), 1.40 (t, J = 6.25 Hz, 2H), 1.38-1.25 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H).

2.2.2. Synthesis of 3-formyl-N-hexyl carbazole 2

After POCl₃ (9.1 ml, 100.0 mmol) was added dropwise into DMF (81.0 ml, 100.0 mmol) under stirring in ice-water bath, the reaction mixture was stirred for another 40 min and brought to room temperature under stirring for 1 h. N-hexyl carbazole **3** (2.51 g, 10.0 mmol) in 1, 2-dichloroethane (50.0 ml) was added dropwise and then the reaction mixture was refluxed for 24 h. After cooling to room temperature, the mixture was poured into ice water. The mixture was neutralized with 50% NaOH aqueous solution and then extracted with CH₂Cl₂ (4×50 ml). The

combined organic phase was washed with saturated sodium bicarbonate solution and saturated sodium chloride solution, dried with anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure and the crude product was purified by silica chromatography using petroleum ether: ethyl acetate = 3:1 as eluent to give white powder, 4.17 g, yield, 75.0%. ¹H NMR (500 MHz, CDCl₃, ppm) δ : 10.10 (s, 1H), 8.61(d, *J* = 0.5 Hz, 1H), 8.16 (d, *J* = 3.75 Hz, 1H), 8.02-8.00 (m, 1H), 7.55-7.52 (m, 1H), 7.47 (t, *J* = 8.25 Hz, 2H), 7.34-7.31 (m, 1H), 4.33(t, *J* = 7.25 Hz, 2H), 1.92-1.86 (m, 2H), 1.41-1.35 (m, 2H), 1.34-1.26 (m, 4H), 0.86 (t, *J* = 7.25 Hz, 3H).

2.2.3. Synthesis of 2-amino-3-((Z)-((9-hexyl-9H-carbazol-3-yl) methylene)amino)maleonitrile (sensor 1)

A mixture of 30 ml ethanol, 2, 3 - diaminomaleonitrile (0.648 g, 6 mmol) and 9-hexyl-9*H*-carbazole-3-carbaldehyde (0.837 g, 3 mmol) in a 100 ml flask was stirred at 70°C for 6 h. The solvent was distilled off and residue was chromatographed over silica gel using petroleum ether/ethyl acetate (3:1) to afford sensor **1** with a yield of 68.8%. ¹H NMR (500MHz, CDCl₃, ppm) δ : 8.58 (s, 1H), 8.53 (d, *J* = 0.5 Hz, 1H), 8.14 (d, *J* = 4.0Hz, 1H), 7.97-7.95 (m, 1H), 7.54-7.51 (m,1H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 7.25 Hz, 2H), 7.26 (s, 1H), 5.01 (s, 2H), 4.32 (t, *J* = 7.25 Hz, 2H), 1.90-1.86 (m, 2H), 1.41-1.34 (m, 2H), 1.33-1.26 (m, 4H), 0.865 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃, ppm): δ = 160.07, 143.09, 141.02, 126.86, 126.61, 126.03, 123.36, 122.75, 120.66, 120.09, 114.00, 112.68, 109.48, 109.34, 109.21, 43.39, 31.49, 28.91, 26.91, 22.51, 13.98 ppm. IR (KBr): 3435.30, 3330.26, 2923.87, 2857.53, 2228.29, 2193.72, 1684.55, 1599.90, 1467.60, 1368.92, 1237.25, 1126.51, 962.91, 895.09, 805.00, 746.44, 603.42, 563.54, 465.21 cm⁻¹. HRMS: m/z: found [M + Na]. 392.45, molecular formula C₂₃H₂₃N₅, requires [M + Na] 392.18.

2.3. Detection of sensing properties of sensor 1 for copper ions

2.3.1. Selective experiments

Both absorbance and fluorescence spectra were used to investigated the selective response of sensor 1 to metal ions. Sensor 1 (184.5 mg, 0.5 mmol) was dissolved in acetonitrile (CH₃CN, 100 ml) to get the 5×10^{-3} M stock solution. And then, 0.2 ml stock solution were taken with microliter

