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SELECTIVE FLUORESCENCE SENSING OF THE Cu^{2+} IONS USING NEWLY SYNTHESISED BIS-NAPHTHALENE HYDRAZONE PROBES AND APPLICATION IN MONITORING ENVIRONMENTAL SAMPLES

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ABSTRACT

Bis-naphthalene hydrazone-based three-fluorophore probes (BNH) were synthesised from the hydrazine and 1-hydroxy-2-naphthaldehyde in a Schiff base reaction. The synthesised probes were characterised using NMR, HRMS, CHNS and other spectroscopic techniques. The synthesised probes, owing to their extended conjugation, exhibited excellent fluorescence emission at 564-598 nm. The synthesised probes showed selective binding to Cu^{2+} ions in the presence of other matrix ions; thus, a photoluminescence study was performed to detect and measure Cu^{2+} ions selectively in a variety of samples. The probe's sensitivity was investigated with respect to the roles of different polar and non-polar solvents, temperature, pH, and matrix effects. The developed method was validated, and the binding energy, detection limit and quenching constant were calculated. Additionally, the geometries of the synthesised probes and the probe-metal ion complex, as well as the orbital energies, were determined using DFT calculations to support the excellent sensing capacity of the synthesised probes.

Keywords: Fluorescent Probes, Hydrazones, Naphthalene, Chemosensing, River Samples, DFT.

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INTRODUCTION

Copper (Cu) is a transition metal essential for biological and industrial applications. In the natural environment, copper commonly exists in +1 and +2 oxidation states; among them, divalent Cu is the more stable and environmentally relevant form, especially for aqueous systems.[1,2] Due to its excellent physicochemical properties, Cu is widely used in electrical wiring, construction materials, alloys, electronics, catalysts, pigments, pesticides, fertilisers, and medical antimicrobial surfaces, among other applications.[3-7] Cu^{2+} ions are considered essential trace micronutrients and thus play a crucial role in biological systems, including human, animal, and plant systems.[8] Cu is necessary for numerous biocatalysts, such as cytochrome c oxidase, superoxide dismutase, tyrosinase, and ceruloplasmin, which are involved in respiration, antioxidant defence, iron metabolism, and connective tissue and neurotransmitter formation.[9,10] Also, in biological systems, Cu is involved in the production of haemoglobin, bone formation, dopamine synthesis, regulation of nerve activity, gene expression, and the improvement of protein structure and function.[11-14] In plants, Cu regulates photosynthesis, electron-

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transport reactions, and lignin biosynthesis.[15] However, the excessive intake of Cu^{2+} is hazardous to animals and plants and causes several illnesses in humans, such as heart disease, metabolic and genetic problems, obesity, diabetes, and Wilson's disease.[16,17] Thus, international health organisations such as the WHO define permissible exposure limits for drinking water (2.0 mg/L).[2] Water pollution is the primary cause of excessive Cu^{2+} ion intake. Industrial processes and products, such as electroplating, mining, pipelines, household appliances, pesticides, and fungicides, are the main drivers of global Cu^{2+} pollution.[2,18,19] Due to a minor concentration difference between the toxicity and essentiality of Cu^{2+}, monitoring and controlling Cu^{2+} concentration at quantitative and qualitative levels in environmental and biological systems is of significant scientific and public health importance. Therefore, sensing, monitoring, and removal of excess Cu^{2+} ions have attracted considerable attention in environmental chemistry, analytical sensing, and water purification research. Therefore, in this report, a fluorescence sensing method for measuring Cu^{2+} ion concentration at quantitative and qualitative levels has been developed. Three reactive fluorescent ligands/probes (BNH-1 to 3) based on bis-naphthalene hydrazone derivatives were prepared in a Schiff base reaction. Naphthalene is a fused-ring aromatic system that possesses high π-electron delocalisation, allowing easy synthesis of a variety of derivatives and thus has been used in different photochemical and medicinal applications such as the synthesis of chemosensors, LEDs, solar plates, electronic devices, pharmaceuticals, cosmetics, paint, pigments, dyes and fungicides, etc.[20-22] Synthesised probes were used to investigate the concentration of Cu^{2+} ions in aqueous samples from different regions of India. The electronic effect of the attached electron-donating and withdrawing groups on the probes were investigated. The stable conformer, energy and LUMO-HOMO orbitals of the probes and probe-Cu^{2+} ion complexes were developed using DFT calculations. The method was validated, and the binding energy, screening constant, quenching constant, LOD and LOQ were calculated.

