Journal of Fluorescence (2019) 29:1153–1159
https://doi.org/10.1007/s10895-019-02406-z

ORIGINAL ARTICLE

A Fluorescent Sensor for Cu^{2+} Ion with High Selectivity and Sensitivity Based on ICT and PET

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Received: 14 May 2017 / Accepted: 2 July 2019 / Published online: 16 August 2019

Abstract

A novel fluorescent sensor (L) based on 1,8-naphthalic anhydride has been developed which can selectively detect Cu^{2+} in CH_3CN medium over other metal ions at 408 nm in the fluorescence spectra. When Cu^{2+} was added into L, L showed fluorescent turn-off by coordinating with Cu^{2+}. A fresh absorption band was found at the position of 290 nm as was a red-shifted absorption band from 356 nm to 376 nm in UV-vis spectra which might be attributed to the intramolecular charge transfer (ICT). Meanwhile, L-Cu^{2+} showed fluorescence quenching via photoinduced electron transfer (PET). The complexation ratio was proposed to be 1:1 which was determined by Job's plot, fluorescence titration and ^1H NMR titration. The detection limit was 9.1×10^{-8} mol·L^{-1}, a satisfying level to detect Cu^{2+} in the micromolar scale. Corresponding molecular geometries, orbital energies and electron contributions of sensor L were calculated by the DMol3 program package using the density functional theory.

Keywords 1,8-naphthalic anhydride · Fluorescent sensor · Cu^{2+} ions · Density functional theory

Introduction

The trace element copper is the third most abundant element in the human body after iron and zinc and plays an important physiological role in many biological systems [1]. Moreover, copper, as an important catalytic cofactor, has great effects on redox chemistry for proteins required for growth and development [2, 3]. However, copper is one of the toxic metals and high levels of copper ions cause neurodegenerative diseases such as Alzheimer's, Parkinson's and it is also suspected of causing amyloidal precipitation and toxicity [4–11]. Both a deficiency and an excess of copper can lead to health problems. Thus, a convenient and fast way to detect Cu^{2+} ions is particularly important. Until now, many analytical methods have been developed to sensitively and reliably detect Cu^{2+}. These include atomic absorption spectrometry, inductively coupled plasma-atomic emissions spectrometry and inductively coupled plasma-mass spectrometry [12]. Compared to these approaches, the fluorescent sensors are a very important and effective way to detect metal ions and are the subject of much current experimental interest due to their several outstanding advantages e.g. low cost, simplicity, high selectivity, good sensitivity and high response speed [13].

Currently, 1,8-naphthalic anhydride and its derivatives are employed extensively as potential fluorophores for several metal ions because of their good photo stability, strong fluorescence, high quantum yield, large Stocks shift and easy modification [14–17]. Schiff bases, which contain nitrogen-oxygen-rich coordination environments are known to be good ligands for metal ions [18, 19]. Keeping these in mind, herein, we report a new Schiff-base fluorescent sensor, L, derived from 1,8-naphthalic anhydride (Scheme 1). It exhibits high sensitivity and selectivity for Cu^{2+} in CH_3CN solution based on the PET and ICT mechanism. Additionally, L has been characterized by different spectroscopic techniques.

Experimental

Apparatus

^1H NMR and ^{13}C NMR spectra were measured on a Bruker Avance instrument operating at 500 MHz and 126 MHz respectively, and chemical shifts were reported in ppm using

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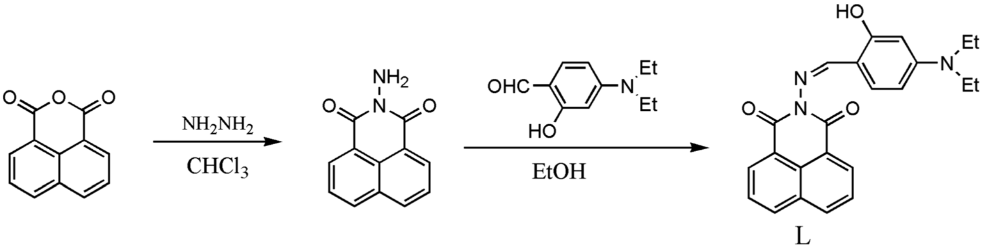
Scheme 1 The synthetic route of sensor L



tetramethylsilane (TMS) as internal standard. UV-vis absorption spectra were determined on a UV-3900 spectrophotometer. Fluorescence spectra were recorded with a Hitachi F-4600 spectrophotometer equipped with quartz cuvettes of 1 cm path length. MS spectra were measured on a Shimadzu GC-17A, QP-5000 GC/MS spectrometer. C, H and N analyses were obtained using an Elemental Vario-EL automatic elemental analysis instrument.

