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A fluorescent colorimetric vanillin di-Schiff base chemosensor for detection of Cu(II) and isolation of trinuclear Cu(II)–dihydrazide†

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In this work, we developed a simple fluorescent colorimetric chemosensor **H₂L** [6,6'-(1,1')-hydrazine-1,2-diylidene bis(methanylylidene) bis(2-(6-methoxy)) phenol] for rapid detection of Cu²⁺ in aqueous solution. The method for synthesis of **H₂L** is very simple and environment friendly. This organic Schiff base probe was characterized by ¹H-NMR, FT-IR and ESI-MS spectroscopy along with single-crystal XRD analysis. It exhibited binding-induced colour change with Cu²⁺ ion from colourless to intense yellow and fluorescence enhancement. The LOD values of **H₂L** towards Cu²⁺ were calculated to be 7.1 × 10⁻⁸ M (colorimetrically) and 3.8 × 10⁻⁸ M (fluorometrically). The interactions between **H₂L** and Cu²⁺ were studied by Job's plot, ESI-MS, FT-IR spectroscopy and DFT calculations. The crystal structure of the L–Cu²⁺ adduct was also determined by single-crystal X-ray analysis, and it was found that two molecules of **L** coordinate with three molecules of Cu²⁺ ions. The receptor **H₂L** could operate in a wide pH range and can be successfully applied for detection and quantification of Cu²⁺ ions in environmental samples and logic applications.

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Introduction

Copper(II) is one of the essential metal ions in the human body, and it plays an important role in various biological and environmental processes.^{1–5} The first-row transition element Cu is the third most important tracer element in biological processes, and Cu concentrations up to 1.3 mg L⁻¹ in drinking water are tolerable, according to the U.S. Environmental Protection Agency (EPA).^{6–8} Cu(II) is present in hemocyanin, which functions as oxygen transporter in insects. In the human body, Cu(II) is present in several enzymes, including superoxide dismutase, tyrosinase, and dopamine hydroxylase. In cytochrome *c* oxidase and ferritin, Cu(II) presents in combination with another metal, iron (Fe²⁺). Although excess copper can result in mitochondrial damage, DNA breakage, neuronal injury, and Alzheimer's and Wilson's diseases, deficiency of Cu(II) causes serious illness such as anemia, as it is an important part of ceruloplasmin.^{9–13} Major concentrations of Cu(II) are found in the liver, kidney, heart and brain. Cu(II) is a key component in the body needed for regulation of many biological functions. However, its presence in excess affects some biologically

important enzymes by binding with their active sites and displacing other nutrient minerals.^{14–17} Cu(II) can disturb nutrient absorption and transport, thus destroying plant and aquatic ecosystems.^{18–20}

Conventional methods such as atomic absorption spectrometry (AAS),^{21,22} inductively coupled plasma–mass spectrometry (ICP-MS),²³ flame atomic absorption spectrometry (FAAS)^{24,25} and electrochemical assays^{26,27} are generally used for the detection of metal ions like Cu²⁺. Although these traditional methods are responsive, accurate and selective, they require special sampling techniques, manual operation, and professional handling, and they are time consuming, expensive and sometime inconclusive. To overcome these problems, researchers are searching for easily employable optical sensors.

Optical sensors have several advantages such as sensitivity, selectivity, cost effectiveness and simplicity.^{28,29} Colorimetric and fluorometric optical chemosensors offer real-time analyte recognition, easy operation, controllability and lower response time.^{30–34} A variety of colorimetric and fluorometric sensors for Cu(II)^{35–44} have been reported. Despite these, considering the effectiveness and low detection limit, the design and synthesis of more effective chemosensors are still desirable.

In our ongoing research, we have already reported several chemosensors for Cu(II) based on amides,⁴⁵ triazole,⁴⁶ dihydrazone,⁴⁷ dihydrophenylquinazolinone,⁴⁸ thiosemicarbazone,⁴⁹ and benzohydrazide.^{50,51} Taking another step in the process of searching for suitable chemosensors, we focused on

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dihydrazone-based chemosensors. Herein, we have successfully designed and synthesized 6,6'-((1,1')-hydrazine-1,2-diylidene bis(methanylylidene)) bis(2-(6-methoxy)) phenol (**H₂L**), a dihydrazone of *ortho*-vanillin and hydrazine, which selectively and sensitively detects Cu²⁺ both colorimetrically and fluorometrically among a series of different metal ions.

Experimental

General information

All the required materials used for synthesis were obtained from Sigma-Aldrich and directly used. Analytical-grade solvents were used for the overall experiments, and freshly prepared double-deionized water was used for dilution and preparing *tris* HCl buffer (10 μM, pH = 7.2) solution. The metal ion solutions were prepared from their nitrate salts. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker DRX spectrometer operating at 400 MHz in CDCl₃ solvent, and chemical shifts were recorded in ppm relative to TMS. Melting point was determined on an X-4 digital melting-point apparatus and was not corrected. Absorption spectra were recorded on a Shimadzu UV 1800 spectrophotometer using 10 mm path length quartz cuvettes with the wavelength in the range of 200–800 nm. Fluorescence spectra were recorded on a Hitachi spectrophotometer. Electro-spray ionisation mass spectra (ESI-MS) were recorded on a Waters mass spectrometer using the mixed HPLC grade solvent methanol and triple distilled water. The pH adjustments were monitored using a digital pH meter (Merck) in buffer solution and adjusting with dilute hydrochloric acid and sodium hydroxide. Solutions of the receptor **H₂L** (1 × 10⁻⁵ M) and metal salts

(1 × 10⁻⁴ M) were prepared in methanol-*tris*-HCl buffer (10 mM, pH 7.2) medium (1 : 1 v/v) and H₂O, respectively.

