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2, 2'-(hydrazine-1, 2-diylidenedimethylylidene) bis (6-isopropyl-3-methylphenol) Based Selective Dual Channel Chemosensor for Cu^{2+} in Semi-Aqueous Medium

Umesh Fegade[a], Anu Saini[d], Suban K. Sahoo[b], Narinder Singh[c*], Ratnamala Bendre[a*], Anil Kuwar[a]*

[a]School of Chemical Sciences, North Maharashtra University, Jalgaon, 425001 (MS) India.

[b]Department of Applied Chemistry, SV National Institute of technology, Surat (Gujrat), India

[e]Department of Chemistry, Indian Institute of Tecnhology, Ropar, Rupanagar, Punjab, India

[d]Centre for Nanoscience and Nanotechnology (UIEAST), Panjab University, Chandigarh, India

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*Corresponding authors. Tel.: +91-257-2257432; fax: +91-257-2257403; E-mail: kuwaras@gmail.com, bendrers@rediffmail.com, nsingh@iitrpr.ac.in

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Abstract

A novel substituted salicylaldehyde derivative, 2, 2'-(hydrazine-1, 2-diylidenedimethylylidene) bis (6-isopropyl-3-methylphenol) based receptor **1** was synthesized and characterized using various spectroscopic techniques. The UV-Visible spectroscopic studies of receptor **1** revealed selectivity for Cu^{2+} in the presence of other metal ions by showing change in colour from colourless to yellow. Red shift was observed with appearance of new absorption band at 450 nm in CH_3OH/H_2O (60:40, v/v). Similarly the fluorescence studies of receptor **1** displayed "turn-off" recognition properties for Cu^{2+} ion with 1:1 binding stoichiometry and the detection limit was found to be 50 nM.

Introduction

The design and synthesis of chemosensors with high selectivity and sensitivity for transition metal ion like copper is captivating great interest in the field of supramolecular chemistry due to its significant importance in material sciences and biology [1-3]. Copper, the third most essential trace nutrient in the human body and plays a vital role in physiological processes of organism including connective tissue development, bone and blood formation However, free Cu^{2+} is potentially toxic to aquatic life, both acutely and chronically. For example, micro-organisms are affected even at a micromolar concentration of Cu^{2+} ions. In human, brain concentrates heavy metal ions like copper for the metabolic utilization. Copper finds great importance for the normal development and working of brain. As copper is a cofactor of many enzymes so it actively participates in many physiological pathways in brain activities. Any disturbance in copper uptake leads to neurodegenerative disorders. Cloning of the genes may be liable for the two major genetic disorders of copper metabolism in human, and it has been observed that Menkes and Wilson diseases are resulted due to excessive intracellular copper transport [4-9]. Also, Cu^{2+} can react with molecular oxygen to form reactive oxygen species (ROS), which can damage lipids, nucleic acids and proteins. The significant physiological relevance of Cu^{2+} and its associated biomedical implications has resulted considerable interest for the designing of highly selective and sensitive copper chemosensors.

Noncyclic compounds containing multiple coordination sites have gained considerable attention recently because of their ability to complex different ionic and /or neutral molecules [10-16]. Such noncyclic derivatives of hydrazide show interesting coordination properties toward transition metal ions due to the presence of several potential coordination sites [17]. The proton of -NH group becomes more labile when condensation reaction between terminal NH_2 group and aldehyde or ketone takes place and resulting acyl hydrazones react with metal ion in enol form [18-20]. This type of receptor has strong coordination ability for metal ions making them excellent candidate for cation sensing. In the present investigation, we report Cu^{2+} ion via UV-Vis and fluorescence spectroscopic techniques using a chemosensor based on derivatized Salicyaldehyde and hydrazine hydrate. The substituted Salicyaldehyde was selected based on the natural occurrence of parent compound thymol (2-hydroxy-3-isopropyl-6-methyl benzene) and its broad spectrum of various activities [21, 22].