Materials

All reagents were purchased commercially and were of grade quality and without any further purification. The stock solutions of metal ions were prepared from nitrate salts. The ligand L was synthesized in the laboratory. Double-distilled water was used throughout the experiments.

Synthesis

Synthesis of N-Aminonaphthalimide

N-aminonaphthalimide was synthesized according to a published method [20].

Synthesis of Sensor L

N-aminonaphthalimide (0.318 g, 1.5 mmol) was dissolved in ethanol (20 mL), then 4-(Diethylamino)salicylaldehyde (0.290 g, 1.5 mmol) was added to the above solution (Scheme 1). The solution was refluxed for 8 h with continuous stirring. The solution was cooled to room temperature and the solvent was evaporated under pressure to obtain a yellow crude product. It was purified by recrystallization in ethanol to give the light yellow solid L (0.477 g, 81%). ^{1}H NMR (500 MHz, DMSO, ppm) δ 11.20 (s, 1H), 8.63 (s, 1H), 8.52 (dd, 4H), 7.91 (t, 2H), 7.40 (d, 1H), 6.37 (dd, 1H), 6.19 (d, 1H), 3.42 (q, 4H), 1.14 (t, 6H). ^{13}C NMR (126 MHz, DMSO, ppm) δ 171.74, 162.23, 161.62, 152.96, 135.42, 134.01, 132.29, 131.89, 128.21, 127.70, 123.35, 106.47, 105.14, 97.92, 44.87, 13.39. Elemental analysis calculated for $C_{23}H_{21}O_3N_3$: N: 10.85% C: 71.32% H: 5.43% found: N: 10.87% C: 71.42% H: 5.43%. MS (ESI): calculated for $C_{23}H_{21}O_3N_3$: $[M+H]^+$ (m/z): 388.42; found: 388.32.

Results and Discussion

UV–vis Studies of Sensor L

The binding ability of L toward Cu^{2+} was initially evaluated by UV-vis spectral analysis. Figure 1 shows the absorption spectrum of L with different metal ions in CH_3CN medium. Free ligand L exhibited a main absorption at 356 nm. Upon addition of a constant amount (10equiv.) of Cu^{2+} ion to L, a new absorption band appeared at 290 nm and a red-shifted absorption from 356 nm to 376 nm which might be attributed to the ICT. The other metal ions did not show any optical changes with L. From this, it is clear that L can selectively detect Cu^{2+} in the presence of other metal ions. Then, with the stepwise addition of Cu^{2+} ions (0-1equiv.) to L, the corresponding absorption band gradually increased at 290 nm (Fig. 2) and reached a maximum at 1.0 equiv. of Cu^{2+}. This implies the formation of a 1:1 complex between L and Cu^{2+}. These observations might be attributed to the chemical interaction of L with Cu^{2+}.

Fluorescence Studies of Sensor L

Selectivity is a very important parameter for evaluating the performance of a new fluorescent sensor [21–23]. Therefore,