X-Ray data collection and structure determination

Single-crystal X-ray data were collected using MoKα (λ = 0.7107 Å) radiation on a Bruker APEX II diffractometer equipped with a CCD area detector. Data collection, data reduction, and structure solution/refinement were carried out using the software package of SMART APEX.⁵² The structures were solved by direct methods (SHELXS-97) and standard Fourier techniques, and refined on F² using full-matrix least-squares procedures (SHELXL-97) using the SHELX-97 package⁵³ incorporated in WinGX.⁵⁴ In most cases, non-hydrogen atoms were treated anisotropically. Hydrogen atoms were fixed geometrically at their calculated positions following the riding atom model. The crystallographic data of **H₂L** and its Cu(II) complex (**1**) are listed in Table 1. Structural information for **H₂L** and **1** was deposited at the Cambridge Crystallographic Data Center (CCDC 2087278 and 2087279, respectively).

Synthesis of **H₂L**

The ligand was synthesized by following a reported procedure.⁵⁵ *o*-Vanillin (0.304 g, 2 mmol) was dissolved in 10 mL dry methanol, and to this 0.05 g hydrazine hydrate (1 mmol) was added dropwise with constant stirring. Then, the reaction mixture was refluxed for 4 h under dry condition. After reflux, the reaction mixture was kept in air for evaporation; a light-yellow solid crystal separated out. Yield, 0.255 g, 85%. Anal. calc. For C₁₆H₁₆N₂O₄: C, 63.99; H, 5.37; N, 9.33%. Found: C, 63.86; H, 5.49; N, 9.26%. ESI-MS: *m/z* 301.1 (**H₃L**⁺) (Fig. S1, ESI[†]).

Table 1 Crystallographic data and refinement parameters of the probe **H₂L** and its trinuclear Cu(II) complex (**1**)

Identification code	H₂L	1
Empirical formula	C ₁₆ H ₁₆ N ₂ O ₄	C ₃₂ H ₃₂ Cu ₃ N ₆ O ₁₆
CCDC number	2087278	2087279
Formula weight	300.31	947.25
Temperature/K	296.15	296.45
Crystal system	Monoclinic	Triclinic
Space group	<i>P</i> 2 ₁	<i>P</i> $\bar{1}$
<i>a</i> /Å	5.701(3)	8.157(7)
<i>b</i> /Å	17.733(10)	9.902(9)
<i>c</i> /Å	6.535(4)	11.983(9)
α /°	90	75.79(2)
β /°	106.388(9)	85.79(2)
γ /°	90	87.08(3)
Volume/Å ³	633.8(6)	935.2(14)
<i>Z</i>	2	1
ρ_{calc} /g cm ⁻³	1.574	1.682
μ /mm ⁻¹	0.115	1.771
<i>F</i> (000)	316.0	481.0
Crystal size/mm ³	0.28 × 0.21 × 0.19	0.32 × 0.24 × 0.18
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2θ range for data collection/°	4.594 to 53.832	4.81 to 50.75
Index ranges	-7 ≤ <i>h</i> ≤ 7, -22 ≤ <i>k</i> ≤ 22, -8 ≤ <i>l</i> ≤ 8	-9 ≤ <i>h</i> ≤ 9, -11 ≤ <i>k</i> ≤ 10, -14 ≤ <i>l</i> ≤ 14
Reflections collected	8607	6361
Independent reflections	2687 [<i>R</i> _{int} = 0.0634, <i>R</i> _{sigma} = 0.0851]	3325 [<i>R</i> _{int} = 0.0866, <i>R</i> _{sigma} = 0.1465]
Data/restraints/parameters	2687/13/203	3325/0/262
Goodness-of-fit on <i>F</i> ²	1.240	1.024
Final <i>R</i> indexes [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> ₁ = 0.0537, <i>wR</i> ₂ = 0.1114	<i>R</i> ₁ = 0.0748, <i>wR</i> ₂ = 0.1706
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.1483, <i>wR</i> ₂ = 0.1456	<i>R</i> ₁ = 0.1347, <i>wR</i> ₂ = 0.2081
Largest diff. peak/hole/e Å ⁻³	0.21/-0.25	0.71/-0.84



FTIR/cm⁻¹ (KBr): 3275 (vb, O–H), 1662 (vs, C=N), 1589 (m), 1446 (m), 1217 (s), 984 (m), 864 (m), 686 (s) (Fig. S2, ESI[†]). ¹H NMR (400 MHz, CDCl₃, TMS): δ 11.55 (s, 2H, –OH), 8.71 (s, 2H, –C=NH), 7.20–6.91 (m, 6H, ArH), 3.95 (s, 6H, OCH₃) (Fig. S3, ESI[†]). ¹³C NMR (100 MHz, CDCl₃, TMS): δ 164.79, 149.75, 148.39, 124.08, 119.42, 117.39, 115.22, 56.24 (Fig. S4, ESI[†]).

Synthesis of H₂L–Cu²⁺ complex [Cu₃L₂(NO₃)₂(H₂O)₂] (1)

An aqueous–methanolic solution (10 mL, 1:1 v/v) of Cu(NO₃)₂·9H₂O (0.166 g, 0.5 mmol) was added dropwise to the hot stirring solution of H₂L (0.30 g, 1 mmol) in methanol–H₂O (10 mL, 1:1 v/v). Immediate colour change was observed from colourless to reddish yellow. The solution was then stirred for 5 min at room temperature. The clear solution was kept for evaporation to obtain a red crude product. Yield, 0.510 g, 53.61%. The obtained product was recrystallised through diffusion from different phases *via* making a series of setups with different concentrations in DCM and diethyl ether; a red crystal suitable for single-crystal XRD analysis was obtained. Anal. calc. for C₃₂H₃₄N₆O₁₆Cu₃: C, 40.49; H, 3.61; N, 8.85%. Found: C, 40.65; H, 4.43; N, 8.97%. ESI–MS: *m/z* 722.5 (2L + 3Cu²⁺) (Fig. S5, ESI[†]). FTIR/cm⁻¹ (KBr): 3064 (wb), 1650 (vs), 2565 (s), 1275 (m), 1178 (s) (Fig. S6, ESI[†]).

