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A simple 2,4-dihydroxyacetophenone based fluorescent chemosensor for detection of copper(II) ion: An Experimental and DFT study

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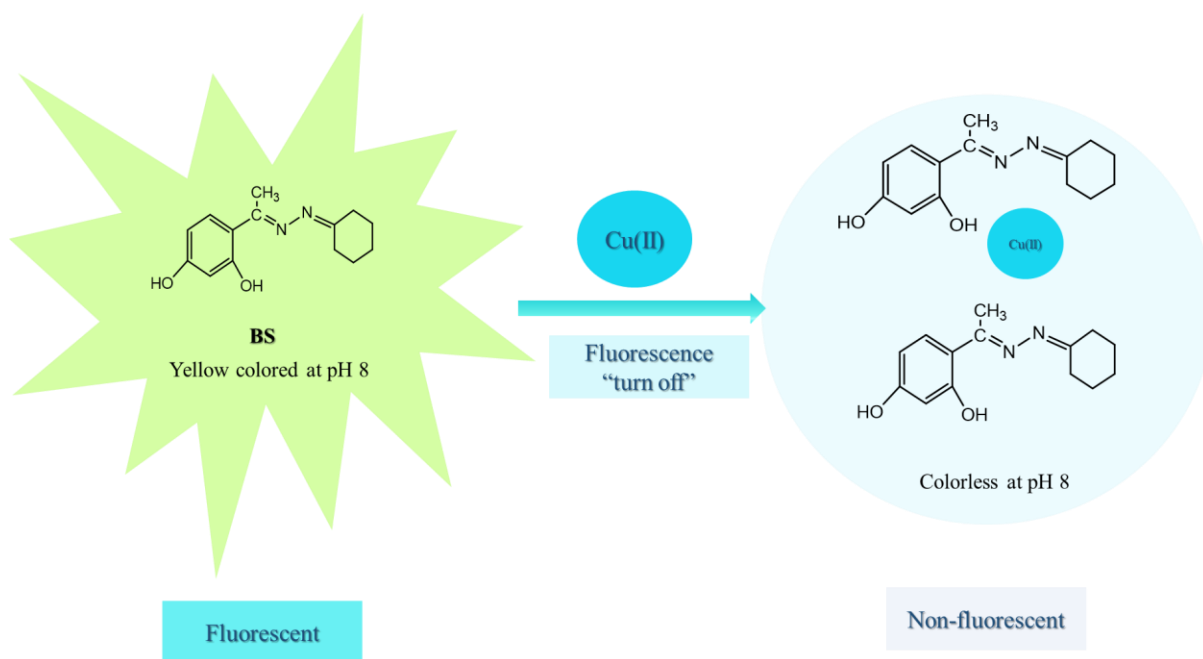
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Highlights

- A fluorescent bis Schiff's base (**BS**) was investigated as a chemosensor for the determination of Cu(II) ions.
- The BS showed remarkable results at pH 8 with LOD and LOQ equal to 0.02 and 0.05 $\mu\text{g mL}^{-1}$ respectively.
- The proposed method was found to be selective and interference free.
- The percent recovery of Cu(II) from water samples was found in the range of 90.17 ± 3.33 to 101.75 ± 2.43 %.

Graphical Abstract



Abstract

The detection of copper ions is of significant importance due to their widespread use and potential toxicity at elevated concentrations. In this study, a bis Schiff's base compound; 4-(1-(cyclohexylidenehydrazono) ethyl) benzene-1,3-diol (**BS**) was used for the first time as a "Turn off" fluorescent chemosensor of Cu(II) ion in water samples with characteristics of aggregation induced enhanced emission (AIEE) at 510 nm upon excitation at 405 nm. **BS** showed high selectivity towards Cu(II) ions with considerable fluorescence quenching and was found interference free in the presence of other metal ions in mixed solution of methanol-water (v/v,1:9) at pH 8. Fluorescence intensity of **BS** was observed to decrease due the formation of non-fluorescent complex (**Cu@BS**) due to the paramagnetic effect from spin-orbit coupling and partial d shell of Cu (II). DFT simulations were performed to gain theoretical insight into the interaction of Cu(II) with **BS**. The value calculated for limit of detection (LOD) was 0.26 μM , and percent recovery of above 90% was achieved from spiked water samples. This novel turn-off fluorescent sensor offers a simple, cost-effective, and reliable method for the rapid detection of copper ions, making it a valuable tool for environmental monitoring and ensuring the safety of water resources.

Keywords: Chemosensor, Environmental monitoring, Fluorescence quenching, Heavy metal, Trace analysis

1.0. INTRODUCTION

Metals have played a crucial role in human civilization since the Bronze Age, significantly shaping the development of human life [1]. Copper, a transition metal, is abundant in the Earth's crust and has been used extensively for over 5,000 years, initially in tool-making [2]. Today, copper's applications have expanded across various domains, including its use in the electrical industry [3], in alloy production, catalysis, electroplating, and fertilizer production [4]. Copper also serves as a bactericide [5] and is an essential trace element necessary for maintaining the normal functioning of the central nervous system, cardiovascular system, hematopoietic function, and enzyme regulation [6-9]. Copper is also considered as an environmental pollutant that may affect human health directly or indirectly (via food chain) [10-13]. The diseases caused by Cu(II) due to excessive intake or deficiency are Alzheimer's disease, Parkinson's and Wilson diseases, and Amyotrophic lateral sclerosis (ALS) [14-17]. The WHO sets a maximum level of Cu(II) in drinking water to be 31.4 μM [18].

Various analytical techniques were developed to assay Cu(II) in environmental and food products. These methods are atomic absorption spectroscopy (AAS), gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) [19, 20]. However, they have limitations of expensive instruments, high cost, long analysis time and complexity in operation. As compared to other analytical techniques the fluorometric method has got great attention especially in metal ion detection due to fast and easy analysis, sensitivity, repeatability and low cost [21, 22]. To date, a series of publications belonging to optical sensing of Cu(II) have been published. These methods include either absorbance, reflectance or colorimetric and fluorimetric methods. Optical chemosensors based on fluorescence have advantages over reflectance and absorbance in terms of sensitivity and few are reported for determination of Cu(II) ions. Calcein, and rhodamine derivatives have been used as fluorophore for sensing of Cu(II) but the main issue concerning these chemosensors is their nonselective nature [23]. A number of other fluorescent chemosensors have been worked out for sensing of Cu(II) ions, being limited by low functionality in aqueous media. Chemists are searching to develop such type of chemosensors which show high efficiency of determination of Cu(II) in different samples [24].

Organic compounds such as *Bis*-Schiff bases, azines and di-imines which are often synthesized by mixing aliphatic or aromatic aldehydes with aniline or aliphatic amines have become more significant in recent years due to their biological applications [25], and as chemosensors in analytical chemistry [26]. Bis Schiff's base compounds have general formula $R_1HC=N-N=CR_2$. [27, 28]. Schiff's base probes exhibit high fluorescence in aqueous media due to the elimination of aggregation-caused quenching [29, 30]. In solvents where Schiff bases, particularly those derived from hydroxyaromatic aldehydes, have maximum solubility, the C-N bond can rotate freely. This free rotation facilitates non-radiative decay, leading to weak fluorescence. However, in solvents with poor solubility, the situation is reversed: aggregation-induced emission occurs, leading to an enhancement in fluorescence intensity. This enhancement is attributed to the intramolecular hydrogen bonding between the nitrogen atom and the hydroxyl group, which activates the excited-state intramolecular proton transfer (ESIPT) process [31, 32].

In the current study, a bis-Schiff base compound; 4-(1-(cyclohexylidenehydrazono) ethyl) benzene-1,3-diol fluorescent chemosensor (**BS**) has been reported that exhibits both aggregation-induced enhanced emission (AIEE) and excited-state intramolecular proton transfer (ESIPT) characteristics. This **BS** chemosensor has been applied for the first time for quantitative determination of Cu(II) in aqueous media, using fluorescence spectroscopy.

2.0. Experimental

2.1. Chemicals

All the solvents, chemicals, and salts of different metals were obtained from Sigma Aldrich, BDH and Merck and utilized without further purification. The chemical reagents used for preparation of Britton Robinson buffer were glacial acetic acid (ACS reagent, 99.7%), phosphoric acid (85%), boric acid (ACS reagent grade) and NaOH (Sigma-Aldrich, Germany). The bis Schiff's base derivative (**BS**) synthesized as reported [33] was thankfully received from Bio-organic and Medicinal Lab, Department of Chemistry, University of Malakand and used as optical chemosensor.