Experimental

All LR grade 99% pure have been used for synthesis and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Varian NMR mercury System 300 spectrometer operating at 300 and 75 MHz respectively in $CDCl_3$ using Me_4Si as an internal standard. The fluorescence and UV-visible spectra were recorded on Fluoromax-4 spectrofluorometer and Shimadzu UV-24500 in the range of 200-600 nm respectively, at room temperature at 28 °C using 1 cm cell. All the spectral experiments were performed in a mixed solvent system CH_3OH/H_2O (60:40, v/v). This solvent mixture is used because solubility issues of ligand were solved and receptor 1 works at its best in this solvent system**.**

Sample preparation

All stock and working solutions were prepared in ultrapure water and spectroscopic grade methanol. A stock solution of receptor **1** ($c = 5 \times 10^{-3}$ M) was prepared in CH_3OH/H_2O (60:40, v/v) solution, and the corresponding working solutions ($c = 5 \times 10^{-6}$ M) were prepared simply by diluting with CH_3OH/H_2O (60:40, v/v). Similarly, the stock solutions of all metal ions ($c = 5 \times 10^{-3}$ M) were prepared in CH_3OH/H_2O (60:40, v/v) and the corresponding working solutions ($c = 5 \times 10^{-5}$ M) were prepared by diluting with CH_3OH/H_2O (60:40, v/v).

Photophysical Studies

The cation binding studies were performed on UV-visible spectrophotometer using different metal ions (Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Na^{+}, K^{+}, Ba^{2+} and Al^{3+}) with receptor **1** in CH_3OH/H_2O (60:40, v/v) at room temperature. The ability of receptor **1** to bind selectively to a particular metal ion was investigated by performing titrations. Titrations confirmed the linear relationship with the selective metal ion and the change in absorbance intensity was used for the calculation of linearity range with correlation coefficient. These titrations were accomplished through addition of metal salt solution in small aliquots ($c = 5 \times 10^{-5}$ M) to a solution of receptor **1** ($c = 5 \times 10^{-6}$ M) in CH_3OH/H_2O (60:40, v/v) in 10 ml volumetric flask. The absorbance intensity was recorded in the range of 200-600 nm alongside a reagent blank.

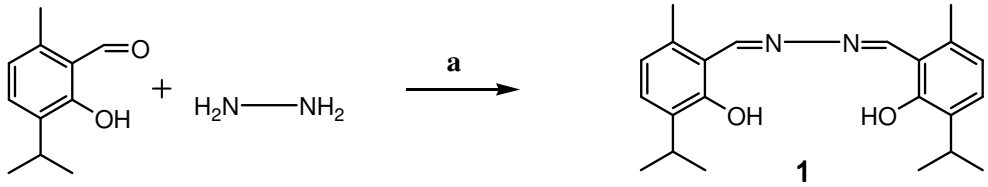
Similarly, metal binding test was carried out on Fluoromax-4 spectrofluorometer in CH_3OH/H_2O (60:40, v/v) at room temperature. The fluorescence intensity was recorded at $\lambda_{ex}/\lambda_{em}$ = 405/585 nm alongside a reagent blank. The excitation and emission slits were both set to 5.0 nm. Titrations between receptor **1** and Cu^{2+} were used to evaluate association constant (K_a) and limit of detection. These titrations were carried out by successive addition

of metal salt solutions (c = 5 × 10^{-5} M) to a solution of receptor **1** (c = 5 × 10^{-6} M) in 10 ml volumetric flask.

The stoichiometry of the complex formed was determined by preparing the solution of receptor **1** and Cu^{2+} in the ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These solutions were shaken and then the fluorescence spectrum was recorded. The plot of [HG] versus [H]/ ([H] + [G]) was used to determine the stoichiometry of the complex formed where [HG] is the concentration of complex, [H] is host concentration and [G] is the Cu^{2+} concentration. The fluorescence intensity at 485 nm was used for calculations. The concentration of [HG] was calculated by the equation of [HG] = $\Delta I/I_o$ × [H].

Synthesis of receptor 1

Hydrazine hydrate (0.05 g, 1.0 mmol) was added to a solution 2-hydroxyl-3-isopropyl-6-methylbenzaldehyde (0.35 g, 2.0 mmol) in ethanol (50 mL), and the mixture was refluxed for 8 hours at 80 °C. The yellow coloured solid obtained at room temperature was filtered and dried which was further purified by recrystallization (83 % Yield) [30]. IR (KBr, cm^{-1}): 3420 (-OH), 1600 (-C=N-); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.26-1.29 (d, J=7.2 Hz, 12H, $4CH_3$), 2.37 (s, 6H, Ar-CH_3), 3.33 (heptet, J= 7.2 Hz, 2H, 2CH-Me_2), 6.68-7.11 (d, J=7.3 Hz, 4H, Ar-H), 9.10 (s, 2H, CH=N); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.3, 24.6, 27.4,24.3, 117.4, 120.8, 129.3, 136.3, 137.9, 149.2, 156.3.



Scheme 1 Synthesis of receptor **1** (**a** = Ethanol, 8 hrs reflux).