Computational details

The program package GAUSSIAN-09 Revision C.01 was employed for all calculations.⁵⁶ The gas phase geometries of the compound were fully optimized with symmetry restrictions in singlet ground state and the gradient-corrected DFT level coupled with B3LYP.⁵⁷ Basis set LanL2DZ was used for the whole molecules H₂L and [Cu₃L₂(NO₃)₂(H₂O)₂] (1). The HOMOs and LUMOs of molecular ions were calculated with the same basis set and functional.

Preparation of stock solution for photophysical measurements

Receptor H₂L solution was prepared initially at the concentration of 1 × 10⁻³ M in 10 mL methanol–*tris* HCl buffer medium (10 mM, pH 7.2) solution (1:1 v/v), then diluted to the desired concentration. Stock solutions of guest ions were prepared separately from their nitrate salts (except for the sulphate salt of Fe²⁺, ammonium heptamolybdate salt for Mo⁶⁺ and the potassium salts for Cr⁶⁺ and Mn⁷⁺) at a concentration of 1 × 10⁻³ M in 10 mL double-deionised water and further diluted to their desired concentrations. After mixing H₂L with each of the metal ions for a few seconds, absorption and fluorescence spectra were obtained at room temperature.

Job's plot measurements

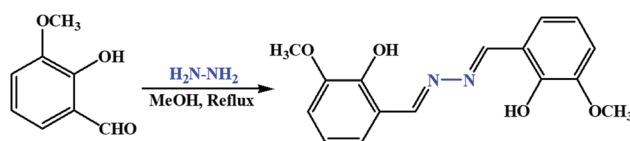
A methanol–*tris*-HCl buffer (1:1 v/v, 10 mM, pH 7.2) solution containing H₂L (10 μM) and an aqueous solution of Cu(NO₃)₂ were prepared separately. Then, the mole ratio of H₂L was changed from 0.1 to 0.9 in such a manner that the sum of the total volume of metal ions and H₂L remained constant (2 mL). All solutions were diluted to 3 mL. After shaking them for a minute, UV–vis spectra were obtained at room temperature.

Results and discussion

Structure establishment of ligand (H₂L)

The condensation reaction between *o*-vanillin and hydrazine in a 2:1 proportion produced Schiff base receptor H₂L as a yellow solid in good yield, and it was mainly characterised by elemental analysis, FTIR spectra, ESI–MS, ¹H–NMR spectra and single-crystal XRD analysis. The compound showed excellent solubility in common solvents such as acetonitrile (ACN), dimethyl sulphoxide (DMSO), methanol (MeOH) and dichloromethane (DCM) but not in pure water (Scheme 1). In our present study, methanol is used to improve water solubility of the probe H₂L. To investigate the stability of ligand H₂L in 1:1 MeOH–H₂O medium at room temperature, ESI–mass spectrum was recorded (Fig. S1, ESI[†]). Here, the presence of an intense molecular ion peak at *m/z* = 300.1 confirms the solution stability of H₂L. In the FTIR spectra of H₂L, the broad band appearing at 3480 cm⁻¹ is due to phenolic OH, and the sharp peak at 1620 cm⁻¹ is due to the C=N group. From the ¹H–NMR spectra, characteristic singlet peaks were obtained at 11.62, 9.89 and 1.32 ppm respectively due to –OH, –C=N and –OMe groups, along with peaks between 7.62 to 7.36 ppm for aromatic protons. The compound H₂L displayed intense absorption bands at 313 nm and 225 nm, and a small hump at 390 nm, which imparts its very light-yellow color in 1:1 aqueous methanol. Here, the band at 313 nm and 225 nm originated because of the n–π* and π–π* electronic transitions. Also, in the fluorescence study of the receptor H₂L in this medium, a broad maximum was obtained around 360 nm when excited at 310 nm.

Slow evaporation of a moderately concentrated methanolic solution of the probe H₂L yielded a fine, needle-shaped crystalline form suitable for single-crystal XRD. The shortest imine-based salen derivative, the probe H₂L is a 3-methoxy 2-hydroxyaldazine in which the two units of salicylaldehyde derivative are linked directly through imine nitrogen atoms. There are rotational degrees of freedom about the central N–N bond. H₂L is crystallized in the monoclinic system with space group P2₁. The SCXRD studies revealed that L molecule is planar. The ORTEP view of H₂L with the atom numbering scheme is shown in Fig. 1. The bond length and bond angles are within the expected ranges. The methoxy groups, as well as the hydroxyl groups, are anti to each other. So, the molecule of H₂L adopts an anti-configuration in solid state. The N1–C1 (1.240 Å) and N1–N2 (1.346 Å) distances indicate agreement to double and single bonds, respectively. The torsion angle N1–C1–C2–C2 [–175.4°] specifies the molecule is essentially planar. Intra-molecular O3–H···N1 and symmetry equivalent O4–H···N2 hydrogen bonds play an important role in stabilizing the geometry of H₂L, and in the crystal, the molecules are



Scheme 1 Synthesis of the probe H₂L.



