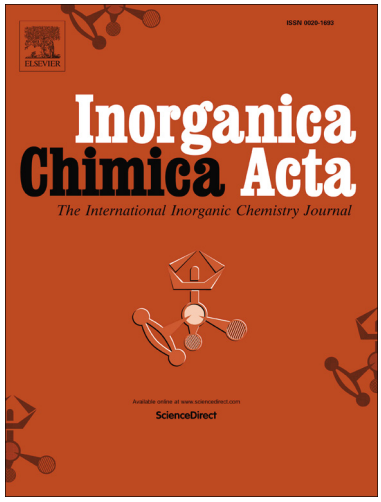

Research paper

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Colorimetric Indicators for Specific Recognition of Cu^{2+} and Hg^{2+} in Physiological Media: Effect of Variations of Signaling Unit on Optical Response

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ABSTRACT

Easy to synthesize probes are designed using bispicolyl moiety as the receptor unit with two different signaling moieties, anthraquinone and bisindolyl. Both the compounds show 'naked-eye' sensing of Cu^{2+} and Hg^{2+} in ~100% aqueous medium. Not only selectivity, the compounds also show high sensitivity towards the target metal ions, as evident by their sufficiently low detection limits. The present system can detect Cu^{2+} as low as 0.06 ppm and Hg^{2+} up to 0.03 ppm from visible color change. The sensors are found equally effective in the detection of metal ions even in the protein-bound state. The impact of the signaling unit on the sensing properties of probes having same binding units has been observed and investigated. Mechanistic investigations revealed that variations in signaling unit influence the pK_a of the bispicolyl nitrogens, which subsequently controls the stoichiometry of the probe-metal ion complexes as well as their luminescence property. Additionally, high specificity and good accuracy with recovery values ranging from 98.0 to 104.0 % were obtained during Cu^{2+}/Hg^{2+} estimation in spiked water samples. Low cost, reusable paper strips were developed for on-site detection of the metal ions which does not require the assistance of an optical instrument.

KEYWORDS: Naked-eye sensing, Signaling unit, toxic metal ions, Albumin protein, Paper strips

Highlights:

- Naked-eye sensing of Cu^{2+} and Hg^{2+} in physiological media
- Role of signaling unit in determining the stoichiometry of probe-metal ion complexes
- Estimation of toxic metal ions in natural water samples and also in presence of serum albumin

- Low-cost reusable paper strips for on-location detection purpose

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1. INTRODUCTION

Transition metals, up to a certain level, are essential in human body due to their relevance in biological systems. Among these copper is the third most abundant transition metal ion found in the human body. It takes part in a number of physiologically relevant redox processes.[1] The serum albumins (human, bovine) have specific Cu^{2+} binding site and act as a Cu^{2+} transport protein in blood plasma.[2] But at the same time, short term exposure of high level of Cu^{2+} produces gastrointestinal disturbance, while a longer exposure triggers a liver or kidney damage.[3] 'Free' copper is an indicator of Wilson's disease and Menkes' kinky hair syndrome.[4] Moreover, copper ions in free state catalyses the formation of reactive oxygen species and causes oxidative damage of cellular components.[5] Therefore regulation for allowed levels of copper in drinking water has been imposed. The maximum permitted limit of the Cu^{2+} ion in drinking water is <1.3 ppm (EPA).[6] On the other hand, mercury is one of most toxic metal ions heavily engaged in different chemical industries, as it irreversibly inhibits the activities of different selenium or sulfur-dependent enzymes. Thus the exposure to excess Hg^{2+} ion results in severe kidney problems as well as neurological disorders.[7] Mercury enters in the food chain through organomercury species.[8] Because of the high biomagnification of Hg^{2+} ion in aquatic food chain, its permitted limit in drinking water is only 2 ppb.[6] There are many sensors which report the detection of Cu^{2+} and Hg^{2+}.[9] But many of these work only in organic media and often fail to work in water. Therefore there is an urgent need of developing sensors that can detect these ions in water as well as in biological media. The selective as well as sensitivity has always been the primary concern while designing new sensing probes. However, the efficiency of the probes get affected by the electronic characteristic of the signaling as well as receptor moiety, overall structure and the surrounding environment.[10] Though plenty of work has appeared in the literature addressing the effects of surrounding enviroment (polarity, pH, viscosity of the medium) , till date, no systematic effort has been made to explore the role of signaling moiety

on the metal ion sensing behavior of small molecular probes. Considering this, herein we have investigated how the structural and electronic changes in the signaling unit affect the metal ion-chelating behavior of the receptor moiety.

For this, we chose tridentate 2-substituted pyridine based (picolyl) ligands, which are known for binding various metal ions.[11] Metal ion coordination leads to changes in the electronic state of the bispicolyl moeity, which subsequently alter the optical properties of the covalently-linked signaling units. We have coupled this bispicolyl amine (BPA) moiety with two different signaling units based on i) anthraquinone (**1**) and ii) bisindolylmethane (BIM) moiety (**2**) (Scheme 1). Both the probes experience metal ion-triggered modulation in the intramolecular charge transfer interaction and allow ratiometric 'naked-eye' sensing of Cu^{2+} and Hg^{2+} at pH 6.5. On the contrary, specific binding is observed solely with Cu^{2+} at pH 7.4. However, the mode of metal ion binding is found to be dependent on the nature of the signaling moiety. Compound **1**, with anthraquinone as the signaling unit, shows 2 : 1 binding with metal ions, while compound **2** having bis-indolyl unit forms a 1 : 1 complexes with metal ions. Another striking observation was made during the fluorimetric studies, where compound **1** and **2** respectively showed 'turn-on' and 'turn-off' response with both Cu^{2+} and Hg^{2+}. Additionally, color change with Cu^{2+} was also observed in presence of the excess of serum albumin at physiological pH. We further checked the applicability of these probes for the detection of the Cu^{2+} and Hg^{2+} ions in the real-life water samples. Furthermore, low-cost paper strips were prepared for rapid, on-site detection of these toxic metal ions even at distant locations.