2.2. Instrumentation

Fluorescence studies were conducted using a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a 150-watt xenon lamp and a 1.0 cm quartz cell. pH measurement was done using

an analog pH meter (Model PHS-3BW, 2908 N, Chicago, USA). Mass measurement was done with the help of electrical digital balance (Shimadzu-ATY 224, Japan). A spectrophotometer (Biobase BK-S360, China) was used for measurement of absorbance using quartz cells having 1.0 cm path length. Solutions were heated up to the required temperature using an electrical thermostatic water bath (DSB- 1000T, Taiwan).

2.3. Solutions

Stock solutions of the corresponding metal salts ($100\ \mu\text{g mL}^{-1}$) were prepared in distilled water and $100\ \mu\text{g mL}^{-1}$ stock solution of **BS** was prepared purely in methanol and then working solutions of **BS** and metal ions were prepared by taking required volume of stock solution diluted with distilled water. An acid solution of 0.04 M of acetic acid, boric acid and phosphoric acid were mixed in 500 mL volumetric flask to make Briton Robinson Buffer for pH study. The buffers of required pH were obtained by adding appropriate volume of NaOH solution (0.2 M) to required volume of acid mixture and adjusting the pH accordingly.

2.4. Procedure

In a number of volumetric flasks (5.0 mL), 0.5 mL of $100\ \mu\text{g mL}^{-1}$ of **BS** and variable volume of Cu(II) ($10\ \mu\text{g mL}^{-1}$) in the range of 0.05 to 0.6 mL and 2.0 mL of buffer of pH 8 were mixed and dilution was done up to the mark with distilled water. Fluorescence intensity at $\lambda_{\text{em}} = 510\ \text{nm}$ was measured for the reagents blank as well as for the respective test solution. F°/F was calculated in each case and plotted against the concentration of Cu (II)solution. F° represents the FI of blank solution having **BS** and buffer while F is the FI of **BS**, buffer and Cu(II) solution mixture. Fluorescence measurements were taken against the respective blank and results recorded.

2.5. Optimization studies

2.5.1. Effect of solvent on fluorescence quenching of BS by Cu(II)

The fluorescent behavior of **BS** compound and its interaction with copper ions was investigated in different solvents. These solvents were DMSO, ethanol, methanol, acetonitrile, DMF and water. The required volume (0.5 mL) of **BS** solution ($100\ \mu\text{g mL}^{-1}$) and 0.5 mL of $10\ \mu\text{g mL}^{-1}$ of Cu(II) were taken in a series of 5 mL volumetric flasks and diluted with different solvents separately. Similarly, the blank solutions in different solvents were made without the addition of Cu(II) to

investigate solvent effect and “Aggregation Induced Enhanced Emission” (AIEE) properties in solvents especially in water. Fluorescent intensity was measured and $F^0 - F$ was calculated. $F^0 - F$ was plotted against each solvent.

2.5.2. Kinetic stability and reaction time

Kinetics of **BS** and **Cu** interaction as well as stability of **BS** was investigated as a function of time for 1 hour. The amount of **BS** ($10\ \mu\text{g mL}^{-1}$) and $1.0\ \mu\text{g mL}^{-1}$ of Cu(II) in water were kept constant throughout the study, measured fluorescence after interval of 10 min and $F^0 - F$ versus time, curve was constructed.

2.5.3. Effect of pH

To determine the chemosensor efficiency for metal ions determination, it is important to investigate the effect of pH. For solution preparations, 2.0 mL of buffer having pH 2,4,6,8,10 and 12 were taken and added to the solution of **BS** ($10\ \mu\text{g mL}^{-1}$) as well as to mixture of **BS** ($10\ \mu\text{g mL}^{-1}$) and Cu(II) ($1.0\ \mu\text{g mL}^{-1}$). Dilution was done with distilled water and FI was measured against blank solution in each case.

2.6. Interference effect

For exploring the selectivity of the developed method, solutions ($100\ \mu\text{g mL}^{-1}$) of different metal ions were prepared. In a number of volumetric flasks (5.0 mL) having 0.5 mL of **BS** ($100\ \mu\text{g mL}^{-1}$) and 0.5 mL of Cu(II) ($10\ \mu\text{g mL}^{-1}$), 1.0 mL of different metal solutions ($10\ \mu\text{g mL}^{-1}$) were taken to make concentration twice to that of Cu(II). Blank solution of **BS** and Cu(II) was prepared without the addition of other metal ions. Solutions having same concentration of BS and other metal ions as mentioned above were also prepared (3:1) for study of interaction of chemosensor **BS** with other metal ions including Mn^{2+} , Cu^{2+} , Pb^{2+} , Cd^{2+} , Ni^{2+} , Ag^+ , Zn^{2+} , Hg^{2+} , Co^{2+} , and Fe^{2+} . Based on the significant interaction of **BS** with Cu(II) in comparison to other metals, qualify the chemosensing application of **BS** for Cu(II) determination.

2.7. Computational Approach

The 3D chemical structure of **BS** was modeled and optimized in DMol3 code using materials studio package. The Generalized Gradient Approximation (GGA) and Perdew Burke Ernzerhof (PBE) exchange correlation functional, and double numerical polarization (DNP) as basis set were

used with energy cutoff 4.5 Å [34]. The optimized structure of **BS** was further used to investigate electronic properties and its interaction with Cu(II).

2.8. Applications

To explore the applicability of the proposed method, spiking sample study was carried out. Tap water and river water was collected randomly from place-to-place nearby University area. All the water samples were filtered and to 2.0 mL of each sample, added to known volume (0.5 mL) of Cu(II) from stock solutions having concentration of 5.0 and 10.0 $\mu\text{g mL}^{-1}$ respectively, in 5.0 mL volumetric flasks. Then to each sample solution, 0.5 mL of **BS** ($100 \mu\text{g mL}^{-1}$) and 2.0 mL of buffer of pH 8 were added and diluted up to the mark with sample water. The sample blank was also prepared without addition of Cu(II). The experiment was done in replicate.

3.0. Results and Discussion

3.1. Fluorescence study of the interaction of various metal ions with BS

The interaction of BS chemosensor with Cd^{2+} , Pb^{2+} , Hg^{2+} , Cu^{2+} , Mn^{2+} , Ni^{2+} , Ag^+ , Zn^{2+} , and Co^{2+} metal ions were investigated using fluorescence spectroscopy. All the test metals and **BS** were taken in 1:1 i.e. $1.0 \mu\text{g mL}^{-1}$ each. Upon excitation at 400 nm, **BS** shows fluorescence at 510 nm. It was observed when the solution of above metal ions was added to the BS solution, only Cu(II) ions could significantly decrease fluorescence intensity at 510 nm as compared with other metal ions and thus **BS** was selected as fluorogenic chemosensor for selective determination of Cu(II). The results are shown in figure 1.

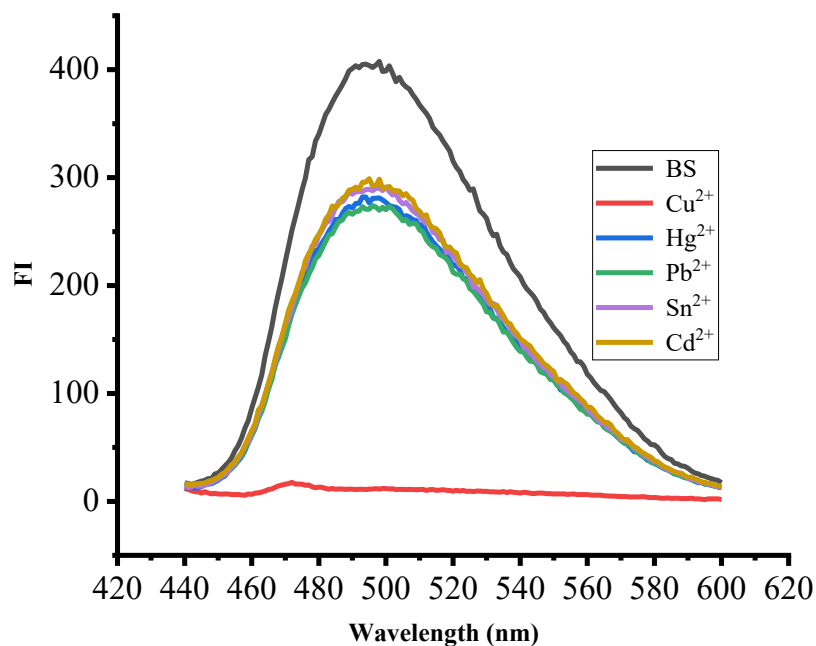


Fig. 1. Fluorescence quenching effect of metal ions on BS

3.2. Optimization study

3.2.1. Effect of BS chemosensor concentration on quenching by Cu(II)

At constant concentration of copper ions ($1.0 \mu\text{g mL}^{-1}$), the quenching rate of **BS** chemosensor in the concentration range of 4.0 to $10 \mu\text{g mL}^{-1}$ was found to increase with the increasing concentration of **BS** and becomes constant at $8.0 \mu\text{g mL}^{-1}$. Beyond $8.0 \mu\text{g mL}^{-1}$, quenching was found to remain unaffected with the above-mentioned concentration of Cu(II). Thus $8.0 \mu\text{g mL}^{-1}$ of **BS** was selected optimum for further studies. The results are shown in figure 2.