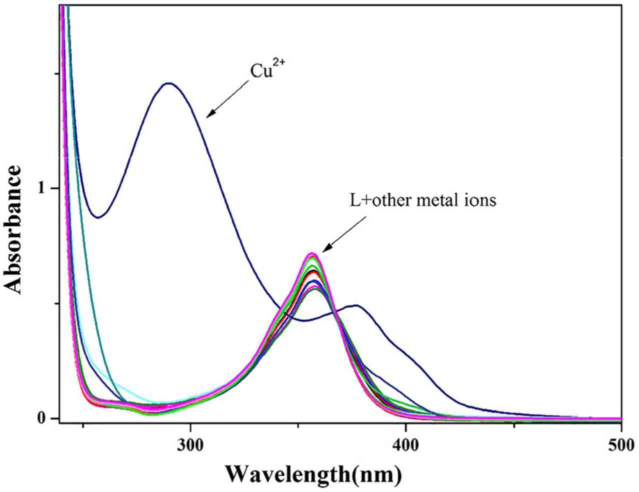


Fig. 1 UV–vis absorption spectra of sensor L (10 μM) in the absence and presence of different metal ions (10equiv.) Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Al^{3+}, Fe^{3+}, Pb^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Sr^{2+}, Zn^{2+}, Sn^{2+}, Cd^{2+}, Hg^{2+}, Ag^+, Cr^{3+}, Fe^{2+} and Cu^{2+} at room temperature

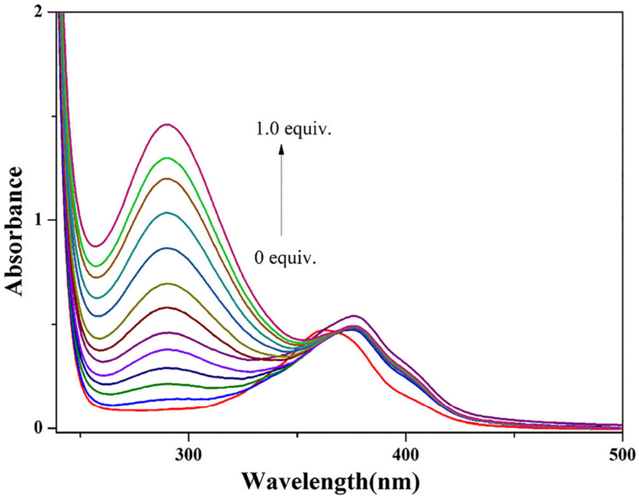


Fig. 2 UV–vis absorption spectra of sensor L with various concentrations of Cu^{2+}

the sensing properties of sensor L toward different metal ions were investigated systematically by fluorescence experiments. As shown in Fig. 3a, the free chemosensor L presented a strong fluorescence emission at 408 nm when it was excited at 308 nm. Upon addition of 10 equiv. of Cu^{2+} ion to L, a clear fluorescence quenching was observed. But the interaction of the other mentioned metal ions with L showed very little fluorescence intensity changes.

To validate the high selectivity of L toward Cu^{2+}, the fluorescence competitive response of the other metal ions was also investigated. This was studied by treating L with 10 equiv. of Cu^{2+} in the presence of 10 equiv. of each of the other metal ions. As shown in Fig. 3b, the response of sensor L for Cu^{2+} was affected minimally by the competing metal ions. This suggests that sensor L has a predominant recognition and selectivity for Cu^{2+}, thus making it a good potential sensor in the detection of Cu^{2+}.

Fluorescence Titration of Sensor L with Cu^{2+}

Based on its evident selectivity, quantitative analysis of the response of L to Cu^{2+} concentration was studied by fluorescence titration. As seen in Fig. 4, upon addition of increasing concentrations of Cu^{2+}, the emission intensity at 408 nm decreased gradually and showed a linear decrement. Simultaneously, titration experiment results indicated a good linear dependence relationship between the fluorescence intensity of L and Cu^{2+} concentration (insert in Fig. 4), which suggests that the quantitative detection of Cu^{2+} by L is applicable. More importantly, the fluorescence intensity reached a minimum when 1equiv. of Cu^{2+} was added to L. Thus suggesting that a new compound was formed by a 1:1 association between L and Cu^{2+}.