pipette and added into 100 ml volumetric flask. After the CH₃CN was added to the volumetric scale mark, the 1×10^{-5} M solution of sensor 1 was obtained. 0.25 mmol of Cu(NO₃)₂·3H₂O, Ni(NO₃)₂·6H₂O, Al(NO₃)₃·9H₂O, Zn(NO₃)₂·6H₂O, Cr(NO₃)₃·9H₂O, Cd(NO₃)₂·4H₂O, Fe(NO₃)₃·9H₂O, Co(NO₃)₂·6H₂O, Mg(NO₃)₂·6H₂O, KNO₃, AgNO₃, BaCl₂·2H₂O, MnCl₂, HgCl₂, PbCl₂, FeSO₄·7H₂O, CaCl₂ and SrSO₄ were dissolved into deionized water (50 ml) to make 5×10⁻³ M metal ion solutions, respectively. For selective test, the each test solutions were prepared by placing 0.05 ml of the each metal solution (5×10⁻³ M) into 2.5 ml sensor 1 solution (1×10⁻⁵ M), respectively. In the mixture solutions, the metal ion is 10 equiv. of sensor 1. After mixing them for three minutes, UV–vis absorbance and fluorescence spectra were taken at room temperature.

2.3.2. Absorbance and fluorescence titration

For each titration solution with different molar ratio of Cu^{2+} to sensor 1 was prepared by mixing different volumes [µL] of Cu^{2+} and sensor 1 stock solutions, and then the mixing solution was diluted using CH₃CN to 2.5 mL. The concentration of sensor 1 in every solution was kept at 1×10^{-5} M. The absorbance and fluorescence spectra were taken at room temperature after finishing the solution preparation for three minutes.

2.3.3. Job' plot measurements

The stoichiometry of complexes can be obtained using Job' plot method via the measurement of absorbance and fluorescence spectra. Job' plot was drawn based on the measurement of a series of solutions in which the molar concentrations of ligand and metal ion vary but their sum remains constant. The absorbance or fluorescence of each solution is measured and plotted the maximum value of absorbance or emission against the mole fraction of ligand or metal ion. The maximum or the inflection point on the Job' plot appear at the mole ratio corresponding to the combining ratio of the complex.

3. Results and Discussion

3.1. Synthesis and structural characterization

The general synthetic procedure is given in Scheme 1. The target sensor 1 was easily synthesized by the reaction of 2, 3-diaminomaleonitrile and 9-hexyl-9H-carbazole-3-carbaldehyde. The structure of sensor 1 was well characterized by IR, ¹H NMR, ¹³C NMR, and HRMS analyses. The alkyl connected on the N atom of carbazole unit is aimed to increase the solubility in solvents.

3.2 Fluorescent recognition of Cu^{2+}

There are five nitrogen atoms with lone pair electrons in sensor 1, it is anticipated that sensor 1 can coordinate with metal ions and further change its fluorescent property. The preliminary fluorescence spectra studies revealed that sensor 1 exhibited a better selectivity to Cu²⁺ in CH₃CN solution. Fig. 1 presents the fluorescence spectra of sensor 1, compound 2 (aldehyde carbazole) and their mixed solutions with Cu2+ in CH3CN solutions, the concentration of sensor 1 and compound 2 are kept at same as 1×10^{-5} M. The results obviously indicates that Cu²⁺ endows the large enhancement effect on the fluorescence of sensor 1 with the 70 nm red-shift compared with the maximum fluorescence peak of sensor 1. At the excitation wavelength of 298 nm, sensor 1 presented two main emission peaks at 335 and 381nm, compound 2 also showed two emission peaks at 334 and 430 nm. Their emissions are weak under measurement condition and relative intensity are just larger than 100, which is probably due to the C=N isomerization [40]. In the same concentration and measurement condition, after addition of 10 equiv. Cu²⁺, the fluorescence spectrum of compound 2 had not obvious change and its intensity did not increase or decrease, which indirectly indicated that compound 2 did not have obvious interaction with Cu^{2+} . However, upon the addition of Cu^{2+} into the sensor 1 solution, a new emission peak at 450 nm appeared and its intensity was dramatic large to be 4800, it should be ascribed to the complex of sensor 1 with Cu^{2+} . Before addition of Cu^{2+} , the relative fluorescence intensity at 450 nm of sensor 1 was just 30, but it increased to 4800 after addition of 10 equiv. Cu²⁺. The fluorescence enhancement was up to 160-fold, which also confirmed the complex of sensor 1 with Cu^{2+} has large fluorescence efficiency. The solution of sensor **1** possessed a quantum yield of 0.01 (1×10^{-5} M) in CH₃CN, after addition of 10 equiv. Cu^{2+} , the quantum yield increased to 0.22. Meanwhile, the difference of fluorescence spectra of sensor 1 with compound 2 upon the addition of Cu^{2+} also showed that the

coordination effect of sensor **1** with Cu^{2+} comes from diaminomaleonitrile moiety but not carbazole and aldehyde units. The fluorescence images of sensor **1** before and after addition of Cu^{2+} in Fig. **1** also illustrated the large fluorescence enhancement after addition of Cu^{2+} .