Results and Discussion

Synthesis and characteristics of 1

The receptor **1** was synthesized by the Schiff base condensation of hydrazine hydrate with two moles of 2-hydroxyl-3-isopropyl-6-methylbenzaldehyde in ethanol (**Scheme 1**). The compound was characterized using various techniques [30].

Recognition studies of receptor 1

The absorption behaviour of receptor **1** (c = 5 x 10^{-6} M) with various metal ions was studied in CH_3OH/H_2O (60:40, v/v). The absorption spectrum of receptor **1** showed two maxima at 325 nm and 375 nm (**Figure 2**). With the addition of Cu^{2+}, the band at 375 nm disappeared and a new peak developed at 450 nm .The reason behind the development of

new peak at 450 nm can be the possible charge transfer between the receptor **1** and Cu^{2+} (**Figure 2**). The spectral shift also clearly delineated that the core functionality provided by **1** was suitable for the selective encapsulation of Cu^{2+}. The effect of Cu^{2+} was so substantial that it could also be detected by the naked-eye with a distinct solution colour change from colourless to yellow. In general, the cations such as Fe^{3+}, Ni^{2+} and Co^{2+} are known to interfere in Cu^{2+} ion detection. However, in the present study, no significant change in colour of the solution **1** was observed on addition of these interfering cations [23].

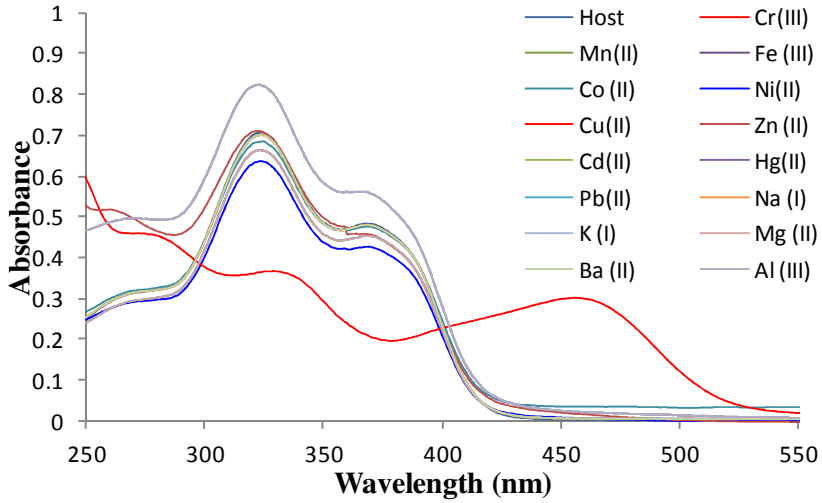


Figure 2 Changes in absorbance spectrum of receptor **1** ($c = 5 \times 10^{-6}$ M) in the presence of different metal ions ($c = 5 \times 10^{-5}$ M) in CH_3OH/H_2O (60:40, v/v).

For in-depth study about the sensing ability of Cu^{2+} ion, titrations were performed by addition of small amount of Cu^{2+} to solution of receptor **1** ($c = 5 \times 10^{-6}$ M). Upon successive addition of Cu^{2+} ion, there was decrease in absorbance at 375 nm and increase in absorbance at 450 nm with two isosbestic points at 295 nm and 405 nm (**Figure 3**). To confirm the relationship between the absorbance intensity and concentration of Cu^{2+}, a graph was plotted between A_{450}/A_{375} vs [Cu (II)] (**Figure 3 inset**). The linear dependence of concentration of Cu^{2+} authenticated that the receptor **1** could be utilized for the quantitative determination of Cu^{2+} ion.