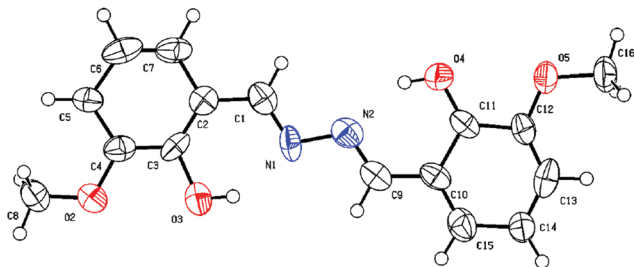


Fig. 1 ORTEP diagrams of **H₂L** showing the atom-labeling scheme and 50% thermal ellipsoids.

interlinked and stabilized through C–H··· π (C4–C6) interactions in addition to van der Waals forces (Fig. S7, ESI[†]).

Single-crystal structure of the Cu(II) complex of **H₂L** (**1**)

1 crystallized in the triclinic system with space group P_1 . The SCXRD studies revealed that **1** is trinuclear. The ORTEP view of **1** with the atom numbering scheme is shown in Fig. 2. In the crystal structure of the Cu(II) complex, two different coordination environments of the Cu(II) centres are observed with two di-deprotonated ligands of **H₂L** (C₁₆H₁₆N₂O₄) and three copper(II) ions. Two peripheral Cu(II) ions are fifth coordinated distorted trigonal bipyramidal, while the inner Cu(II) centre is sixth coordinated octahedral geometry. The TBP geometries of the peripheral Cu(II) ions have also been confirmed by the calculations of Addison parameter value $\tau = 0.883$ ($\tau = \beta - \alpha/60$).⁵⁸ Fifth coordination of each of the two peripheral Cu(II) ions is satisfied by one imine–N atom, two phenoxide O atoms, and one O-atom of one –OCH₃ group of two different molecules of the ligand **H₂L** and the O atom of the coordinated H₂O molecule, with the corresponding bond distances Cu(II)–N(imine) = 1.934 Å, Cu(II)–O(phenoxide 1) = 1.875 Å, Cu(II)–O(phenoxide 2) = 1.974 Å, Cu(II)–O(OCH₃) = 2.083 Å and Cu(II)–O(OH₂) = 2.122 Å. The two peripheral Cu(II) ions are above and below the plane of the inner Cu(II) centre. The di-deprotonations of the two ligands (**H₂L**) balance the charges of the peripheral Cu(II) centres. The sixth coordination of the two inner Cu(II) ions is satisfied by two imine –N atoms and two phenoxide O atoms of different ligand molecules and two

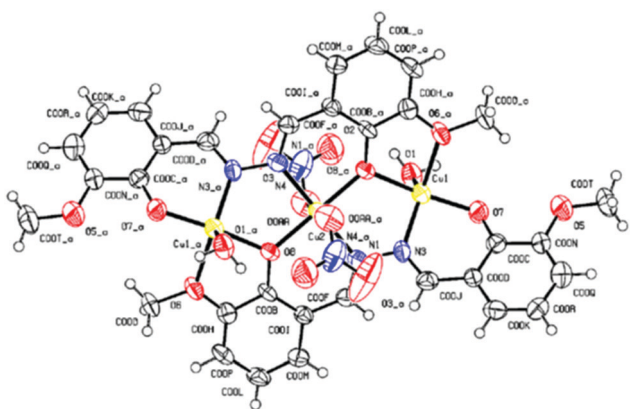


Fig. 2 ORTEP diagrams of **1** showing the atom-labeling scheme and 50% thermal ellipsoids.

Cu(II)–O(NO₃) bonds, with the respective distances of 1.978 Å, 1.949 Å and 2.516 Å. All the bond distances are in good agreement with previously reported structures. Three copper(II) ions are not in a straight line in the complex, and the atomic distance of Cu···Cu is 3.344 Å. Both Cu–O–Cu bond angles are 116.96°, whereas the Cu–N–N–Cu torsional angles are 30.18°. The metal ions are surrounded by the organic ligand and do not interact with one another. There is perfect face-to-face π – π stacking with a distance 3.755 Å, and in the crystal structure of **1**, there are several OH···H hydrogen-bonding interactions between coordinated H₂O molecules and NO₃ counter-ions (Fig. S8, ESI[†]).

Absorption selectivity studies

Initially, the selectivity of chemosensor **H₂L** towards various metal ions was observed by naked eyes, along with absorption studies at room temperature in methanol–tris-HCl buffer (1:1 v/v, 10 mM, pH 7.2) solution. On systematic addition of 5 equiv. of different metal ions (e.g., Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Pb²⁺, Mo⁶⁺, Cr³⁺, Ag⁺, Al³⁺, Mn²⁺, Fe²⁺, Cr⁶⁺ and Mn⁷⁺) to a fixed amount of **H₂L**, no colour change from colourless to yellow (Fig. 3) and no spectral change (Fig. 4) were observed in all cases except Cu²⁺ and Mo⁶⁺. An intense peak, developed at 274 nm, and increased absorbance intensity at 313 nm for **H₂L** were observed in presence of 5 equiv. Mo⁶⁺ ion; in the case of the addition of Cu²⁺ ion, a new peak developed at 434 nm. In the competitive experiments, the metal (Cu²⁺)-dependent absorption spectra of **H₂L** was not disturbed in the presence of the remaining metal ions. The characteristic absorption band at 434 nm for **H₂L**–Cu²⁺



Fig. 3 Colour change in visible light of probe **H₂L** on addition of different metal ions.