Fig 1. Fluorescence spectra comparison of sensor **1**, compound **2** (1×10^{-5} M) and their mixed solutions with Cu²⁺ ions (10 equiv.) in CH₃CN solutions and fluorescence images of sensor **1** before and after addition of Cu²⁺ ions.

3.3 Selectivity

High selectivity toward target analyte over the other potentially competitive species is a very important parameter to evaluate the performance of a chemosensor. Therefore, the fluorescence response of sensor 1 solution $(1\times10^{-5} \text{ M})$ in CH₃CN solutions toward various metal ions (10.0 equiv.) was conducted and the results were shown in Fig. 2. Free sensor 1 showed a very weak emission with two peaks at 335 and 381 nm under the excitation of 298 nm light, upon addition of 10.0 equiv. of Cu²⁺ into the sensor 1 solution, a new emission band centered at 450 nm appeared and displayed a dramatic fluorescence enhancement (Fig. 2a). Other metal ions such as K⁺, Ag⁺, Mn²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Pb²⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Hg²⁺, Fe²⁺, Fe³⁺, Al³⁺ and Cr³⁺ (10.0 equiv. of each) did not induce significant fluorescence emission changes under the identical conditions (Fig. 2b). Some of these potentially competitive metal ions also induced a new emission peak at 450 nm but the intensities were very weak, which may be due to their very weak interaction with sensor 1. These observations indicate that sensor 1 has an excellent selectivity to Cu²⁺ ion in CH₃CN solutions.



Fig 2. Fluorescence response of sensor 1 (1×10⁻⁵ M) in CH₃CN towards to various metal ions (10.0 equiv.).

This fluorescence enhancement could be ascribed to the complex formation between sensor **1** and Cu^{2+} . The fluorescence of sensor **1** is weak in CH₃CN solution due to C=N isomerization which is the predominant decay process of the excited state. Upon addition of Cu^{2+} , the coordination of sensor **1** with the metal ion inhibits the C=N isomerization. On the contrary, other ions fail to form such framework and did not increase the fluorescence intensity, which may be due to the unsuitable coordination geometry conformation of the Schiff-base unit of sensor **1** and the inappropriate ion radius and insufficient binding energy of these metal ions.

The anti-jamming ability is also important for one sensor to be used in practical applicability, the possible interferences by metal ions including K⁺, Ag⁺, Mn²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Pb²⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Hg²⁺, Fe²⁺, Fe³⁺, Al³⁺ and Cr³⁺ were measured through competitive experiments. The competition experiment was carried out by monitoring the change of fluorescence intensity at 450 nm upon addition of 10 equiv. Cu²⁺ and 10 equiv. other metal ions to a solution of sensor **1**, the results are shown in Fig. 3. Although exists of a certain degree of perturbation caused by the above competitive metal ion, the same concentration of Cu²⁺ still enhanced the fluorescence intensity of sensor 1 near to 100-fold of its original value. This result indicated that the exists of the competitive metal ion could not interfere detection of sensor 1 for Cu²⁺, in other words, the sensing of sensor **1** toward Cu²⁺ has the high selectivity and sensitivity.

Therefore, it is safe to say that sensor 1 is capable of fluorescent recognition of Cu^{2+} with high selectivity from different interference metal ions.



Fig 3. (a) The fluorescence intensity changes of sensor 1 (1×10^{-5} M) to various metal ions. The black bars represent the fluorescence intensity of sensor 1 in the presence of miscellaneous metal ions (10 equiv.), the red bars represent the fluorescence intensity of the above solution upon further addition of 10 equiv. of Cu²⁺ (λ em =450 nm). (b) Fluorescence images of sensor 1 after addition of different metal ions. (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article.)