2. EXPERIMENTAL SECTION

2.1. Material and methods: All reagents, including starting materials, and silica gel (for TLC and column chromatography) were obtained from the best known commercial sources and used without purification. FT-IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX system and were reported in cm^{-1}. ^{1}HNMR and ^{13}C-NMR spectra were recorded with a Bruker Advance DRX 400 spectrometer operating at 400/300 and 100/75 MHz for ^{1}H and ^{13}C NMR spectroscopy respectively. Mass spectra were recorded on Micro mass Q-TOF Micro TM spectrometer.

2.2. Spectroscopic studies: UV−vis and fluorescence spectra were recorded on a Shimadzu model 2100 UV-vis spectrometer and a Cary Eclipse spectrofluorimeter respectively. In the emission experiments, the slit widths were kept 10 nm and 10 nm for the excitation and emission channel respectively. The excitation wavelength was chosen as 480 and 465 nm for **1** and **2** respectively. For sensing in different buffered media, solutions of different pH (HCO_2Na/HCl buffer for pH 2-4.0, CH_3CO_2Na/HCl buffer for pH 4.5-6.5, Tris/HCl for pH 7-9 and $Na_2B_4O_7{\cdot}10H_2O$/NaOH for pH 9.5-10.5) were prepared. Sensing of metal ions in aqueous medium was carried out by adding 10 μL DMSO solution of either **1** or **2** from a stock (1×10^{-3} M) in buffered medium to make the final volume of 1 mL ([**1**]=[**2**]=1×10^{-5} M) followed by addition of aqueous solution of metal ions. The final concentration of DMSO in all the studies were less than 1%.

2.3. Preparation of paper strips for sensing: To prepare dye-coated filter paper, a methanolic solution of compound **1** (1 mM) was drop cast onto the filter paper to form a rectangular luminescent spot with a size of about 1 x 1.5 cm. The solution was completely absorbed on the filter paper within 20 min and then the filter papers were left overnight for air-dry. To check metal ion sensing, the dye-coated paper strips were exposed to aqueous solutions of different metal ions. For recovery studies, the metal ion treated paper strips were dipped into EDTA (1 mM) solution for ~5 min between each reading. Similarly, iodide solution (1 mM) was utilized to discriminate between Cu^{2+} and Hg^{2+}.

3. RESULTS AND DISCUSSION

3.1. Synthesis and photophysical properties of receptors: The two sensors were synthesized using a simple synthetic strategy as shown in Scheme 1. The key starting material *p*-BPA benzaldehyde was being synthesized via Vilsmeier-Haack reaction, and then coupled with either 1, 2-diaminoanthraquinone to afford **1** or with 2 equiv. of indole followed by oxidation using DDQ to get **2** (See ESI†). All of the compounds were adequately characterized using FT-IR, mass, ^{1}H and ^{13}C NMR spectral methods (See ESI†). To evalute the metal ion binding ability of the probes, Uv-visible spectra of compounds were monitored at different pH in buffered medium. The two pK_a of the bispicolyl (BPA) unit were determined as 3.6 and 8.6 for the protonation of pyridine and the tertiary nitrogen respectively for **1** (Fig. S1a, ESI†). For

compound **2**, the pK_a values were estimated as 3.9 and 6.5 respectively (Fig. S1b, ESI†). These pK_a values indicate that at pH 6.5, the tertiary nitrogen of compound **1** will be more protonated compared to the compound **2**. On the other hand, at pH 6.5 bisindolyl site of compound **2** will be in the partially protonated state.[12] Both these factors module the internal charge transfer states of the compounds in different ways. Protonation at the bispicolyl end restricts the charge transfer process in **1**, while protonated bisindolyl group of **2** makes the process energetically more favorable. Thus the position of ICT band of compound **2** (λ_{max} 510 nm) was found to be more redshifted compared to **1** (λ_{max} 487 nm). This was also supported by the HOMO-LUMO energy gap of **1** (2.5 eV) and **1**-H^+ (3.7 eV) (Fig. S2, ESI†).[13] Moreover, microscopic images (AFM) indicated that compound **2** also forms self-assembled random aggregates at pH 6.5 (Fig. S3, ESI†). Variable temperature and concentration-dependent emission studies further substantiate this fact (Fig. S4, ESI†). Both these factors together markedly affect the selectivity as well as sensitivity of thr probe compounds towards metal ions.

3.2. Metal ion sensing behavior in the aqueous medium: The metal ion sensing properties of each probe was explored both at pH 6.5 and 7.4 in 0.05 M HEPES buffer medium. Compound **1** showed interaction with Cu^{2+} and Hg^{2+} at pH 6.5 with a prominent color change from red to yellow (Fig. 1a and S5a, ESI†). However, at pH 7.4, a selective binding with Cu^{2+} was observed (Fig. 1b and S5b, ESI†). Most importantly, no such color change was witnessed when other metal ions, such as Ag^+, Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Al^{2+}, Cr^{3+}, and Fe^{3+} were added as controls to the aqueous solutions of either **1**. The changes in the color of **1** upon addition of Cu^{2+} and Hg^{2+} are shown in the Fig. 1. The UV-Vis titration of **1** with increasing Cu^{2+} at pH 7.4 led to decrease in absorbance at 497 nm with a concomitant increase at 433 nm (Fig. S6, ESI†). The presence of an isosbestic point at 453 nm indicates the existence of a one-to-one equilibrium between **1** and the Cu^{2+} ion. The plot of absorbance ratios at 435 and 505 nm *vs.* [Cu^{2+}] gave a straight line indicating a visible, ratiometric detection of Cu^{2+} at physiological pH. Job plot analysis under similar condition showed a 2:1 complexation between **1** and Cu^{2+} ion (Fig. S7, ESI†).[14] On the other hand, titration studies at pH 6.5 showed that there is a blue shift of ~80 nm with both the Cu^{2+} and Hg^{2+} ions (Fig. 1c & 1d). The Job plots indicated

the formation of 2:1 complexes with either ion in this condition (Fig. S8, ESI†). Thus, we realized that both the selectivity as well as sensitivity towards metal ions largely depends on the pH of the medium. At pH 7.4, the selectivity gets improved and exclusive interaction was observed with Cu^{2+}. However, the extent of metal ion-induced blue shifts of CT band ($\Delta\lambda$ ~ 80 nm at pH 6.5 and $\Delta\lambda$ ~ 65 nm at pH 7.4) were reduced when the pH of medium was changed from 6.5 to 7.4. Further, association constant of **1** was calculated (for the 2:1 complex) with each of Cu^{2+} and Hg^{2+} at both pH 6.5 and 7.4 in HEPES buffer medium (Fig. S9, ESI†).[15] The detection limit for Cu^{2+} was estimated to be ~0.06 ppm from UV-Vis spectral titration of the compound **1** (Table S1, ESI†). Thus with **1**, one can detect Cu^{2+} even ~21 times lower than the permitted limit of Cu^{2+} in water and ~0.15 ppm level of Hg^{2+}.[16]