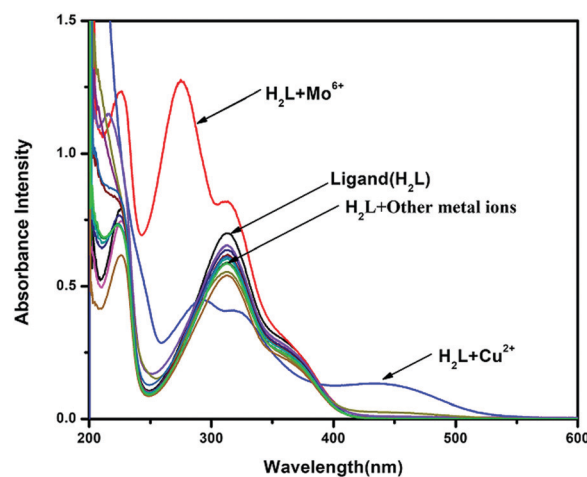


Fig. 4 Absorption spectra of **H₂L** (10 μ M) changes in the presence of 5 equiv. of different metal ions.



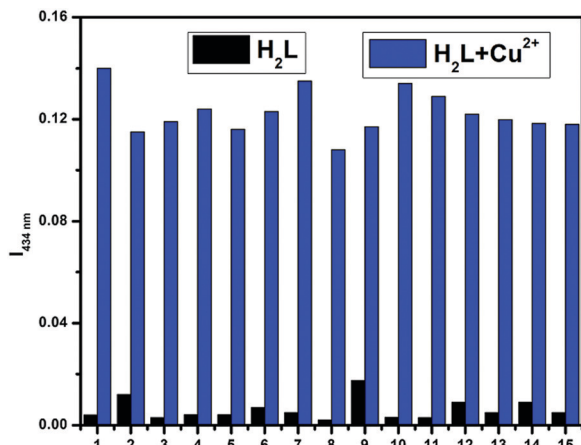


Fig. 5 Competitive experiment of $\text{H}_2\text{L}-\text{Cu}^{2+}$ with other metal ions where 1 = H_2L , 2 = Mo^{6+} , 3 = Cd^{2+} , 4 = Pb^{2+} , 5 = Fe^{3+} , 6 = Fe^{2+} , 7 = Ni^{2+} , 8 = Al^{3+} , 9 = Zn^{2+} , 10 = Co^{2+} , 11 = Hg^{2+} , 12 = Cr^{3+} , 13 = Ag^+ , 14 = Cr^{6+} , 15 = Mn^{7+} .

remained almost consistent, with negligible loss of intensity, when 5-fold higher concentrations of interfering ions were added in the host-guest solution. Even the 10-fold higher concentration of Mo^{6+} ion does not interfere with the absorption of $\text{H}_2\text{L}-\text{Cu}^{2+}$ (Fig. 5).

Absorption titration and LOD

In the absorption titration experiments, upon incremental addition of aqueous Cu^{2+} , the absorbance intensity at 313 nm steadily decreased with the generation of a new peak at 434 nm, accompanied by 121 nm bathochromic shift. Thus, two clear isosbestic points at 355 nm and 390 nm developed, which indicate that H_2L and its Cu^{2+} adduct are present in equilibrium (Fig. 6a). The limit of detection (LOD) for Cu^{2+} was calculated from these titration curves by linear fitting of the intensity at 434 nm vs. $[\text{Cu}^{2+}]$. The obtained LOD value is 7.1×10^{-8} M for Cu^{2+} , calculated using the formula $3(\text{SD}/S)$, where 'SD' is the measured standard deviation of the blank sample and 'S' is the slope of the linear fitted calibration curve (Fig. 6b).

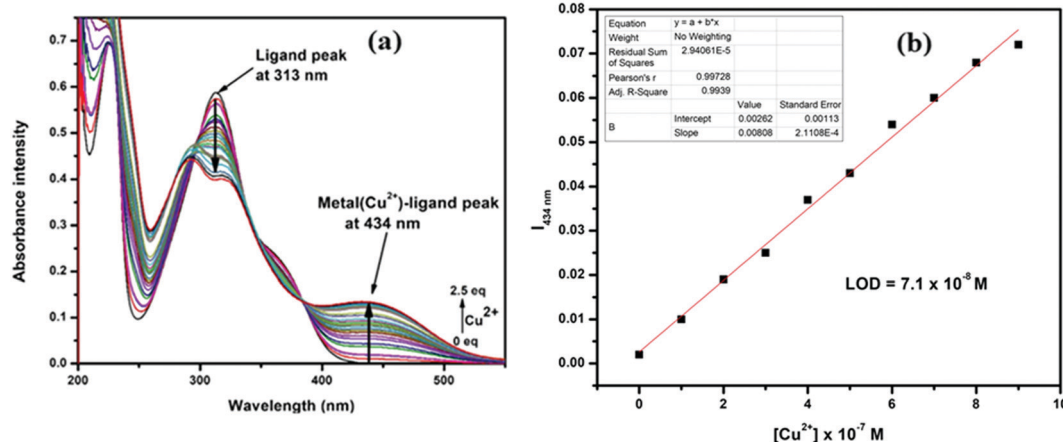


Fig. 6 (a) UV-vis titration of H_2L with Cu^{2+} , (b) detection limit.

Stoichiometry and association constant

Conventional Job's continuation method of variation indicates that two H_2L molecules can coordinate with three Cu^{2+} ions (Fig. S9, ESI[†]), which was further established by ESI-MS analysis. The base peak was obtained at $m/z = 722.5$, which corresponds to $[\text{Cu}_3\text{L}_2]^{2+}$ (Fig. S5, ESI[†]). To support the Cu_3L_2 complexation in solid phase, isolation in single-crystal form is also necessary, as it is more acceptable in current research. Thus, sincere efforts were made to isolate the $\text{L}-\text{Cu}^{2+}$ complex in the form of single crystal at the experimental condition. Mixing of each receptor-analyte in a 2:1 molar ratio, a block-shaped red crystal was separated out, which was suitable for single-crystal XRD (*vide supra*).