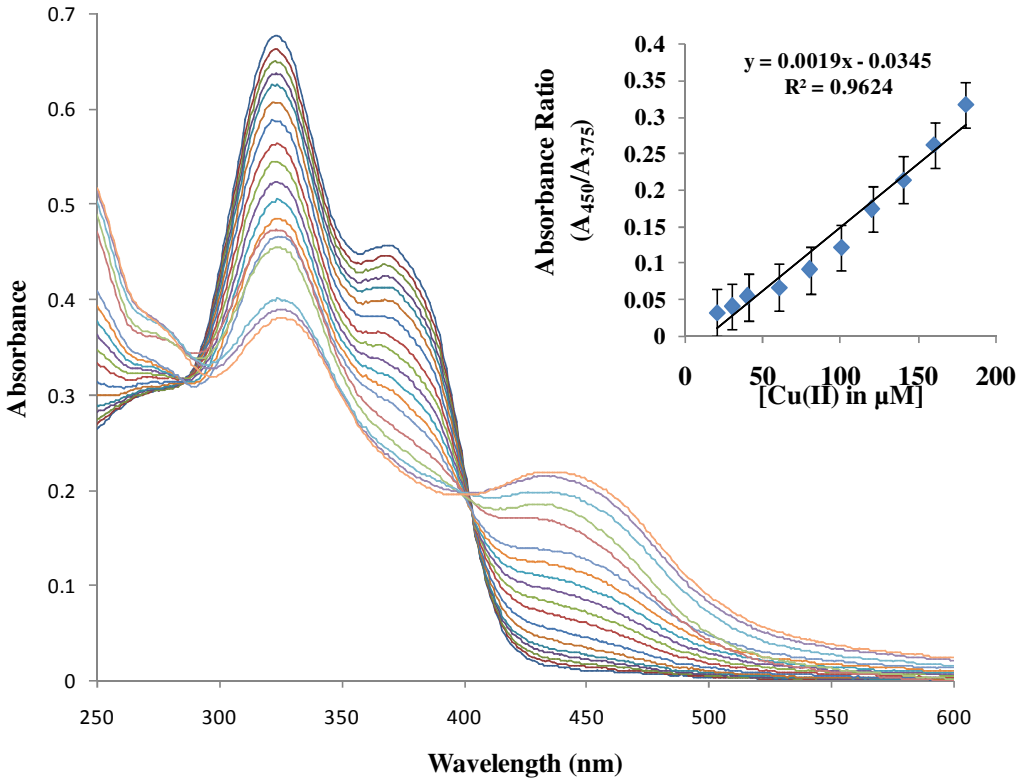


Figure3 Change in absorption profile of receptor **1** (c = 5 × 10^{-6} M) upon gradual addition of Cu^{2+} (c = 5 × 10^{-5} M) in CH_3OH/H_2O (60:40, v/v). Inset showing the normalized response of absorbance signal with regression 0.962.

The charge transfer process during the encapsulation of Cu^{2+} by receptor **1** was investigated by density functional theory (DFT) calculations by applying the B3LYP functional, and the basis sets 6-31G** (for C, H, N and O atoms) and LANL2DZ (for Cu atom) available in the computational code Gaussian 09W [24]. The optimized structure of receptor **1** and its complex with Cu^{2+} is shown in **Figure 4a**. On complexation of **1** with Cu^{2+}, lowering in the interaction energy by -106.67 kcal/mol was observed which indicated the formation of a stable complex with the calculated bond lengths for Cu-N and average Cu-O of 2.044 Å and 1.876 Å respectively. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of **1** was distributed uniformly in the entire molecule (**Figure 4b-c**). However, analysis of frontier molecular orbitals (FMOs) plots of **1**:Cu^{2+} complex indicated the intramolecular charge transfer (ICT) occurred between the receptor **1** and Cu^{2+}. Also, the band gap between the HOMO and LUMO of **1** was lowered on complexation which confirmed the observed red-shift in the absorption band.

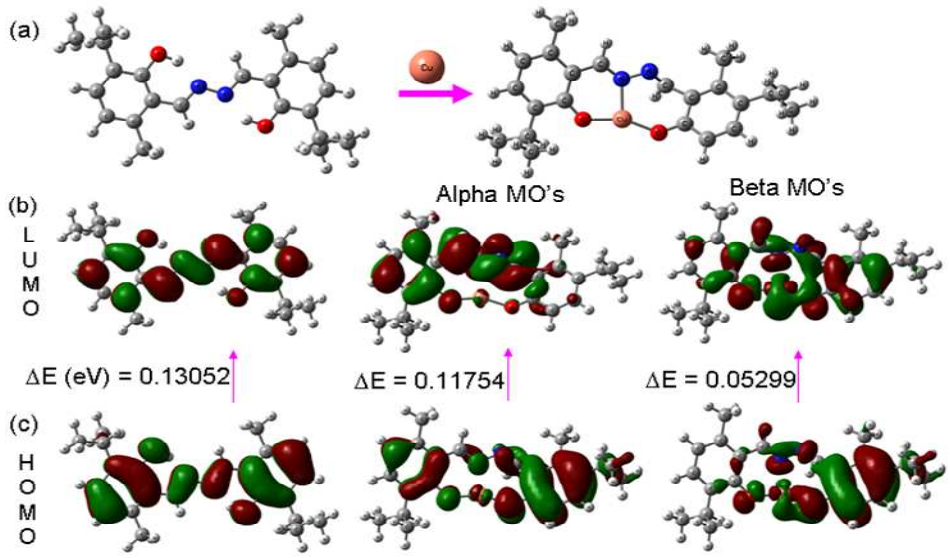


Figure 4 DFT computed (a) optimized structure of receptor **1** and its complex with Cu^{2+}, and the (b) LUMO and (c) HOMO diagrams of **1** and its **1**-Cu^{2+} complex.