On the contrary, compound **2** possessing the BPA and a BIM moiety showed an altered complexation behavior with the above ions. At pH 6.5, it showed similar interaction with both Hg^{2+} and Cu^{2+} ion (Fig. 2a & S10a, ESI†), but did not show any interaction with metal ions at pH 7.4 (Fig. S10b, ESI†). The complexation of **2** resulted in a decrease in the UV-Vis spectral intensity of the 497 nm band and the emergence of a new band at 403 and 438 nm with Cu^{2+} and Hg^{2+} ions respectively (Fig. S11-S12, ESI†). The titration studies showed ratiometric sensing for both Cu^{2+} and Hg^{2+}. Job plot analysis at this condition indicates that compound **2** formed complexes at 1:1 stoichiometry with both Cu^{2+} and Hg^{2+} ion (Fig. 2b & S13, ESI†). The association constants were calculated using Benesi-Hildebrand equation (Fig. S14, ESI†). The detection limit of **2** was estimated to be ~0.07 ppm and ~0.03 ppm respectively for Cu^{2+} and Hg^{2+} based on UV-vis titrations (Table S1, ESI†). However, both probes showed reversible binding with the metal ions when EDTA was used. Thus using EDTA, the sensors could be used multiple times for detection (Fig. 2c-d, S15, ESI†). A comparison study with some of the structurally-related optical probes has been made in terms of their interaction medium, selectivity, optical response and detection limits to evaluate the potential of the present system (Table S2, ESI†).[17]

The fluorescence responses of both the probes towards various metal ions were checked.

Compound **1** was faintly fluorescent at pH 6.5, but the addition of both Cu^{2+} and Hg^{2+} ions resulted in the 'turn on' emission (Fig. 3a & S16a, ESI†). Compound **2** showed fluorescence emission at 567 nm upon excitation at 465 nm. The fluorescence was however quenched by both Cu^{2+} and Hg^{2+} ions (Fig. 3b & S16b, ESI†). Most importantly, the other metal ions had negligible effect on the emission intensity of both the ligands (Fig. S17, ESI†).

3.3. Mechanistic insight into the metal ion binding profile: ^{1}H NMR spectral titrations were employed to understand the binding mode. ^{1}H NMR titration of **1** with Cu^{2+} resulted in a complete quenching of all the peaks. This may be due to a paramagnetic behavior of Cu^{2+} ion when complexes with **1**. The NMR titration of **1** with Hg^{2+} ion, however, resulted in a downfield shift of all the protons with maximum changes to the protons of the BPA moiety (Fig. 3c). It indicates that the metal ion are bound to the picolyl moiety. The ^{1}H NMR titration of **2** with both metal ions showed similar downfield shifts in ^{1}H-NMR (Fig. S18-S19, ESI†). The complexations of both **1** and **2** with metal ions were further verified by mass spectrometry. Each probe furnished mass spectral peaks due to the respective metal complexes of the stoichiometry consistent with Job plots (Fig. S20, ESI†). Based on the stoichiometry data derived from the Job plots, NMR titrations and mass spectra, it may be concluded that the **1** forms an octahedral complex with Cu^{2+}, whereas **2** forms a tetrahedral complex (See DFT optimized structures of **1** and **2** with Cu^{2+} and Hg^{2+} as well, Fig. 4a).[18]

3.4. Signaling unit-controlled alteration in sensing behavior: Thus both the probe showed preferential interactions with both Cu^{2+} and Hg^{2+} ions at pH 6.5 and selective detection of Cu^{2+} at physiological pH 7.4. The mechanism of interaction indicates that both the probes interact with metal ions through the bispicolyl unit. Inspite of having identical binding site, the compounds showed significant differences in their metal ion-chelating properties: i) **1** showed 1:2 stoichiometry with metal ions whereas, **2** showed 1:1 stoichiometry; ii) **1** showed an increase in emission intensity upon addition of metal ions, while quenching of emission intensity was observed with **2**. The changes in the medium of detection (either solvent system or the pH of medium) are known to influence the binding properties of the probe towards the metal ions.[19] Here we observed even the change in signaling unit affects the binding properties

of the same binding site.

As described earlier, compound **2** formed self-assembled nanostructure in aqueous medium owing to its highly ampiphatic nature. Due to aggregation, some of the binding sites of **2** will be in the buried state and will be inaccessible to the incoming metal ions. Thus for compound **1**, saturations in optical signal were achieved upon addition of ~15 μM of metal ions, while for compound **2**, the amount was as high as ~40 μM. Moreover, compound **1** exhibited ~80 nm blue shift in ICT band, while with **2**, the addition of metal ions induce a smaller shift of ~14 nm of the CT band. On the other hand, from pH variation studies, we assumbed that the tertiary nitrogen center (of BPA unit) of **1** is in protonated form at pH 6.5 and therefore will not participate in metal ion binding process. Thus, to provide proper coordination sites for metal ions, two molecules of **1** is required (it showed 2:1 stoichiometry with both the metal ions). To confirm this we have determined the stoichiometry of interaction between **1** and metal ion (Cu^{2+}) in acetonitrile medium where no protonation is possible. In CH_3CN medium, **1** showed 1:1 interaction with metal ion (Fig. 4b). In a similar way, we have determined the stoichiometry interaction of **2** with metal ion at pH 4.0, where the tertiary nitrogens of the BPA unit will mostly be in the protonated state. At this pH, compound **2** showed 2:1 interaction with metal ion (Fig. S21a, ESI†). Thus from the above shred of observations, it was clear that the electronics as well as orientation of the signaling meoity markedly influence the protonation equilibrium of the bispicolyl moeity, which inturns affect the sensitivity of the compounds towards metal ions as well as the stoichiometry of probe-metal ion complexes (Fig. 5).