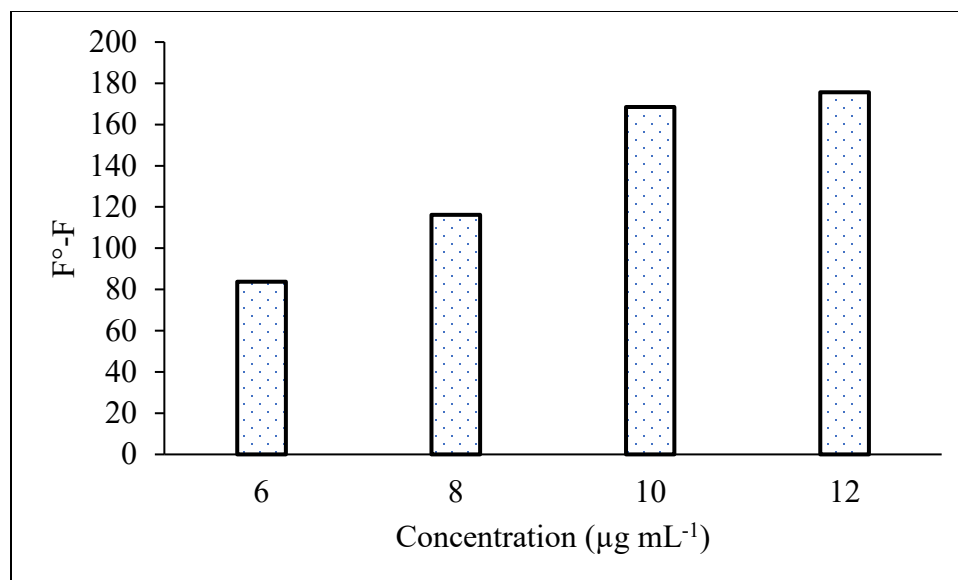


Fig. 2. Quenching versus concentration of BS under constant concentration of Cu(II)

3.2.2. Effect of Solvent on quenching of FI of BS by Cu(II)

The influence of various solvents on quenching was investigated using DMSO, DMF, Acetonitrile, methanol, ethanol and water as solvents. **BS** is soluble in organic solvents such as methanol, DMSO and other. In pure organic solvent it was observed to show less fluorescence and consequently less quenching by Cu(II) ions. When the analysis was carried out in 9:1 water and methanol, enhanced fluorescence of **BS** and thus greater quenching by Cu(II) was observed. The reason for the above-mentioned behavior of **BS** is “Aggregation Induced Enhanced Emission (AIEE) effect and “Excited state of Intramolecular Proton Transfer” (ESIPT) from -OH to imine group in aqueous solvent system [35]. The results are shown in figure 3.

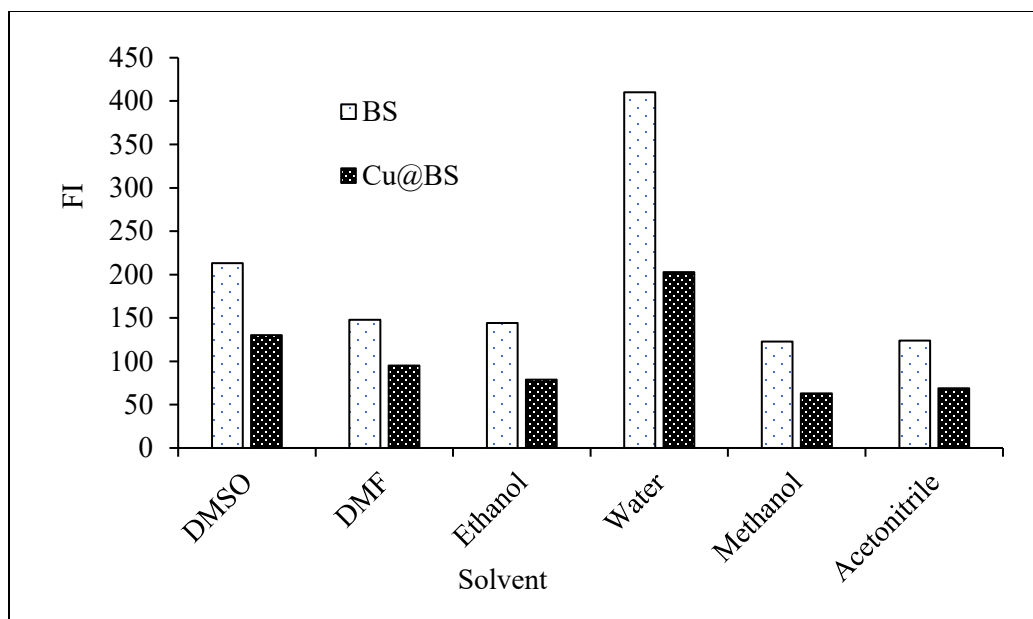


Fig.3. Effect of solvent on quenching

3.2.3. Effect of pH

Under strong acidic or basic conditions, Schiff's base compounds undergo hydrolysis reactions. To optimize pH conditions, the pH of the medium was varied in the range of 2.0 to 12.0 (Fig. 4). It was observed that **BS** showed low emission intensity and no significant fluorescence quenching at 510 nm in the pH range of 2 to 6. This indicates that the compound acts as a Lewis base and is not suitable for complex formation with Cu(II) ions in this pH range due to the protonation of functional groups such as (-OH) and (-C=N) [36]. Similarly, phenol is non-fluorescent in acidic media, while in neutral and basic media, it exhibits fluorescence [37]. However, higher emission intensity was observed in the pH range of 8-10 that can be attributed to the ionization of (-OH) groups in basic media. This ionization improves the conjugation and binding ability of **BS** towards Cu(II) ions, leading to maximum quenching [38, 39]. Therefore, the study was conducted in a slightly basic medium at pH 8 for optimal determination of Cu(II). The effect remained consistent at higher pH levels.