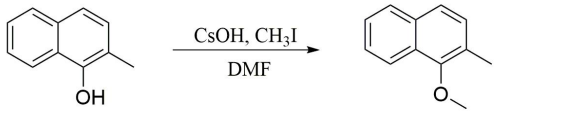
EXPERIMENTAL

Material and Methods

2-Methyl-1-naphthol was purchased from TCI (Japan). Tosyl chloride, 4-DMAP, monohydrate caesium hydroxide(CsOH), iodomethane, and monohydrate hydrazine were purchased from Sigma-Aldrich. SeO_2, dioxane, DMF, $NaHCO_3$, K_2CO_3, NaOH, methanol, ethanol, dichloromethane (DCM), ethyl acetate, chloroform, acetone and other solvents were purchased from Avra Chemicals (India). River water samples were collected from various regions of India.

Instruments and Equipment

The synthesis products were examined with a Shimadzu UV-2450 spectrophotometer, an Agilent fluorescence spectrometer, an Agilent FT-IR spectrophotometer, a Jeol 500 MHz NMR instrument, a Bruker HD high-resolution mass spectrometer, and an elemental analyser.



2-methylnaphthalen-1-ol (**Compund 1**) 1-methoxy-2-methylnaphthalene (**Compund 2**)

Fig.-1: Synthesis of the Compound 2.

Synthesis of the 1-Methoxy-2-methylnaphthalene (2)

A solution of 2-methyl-1-naphthol (475 mg, 3 mmol; compound 1) in anhydrous DMF was added to a 10 mL solution of monohydrated caesium hydroxide (495 mg, 3 mmol, 1 equiv). Then iodomethane (0.23 mL, 2.2 mmol, 1.2 equiv) in anhydrous DMF was added to the reaction, and the mixture was stirred for 24 hours at room temperature. The reaction solution was filtered, DMF was removed over crushed ice, and the mixture was then extracted with 1 N NaOH and ethyl acetate.[23] The extracted organic layer was then dried under reduced pressure and yielded (82%) solid compound 2 (Fig.-1). Synthesised compound 2 was characterised by NMR, IR, HRMS, and CHNS and found to be identical to the literature reports.[23]

Synthesis of 1-Hydroxynaphthene-2-carbaldehyde (3) and 1-Methoxynaphthene-2-carbaldehyde (4)

SeO_2 (444 mg, 4 mmol) was added to a solution containing 2-Methyl-1-naphthol (compound 1, 475 mg; 3 mmol) in dioxane, and this reaction mixture was refluxed under stirring conditions for 3 hours.[24,25] After the reaction, the mixture was extracted with DCM and water, and the organic phase was collected and

dried under vacuum. The residue was then purified using column chromatography to give 376 mg of 3 (67%). A similar method was used to prepare compound 4 from 1-methoxy-2-methylnaphthalene (compound 2; 348 mg; 62%). Synthesised compounds 3 and 4 (Fig.-2) were characterised with NMR, IR, HRMS and CHNS and found identical to the literature reports.[24,25]

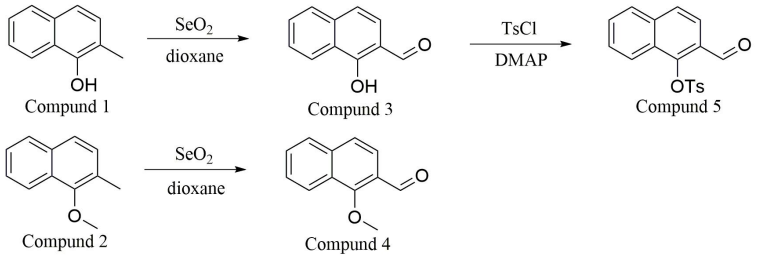


Fig.-2: Synthesis of Compounds 3, 4 and 5.