The change in turn-on and turn-off behaviour of fluorescence with the change in stoichiometry is known.[20] Here the results indicated the similar phenomenon; when the probes form 2:1 complex preferably with the metal ions, they show turn on emission while 1:1 interaction results in turn off emission. Further, we checked their emission response towards the metal ions with varying pH. It has been known that the emission property of the sensors with bispicolyl binding site changes with change in pH.[21] Interestingly, we found that both the compounds showed a similar trend with a change in pH (Fig. 4c & S21b, ESI†). The addition of metal ions to both the molecules showed an increase in emission intensity at the pH range where the nitrogens are

protonated. However, at higher pH, where both the nitrogens are deprotonated showed a decrease in emission intensity upon addition of metal ions (Fig. S22, ESI†).

3.5. Sensing of metal ions in presence of serum albumin protein: Mammalian serum albumin has a specific Cu^{2+} ion binding site and is proposed to act as a Cu^{2+} transport protein in blood plasma.[22] Thus the ability of probes to sense protein-bound Cu^{2+} can be extremely important to probe the transportation pathways inside the cells through different membrane-bound carrier proteins or mechanistic investigations. On the other hand, binding of metal ions like Hg^{2+}, Ni^{2+} and Co^{2+} to HSA can occur during acute intoxication. Thus detection of such metal ions in the bound state with HSA has high clinical importance. To detect the presence of endogenous Cu^{2+} ion in the human serum albumin (HSA), the sensing ability of **1** and **2** were checked in HSA. Even in concentrated (0.1 mg/ml) BSA, **1** showed a ratiometric detection of Cu^{2+} at biological pH of 7.4 (Fig. 6a). Both the sensors showed ratiometric sensing of either Cu^{2+} or Hg^{2+} in HSA at pH 6.5 (Fig. S23-S24, ESI†).

3.6. Sensing of metal ions in natural water samples: Though metal ions play important roles in maintaining a wide range of physiological processes, their excess intake may cause severe health problems, even can lead to death. Considering this, here we have collected water from institute supply system and determined presence of toxic metal ions, such as Cu^{2+} and Hg^{2+}. In both the cases, the water samples spiked with different amounts of toxic metal ions (0-2.5 ppm) were subjected to spectral analysis without any purification (Fig. S25, ESI†). All the analytical parameters, such as recovery values, standard deviations were calculated based on the changes in absorbance values at 490 nm (Fig. S25, ESI†).[23] A standard equation of $Y = 0.980-0.0474\ x$ was used for the quantification of Cu^{2+} and $Y = 1.0417-0.0344\ x$ for estimation of Hg^{2+} in the spiked samples (x = conc. of analyte in μM). In most of the cases, the recovery values varied from 98% to 105% with relative standard deviations (RSDs) in the range of 2.3 – 8% (Fig. 6c, Table S3, ESI†). Small standard deviation values indicate a high accuracy of the present method.

3.7. Sensing of metal ions using dye-coated paper strips: Considering the severity of metal toxicity in drinking water and the much needed tools for on site detection of ions, we have

developed an alternative strategy for rapid, on-location sensing of Cu^{2+} and Hg^{2+} using low-cost paper strips.[24] A gradual change in color from red to yellow was observed when the strips were dipped into either Cu^{2+} or Hg^{2+} solutions (0–4 μM) (Fig. 6b). Thus, using the current method, toxic metal ions as low as ~1 μM can be identified from the visible color change. The selectivity experiment was performed by applying other metal ion solutions as controls onto these paper strips. The results of such study clearly indicate that the present system is fairly selective towards Cu^{2+} and Hg^{2+} and will not face significant interference from other competing metal ions. Additionally, the color strips can be reused by subsequently washing them with dilued EDTA solution (at least for 5 cycles) (Fig. S26a, ESI†). Further, to differentiate between Cu^{2+} and Hg^{2+}, we have employed the aqueous solution of iodide as a 'selective masking agent'. When the metal ion-treated paper strips were exposed to the iodide solution, the original red color could be recovered specifically for Hg^{2+} ion (Fig. S26b, ESI†). Since in this case, no sophisticated instrument is required (naked-eye sensing), people with limited knowledge in science will be able to use these strips without much difficulty. Further, the reusability will significantly lower the cost of these strips.

4. CONCLUSION

In summary, we have synthesized bispicolyl based small molecular probes comprising with two different chromophore (signalling) units. Both the compounds allow naked eye, ratiometric sensing of Cu^{2+} and Hg^{2+} in buffered aqueous medium and in various biological conditions. The attributes of pH of the medium of detection on the metal ion sensing behavior is demonstrated, where a fairly selective response was observed for Cu^{2+} at physiological pH. Overall, the present system can detect Cu^{2+} as low as 0.06 ppm from visible color change, while the detection limit was even lower for Hg^{2+}, only 0.03 ppm in aqueous medium. The study also revealed that the modes of metal ion binding largely depend on the nature of the signaling unit. Though for both the probes, it is evident that metal ion coordination occurs only through bispicolyl unit, with compound **1** we observe 2:1 binding interaction, while for **2**, it is 1:1 complexation. Moreover, probe **1** shows 'turn-on' detection of target metal ions, whereas with **2**, 'turn-off' response was witnessed. The signaling unit controls the protonation equilibrium

of the bispicolyl unit to a significant extent, which likely influences the self-assembly of the probe molecules in buffered medium as well as their metal ion binding property. Thus the present study provides an insight on how the same binding moiety behaves differently just by changing the signaling unit. Additionally, the probes were utilized for estimation of toxic metal ions in natural water samples. Low-cost dye-coated paper strips were designed for on-location detection purpose. Therefore new probes of such kind will not only offer significant practical utility in terms of naked-eye sensing but also added a new understanding on the mechanism of interaction.