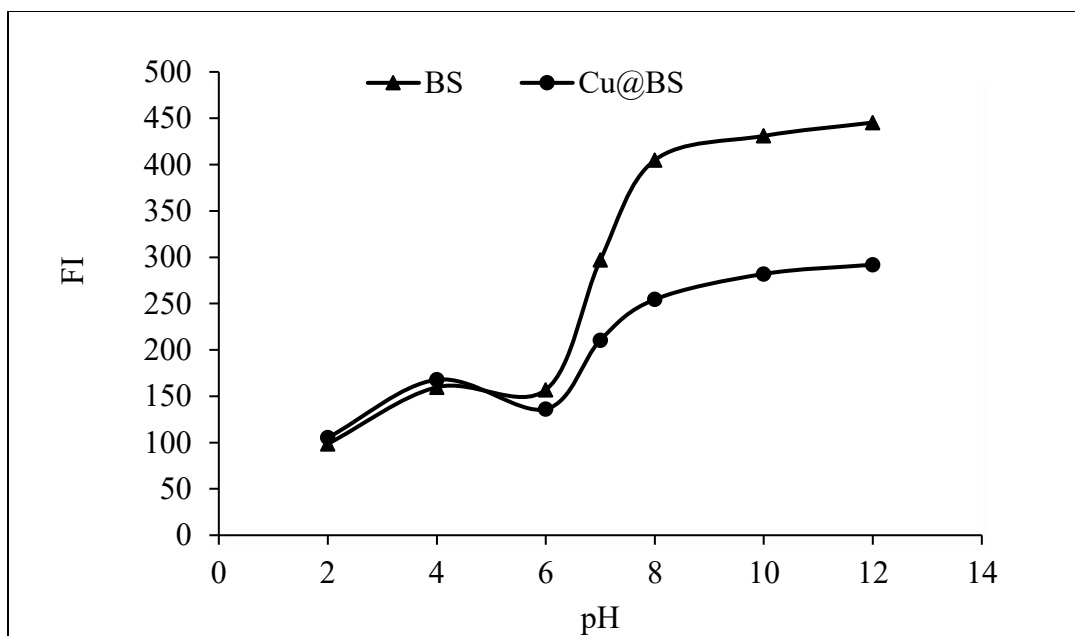


Fig. 4. Effect of pH on the FI of BS and Cu@BS

3.2.4. Kinetic stability and reaction time

The fluorescent intensity of **BS** was found to decrease to a little extent with the passage of time that can be considered negligible. **BS** probe bears (-OH) groups which formed H-bonding with water and thus caused stability in the compound as shown in Fig 5. Similarly, when Cu(II) was added to **BS**, its interaction ability remains same with the passage of time while stability was improved by Cu(II) addition. The little decrease in FI was due to the poor solubility of the **BS** compound leading to higher aggregation while **BS-Cu** formed stable complex and remain unchanged with time.

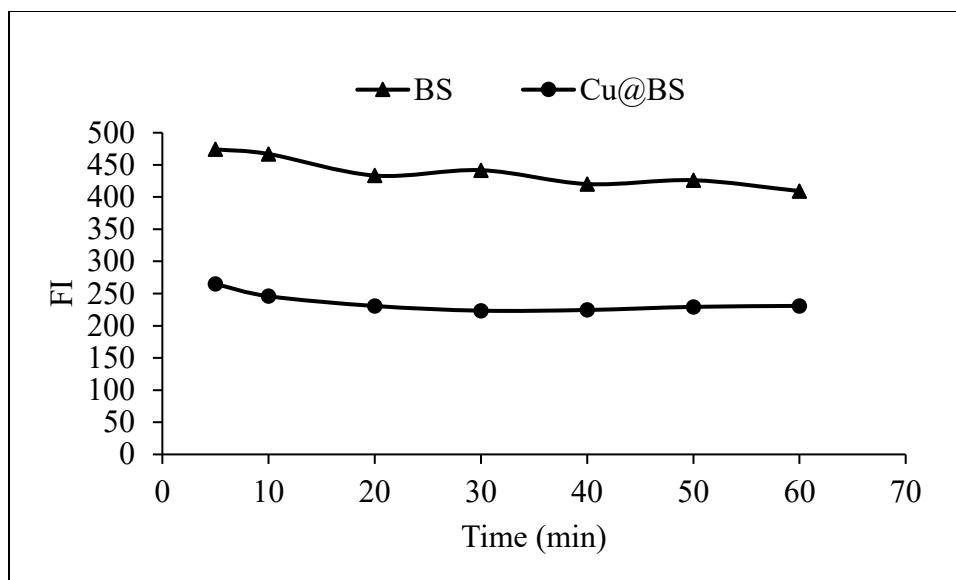


Fig.5. Kinetic study of BS and Cu@BS

3.2.6. Temperature effect on extent of quenching

The fluorescence intensity of BS decreases with the increase of temperature causing lower quenching rate by Cu(II) ions at higher temperature. The possible explanation is the increase in molecular collision at higher temperature that led to non-radiative energy lost. Therefore, all the studies were conducted at room temperature. The results are shown in figure 6.

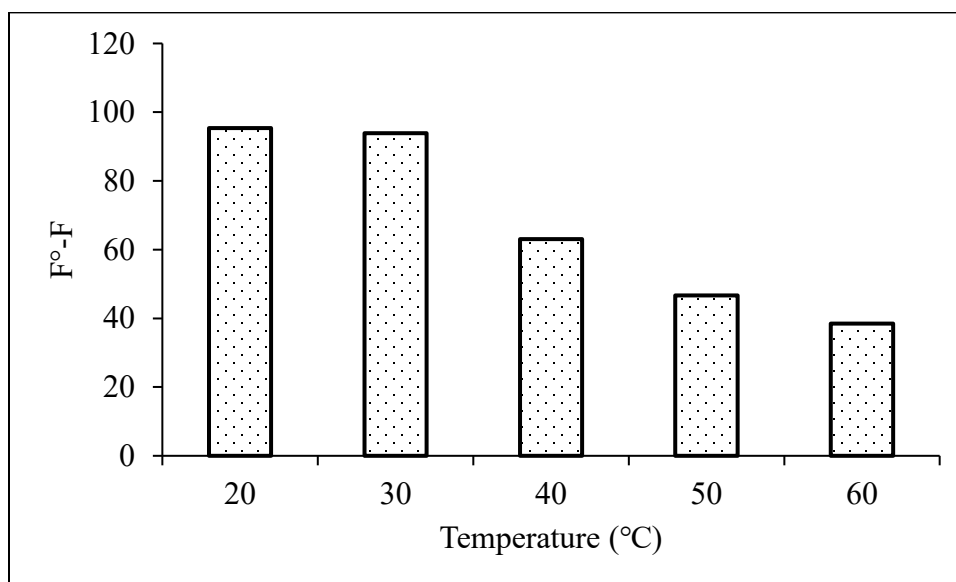


Fig. 6. Effect of temperature on extent of quenching by Cu(II)

3.2.7. Interference effect

To explore the selectivity of BS as chemosensor for Cu(II) ions, interference study was carried out. Co-existing interference study of metal ions including Ni²⁺, Pb²⁺, Co²⁺, Cr³⁺, Zn²⁺, Cd²⁺, Mn²⁺, and Hg²⁺ with Cu(II) showed that BS is selective probe only for target analyte of Cu(II) in the environment of interfering ions. In addition, only Cu(II) visually produced clear detection and make the solution colorless from yellow (Detail is given in colorimetric study). The results are shown in Fig.7

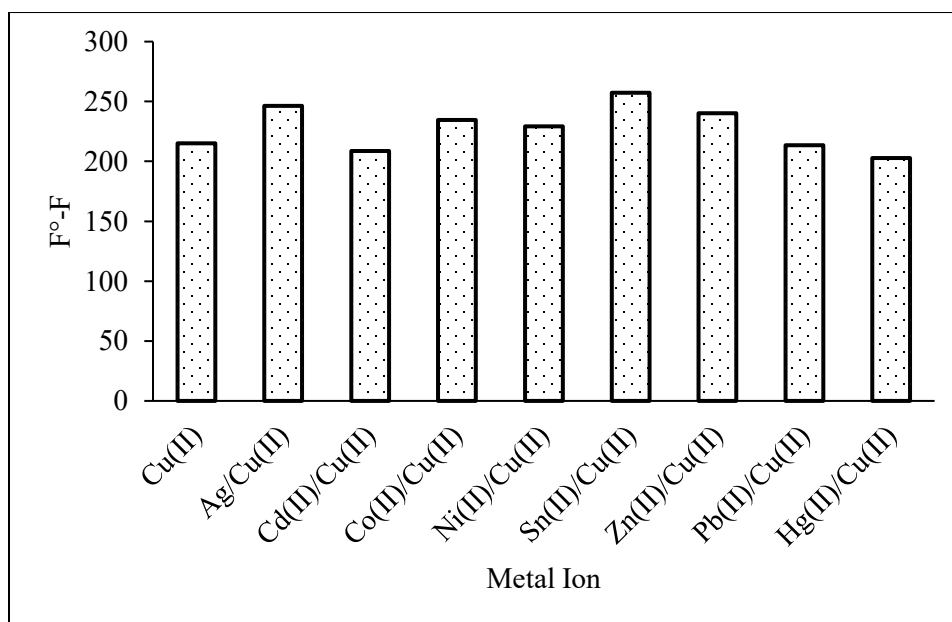


Figure 7. Co-existing interference effect of different metal ions on Cu(II) quenching

3.2.8. Effect of concentration of Cu(II) on Fluorescence quenching

Titration experiment was performed against Cu(II) ion concentration by increasing its concentration from 0.05 to 1.2 $\mu\text{g mL}^{-1}$. By plotting the values of fluorescence intensity versus Cu(II) concentration as shown in Fig. 8a and 8b, it was observed that FI of **BS** is regularly quenched by increasing the concentration of Cu(II). At lowest quantifiable concentration of Cu(II) i.e. 0.05 $\mu\text{g mL}^{-1}$, replicate analysis was performed. The value calculated for limit of detection (LOD) using formula $\text{LOD} = 3.3\sigma/S$ was found to be 0.015 $\mu\text{g mL}^{-1}$ and limit of quantification (LOQ) using formula $\text{LOQ} = 10\sigma/S$ was found to be 0.05 $\mu\text{g mL}^{-1}$ respectively. Herein, the sign " σ " denote sample standard deviation and the letter "S" shows slope of the calibration curve. The analytical figures of merit are given in table 1.