The specificity of receptor **1** for the detection of Cu^{2+} in the presence of other interfering metal ions was done by addition of 1 equiv. of Cu^{2+} to receptor **1** in the presence of 2 equiv. of all other metal ions. As shown in **Figure 5**, the results indicated that the miscellaneous competitive cations did not lead to any significant spectral change. The data clearly suggests that there is no interference of other metal ions for the sensing of Cu^{2+}.

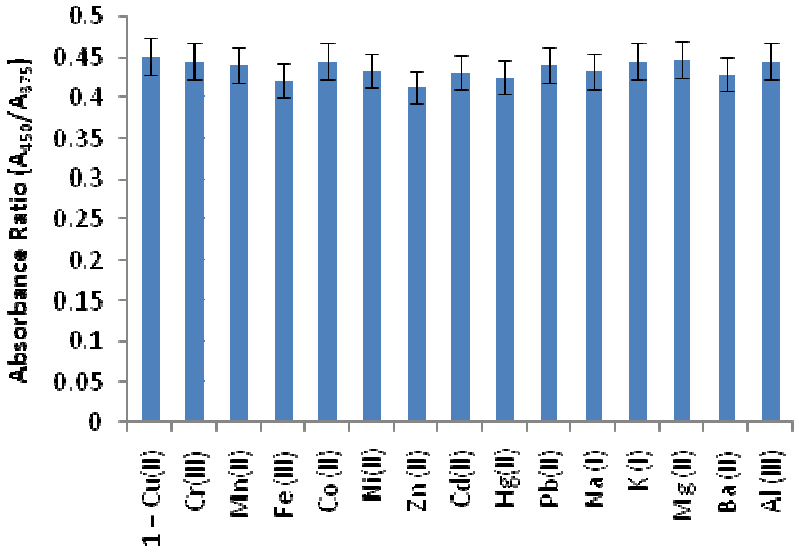


Figure 5 Absorbance ratio (A_{450}/A_{375}) of receptor **1** ($c = 5 \times 10^{-6}$ M) with 1 equiv. of Cu^{2+} and 2 equiv. of the metal ion stated.

The fluorescence properties of receptor **1** were studied upon addition of various metal nitrate ($c = 5 \times 10^{-5}$ M) at an excitation wavelength of 405 nm in CH_3OH/H_2O (60:40, v/v). With the addition of different metal nitrate salts no significant changes were observed but on adding Cu^{2+}, there was quenching in the broad spectrum peak at 585 nm (**Figure S1**). To investigate the sensing capability of receptor for Cu^{2+} ion, titration was carried with incremental addition of Cu^{2+} to receptor **1. Figure 6** displays the fluorescence intensity is quenched with the successive addition of Cu^{2+} ion. Receptor **1** contains an intramolecular hydrogen bond between the phenolic-OH and the nitrogen of the imine group that undergoes excited-state intramolecular proton transfer (ESIPT) and yields a normal emission at 585 nm from the proton transfer tautomer [25]. The quenching of emission at 585 nm was observed due to the involvement of phenolic-OH groups of receptor **1** in complex formation with Cu^{2+} which inhibited the ESIPT phenomenon.