Synthesis of 1-Tosylnaphthene-2-carbaldehyde (5)

175 mg 1-hydroxynaphthene-2-carbaldehyde (compound **3**; 1 mmol) and 210 mg tosyl chloride (1.1 mmol) were dissolved in 30 mL dry ether, and under stirring, a catalytic amount of 4-DMAP was added to it. The reaction was allowed to proceed to room temperature, yielding 98% (320 mg) of the desired compound **5** (Fig.-2). Synthesised compound **5** was characterised by NMR, IR, HRMS, and CHNS, and was found to be identical to the literature reports.[25]

Synthesis of Bis-Naphthalene-hydrozones Probes

A reaction solution was prepared by adding hydrazine monohydrate (291 µL, 6.0 mmol) and a 1-hydroxy-2-naphthaldehyde (compound **3**; 516 mg, 3 mmol) in methanol at 0°C, and the mixture was stirred for 24 hours at room temperature. After the reaction, the mixture was extracted with DCM and water, and the obtained residue was dried under vacuum.[24.] The solid residue was then passed through column chromatography (chloroform/methanol = 10/1) to get the pure product (**BNH-1**; 62% yield). Similarly, **BNH-2** and **BNH-3** were prepared (Figure 3), but compounds **4** and **5** were used, respectively, instead of compound **3**. All probes were characterised using NMR, HRMS, and CHNS.

BNH-1: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.54 (2H,s), 7.84 (2H,d), 7.78 (2H,d), 7.71 (2H,d), 7.58 (4H,m) and 7.46 (2H,m). ^{13}C-NMR (151 MHz, $CDCl_3$) δ 155.94, 154.36, 136.93, 129.40, 128.21, 127.21, 125.83, 124.81, 124.38, 123.92 and 116.70. Anal. calcd (found)% for $C_{22}H_{16}N_2O_2$: C, 77.63 (77.46); H, 4.74(4.56); N, 8.23(8.42). HRMS: 341.13 ($M+H^+$).

BNH-2: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (2H,s), 8.14 (2H,d), 7.80 (2H,d), 7.76 (2H,d), 7.54 (2H,d), 7.51 (2H,m), 7.42 (2H,m) and 3.89 (6H,s). ^{13}C-NMR (151 MHz, $CDCl_3$) δ 155.51, 152.77, 135.76, 128.03, 127.90, 127.70, 127.52, 126.48, 126.28, 124.71, 123.77 and 63.13. Anal. calcd (found)% for $C_{24}H_{20}N_2O_2$: C, 78.24(78.11) ; H, 5.47(5.28); N, 7.60(7.78). HRMS: 369.16 ($M+H^+$).

BNH-3: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.58 (2H,s), 8.30 (2H,d), 7.783 (2H,d), 7.75 (6H,d), 7.57 (4H,m), 7.35 (4H,m) and 2.39 (6H,s).^{13}C-NMR (151 MHz, $CDCl_3$) δ 159.52, 154.74, 148.80, 142.11, 135.78, 132.09, 129.93, 129.61, 128.60, 128.04, 127.58, 126.77, 126.59, 123.79 and 21.56. Anal. calcd (found)% for $C_{36}H_{28}N_2O_6S_2$: C, 66.65 (66.28); H, 4.35 (4.47); N, 4.32 (4.12); S, 9.88 (10.07). HRMS: 649.14 ($M+H^+$).

Fluorescence Studies

Probe BNH-1 was selected as representative for fluorescence studies in this report. For fluorescence studies, a successive amount of the solution of metal ions (1×10^{-3} M) in water-buffer was added to the solution of probe BNH-1 (5×10^{-5} M in MeOH), and the resulting solution was then examined on a fluorescence spectrometer. Similarly, other probes were evaluated for fluorescence detection in the presence of metal-ion samples.

RESULTS AND DISCUSSION

Synthesis of the Naphthalene Carbaldehyde Derivatives

The 2-Methyl-1-naphthol (compound **1**) was modified to obtain three aldehyde derivatives (compounds 3-5). Compound **2** was prepared by an etherification reaction of 2-Methyl-1-naphthol (compound **1**) and Iodomethane in the presence of caesium hydroxide monohydrate (CsOH), which acts as the catalyst.[26] Cs binds to Iodomethane and enhances its reactivity toward substitution reactions, thus yielding a high yield of the compound **2** under simple stirring conditions (Fig.-1). The aldehyde derivatives of compounds **1** and **2** were prepared by oxidation with SeO_2, which oxidised the methyl group attached to the naphthalene ring. SeO_2 is a strong oxidising reagent and commonly used in the preparation of alcohols, aldehydes and carbonyls at the active carbon positions.[27] SeO_2 binds to the active carbon position and inserts an oxygen atom into the active site under simple reflux conditions, yielding a high percentage yield.[27] Thus, compounds **3** and **4** were obtained in high yield (62-65%). Compound **5**, the tosyl derivative of compound **2,** was prepared from compound **2** in a tosylation reaction with tosyl chloride (Fig.-2). Tosyl chloride, in the presence of an acyl functional group, is a highly reactive molecule toward the nucleophilic substitution reactions,[28] and thus is easily substituted with the hydroxyl group of compound **3** in the presence of a catalytic amount of the 4-DMAP, which accelerates the reaction by preparing the pyridine-tosylate intermediate, and produced nearly 100% yield of the desired compound **5**.