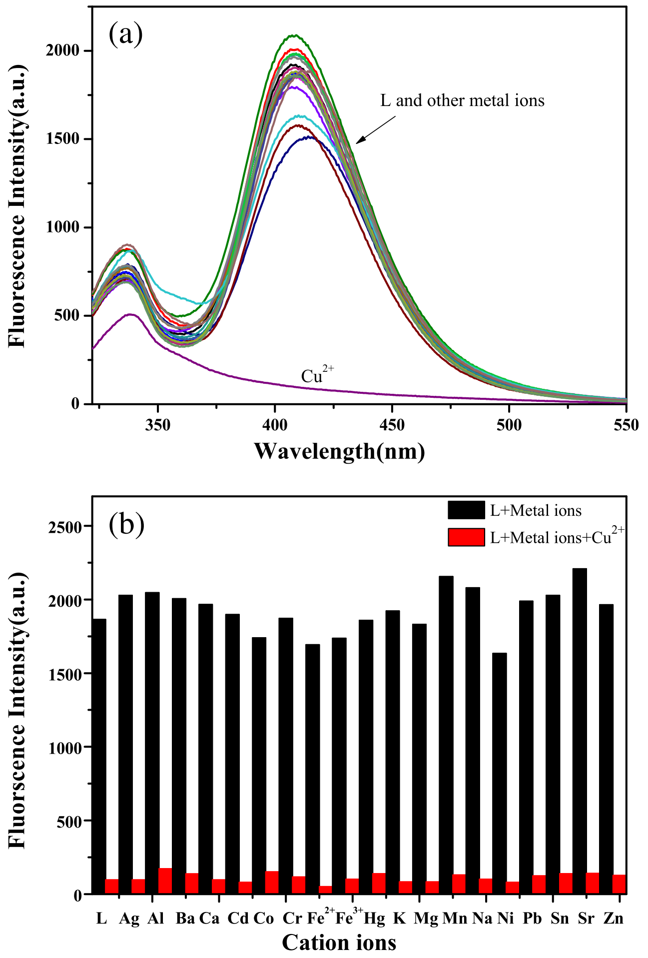


Fig. 3 **a** Fluorescence spectra of L upon addition of different metal ions. **b** Competitive fluorescent selectivity toward Cu^{2+} in the presence of different cations (10 equiv. of Cu^{2+} and 10 equiv. of Ag^+, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sn^{2+}, Sr^{2+}, Zn^{2+})

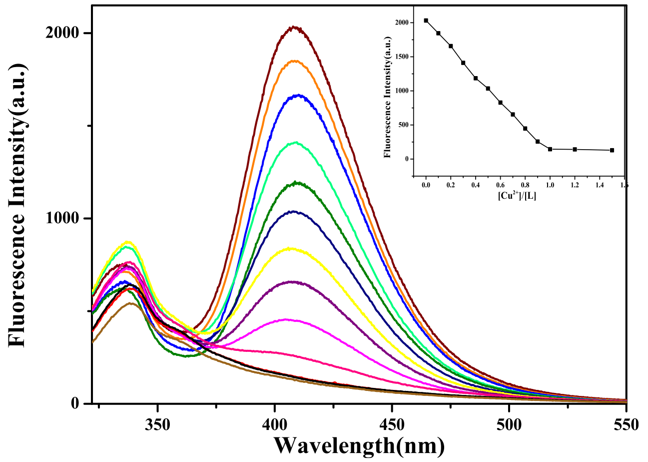


Fig. 4 Fluorescence spectra of L (10 μM) upon the titration of Cu^{2+} (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2 and 1.5 equiv.) in CH_3CN-H_2O. Inset: Fluorescence intensity at 408 nm as a function of Cu^{2+} concentrations

Binding Mode of Sensor L and Cu^{2+}

A proposed binding mode of L with Cu^{2+} ions is shown in Fig. 4 (inset), which primarily demonstrates a 1:1 stoichiometric complexation between sensor L and Cu^{2+}. This was also ascertained by Job's plot. The minimum fluorescence intensity appeared at a mole fraction of 0.5, suggesting the 1:1 complexation between L and Cu^{2+} ions (Fig. 5). Furthermore, complexation was also demonstrated by the appearance of a peak at m/z 477.28 assignable to $[L\text{-}Cu^{2+}\text{-}CN^-]^+$ (calculated for 477.42) in the ESI mass spectrum. On the basis of 1:1 stoichiometry, the association constant K_a of the L-Cu^{2+} complex was determined to be 2.13×10^5 mol·L^{-1} by the Benesi-Hildebrand equation [24].

$$\frac{1}{F_0-F}=\frac{1}{Ka\times\left(F_0-F_{\min}\right)\times\left[Cu^{2+}\right]}+\frac{1}{F_0-F_{\min}}$$

Where F_0, $F_{\min}$ and F are the fluorescence intensities in the absence of Cu^{2+}, presence of saturated Cu^{2+} and the fluorescence intensity of the L-Cu^{2+} complex at any given Cu^{2+} concentration. The detection limit (DL) of Cu^{2+} using L was determined from the follow equation: DL = K × SD/S, where K = 3; SD is the standard deviation of the blank solution; S is the slope of the calibration curve (Fig. 6). DL = 9.1×10^{-8} mol·L^{-1} (calculated as three times the standard deviation of blank solution). This DL indicates a good sensitivity of L with Cu^{2+} ions, sufficiently low to detect submicromolar concentrations of Cu^{2+} that are found in the range applicable to many chemical and biological systems.