Considering the binding ratio, the association constant was calculated to be $7.7 \times 10^7 \text{ M}^{-3/2}$ by the linear fitting of Benesi-Hildebrand (B-H) equation $1/(A - A_0)$ vs. $1/[\text{Cu}^{2+}]^{3/2}$ (Fig. S10, ESI[†]). However, the association constant values for other ions were too low to be calculated. This high binding value indicates the strong affinity of Cu^{2+} ion towards the proposed chemosensor H_2L .

pH Experiment

The analyte binding affinity of receptor H_2L is also susceptible to the pH of the working media, as it contains $-\text{OH}$, $>\text{C}=\text{N}$, and $-\text{OMe}$ groups, which are very sensitive to pH. Thus, receptor solutions with pH ranging from 2 to 13 were prepared in separate vials by adjusting with 0.1 M HCl and 0.1 M NaOH. Hence, each vial contains the same amount of H_2L at different pH values; absorption spectra were taken before and after analyte addition. From Fig. 7, the absorbance value of free ligand H_2L and its metal complex gradually increases from neutral to basic medium due to effective electrostatic interaction between the negatively charged H_2L and the positively charged metal ion. Therefore, all the sensing studies were performed at the physiological pH of 7.2, maintained by using *tris*-HCl buffer, where the acid-base sensitive groups cannot be disturbed.

Reversibility studies

The reversible behaviour of the chemosensor H_2L was verified using Na_2EDTA . When the $\text{H}_2\text{L} + \text{Cu}^{2+}$ adduct was treated with



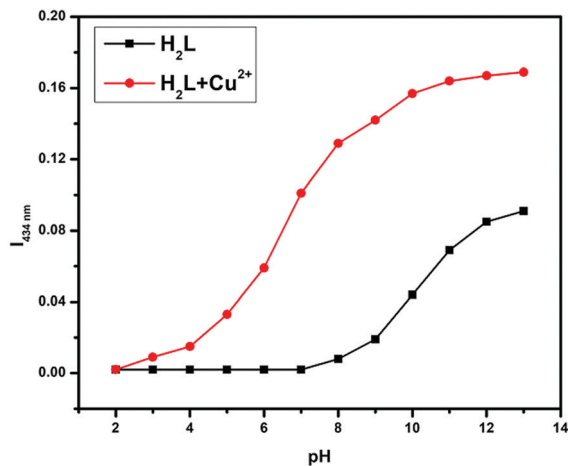


Fig. 7 pH effect of H_2L and $\text{H}_2\text{L}-\text{Cu}^{2+}$ at 434 nm.

Na_2EDTA (Fig. 8), the metal-induced absorption bands at 434 nm immediately vanished, with the simultaneous appearance of the original absorption band of free H_2L at 313 nm, accompanied by visual color change from yellow to colourless. With excessive addition of Cu^{2+} ion into the resultant solution, absorbance intensity was almost recovered. The absorption band switching between H_2L and $\text{H}_2\text{L}-\text{Cu}^{2+}$ was regularly observed for more than 5 cycles with negligible loss of intensity. This observation clearly indicates that metal ions coordinate *via* chelation rather than any acid catalyst irreversible reaction.

Fluorescence property

To check the emission selectivity of H_2L towards metals, steady-state fluorescence experiments were separately performed with Fe^{3+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Mo^{6+} , Cr^{3+} , Ag^+ , Al^{3+} , Mn^{2+} , Fe^{2+} , Cr^{6+} and Mn^{7+} in methanol-*tris*-HCl buffer (1 : 1 v/v, 10 mM, pH 7.2) solution. The receptor H_2L displayed moderate fluorescence at 360 nm when excited at 310 nm (fluorescence quantum yield, $\Phi = 0.007$). This emission intensity was enhanced only in the presence of Cu^{2+} ions (fluorescence quantum yield,

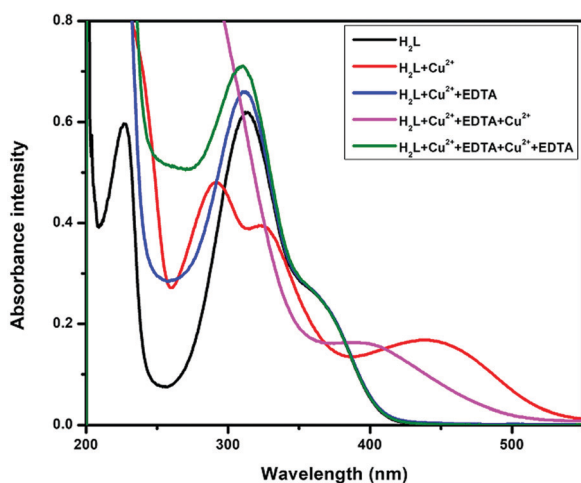


Fig. 8 Reversible investigation of H_2L and Cu^{2+} with EDTA.

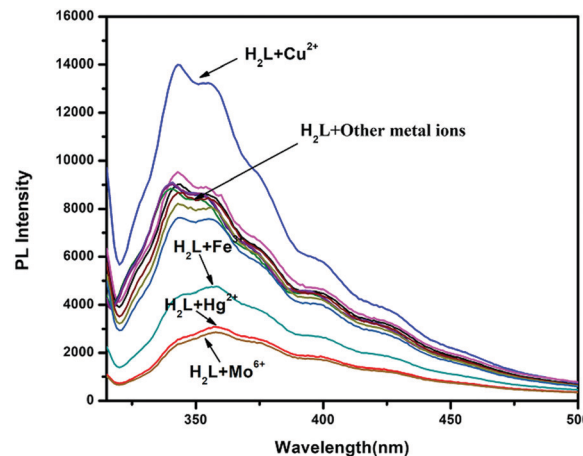


Fig. 9 Fluorescence spectra of H_2L with different cations.