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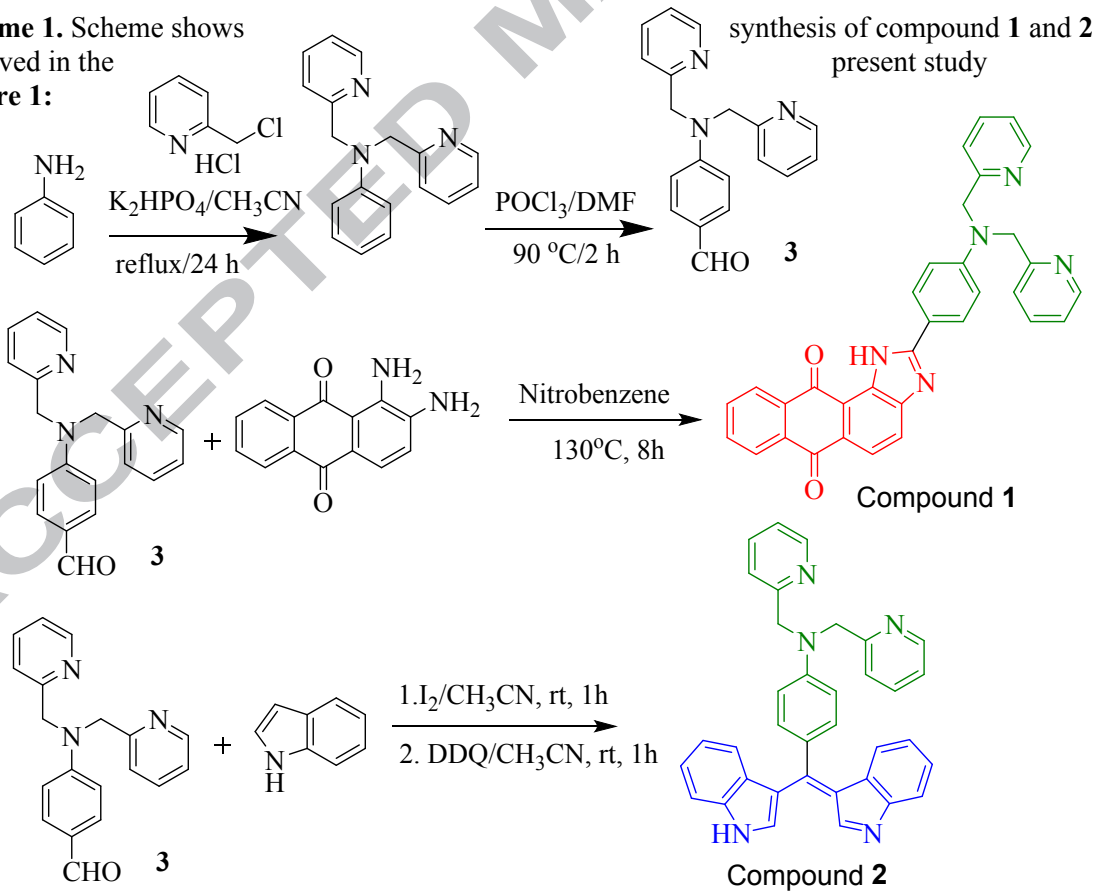
Scheme 1:

Scheme 1. Scheme shows synthesis of compound **1** and **2** involved in the present study

Figure 1:



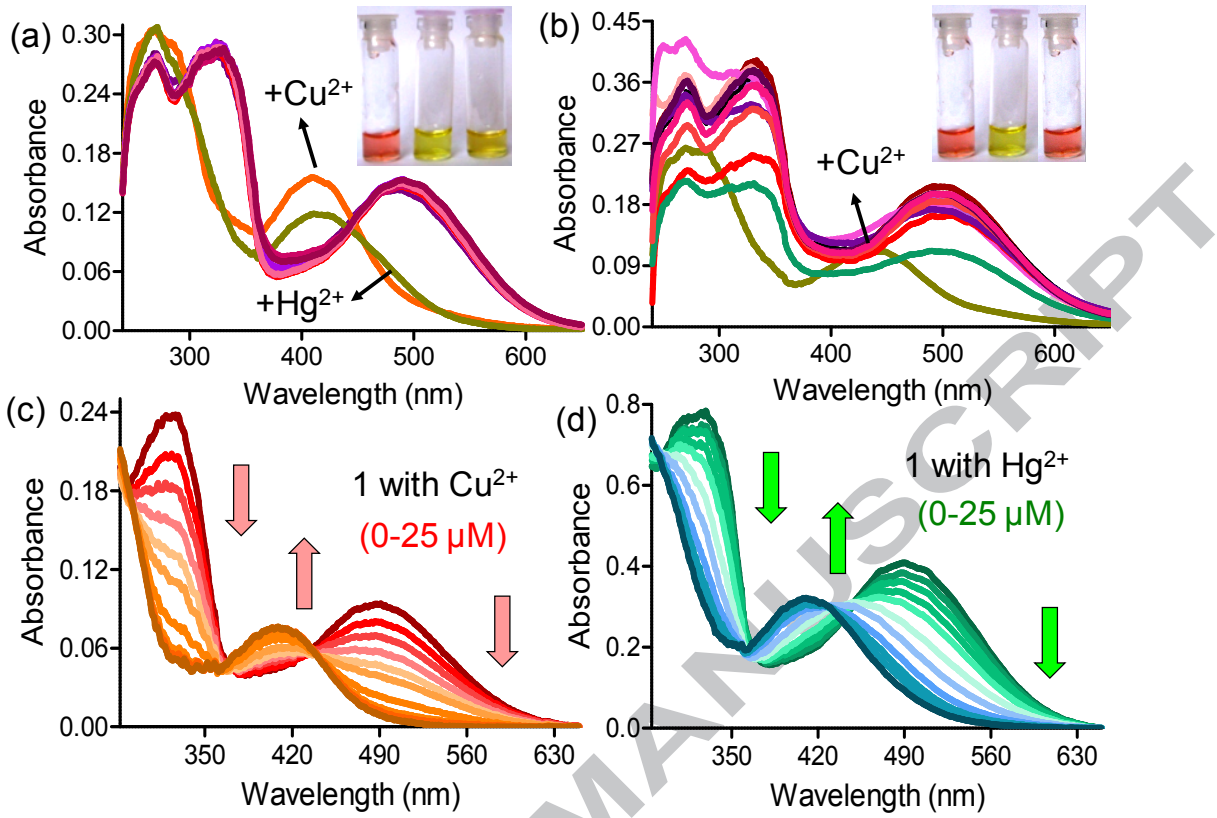


Figure 1. (a) UV-visible spectra of **1** (10 µM) with different metal ions (25 µM) at pH 6.5 in HEPES buffered medium. (b) UV-visible spectra of **1** (10 µM) with different metal ions (25 µM) at pH 7.4 in HEPES buffered medium. (c) UV-visible titration of **1** (10 µM) with Cu^{2+} (0- 25 µM) at pH 6.5 in HEPES buffered medium. (d) UV-visible titration of **1** (10 µM) with Hg^{2+} (0- 25 µM) at pH 6.5 in HEPES buffered medium.