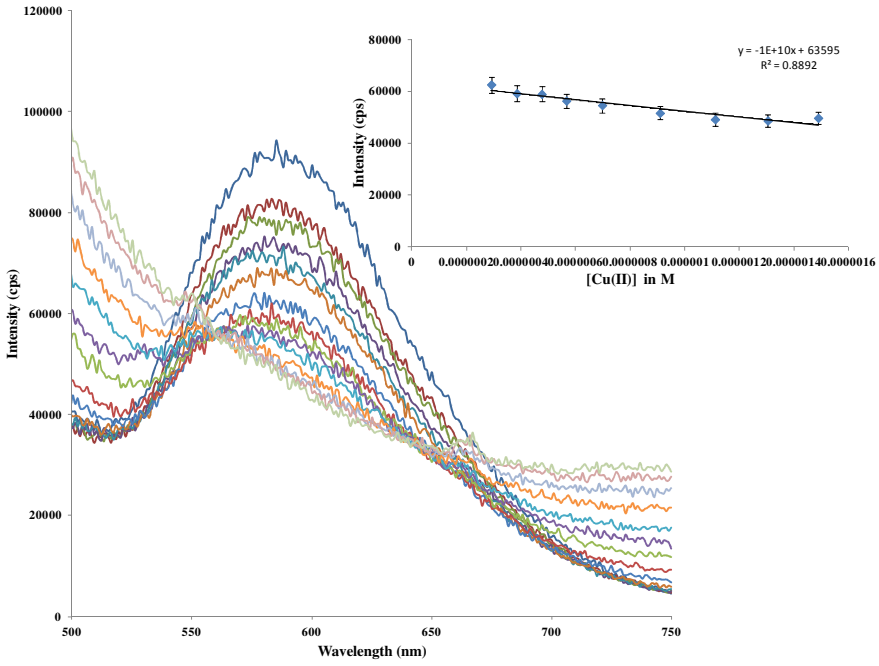


Figure 6 Changes in fluorescent intensity of receptor **1** upon gradually addition of Cu^{2+} ($\lambda_{ex}/\lambda_{em}$= 405/585). Inset showing the response of fluorescence signal with regression 0.96.

The emission intensity of the receptor **1** was linearly proportional to Cu^{2+} concentrations (**Figure 6 inset**) The detection limit was calculated using 3*S/M following the IUPAC criterion where S is the standard deviation of blank signal and M is the slope of the

regression line. The detection limit was found to be 50 nM, which has been compared with some reported Cu^{2+} sensors (**Table S1**).

The stoichiometry of complexation of receptor **1** with Cu^{2+} was studied using continuous variation method (Job's plot) [26]. Job's plot and normalised plot obtained from fluorescence measurements showed the formation of receptors **1** and Cu^{2+} complex in 1:1 ligand to metal ratio (**Figure S2**). This data was further confirmed by mass spectroscopy analysis. ESI–MS data showed the formation 1:1 complex between two deprotonated ligand (receptor **1**) and a metal ion [**1**. Cu^{2+}+2Na^{+}] MW = 458.046; calculated for [$C_{22}H_{26}N_2O_2$ Cu^{2+}+2Na], 458.20). The association constant (K_a) value of the **1**.Cu^{2+} complex was calculated from the fluorescence titration by the Benesi-Hildebrand [27] (**Figure S3**) methodology was 666667 M^{-1} respectively. Further the quenching can be mathematically expressed by the Stern–Volmer Eq. (1), which allows for calculating quenching constants [28].

$$F_0/F = = 1 + k_q \tau_0 [Q] = 1 + K_{sv} [Q] \quad (1)$$

Where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, k_q is the bimolecular quenching constant, τ_0 is the lifetime of the fluorescence in the absence of the quencher [Q] is the concentration of the quencher, and K_{sv} is the Stern–Volmer quenching constant. In the presence of a quencher, the fluorescence intensity is reduced from F_0 to F. The ratio (F_0/F) is directly proportional to the quencher concentration [*Q*]. Evidently:

$$K_{sv} = kq\ \tau_0 \quad (2)$$

$$F_0/F = 1 + K_{sv} [Q] \quad (3)$$

According to Eq. 3, a plot of F_0/F versus [Q] shows a linear graph (**Figure S4**) with an intercept of 1 and a slope of K_{sv}. The linearity observed in **Figure S4** cannot confirm whether the quenching is static or dynamic. This can be explained as dynamic process affects the excited fluorophore but not the ground state which results in no change in absorption spectra during dynamic quenching. In order to reveal the static or dynamic quenching without measurement of fluorescence lifetime, the absorption spectrum was measured carefully to distinguish between static and dynamic quenching. In the present study we observed the change in absorption spectrum which is the criterion for static quenching [29]. Thus our quenching process is static in nature.

Conclusion

We have designed and developed a selective and sensitive chemosensor **1** for the detection of Cu^{2+} in aqueous medium. The detection of Cu^{2+} gave rise to significant UV-Vis

and colour change from colourless to yellow for the easy naked-eye detection. Sensor **1** was not affected in the presence of other interfering metal ions. The 1:1 stoichiometry of the host-guest complex formation was confirmed from Job's plot and mass spectroscopic method. Chemosensor **1** showed fluorescence "turn-off" response for the selective detection of Cu^{2+} with the detection limit down to 50 nM.

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