$\Phi = 0.013$), whereas Mo^{6+} , Hg^{2+} and Fe^{3+} ions showed selective quenching behavior (Fig. 9). The other metal ions showed negligible change in the emission spectra of H_2L , even in the presence of more than 5-fold higher amounts than Cu^{2+} . In the titration experiments, with gradual addition of Cu^{2+} ions to the receptor solution, the emission intensity continuously enhanced and became saturated when $[\text{Cu}^{2+}]$ reached 1.5 equiv. Here, emission was enhanced due to restriction of both PET and C=N-isomerisation process after metal binding; thus, fluorescence was enhanced due to the chelation enhancement fluorescence (CHEF) process. The fluorometric detection limit was $0.038 \mu\text{M}$ (Fig. 10), which is slightly different from values obtained from UV-Vis spectra.

DFT studies on fluorescence properties

The probe H_2L shows a straightforward approach for the selective detection of Cu^{2+} , with enhancement of fluorescence in conjunction with red shift (10 nm) because of chelation-enhanced fluorescence (CHEF) and internal charge transfer (ICT) processes after interaction with Cu^{2+} . The energy gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of H_2L and its $\text{Cu}(\text{II})$ complex (**1**) are 5.55 eV and 2.11 eV, respectively. The complex **1**, compared to H_2L , established easy electronic transition and obtains the additional stability from **1** (Fig. 11). The contours of the electronic distribution in HOMO and LUMO states of these molecules suggest significant energy difference, 3.44 eV, between H_2L and (**1**). Specifically, both the HOMO and LUMO states of the copper complex **1**, in comparison to H_2L , reveal that the electrons are more delocalized in the **1** molecule than the H_2L , in accordance with the barrier of photo-induced electron transfer process, which may result in the enhancement of fluorescence through CHEF.

Application of chemosensor H_2L in real samples

To explore the Cu^{2+} sensing ability of the present chemosensor H_2L in real water samples, we initially tested using laboratory tap water and distilled water samples. The ion concentration of



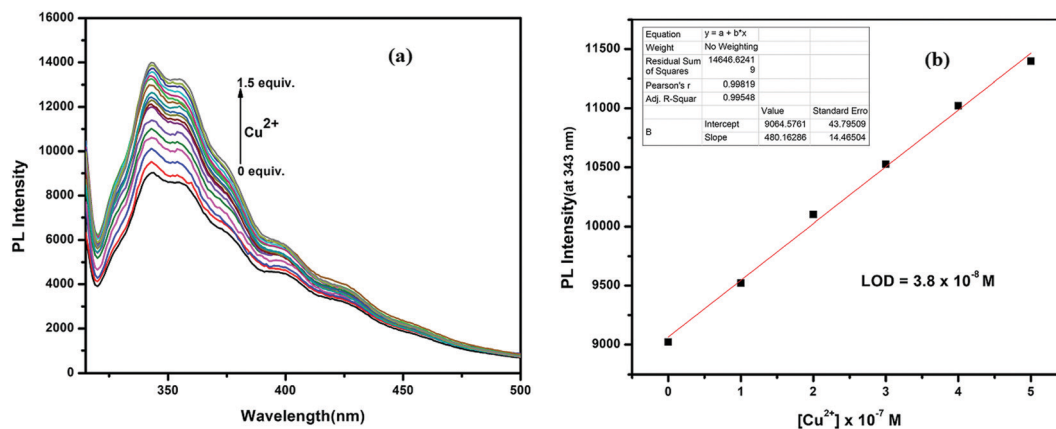


Fig. 10 (a) Fluorescence titration of H_2L with Cu^{2+} and (b) fluorescence detection limit.

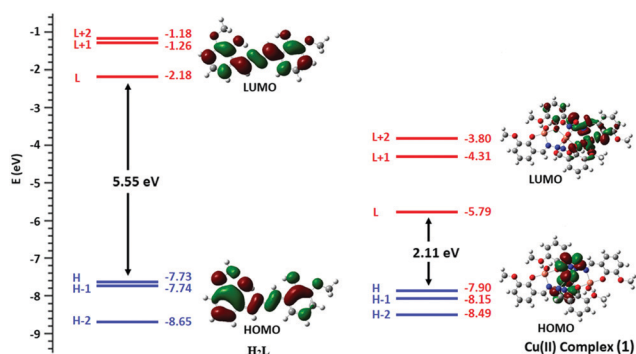


Fig. 11 Energy level diagram for the frontier MOs of H_2L (left) and Cu_3L_2 complex (**1**) (right).

these samples could not be measured by this method as the laboratory tap water and distilled water samples didn't content Cu^{2+} . We then tested the probe H_2L in waste water, and we obtained some results. To check its sensing in different samples, tap water samples artificially contaminated with Cu^{2+} were prepared by adding known amounts of standard Cu^{2+} solutions. The prepared samples were analyzed by the same UV-vis spectrometry method, and the recovery amounts were calculated using the calibration curve (intensity vs. conc.) with the help of Beer-Lambert law. As shown in Table 2, the Cu^{2+} concentrations recovered for each sample are in close agreement with the spiked amounts, with good precision. Thus, the receptor H_2L is suitable for detecting Cu^{2+} ions quantitatively in real water samples, indicating that it could be applied in real environments.