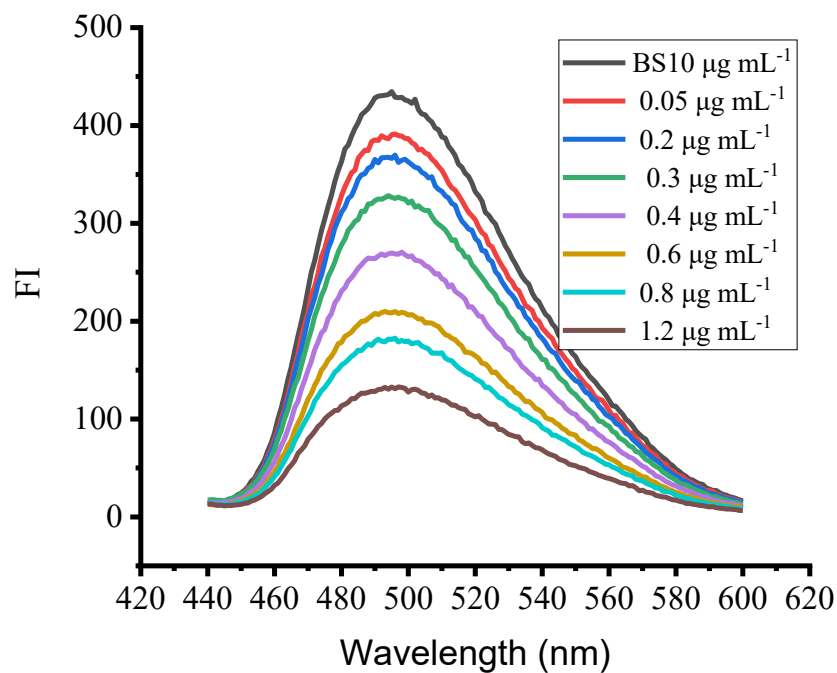


Fig. 8 (a) Effect of Concentration of Cu(II) on quenching of BS ($V_{\text{meth}}:V_{\text{water}} = 1:9$, $\lambda_{\text{ex}} = 400 \text{ nm}$, $\lambda_{\text{em}} = 510 \text{ nm}$)

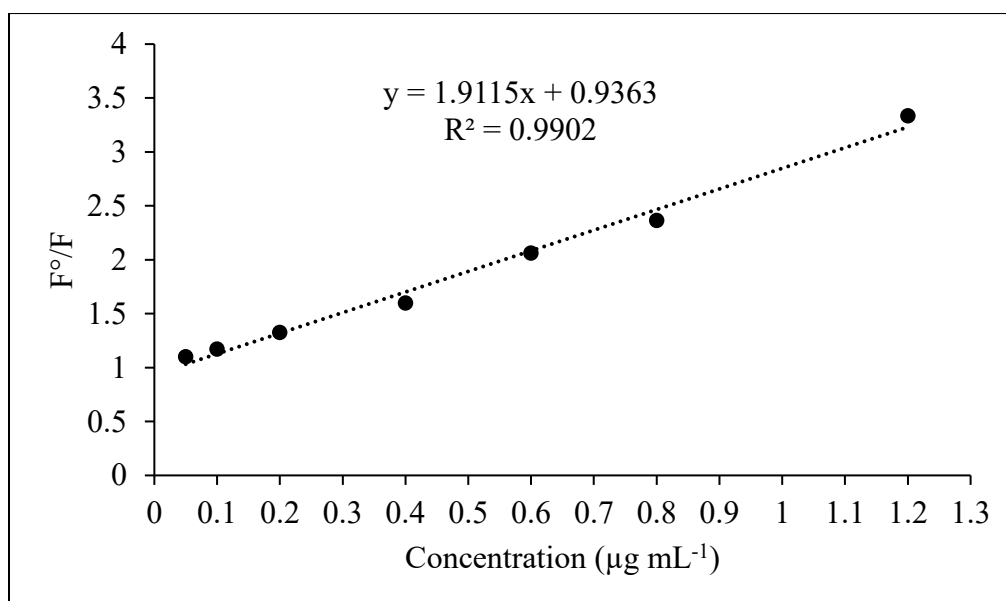


Fig 8. (b) Calibration Curve

Table 1. Analytical figures of the merit

Parameter	Value
λ_{ex} (nm)	400
λ_{em} (nm)	510
Linear range ($\mu\text{g mL}^{-1}$)	0.05-1.2
Straight line equation	$Y=1.911x+0.9363$
LOD ($\mu\text{g mL}^{-1}$)	0.02
LOQ ($\mu\text{g mL}^{-1}$)	0.06
Slope	1.911
Intercept	0.9363
R^2	0.992
SD	0.01
%RSD	7.5

3.2.9. Colorimetric study

The selectivity of **BS** chemosensor was further confirmed through colorimetric study. Solutions of **BS** chemosensor were prepared and tested with different metal ions giving no obvious color change except when Cu^{2+} was added, due to which the solution becomes colorless from yellow at pH 8. The color phenomenon was due to the removal of H^+ from hydroxyl group when the medium is slightly basic, the negative oxygen extends the conjugations through benzene ring towards imine group and impart pale yellow color to **BS**. When Cu(II) solution was added to the **BS** system, the delocalized free electrons are involved in bonding with Cu(II) giving no color to the solution restricting delocalization as shown in figure 9. The UV-visible absorption spectra of the chemosensor **BS** with and without Cu(II) also confirmed that the absorption peak shifted from visible to UV region upon the addition of Cu(II) as represented in figure 10.