3.4 Fluorescence titration

To evaluate the sensing property of sensor **1** toward Cu^{2+} , fluorescence titration experiment of sensor **1** (1×10⁻⁵ M) with Cu^{2+} (0~20 equiv) in CH₃CN solution was carried out. As shown in Fig. 4, upon gradual increasement of Cu^{2+} , the fluorescence intensity of the solution at 450 nm increased. The fluorescence intensity reached to the highest value and the enhancement was almost 160-fold when the 6 equiv. Cu^{2+} was added, see inset in Fig. 4. When more Cu^{2+} was titrated, the fluorescence intensity did not further increase.



Fig 4. Fluorescence response of sensor **1** in CH₃CN solution (1×10^{-5} M) upon addition of Cu²⁺ ($0 \sim 20$ equiv.). $\lambda_{ex} = 298$ nm, $\lambda_{em} = 450$ nm. The inset is the plot of fluorescence intensity at 450 nm of sensor **1** as a function of Cu²⁺ concentration.

Based on the titration data, the binding constant (Ka) of sensor 1 with Cu2+ can be calculated

using the Benesi-Hildebrand plot (Fig. 5a) [41]. Linear fitting of the experiment plot based on the 1:1 binding stoichiometry of sensor **1** and Cu^{2+} was examined and the fitted curve are almost superimposed over the experimental plot with a correlation coefficient over 0.9785, which strongly supports the 1:1 binding stoichiometry of sensor **1** and Cu^{2+} . The binding constant between sensor **1** and Cu^{2+} was evaluated to be 1.26×10^{-6} M⁻¹. The Job's plot further confirmed the 1:1 binding stoichiometry (Fig. 5b).



Fig 5. (a) Benesi-Hildebrand plot of sensor 1 (1×10^{-5}) in CH₃CN solution in the presence of Cu²⁺ ($2.2 \sim 4.0$ equiv.) (R=0.9973). (b) Job's plot from the fluorescence emission spectra of sensor 1 and Cu²⁺ in CH₃CN solution with the total concentration of 5×10^{-5} M. $\lambda_{ex} = 298$ nm, $\lambda_{em} = 450$ nm.

In addition, the fluorescent detection limit of sensor **1** for Cu^{2+} was also evaluated via the calibration curve (Fig. S1) and the standard deviation of the blank solutions. The calibration curve of fluorescence intensity at 450 nm versus $[Cu^{2+}]$ was made and it displayed a linear graph, the linear relationship R is 0.9766. The detection limit C_{LOD} was calculated to be 2.74×10^{-8} M from the following equation: $C_{LOD} = K \times Sb1/S$. Here, K = 3; Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve [42]. The detection limit is much lower than the limit of copper in drinking water (~20 μ M) permitted by US Environment Agency (EPA) [43] and typical concentration of blood copper ($1.57 \times 10^{-5} \sim 2.36 \times 10^{-5}$ M) in normal individuals defined by the U.S. Environmental Protection Agency [44]. This result indicates that sensor **1** is sensitive enough to monitor Cu²⁺ concentration in solution.

The effect of the reaction time on the fluorescence emission of the system was examined and the results are shown in Fig. S2. It can be seen that there is almost no time-dependent effects in the

fluorescence sensing process, the Cu^{2+} solution was added into to sensor 1 solution under hand shake, the emission intensity reaches to its saturation value in just 15 s due to the rapid complex reaction. The solution is uniformly mixed, the emission intensity reaches the saturation immediately. Based on its very fast response, sensor 1, as a fluorescent chemosensor, can provide a very rapid detection of Cu^{2+} .

The reversibility of fluorescence response process of sensor **1** for Cu^{2+} was also investigated with ethylenediamine tetraaetic acid disodium salt (EDTANa₂). After addition of excess EDTA (20 equiv. of Cu^{2+}) to sensor **1** and Cu^{2+} solution, the fluorescence spectrum of the resulted solution restored to the original spectrum of sensor **1** (Fig. S3), which indicates that the Cu^{2+} recognition is complexation process and reversible.

3.5 Colormetric sensoring of sensor 1 for Cu²⁺

The selective response of sensor **1** for Cu^{2+} was also confirmed by the changes of both color and absorbance spectra of sensor 1 solution upon additions of different metal ions. Sensor **1** in CH₃CN solution displayed two absorption bands centered at 290 nm and 398 nm corresponding to the π - π transition of the C=N group and an intermolecular charge transfer (ICT) of the entire conjugated molecule, respectively [40].