Comparison of H_2L with other fluorescent and colorimetric chemosensors

The performance of the sensing action of the probe H_2L towards Cu^{2+} was compared with some other reported chemosensors, as listed in Table 3. Compared to the other systems, our system has several attractive analytical features, such as high sensitivity, wide linear range, high selectivity, lower detection limit, simple operation technology, good solubility,

Table 2 Analysis of Cu^{2+} (μM) in water samples by the developed method (mean \pm standard deviation, $n = 3$)

Sample	Cu^{2+} added	Cu^{2+} found	Recovery (%)	Relative error (%)
Waste water	0	0.72 ± 0.89	—	—
	5	5.13 ± 0.97	102.6	2.6
	10	10.15 ± 1.54	101.5	1.5
Tap water	0	ND	—	—
	5	4.81 ± 1.3	96.2	3.8
	10	10.12 ± 0.93	101.2	1.2
Distilled water	0	ND	—	—
	5	5.02 ± 1.5	100.5	0.4
	10	9.87 ± 1.1	98.7	1.3
	20	19.96 ± 1.5	99.8	1.0

ND = not detected.

sensitive visualization and good practical applicability. Moreover, the synthesis of our proposed chemosensor H_2L requires only two steps and less hazardous reagents, and no hazardous by-product is formed.

Molecular logic gate application

The interesting optical behaviour of the probe H_2L was demonstrated by establishing a molecular logic gate. Herein, the logic characteristics of probe H_2L can also be viewed as a two-input-one-output "IMPLICATION" and "INHIBIT" logic gate in water media. Taking the idea from different absorption intensities in the UV-Vis spectra of developed probe H_2L and its behavior with metal ions, logic operations with Cu^{2+} as chemical input and absorption spectra as a channel output may be achieved. With addition of Cu^{2+} to this ligand, a prominent absorbance band is observed at 434 nm, but upon addition of Na_2EDTA , the absorbance band at 434 nm vanishes. Hence, the INHIBIT logic gate is achieved. On the other hand, at 313 nm, decreased intensity was observed on addition of Cu^{2+} , which reappears on Na_2EDTA addition. Thus, with two inputs, H_2L has the ability to exhibit INHIBIT and IMPLICATION functions *via* absorbance



Table 3 Comparison of H_2L with other reported chemosensors

Type of sensors	Ion sensing	Limit of detection for Cu^{2+} (M)		References
		Colorimetrically	Fluorometrically	
Azine based	Cu^{2+}	7.1×10^{-7}	3.8×10^{-7} (Turn-On)	Present work
	Cu^{2+} , Fe^{3+}	9.8×10^{-7}	—	59
	Cu^{2+}	2.5×10^{-6}	—	60
Dihydrazone based	Cu^{2+}	5.3×10^{-7}	—	48
Chromone-based	Cu^{2+}	4.6×10^{-7}	—	61
Quinoline-based	Cu^{2+} , CN^-	5.3×10^{-7}	—	62
	Cu^{2+} , Fe^{3+}	—	1.35×10^{-7} (Turn-Off)	63
Glycine based	Cu^{2+}	5.0×10^{-7}	—	64
Triazole based	Cu^{2+} , Pb^{2+}	3.7×10^{-6}	1.24×10^{-6} (Turn-On)	46
	Cu^{2+}	—	3.6×10^{-9} (Turn-On)	65
Naphthalene-based	Cu^{2+} , cysteine	1.07×10^{-6}	—	66

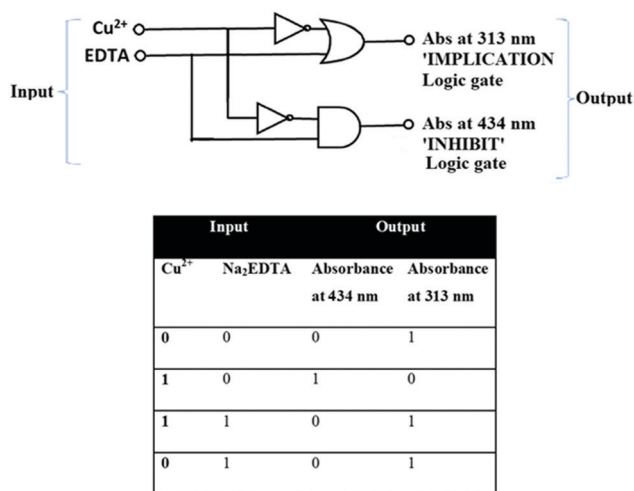


Fig. 12 IMPLICATION and INHIBIT logic gates and truth table.

output. The corresponding truth table, along with a sketch of the logic gate, is presented in Fig. 12.

Conclusions

In summary, we have developed a novel azine-based fluorescent colorimetric sensor H_2L for the selective and sensitive dual detection of Cu^{2+} . The chemosensor established the presence of the cation Cu^{2+} by both UV-visible and fluorescence spectra, with an instant change of colour from colourless to intense yellow. LOD values of H_2L towards Cu^{2+} were calculated to be 7.1×10^{-8} M (colorimetrically) and 3.8×10^{-8} M (fluorometrically). The interaction between H_2L and Cu^{2+} were studied by Job's plot, ESI-MS, FT-IR spectroscopy and DFT calculations. Single-crystal structures both for the ligand H_2L and its $Cu(II)$ complex **1** were reported. Our developed probe H_2L is much more efficient in comparison to other reported ligands. H_2L can operate in a wide range of pH and be successfully applied for detection and quantification of Cu^{2+} in various environmental samples, as well as for constructing INHIBIT and IMPLICATION type molecular logic gates.

Conflicts of interest

Authors declare no conflicts of interest.

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