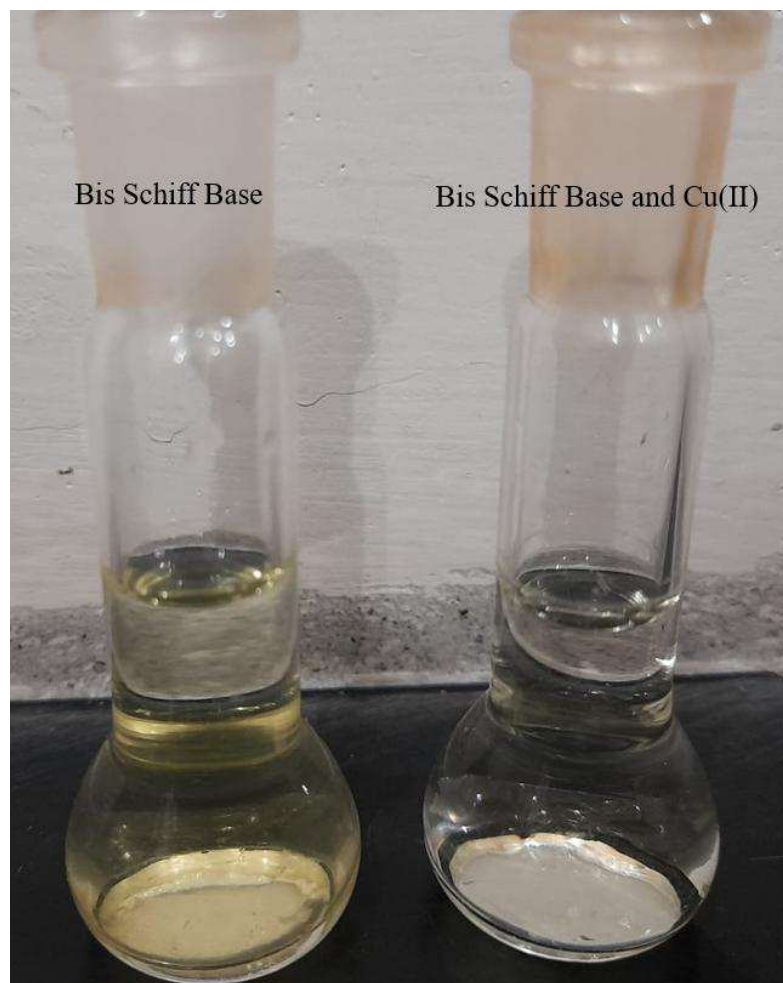


Fig. 9. Colorimetric study of BS & its complexation with Cu

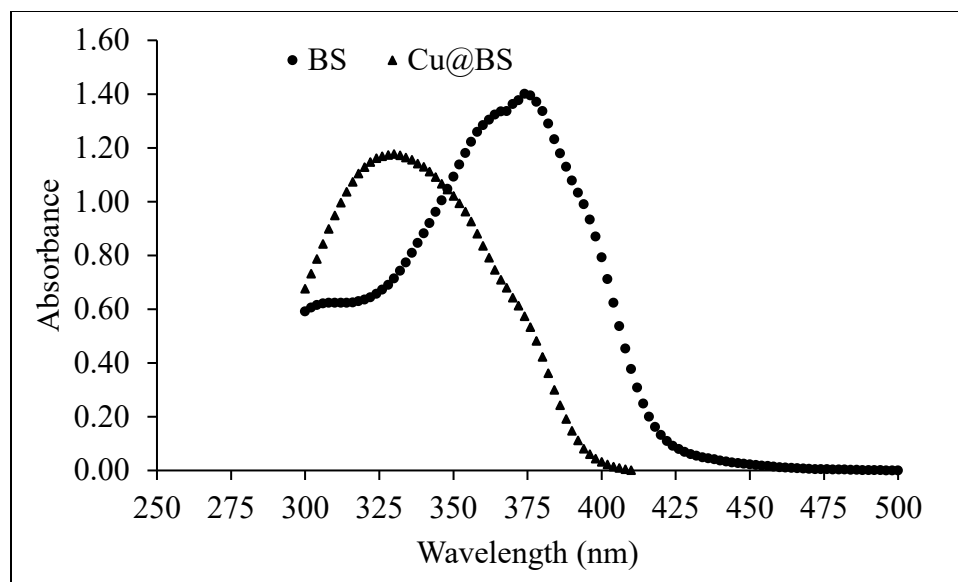
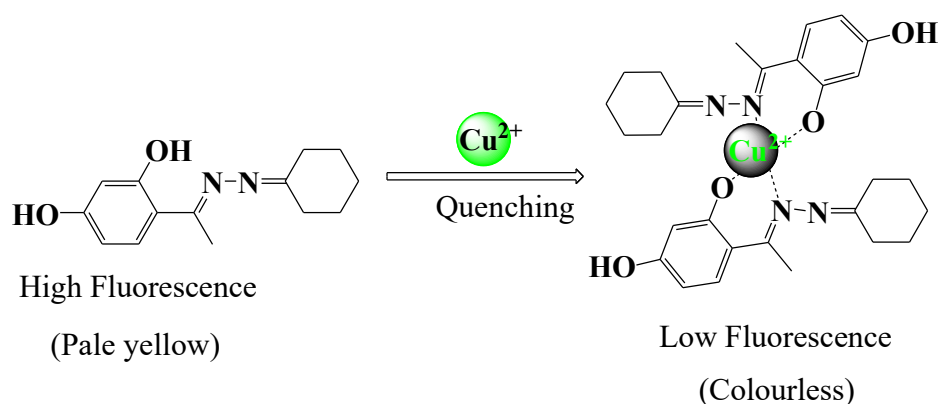


Fig. 10. UV-Visible spectra of BS and Cu@BS

3.2.10. Explanation of proposed sensing mechanism

Bis Schiff's bases are the organic compounds of diimines. This **BS** chemosensor has two imine ($\text{C}=\text{N}-$) functionalities along with a hydroxyl group in their formula as shown in scheme 1.0. The chance of interaction with target analyte Cu(II) is much higher because Schiff's bases form complexes through hetero atoms i.e. N and O [40]. This quenching in fluorescence could be probably due to the paramagnetic effect from spin orbit coupling and partial d shell of the Cu(II) ion leading to the formation of non-fluorescent complex between BS and Cu(II) via electron transfer process from donor to acceptor based on photo induced electron transfer (PET). Here in, the **BS** chemosensor act as Lewis's base and their interaction with Cu^{2+} also restricted the proton transfer from hydroxyl to imine group forming complex. As shown in scheme 1.0, the proposed mechanism indicates that chemosensor **BS** coordinated at 1:2 with Cu^{2+} ions via lone pair electrons of hetero atoms such as N and O.



Scheme 1.0 Sensing mechanism of Cu(II) using BS with Cu^{2+}

3.2.11. Application to water samples

The proposed method was further extended to investigate the potential of the BS chemosensor for the sensing of Cu(II) in spiked water samples including tap and pond water. A known concentration of Cu(I) was added to water samples and subjected to the optimal conditions of the proposed method, the percent recoveries up to $90 \pm 3.3 - 101.7 \pm 2.4$ % were achieved which reveals that the designed probe is applicable for recognition of Cu(II) in water samples. The results are shown in table 2.

Table 2. Quantification of Cu(II) in water samples

Sample type	Amount taken ($\mu\text{g mL}^{-1}$)	F°	F	F°/F	Amount Found ($\mu\text{g mL}^{-1}$)	% Recovery	Average % recovery $\pm$ SD
Tap water	0.5	677.545	372.538	1.818727	0.461	92.35	94.21 $\pm$ 1.72
	0.5	677.545	368.312	1.839595	0.472	94.53	
	0.5	677.545	365.98	1.851317	0.478	95.76	
	1.0	677.545	238.243	2.843924	0.998	99.82	101.75 $\pm$ 2.43
	1.0	677.545	236.454	2.933121	1.044	104.49	
	1.0	677.545	230.998	2.865441	1.009	100.94	
Pond water	0.5	395.817	215.955	1.832868	0.469	93.83	90.17 $\pm$ 3.33
	0.5	395.817	223.555	1.770558	0.436	87.31	
	0.5	395.817	221.102	1.790201	0.446	89.36	
	1.0	395.817	143.612	2.756155	0.952	95.23	97.74 $\pm$ 5.39
	1.0	395.817	135.44	2.922453	1.039	103.93	
	1.0	395.817	144.776	2.733996	0.947	94.07	

3.2.12. DFT results

Density functional theory (DFT) simulations were used to investigate the theoretical interaction of Cu^{2+} with ligand BS. The DFT calculations were performed by DMol3 code [41, 42], using the Perdew Burke Ernzerof functional under the framework of generalized gradient approximation [43]. The DFT Pseduocore Potential basis set was applied for all calculations. The binding energy (E_b) was computed using equation;

$$E_b = E_{\text{complex}} - (E_{\text{Lig}} + E_{\text{Cu}}) \quad (1)$$

Here, the E_{complex} , E_{lig} and E_{Cu} is the total optimized energy of the complex system, ligand and Cu atom.

The optimized geometry, molecular electrostatic potential map (MEP) and HOMO-LUMO plots of the ligand is illustrated in Figure 11. The blue and red zone in MEP shows the most electropositive and most electronegative region of ligand molecule. The MEP confirms that the N and O atoms are hot site for Cu binding due to its high electron rich nature. The HOMO-LUMO gap for ligand is computed as 2.74 eV.