Figure 2.

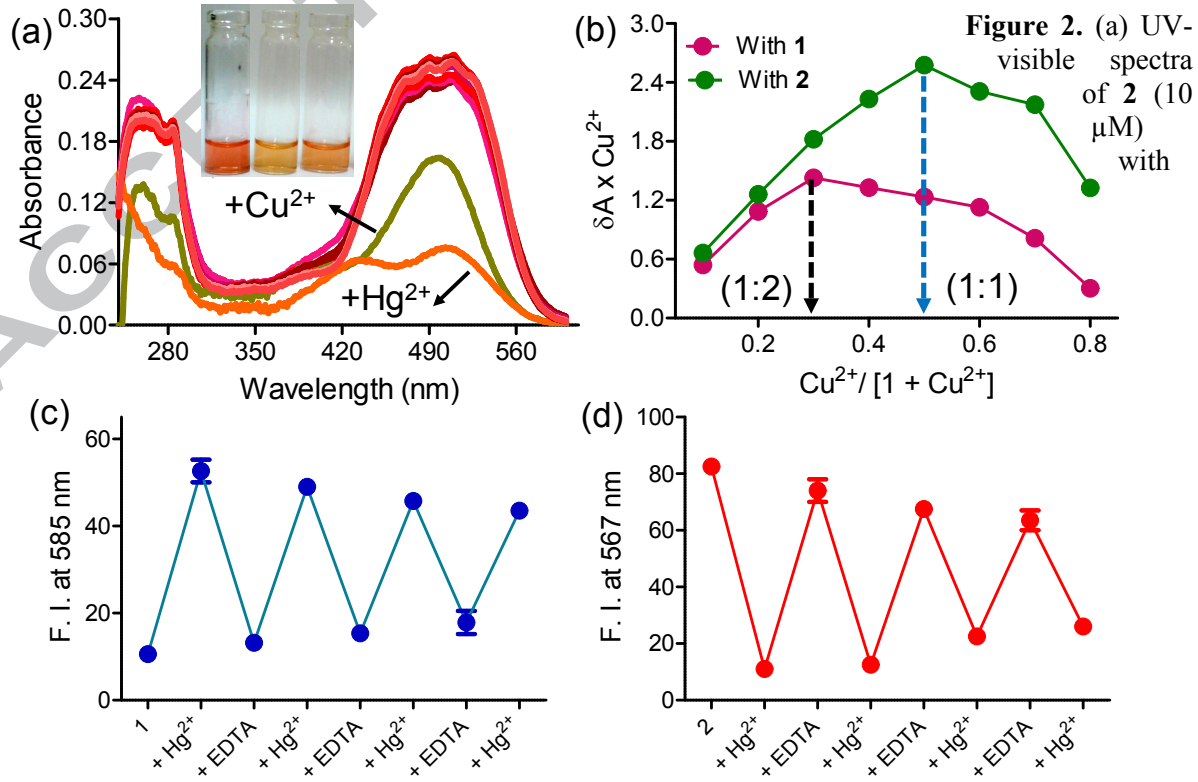
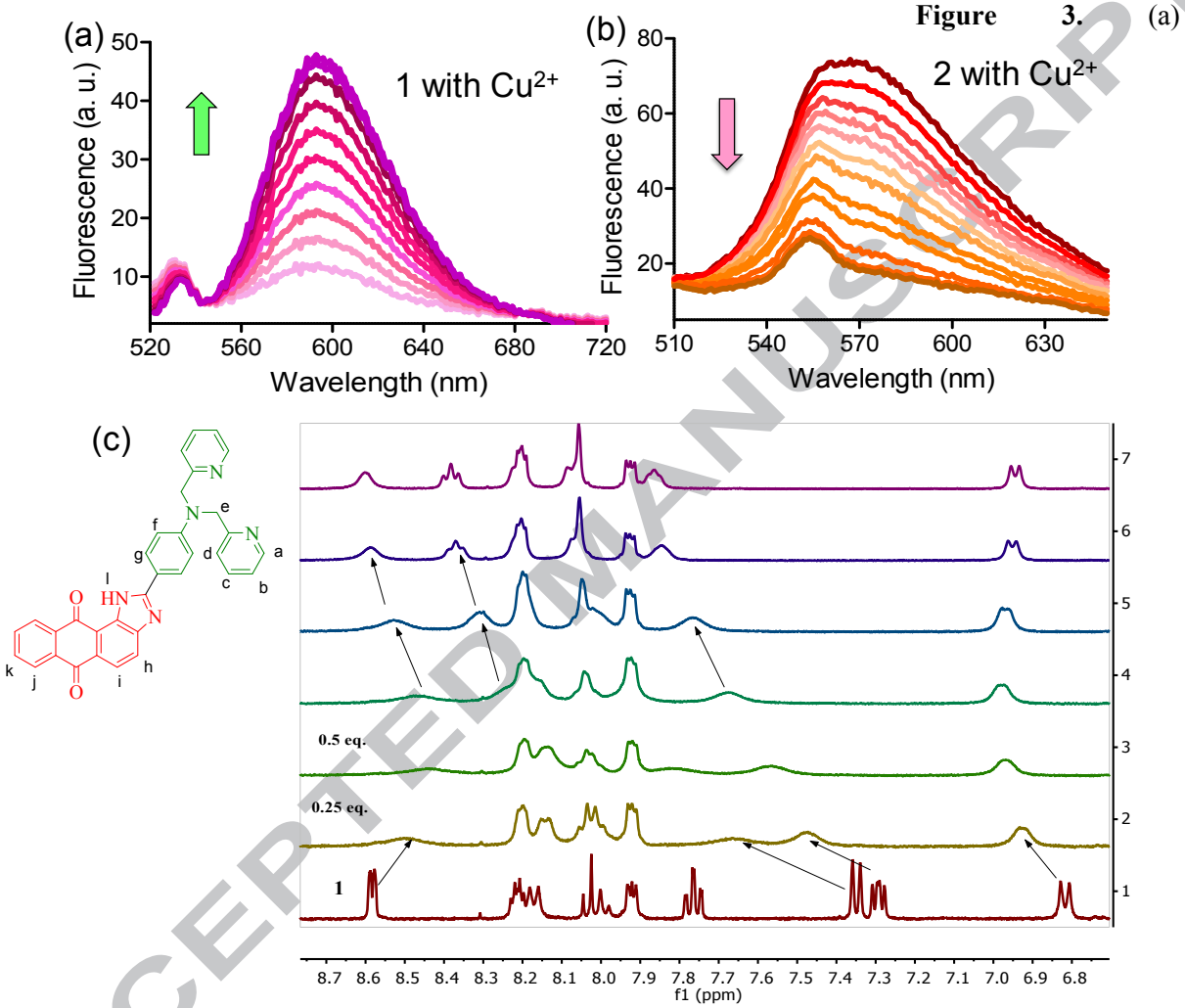