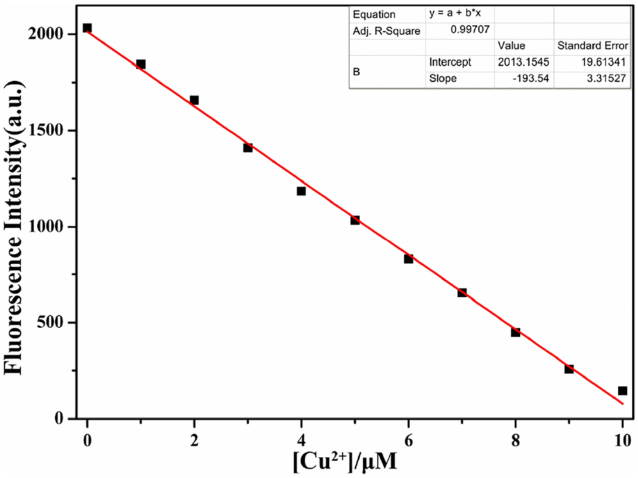


Fig. 6 Plot of fluorescence intensity variation upon varying the concentrations of Cu^{2+} ion from 1 to 10 μM with an excitation of 308 nm and an emission of 408 nm

Effect of Buffer pH

The effect of pH on the fluorescence intensity of sensor L in the absence and presence of Cu^{2+} was carried out in 10 mmol·L^{-1} tris-HCl buffer and investigated in the pH range from 1.67 to 12.59 (Fig. 7). The fluorescence intensity of L-Cu^{2+} was weak and stable in a wide pH range from 2 to 8, which indicated that the sensor L is suitable to act as fluorescent sensor for Cu^{2+}. But the fluorescence intensity of L-Cu^{2+} was increased at pH > 8 due to the L-Cu^{2+} complex breaking down and $Cu(OH)_2$ forming, thus freeing L. In the case of L, the plot of fluorescence intensity versus pH did not display any obvious changes in the pH range from 2 to 12 which suggests that it is stable over the pH range of 2–12 and could work in real samples with very low background fluorescence. Ultimately, the solution at pH = 7.21 was chosen as providing the optimum experimental condition.