Synthesis of BNH Probes

The Bis-Naphthalene-hydrozones probes (BNH 1-3) were prepared in a Schiff base reaction (Fig.-3). Schiff base reactions, which are reversible, yield significant amounts of the desired products (aromatic imines) without a catalyst, under simple stirring conditions or mild heating.[26] The oxygen atom of the aldehyde group is substituted with a more nucleophilic nitrogen of hydrazine in a substitution reaction. As a result, the overall nucleophilic nature of the aldehyde carbon atom was suppressed, but conjugation was enhanced due to the coupling with other aromatic systems. These reactions are used explicitly in the synthesis of organic ligands, organometallics, polymers, covalent organic frameworks, pharmaceuticals, cosmetics, organic dyes, herbicides, etc.[17,26] A methanol solution of 1:2 equivalent monohydrate hydrazine and compound **3,** respectively, was stirred at room temperature to produce BNH-1 in good yield. The twofold concentration of compound **3** in the solution leads to the formation of two imine bonds on both sides of the hydrazine, yielding the desired bisubstituted product. Similarly, the BNH-1 and BNH-3 were synthesised in a Schiff base reaction with monohydrate hydrazine. Decreasing the equivalents ratio from 1:2 (hydrazine and compound **3)** to a lower ratio resulted in a lower percentage yield of the desired product, and the monosubstituted product was obtained mainly. Increasing the concentration ratio of compound **3** increased the percentage yield but left unwanted, unreacted material. Thus, a 1:2 ratio of hydrazine to compound **3** was used for the reaction. Similarly, BNH-2 and BNH-3 were prepared using compounds **4** and **5**. Each probe was characterised by NMR and HRMS, and the disappearance of the aldehyde peak in the NMR data confirmed the synthesis.

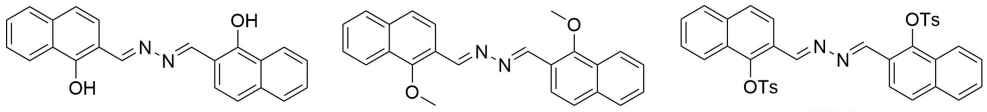


Fig.-3: Chemical Structure of the Synthesised Probes (BNH-1, 2 and 3).

Fluorescence Studies

The naphthalene and its derivatives are well known for their photoluminescence properties due to the conjugated π-electron aromatic system,[29] and attached functional groups to the naphthalene ring used to control or modify the photoluminescence properties to a desired range. Naphthalene-based ligands have been used in the preparation of ligands, chemosensors, reagents for UV-Visible analyses, medicines, pigments, dyes, etc.[20-22,30,31] The photoluminescence properties of the synthesised probes were examined in a solution containing methanol and HEPES buffer (9.5:0.5%). Each ligand (5×10^{-5} M) showed excellent fluorescence emission during the examination, and the fluorescence emission was observed in the range 564-598 nm for the prepared probes. The maximum fluorescence emission was observed for

BNH-1 (592 nm). The different functional groups attached to the prepared probes induce distinct electronic effects, thereby directly influencing their fluorescence emission properties and leading to different emission wavelengths and intensities.