Figure 2. (a) UV-visible spectra of **2** (10 µM) with

different metal ions (40 μM) at pH 6.5 in HEPES buffered medium. (b) Job's plot analysis of **1** and **2** with Cu^{2+} at pH 6.5 in HEPES buffered medium. (c) EDTA-mediated (25 μM) reversibility checking during interaction of **1** (10 μM) with Hg^{2+} (25 μM) at pH 6.5 in HEPES buffered medium. (d) EDTA-mediated (40 μM) reversibility checking during interaction of **2** (10 μM) with Hg^{2+} (40 μM) at pH 6.5 in HEPES buffered medium.

Figure 3.



Fluorescence titration of **1** (10 μM, λ_{ex} = 480 nm) with Cu^{2+} (0-25 μM) at pH 6.5 in HEPES buffered medium. (b) Fluorescence titration of **2** (10 μM, λ_{ex} = 465 nm) with Cu^{2+} (0-40 μM) at pH 6.5 in HEPES buffered medium. (c) ^{1}H-NMR titration of **1** (5 mM) with Hg^{2+} (0-2 equiv.) in DMSO-d_6/D_2O (5:1) mixture medium (the relevant protons were assigned in the structure for convenience).

Figure 4.

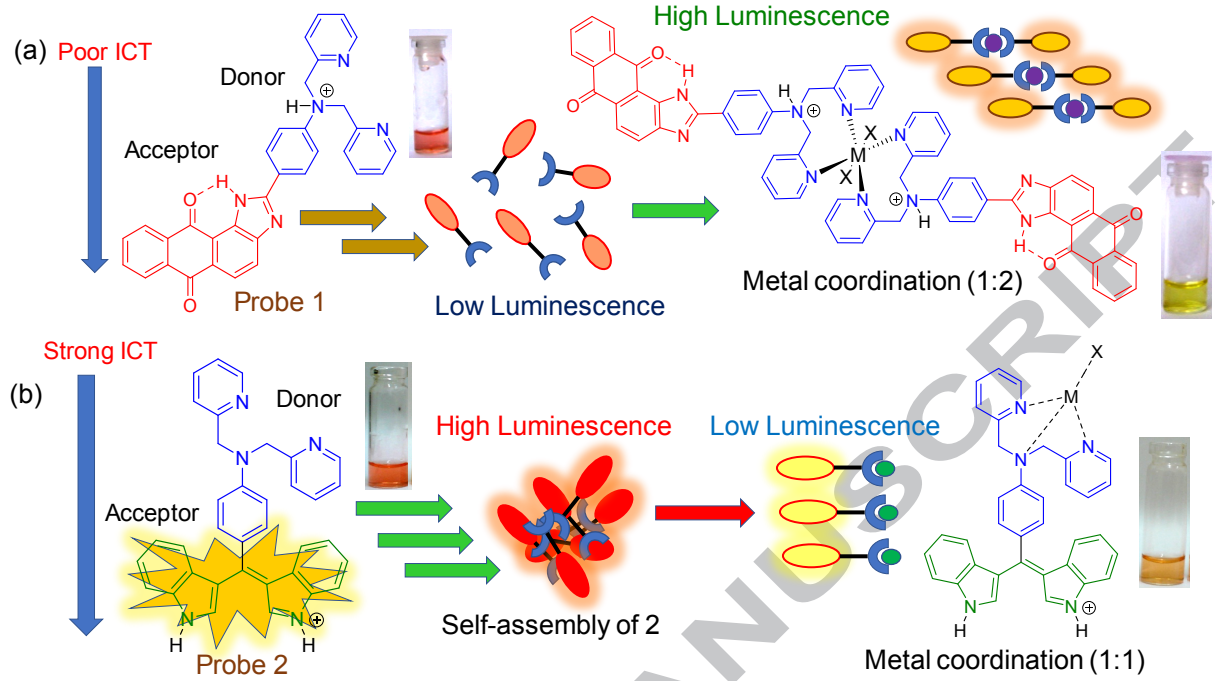


Figure 4. (a) Scheme shows formation of highly fluorescent 1:2 metal ion-probe complex of **1** (10 μM) with Hg^{2+}/Cu^{2+} at pH 6.5 in water. (b) Scheme shows formation of nonfluorescent 1:1 metal ion-probe complex of **1** (10 μM) with Hg^{2+}/Cu^{2+} at pH 6.5 in water.

Figure 5.