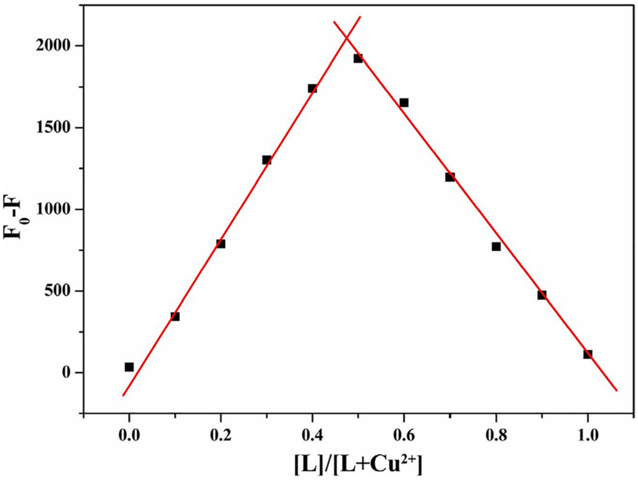


Fig. 5 The Job's plot of sensor L and Cu^{2+}

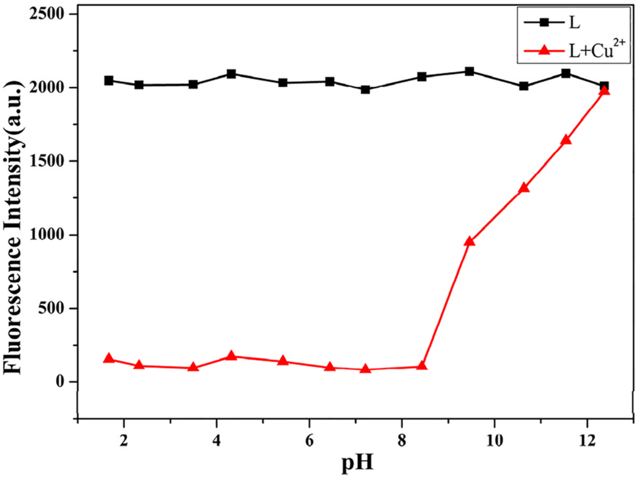


Fig. 7 Fluorescence intensity of L (10 μmol·L^{-1}) in the absence and presence of Cu^{2+} (100 μmol·L^{-1}) in a buffer solution at varying pH

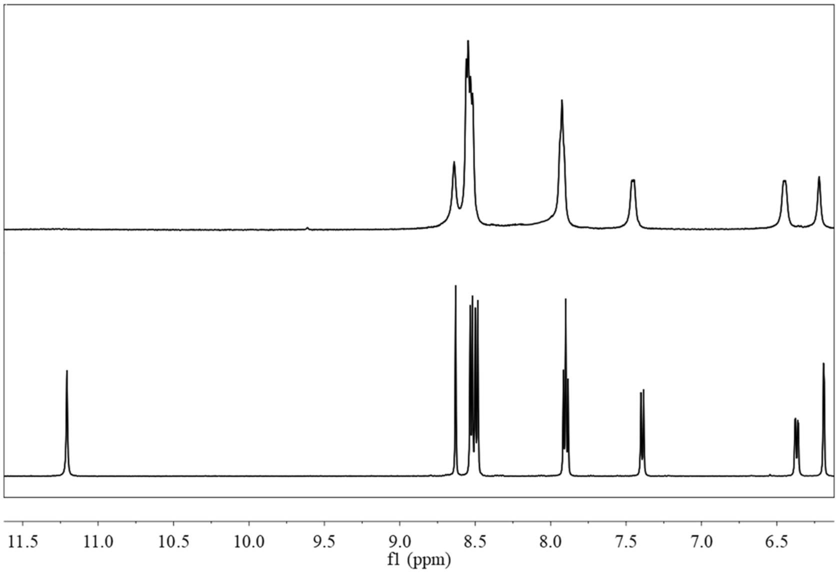


Fig. 8 ^{1}H NMR spectra of L with $Cu(NO_3)_2{\cdot}3H_2O$ in DMSO-d_6: L and L with 1.0 equiv. of Cu^{2+}

^{1}H NMR Titration

In order to obtain a better insight into the response mechanism of L to Cu^{2+}, ^{1}H NMR spectra were analysed in DMSO-d_6. The complexation of L with Cu^{2+} might be expected to reduce the electron density of the coordination sites, resulting in a downfield shift of the nearby proton signals. Significant spectral differences were observed as shown in Fig. 8. The free ligand L exhibited one hydroxy (H_b) signal at 11.21. Upon addition of 1 equiv. of Cu^{2+}, the proton peak of H_b disappeared and the proton peaks of H_i, H_j, H_k displayed apparent downfield shifts (H_i from 7.38 to 7.45, H_j from 6.36 to 6.45, H_k from 6.19 to 6.22), indicating that the oxygen atom of the hydroxy moiety might be coordinated to Cu^{2+} [25–27]. At the same time, H_a underwent a downfield shift of 0.05 ppm and the signals of H_c-H_h all shifted downfield due to the “O” of the carbonyl of chromone coordinating with Cu^{2+}. Meanwhile the signals of the other protons remained relatively unchanged. These spectrum changes suggested that the oxygen atom of the hydroxy, the carbonyl of the 1,8-naphthalic anhydride moiety and the nitrogen atom of the -CH=N- group became coordinated with Cu^{2+}.