After addition of a variety of metal ions (10 equiv.) such as K⁺, Ag⁺, Mn²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Pb²⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Hg²⁺, Fe²⁺, Fe³⁺, Al³⁺ and Cr³⁺ in deionized water (5×10⁻³ M), the absorption intensity did not show obvious change except for Fe³⁺, Pb²⁺ and Cu²⁺ as shown in Fig.6a. The addition of Fe³⁺ and Pb²⁺ just caused the slight absorption increase. As the same as fluorescence response, the absorbance spectra of sensor **1** presented significant change after addition of 10 equiv. Cu²⁺ ions, the absorption band at 398 nm disappeared completely. Meanwhile, the color of the solution became colorless completely from yellowish (Fig.6b), which indicates that sensor **1** can serve as a colorimetric chemosensor for Cu²⁺ ion with high selectivity





Fig 6. (a) Absorption spectral changes of sensor $\mathbf{1}$ (1×10⁻⁵ M) upon the addition of 10 equiv of various metal ions in CH₃CN. (b) The color changes of sensor $\mathbf{1}$ (1×10⁻⁴ M) upon the addition of 10 equiv of various metal ions.

Spectrometric titration experiments can help us to disclose the interaction mechanism of sensor 1 with Cu^{2+} . Therefore, titration of sensor 1 in CH₃CN with Cu^{2+} was subsequently performed. Upon incremental addition of Cu^{2+} (0~20 equiv.) to the sensor 1 solution (1×10⁻⁵ M) results in a stepwise decrease and increase in absorbance at 398 nm and 290 nm, respectively. Meanwhile, a new absorption peak at 320 nm appeared and increased gradually with the addition of Cu^{2+} (Fig.7). The mole extinction coefficient ($\varepsilon = 1.1 \times 10^{-4} \text{ L} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$)) of sensor 1 at 398 nm decreased near to 0. There also existed an isobestic point at 340 nm in the titration spectra. From these results, we could estimate that the complex between sensor 1 and Cu^{2+} formed and the binding effect of sensor 1 with Cu^{2+} was the direct reason for inducing the fluorescence enhancement. In other word, the complexes of sensor 1 with Cu^{2+} have high fluorescent quantum yield.



Fig 7. Changes in the absorption spectra of sensor 1 (1×10^{-5} M) upon addition of Cu²⁺ ($0 \sim 20$ equiv.) in CH₃CN solution and the color change of the solution.

The binding constant was also evaluated using the Benesi-Hildebrand plot via fitting of the absorption data obtained by titration of sensor **1** with Cu^{2+} (Fig. S4) [45]. The absorbance at 398 nm gradually decreased with the successive addition of Cu^{2+} . The Benesi-Hildebrand plot showed a linear line with the correlation coefficient 0.9976 and the binding constant was calculated to be 1.98×10^{-6} . The linear plot also illustrates that sensor **1** form 1:1 stoichiometric complex with Cu^{2+} , and 1:1 binding mode was further confirmed by Job's plot (Fig. S5). On the Job's plot, the inflection point appeared at 0.5 of the mole ratio of $[Cu^{2+}]/[sensor$ **1** $] + [Cu^{2+}]$. These results are consistent with those of fluorescence measurement.

3.6 Proposed binding mode

The response of the fluorescence and absorbance of sensor 1 to Cu^{2+} provided an obvious symbol of interaction and complex formation between sensor 1 and Cu^{2+} with the 1:1 stoichiometry. Comparison of absorbance spectra of carbazole (compound 2 in Scheme 1), sensor 1 and sensor 1-Cu²⁺ in CH₃CN solution can help us to interpret the sensing mechanism of sensor 1 for Cu²⁺ (Fig. S6). Carbazole presents two main absorption peaks at 262 and 293 nm, sensor 1 displays one more absorption peak at 398 nm, which is ascribed to the intermolecular charge transfer (ICT) band of the entire conjugated molecule. So, it can be stated that the absorption peak at 398 nm of sensor 1 is caused by combining of carbazole with diaminomaleonitrile. The absorption peak of sensor 1 at 398 nm disappeared completely but absorption peaks of carbazole did not change obviously after coordination with Cu^{2+} , which state that the coordination unit of sensor 1 with Cu²⁺ is diaminomaleonitrile unit but not carbazole moiety. In addition, the fluorescence spectra of compound 2 also did not change obviously after addition of Cu²⁺ in CH₃CN solution (Fig. 1), which indicated that both aldehyde group and carbazole could not take part in coordination with Cu²⁺. ¹H NMR studies also provide further evidence for the interaction between sensor 1 and Cu^{2+} . Upon the addition of 10 equiv. Cu^{2+} , protons peaks on the sensor 1

broadened and shifted downfield as shown in Fig. S7. Based on the above results, we proposed the

binding mode as in scheme 2.