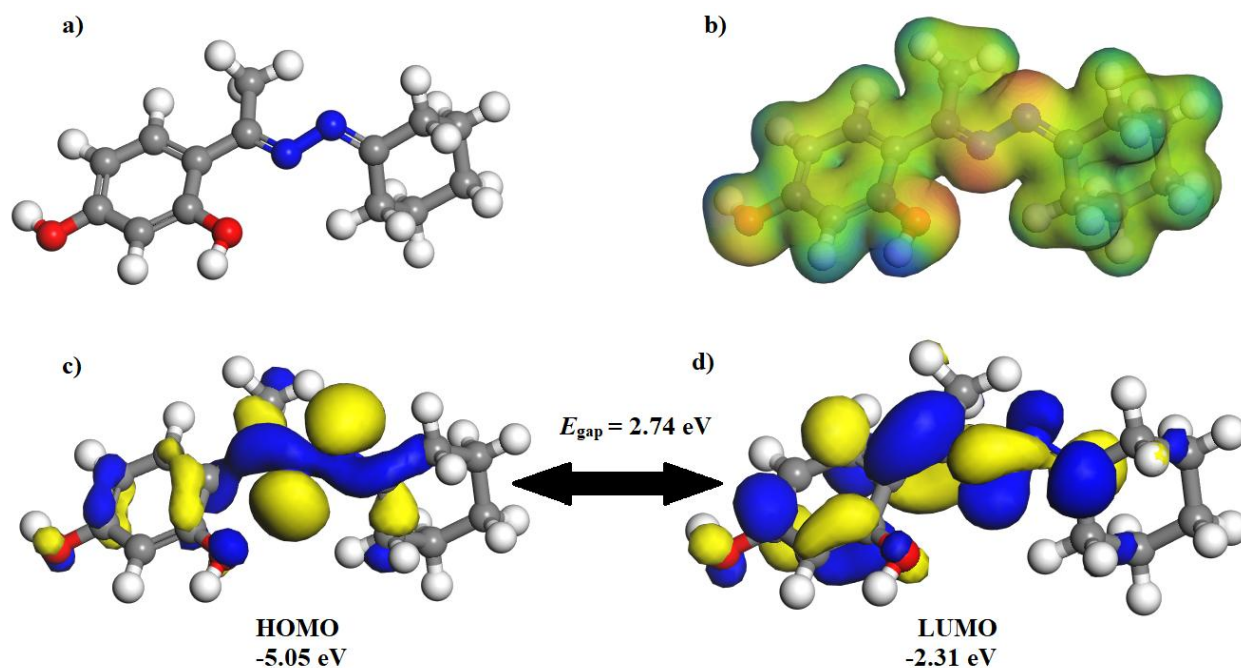
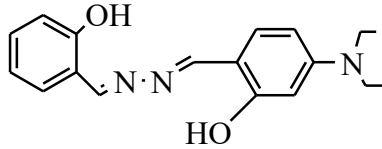
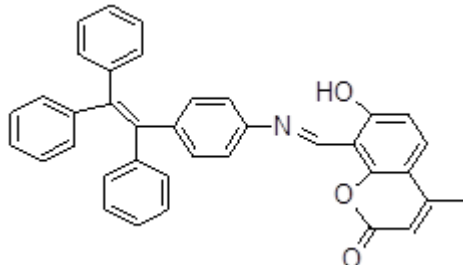
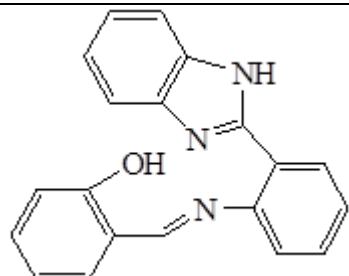
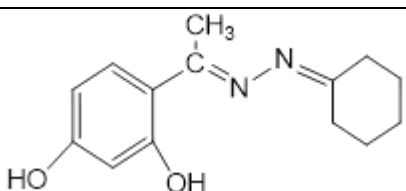


Figure 11. Optimized geometry (a) MEP map (b) HOMO (c) and LUMO (d) plot of ligand

According to the MEP, we simulated two types of complexes for Cu binding with the ligand i.e. complex-1 (where the Cu atom bonded to the N atom of the ligand) and complex-2 (where the Cu atom interacted with the O atom of the OH site of the ligand). The optimized geometry of both complexes is shown in Figure 11. The geometry relaxation shows that the Cu atom makes a Cu—N bond with the N site with a bond distance of 2.01 Å, while the E_b value obtained for this complex is -0.71 eV. The negative binding energy shows that the Cu atom bonded strongly with the N atom of the ligand. The Hirshfeld charge density analysis shows that there is 0.017 e transferred that occurred during the complexation process. In complex-2, the Cu atom makes a stronger Cu—O bond with a bond length of 1.93 Å while due to this bonding the O—H bond ruptured, and the H released and attached to N atom. The binding energy value calculated for this complex is -0.51 eV. This larger E_b shows that the complex-2 is more stable than the complex-1. The charge transfer analysis indicates that is 0.037 e transferred during the complex formation. The results show that this ligand has a greater affinity for sensing Cu atoms. Additionally, it is shown that proton transfer also take place from hydroxyl group to imine known as excited state intramolecular proton transfer (ESIPT) by DFT simulations. Moreover, the stronger reduction in the energy gap value (i.e. from

2.74 eV to 1.73 eV for complex-1 and 1.66 eV for complex-2) of the ligand confirmed the efficient sensor behavior.

3.2.13. Comparison of the reported methods with present work.

Compound Structures	Target ions	Test media	LOD (μM)	Ref.
	Cu(II)	EtOH/HEPES (1/1)	0.34	[44]
	Cu(II)	THP/H ₂ O (1/99)	0.36	[45]
	Cu(II)	CH ₃ OH/Tris-HCl	0.54	[46]
	Cu(II)	CH ₃ OH/H ₂ O (1/9)	0.25	Present work

Conclusion

In the current study an easy, selective and highly sensitive **BS** chemosensor probe have been proposed for the determination of Cu(II) ions in water. The photoproperties of **BS** chemosensor was investigated by fluorometer. Fluorescence quenching and colorimetric change was observed with the formation of complex of **BS** with Cu(II) at pH 8. The maximum concentration of **BS** was used 10 $\mu\text{g mL}^{-1}$ and had no interference effect of other metal ions. The value obtained for SD, RSD and R^2 is 0.01, 7.5 and 7.5 % respectively. Value calculated for limit of detection was 0.02 $\mu\text{g mL}^{-1}$ which is equal to 0.26 μM and allowable concentration of Cu(II) in drinking water as set

by the US= EPA 31.4 μM [18]. The proposed method was successfully applied for determination of Cu(II) ions in different water samples of pond and tap water with % recoveries from 90.17 ± 3.33 to 101.75 ± 2.43 %.

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References

- [1] Cutler CP. Use of metals in our society. *Metal Allergy: From Dermatitis to Implant and Device Failure*. 2018;3-16.
- [2] Pompeani DP, Abbott MB, Steinman BA, Bain DJ. Lake sediments record prehistoric lead pollution related to early copper production in North America. *Environmental Science & Technology*. 2013;47(11):5545-52.
- [3] Zeng L, Miller EW, Pralle A, Isacoff EY, Chang CJ. A selective turn-on fluorescent sensor for imaging copper in living cells. *Journal of the American Chemical Society*. 2006;128(1):10-1.
- [4] Gupta VK, Singh A, Ganjali M, Norouzi P, Faridbod F, Mergu N. Comparative study of colorimetric sensors based on newly synthesized Schiff bases. *Sensors and Actuators B: Chemical*. 2013;182:642-51.
- [5] Lamichhane JR, Osdaghi E, Behlau F, Köhl J, Jones JB, Aubertot J-N. Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. *Agronomy for sustainable development*. 2018;38(3):28.
- [6] Andrews NC. Metal transporters and disease. *Current opinion in chemical biology*. 2002;6(2):181-6.
- [7] Chen J, Ying G-G, Deng W-J. Antibiotic residues in food: extraction, analysis, and human health concerns. *Journal of Agricultural and Food Chemistry*. 2019;67(27):7569-86.
- [8] Prohaska JR. Impact of copper limitation on expression and function of multicopper oxidases (ferroxidases). *Advances in nutrition*. 2011;2(2):89-95.