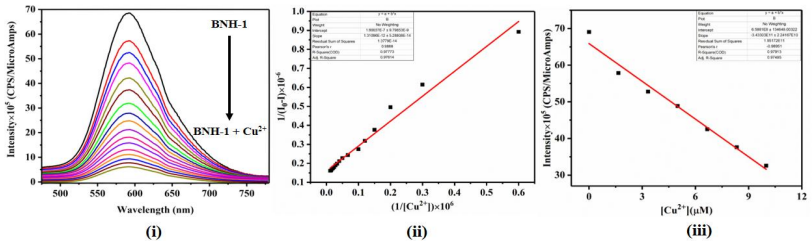


Fig.-4: (i) The Fluorescence Spectra of BNH-1 upon Successive Addition of Cu^{2+} ions Solution; (ii) and (iii) are the Binding Constant and Detection Limit for Cu^{2+}, respectively

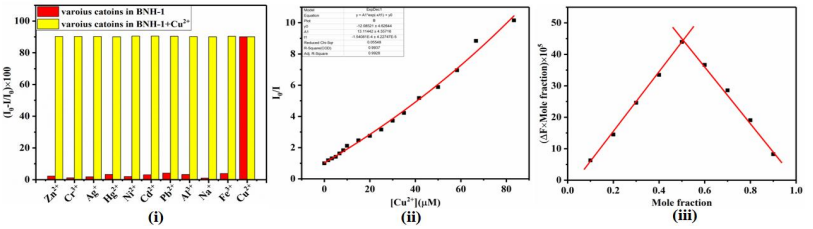


Fig.-5: (i) Analysis of Interfering Study of BNH-1 upon Addition of Different Metal Ions, (ii) and (iii) are the Stern-Volmer Plot and Job Plot for Cu^{2+}, respectively

Sensing of Cu^{2+} Ions

The synthesised probes selectively bind Cu^{2+} ions due to the structural feasibility of forming metal-ion complexes. All probes possess appropriate binding sites, such as bi-imine bonds (hydroazones), hydroxyl groups, methoxy groups, and tosyl groups, which enable covalent and non-covalent interactions with Cu^{2+} ions. Different metal ions solution such as Zn^{2+}, Cr^{3+}, Ag^{+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Al^{3+}, Na^{+}, $Fe^{3+}$and Cu^{2+} were used for fluorescent detection. Except for Cu^{2+}, the fluorescence intensities of the synthesised probes (BNH-1 to 3) in solution showed no discernible change upon addition of these metal ions. The addition of Cu^{2+} to the **BNH-1** solution, as a representative example, resulted in a significant quenching of the fluorescence peaks at 592 nm (Fig.-4(i) shows the fluorescence spectra of BNH-1). Similar fluorescence-based metal-ion detection studies were performed using BNH-2 and BNH-3. Increasing the solution temperature led to a faster fluorescence response than at lower temperatures, and higher and lower pH values disrupted the fluorescence analysis. Thus, for the fluorescence studies, the temperature and pH were maintained at 30 °C and 7.4, respectively. The quenching constants for the detection of Cu^{2+} ions using the representative ligand **BNH-1** were determined from Stern-Volmer plots and were found to be 8.51×10^4 M^{-1}. The combination of static and dynamic quenching in Stern-Volmer plots is responsible for fluorescence quenching.[32] The binding constant of **BNH-1** towards Cu^{2+} ions were determined using Benesi-Hildebrand method, and were found 1.22×10^5 M^{-1} as provided in Fig.-4(ii). The calculated detection limit of the **BNH-1** towards Cu^{2+} is 68.53 nM [Fig.-4(iii)]. Among prepared ligands, **BNH-1** had the lowest detection limit towards Cu^{2+}. Additionally, a competing study of the selective detection of Cu^{2+} using **BNH-1**, in the presence of other cations (Zn^{2+}, Cr^{3+}, Ag^{+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Al^{3+}, Na^{+} and Fe^{3+}) was performed. No interference was observed in the analysis in the presence of the other metal ions [Fig.-5 (i)]. Consequently, the prepared probes (**BNH-1, 2,** and **3**) are extremely selective fluorescence chemosensors for Cu^{2+}. The **BNH-3**, in the presence of the electron-withdrawing tosyl groups, showed lower sensitivity to Cu^{2+} ions than other probes with electron-enriching functional groups (-OH and –OMe). To confirm the stoichiometry and the mechanism of **BNH-1**'s interaction with Cu^{2+}, a Job's plot study was performed.[33] Job's plot of the fluorescence intensity variation at λ_{em} of **BNH-1** against the mole fraction of Cu^{2+} [Fig.-5(iii)] showed that **BNH-1** had the maximum fluorescence intensity at 0.5 mole fraction, suggesting a stoichiometry ratio 1:1.