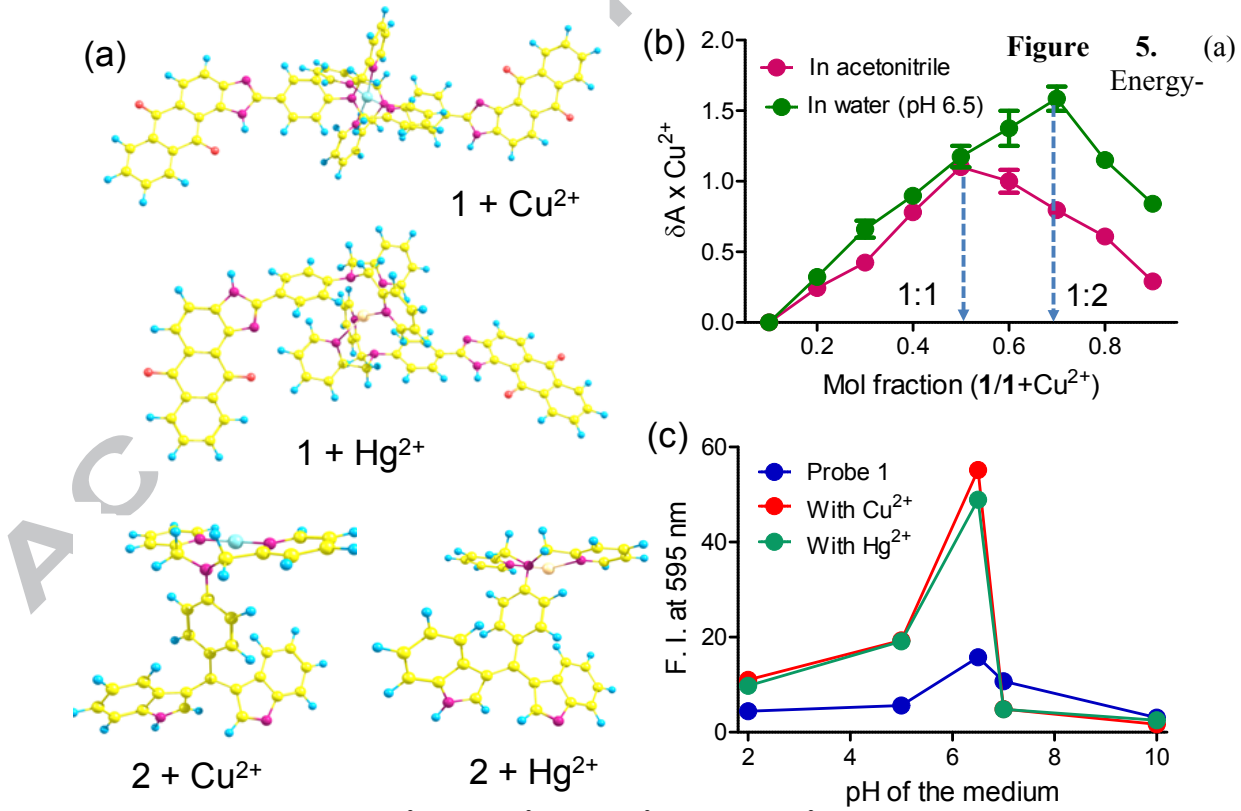
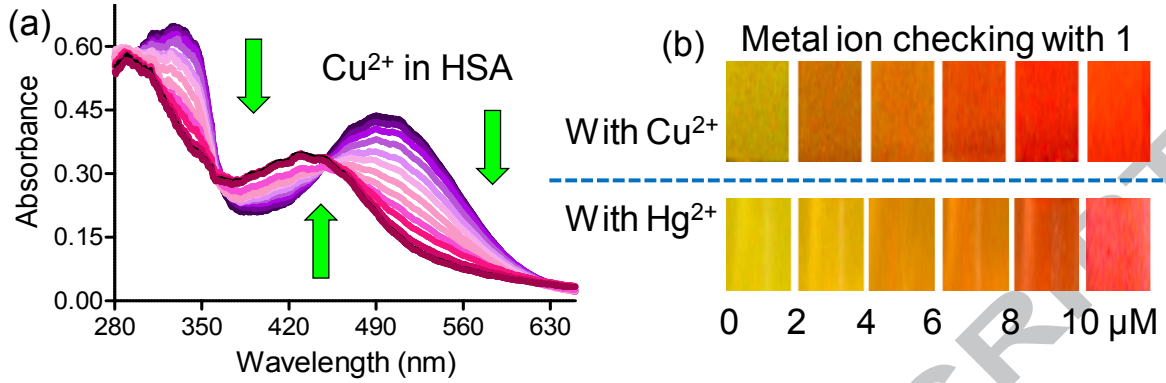


Figure 5. (a) Energy-minimized structures of **1** + Hg^{2+}, **1** + Cu^{2+}, **2** + Cu^{2+} and **2** + Hg^{2+} using B3LYP/LANL2DZ level of theory. (b) Job's plot analysis of **1** with Cu^{2+} at pH 6.5 in HEPES buffer and acetonitrile medium. (c) Change in fluorescence intensity of **1** at 472 nm upon addition of Hg^{2+} and Cu^{2+} in different buffered medium.

Figure 6.



Hg^{2+} added (µM)	Hg^{2+} Calcd (µM) Y = 0.980-0.0474 x			Hg^{2+} found (µM)	Recovery (%)	RSD (%)
0	0	0	0	0	0	0
1.54	1.45	1.32	1.65	1.47	99.9	8.0
3.08	2.92	3.20	2.87	3.00	99.9	6.1
4.62	4.78	4.54	4.92	4.75	100.1	4.2
6.16	5.83	5.92	6.20	5.98	99.6	3.2
7.70	7.38	7.52	7.85	7.58	99.0	3.1

Figure 6. (a) UV-visible titration of **1** (10 µM) with Cu^{2+} (0-25 µM) at pH 7.4 in presence of 0.1 mg/mL HSA. (b) Job's plot analysis of **1** with Cu^{2+} at pH 6.5 in HEPES buffer and acetonitrile medium. (c) Estimation of Hg^{2+} using compound **1** (10 µM) in natural water sample.

Colorimetric Indicators for Specific Recognition of Cu^{2+} and Hg^{2+} in Physiological Media:

Effect of Variations of Signaling Unit on Optical Response

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Highlights:

- Naked-eye sensing of Cu^{2+} and Hg^{2+} in physiological media
- Role of signaling unit in determining the stoichiometry of probe-metal ion complexes
- Estimation of toxic metal ions in natural water samples and also in presence of serum albumin
- Low-cost reusable paper strips for on-location detection purpose