Sensing Mechanism

As the recognition unit, Schiff bases have been widely used for the development of fluorescence sensors based on various sensing mechanisms. The lone pair of electrons from nitrogen move to the 1,8-naphthalic anhydride (acceptor) in sensor L before binding with Cu^{2+} as shown in Fig. 9. The electron transfer from 4-N, N-dimethylamino salicylaldehyde to 1,8-naphthalic anhydride was enhanced after the addition of Cu^{2+}, due mainly to the carbonyl oxygen atom of 1,8-naphthalic anhydride participating in the coordination with Cu^{2+}. Therefore the electron density of 1,8-naphthalic anhydride was reduced. However, the charge transfer in the molecule was enhanced, resulting from an increase in the degree of conjugation as shown in the UV spectrum. This is the process of the ICT mechanism. On the other hand, the copper ions can be also act as electron acceptors. Under the excitation of light, the electrons in the excited state of the sensor L are transferred to the copper ions by the coordinating procession which leads to fluorescence quenching and the PET process happening. In conclusion, the UV and fluorescence spectra changes can be explained by the ICT and the PET mechanisms.

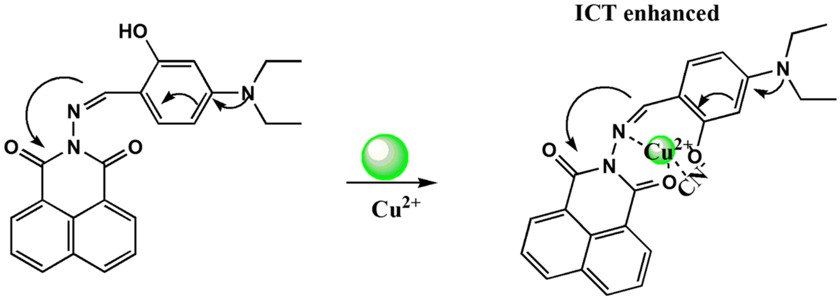


Fig. 9 Proposed mechanisms for recognition of Cu^{2+} by sensor L

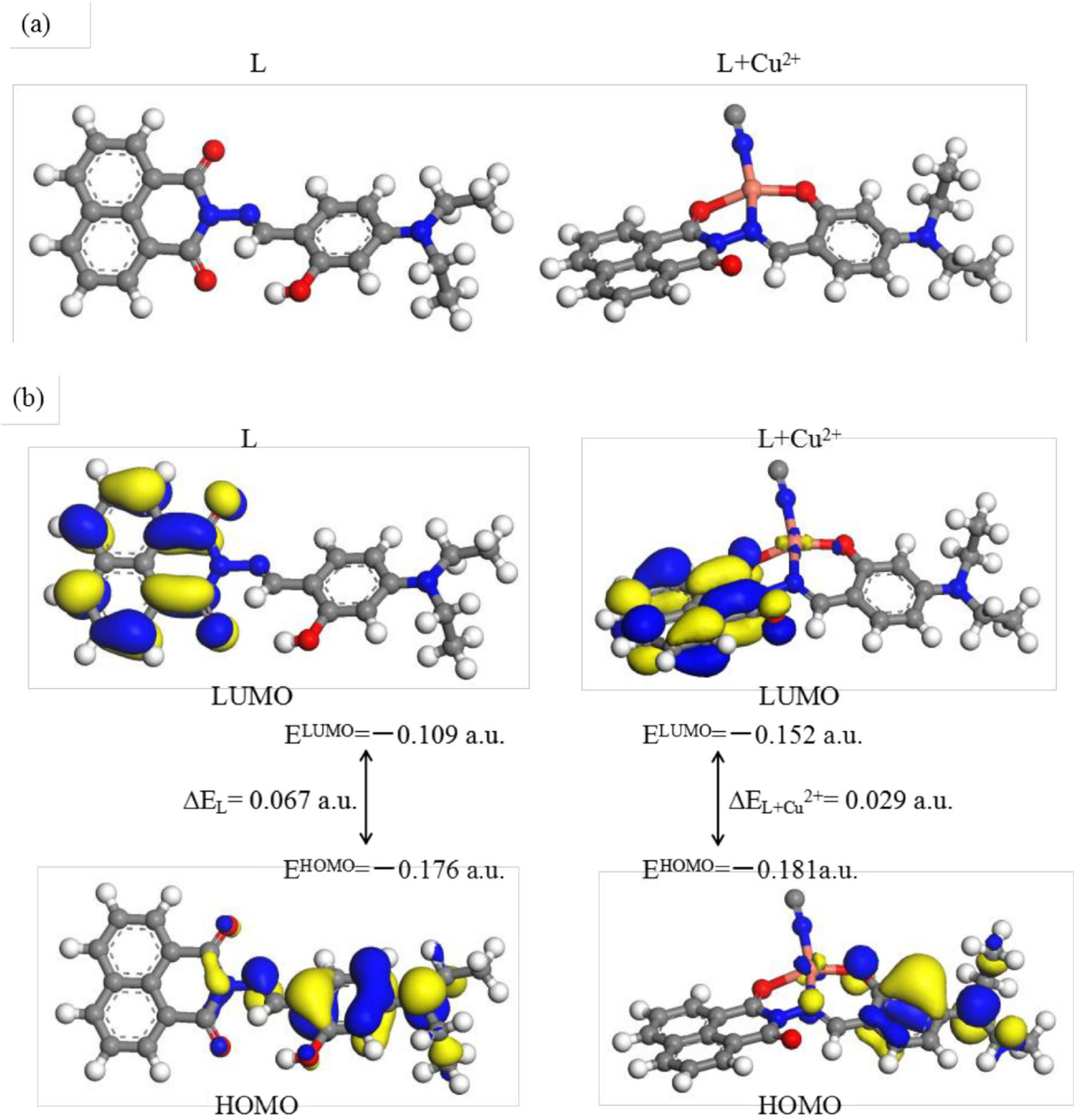


Fig. 10 **a** DFT Optimized structure of the L and L-Cu^{2+} complex evaluated at GGA/PBE level. **b** DFT Optimized structure of HOMO and LUMO orbitals of L and L-Cu^{2+} complex

DFT Calculation

Density functional theory (DFT) electronic structure computations were performed using DMol^3 (Materials Studio 5.5 package), to interpret the intramolecular charge transfer system of sensor L. [28] The Perdew-Burke-Ernzerhof (PBE) function of the generalized gradient approximation (GGA) level with a double numeric polarization basis set was used to calculate the energy level of the frontier molecular orbits [29]. No imaginary frequency of the