DFT and Chemosensing Mechanism

The most stable conformer, binding orientation and orbital energies of the synthesised probes were determined using Gaussian software.[34-36] 3D-optimised structures of the probes show that the hydrazone

in each probe is arranged in a trans orientation, forming a more stable conformation. Probes 1 and 2 are planar, whereas probe 3 is slightly nonplanar due to the presence of a bulky tosyl group (–OTs).

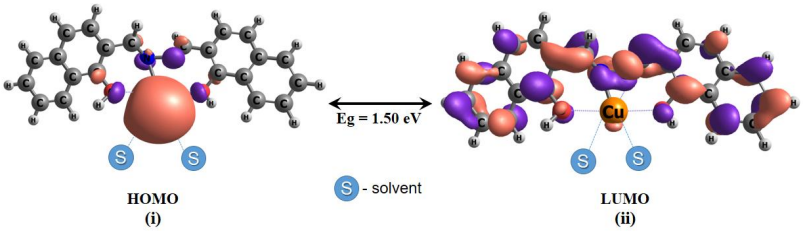


Fig.-6: LUMO-HOMO Energy Orbitals of BNH-1-Cu^{2+} Complex

Also, the calculations show that the LUMO-HOMO band gap is lower in probes BNH-1 (1.502 eV) and BNH-2 (1.681 eV) than in probe BNH-3 (2.062 eV). The extended conjugation in the synthesised probes results in excellent fluorescence emission at 592, 598, and 564 nm for BNH-1, BNH-2, and BNH-3, respectively. The Cu^{2+} ions bind to two nitrogen atoms of the hydrazone and to two hydroxyl groups of the BNH-1, forming a hexacoordinated complex in which solvent molecules stabilise the two vacant sites (Fig.-6). Similarly, the –OMe and –OTs groups of BNH-2 and BNH-3, respectively, take part in the formation of metal ion complexes. The bulky OTs group makes the hydrazone site more crowded, thereby hindering its interactions with Cu^{2+} ions and resulting in a comparatively lower response than other probes (1 and 2). At the same time, the negative electronic effect of the attached tosyl group shifted the fluorescence emission to a lower wavelength. The formation of the metal-probe complex leads to the transformation of the probe's planar structure to a non-planar structure, and metal binding reduces the bonding electrons in the double bond of the hydrazone and breaks the conjugation within the system, resulting in a sharp quenching of fluorescence emission. Similarly, probes 2 and 3 exhibit a turn-off fluorescence response upon forming the Probe-Cu^{2+} ion complex.

Environmental Samples Analysis

The concentration of Cu^{2+} ions in tap water and river water samples collected from different regions of India was analysed using the synthesised probe BNH-1 as a representative. The results of the tap water analysis were found to be similar, as shown in Fig.-4(i). The concentrations of Cu^{2+} ions in water samples from the Ganga, Yamuna, Narmada, Brahmaputra, Chambal, and Banas rivers were 0.053 mgL^{-1}, 0.214 mgL^{-1}, 0.021 mgL^{-1}, 0.015 mgL^{-1}, 0.048 mgL^{-1}, and 0.124 mgL^{-1}, respectively. The obtained results are compared with previously reported literature and found to be accurate.

CONCLUSION

Three bis-naphthalene hydrazone-based fluorescent probes were prepared, and a turn-off detection method was developed for nanomolar detection of Cu^{2+} ions in various aqueous samples (*SDG-6: Clean Water and Sanitation)*. The developed method produces robust results even in the presence of other interfering metal ions, owing to the selective binding of Cu^{2+} ions by the synthesised probes. The detection sensitivity of the probes was affected by the planarity, attached substituents, and their electronic effects. The electron-withdrawing groups in the probe (BNH-3) reduce electron density in conjugated systems, increase the LUMO-HOMO orbital energy gap, and enable fluorescent detection at a lower wavelength with comparatively less sensitivity. The synthesised probes accurately measure Cu^{2+} ions across different samples; thus, they can be used with confidence to detect Cu^{2+} specifically in industrial wastes and environmental samples. The literature reports that naphthalene compounds have great promise as electroluminescent and electron-transport materials, in addition to the chemosensor applications studied here.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-6: Clean Water and Sanitation*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profiles of the authors can be verified from their ORCID iDs, given below:

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