- [9] Linder MC, Hazegh-Azam M. Copper biochemistry and molecular biology. *The American journal of clinical nutrition*. 1996;63(5):797S-811S.
- [10] Hinge SP, Orpe MS, Sathe KV, Tikhe GD, Pandey NS, Bawankar KN, et al. Combined removal of Rhodamine B and Rhodamine 6G from wastewater using novel treatment approaches based on ultrasonic and ultraviolet irradiations. *Desalination and water treatment*. 2016;57(50):23927-39.
- [11] Araya M, Olivares M, Pizarro F. Copper in human health. *International Journal of Environment and Health*. 2007;1(4):608-20.
- [12] Hadjipanagiotou C, Christou A, Zissimos AM, Chatzitheodoridis E, Varnavas SP. Contamination of stream waters, sediments, and agricultural soil in the surroundings of an abandoned copper mine by potentially toxic elements and associated environmental and potential human health-derived risks: a case study from Agrokipia, Cyprus. *Environmental Science and Pollution Research*. 2020;27:41279-98.
- [13] Loland J, Singh B. Copper contamination of soil and vegetation in coffee orchards after long-term use of Cu fungicides. *Nutrient Cycling in Agroecosystems*. 2004;69:203-11.
- [14] Lee HJ, Korshavn KJ, Kochi A, Derrick JS, Lim MH. Cholesterol and metal ions in Alzheimer's disease. *Chemical Society Reviews*. 2014;43(19):6672-82.
- [15] Gaggelli E, Kozlowski H, Valensin D, Valensin G. Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). *Chemical reviews*. 2006;106(6):1995-2044.
- [16] Zou J, Wang X, Zhang L, Wang J. Iron nanoparticles significantly affect the in vitro and in vivo expression of Id genes. *Chemical Research in Toxicology*. 2015;28(3):373-83.
- [17] Raymond KN, Dertz EA, Kim SS. Enterobactin: an archetype for microbial iron transport. *Proceedings of the national academy of sciences*. 2003;100(7):3584-8.
- [18] Uriu-Adams JY, Keen CL. Copper, oxidative stress, and human health. *Molecular aspects of medicine*. 2005;26(4-5):268-98.
- [19] Gumpu MB, Sethuraman S, Krishnan UM, Rayappan JBB. A review on detection of heavy metal ions in water—an electrochemical approach. *Sensors and actuators B: chemical*. 2015;213:515-33.
- [20] Moghaddam MR, Carrara S, Hogan CF. Multi-colour bipolar electrochemiluminescence for heavy metal ion detection. *Chemical Communications*. 2019;55(8):1024-7.

- [21] Kaur B, Kaur N, Kumar S. Colorimetric metal ion sensors—A comprehensive review of the years 2011–2016. *Coordination chemistry reviews*. 2018;358:13-69.
- [22] Walekar L, Dutta T, Kumar P, Ok YS, Pawar S, Deep A, et al. Functionalized fluorescent nanomaterials for sensing pollutants in the environment: A critical review. *TrAC Trends in Analytical Chemistry*. 2017;97:458-67.
- [23] Aksuner N, Henden E, Yilmaz I, Cukurovali A. A highly sensitive and selective fluorescent sensor for the determination of copper (II) based on a schiff base. *Dyes and Pigments*. 2009;83(2):211-7.
- [24] Vojoudi H, Bastan B, Ghasemi JB, Badiei A. An ultrasensitive fluorescence sensor for determination of trace levels of copper in blood samples. *Analytical and bioanalytical chemistry*. 2019;411:5593-603.
- [25] Alam A, Ali M, Latif A, Rehman NU, Saher S, Khan A, et al. Novel Bis-Schiff's base derivatives of 4-nitroacetophenone as potent α -glucosidase agents: Design, synthesis and in silico approach. *Bioorganic Chemistry*. 2022;128:106058.
- [26] Xiong J-J, Huang P-C, Zhang C-Y, Wu F-Y. Colorimetric detection of Cu^{2+} in aqueous solution and on the test kit by 4-aminoantipyrine derivatives. *Sensors and Actuators B: Chemical*. 2016;226:30-6.
- [27] Ayaz M, Alam A, Zainab, Assad M, Javed A, Islam MS, et al. Biooriented synthesis of ibuprofen-clubbed novel bis-schiff base derivatives as potential hits for malignant glioma: In vitro anticancer activity and in silico approach. *ACS omega*. 2023;8(51):49228-43.
- [28] Khan M, Ahad G, Alam A, Ullah S, Khan A, Salar U, et al. Synthesis of new bis (dimethylamino) benzophenone hydrazone for diabetic management: In-vitro and in-silico approach. *Heliyon*. 2024;10(1).
- [29] Fang W, Zhang G, Chen J, Kong L, Yang L, Bi H, et al. An AIE active probe for specific sensing of Hg^{2+} based on linear conjugated bis-Schiff base. *Sensors and Actuators B: Chemical*. 2016;229:338-46.
- [30] Xue X, Xu J, Wang PC, Liang X-J. Subcellular behaviour evaluation of nanopharmaceuticals with aggregation-induced emission molecules. *Journal of Materials Chemistry C*. 2016;4(14):2719-30.
- [31] Fang H, Wang N, Xie L, Huang P, Deng K-Y, Wu F-Y. An excited-state intramolecular proton transfer (ESIPT)-based aggregation-induced emission active probe and its Cu (II)

- complex for fluorescence detection of cysteine. *Sensors and Actuators B: Chemical*. 2019;294:69-77.
- [32] Zhao Y, Wang W, Gui C, Fang L, Zhang X, Wang S, et al. Thermally activated delayed fluorescence material with aggregation-induced emission properties for highly efficient organic light-emitting diodes. *Journal of Materials Chemistry C*. 2018;6(11):2873-81.
- [33] Gul S, Alam A, Assad M, Elhenawy AA, Islam MS, Shah SAA, et al. Exploring the synthesis, molecular structure and biological activities of novel Bis-Schiff base derivatives: A combined theoretical and experimental approach. *Journal of Molecular Structure*. 2024;1306:137828.
- [34] Ismail M, Ahmad R, Latif A, Khan AA, Alam A, Ozdemir FA, et al. Synthesis, antibacterial activities and theoretical study of polyhydroquinoline derivatives. *ChemistrySelect*. 2023;8(41):e202300954.
- [35] Zhang X, Shen L-Y, Zhang Q-L, Yang X-J, Huang Y-L, Redshaw C, et al. A simple turn-off Schiff base fluorescent sensor for copper (II) ion and its application in water analysis. *Molecules*. 2021;26(5):1233.
- [36] Mergu N, Gupta VK. A novel colorimetric detection probe for copper (II) ions based on a Schiff base. *Sensors and actuators B: Chemical*. 2015;210:408-17.
- [37] Sharma B. *Instrumental methods of chemical analysis*: Krishna prakashan media, 1981.
- [38] Soliman EM, Saleh MB, Ahmed SA. New solid phase extractors for selective separation and preconcentration of mercury (II) based on silica gel immobilized aliphatic amines 2-thiophenecarboxaldehyde Schiff's bases. *Analytica Chimica Acta*. 2004;523(1):133-40.
- [39] Ghaedi M, Montazerzohori M, Rahimi N, Biysreh MN. Chemically modified carbon nanotubes as efficient and selective sorbent for enrichment of trace amount of some metal ions. *Journal of Industrial and Engineering Chemistry*. 2013;19(5):1477-82.
- [40] Mei-Bi D, Qi-Long Z, Xing-Cheng Z, Yun-Qian Z, Bi-Xue Z. Synthesis and crystal structure of Schiff base ligand and dinuclear Cu (II) complex. *CHINESE JOURNAL OF INORGANIC CHEMISTRY*. 2008;24(9):1381-6.
- [41] Delley B. An all-electron numerical method for solving the local density functional for polyatomic molecules. *The Journal of chemical physics*. 1990;92(1):508-17.
- [42] Delley B. From molecules to solids with the DMol 3 approach. *The Journal of chemical physics*. 2000;113(18):7756-64.

- [43] Perdew JP, Burke K, Ernzerhof M. Generalized gradient approximation made simple. *Physical review letters*. 1996;77(18):3865.
- [44] Tiwari K, Kumar S, Kumar V, Kaur J, Arora S, Mahajan RK. An azine based sensor for selective detection of Cu^{2+} ions and its copper complex for sensing of phosphate ions in physiological conditions and in living cells. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2018;191:16-26.
- [45] Wang Y, Hao X, Liang L, Gao L, Ren X, Wu Y, Zhao H. A coumarin-containing Schiff base fluorescent probe with AIE effect for the copper (II) ion. *RSC advances*. 2020;10(10):6109-13.
- [46] Liu H, Zhang B, Tan C, Liu F, Cao J, Tan Y, Jiang Y. Simultaneous bioimaging recognition of Al^{3+} and Cu^{2+} in living-cell, and further detection of F^- and S^{2-} by a simple fluorogenic benzimidazole-based chemosensor. *Talanta*. 2016 Dec 1;161:309-19.