optimized geometries of the L and L-Cu^{2+} complex suggested that the optimized geometry represents stable structures which are shown in Fig. 10(a) [30]. Electron distributions and the HOMO and LUMO energy levels of sensor L were calculated as shown in Fig. 10(b). The electron density of HOMO in L as presented in the 4-(Diethylamino)salicylaldehyde moiety of the donor was moved to the 1,8-naphthalic anhydride moiety of the acceptor in LUMO after energy excitation. This observation agrees with a strong migration characteristic of electrons in sensor L which is also shown in the charge migration of the ICT character of sensor L. The LUMO and HOMO energies of L and L-Cu^{2+} were found to be −0.109 a.u. and − 0.176 a.u. for L and − 0.152 a.u. and − 0.181 a.u. for L-Cu^{2+}, respectively. In this regard, the ΔE values between HOMO and LUMO of L and L-Cu^{2+} were 0.067 a.u.and 0.029 a.u. respectively. In other words, compared with ΔE of L, the ΔE of L-Cu^{2+} is reduced, and the conjugate degree and stability of sensor L has been increased. These results indicated that sensor L coordinated with Cu^{2+} to form a complex.

Conclusion

In summary, a simple Schiff-base L has been prepared for the detection of Cu^{2+}. Upon interaction with Cu^{2+}, L exhibited a pronounced fluorescence quenching in the fluorescence emission spectrum due to the conformational restriction and intramolecular charge transfer. Moreover, L can selectively detect Cu^{2+} in the presence of most competing metal ions. The binding mode of the complex has been confirmed by Job's plot analysis, ESI mass spectrometry and ^1H NMR titration and the detection limit was a low 9.1×10^{-8} mol·L^{-1}, indicating that L has potential applications for detecting Cu^{2+} in environmental systems.

Acknowledgements We are grateful to the Nature Science Foundation of China for financial support (Nos. 21265010) and support from Wang Xiaojing and her team at Inner Mongolia University.

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