Scheme 2. Proposed mechanism of fluorescence enhancement of Sensor 1

4. Conclusions

In conclusion, we have developed a new simple fluorescent chemosensor based on Schiff base for Cu^{2+} with high sensitivity and selectivity in CH₃CN solution. The fluorescence intensity of chemosensor was significantly enhanced about 160 fold with the addition of 6 equiv of Cu^{2+} . In addition, it also displayed a greater colorimetric sensing ability for Cu^{2+} via the changes of color and absorbance spectra. Most importantly, both the color and fluorescence changes of the chemosensor solution are significant for Cu^{2+} in the presence of other heavy and transition metal ions (even in high concentration). The low detection limit and fast response time of chemosensor to Cu^{2+} meet the selective detection requirements for biomedical and environmental monitoring application. We expect that this chemosensor provides a potential tool for the detection and quantification of Cu^{2+} ions in environmental samples.

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Figure Caption:

Scheme 1. The synthetic route of sensor 1.

Scheme 2. Proposed mechanism of fluorescece enhancement of Sensor 1.

Fig 1. Fluorescence spectra comparison of sensor **1**, compound **2** (1×10^{-5} M) and their mixed solutions with Cu²⁺ ions (10 equiv.) in CH₃CN solutions and fluorescence images of sensor **1** before and after addition of Cu²⁺ ions.

Fig 2. Fluorescence response of sensor 1 (1×10^{-5} M) in CH₃CN towards to various metal ions (10.0 equiv.).

Fig 3. (a) The fluorescence intensity changes of sensor 1 (1×10^{-5} M) to various metal ions. The black bars represent the fluorescence intensity of sensor 1 in the presence of miscellaneous metal ions (10 equiv.), the red bars represent the fluorescence intensity of the above solution upon further addition of 10 equiv. of Cu²⁺ (λ em =450 nm). (b) Fluorescence images of sensor 1 after addition of different metal ions. (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article.)

Fig 4. Fluorescence response of sensor 1 in CH₃CN solution (1×10^{-5} M) upon addition of Cu²⁺ ($0 \sim 20$ equiv.). λ_{ex} = 298 nm, λ_{em} = 450 nm. The inset is the plot of fluorescence intensity at 450 nm of sensor 1 as a function of Cu²⁺ concentration.

Fig 5. (a) Benesi-Hildebrand plot of sensor 1 (1×10⁻⁵) in CH₃CN solution in the presence of Cu²⁺ (2.2~4.0 equiv.) (R=0.9973). (b) Job's plot from the fluorescence emission spectra of sensor 1 and Cu²⁺ in CH₃CN solution with the total concentration of 5×10^{-5} M. $\lambda_{ex} = 298$ nm, $\lambda_{em} = 450$ nm.

Fig 6. (a) Absorption spectral changes of sensor **1** $(1 \times 10^{-5} \text{ M})$ upon the addition of 10 equiv of various metal ions in CH₃CN. (b) The color changes of sensor **1** $(1 \times 10^{-4} \text{ M})$ upon the addition of 10 equiv of various metal ions.

Fig 7. Changes in the absorption spectra of sensor 1 (1×10^{-5} M) upon addition of Cu²⁺ ($0 \sim 20$ equiv.) in CH₃CN solution and the color change of the solution.

Ultrasensitive and highly selective detection of Cu²⁺ ions based on a new carbazole-Schiff

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Highlights

► Carbazole-based Schiff as fluorescent chemosensor (sensor 1) was successfully synthesized.

▶ Sensor 1 displayed high selective and sensitive fluorescence sensing for Cu²⁺.

Sensor 1 showed high anti-interference ability in the co-existence of other metal ions.

► Sensor 1 also showed the ability of colorimetric sensing for Cu²⁺ ions.

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