Orginal Research Article

Indole derived "turn-on" fluorometric probe for dual detection of Hg²⁺ and Cu²⁺ ions at nanomolar level

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ARTICLE INFORMATION

ABSTRACT

The newly synthesized 3-(phenyl(phenylthio)methyl)-1*H*-indole ligand demonstrates chemosensor activity towards environmental and clinically important metal ions viz. Hg²⁺ and Cu²⁺, *via* fluorescence intensity enhancement. The rigid complex ceases non-radiative channels with respect to the free ligand. Incorporation of water and significant fluorescence enhancement in presence of interfering metal ions make the method superior over others and detect trace amount of metal ions into the aqueous based medium. Also, the detection of Hg²⁺ and Cu²⁺ ions are considered as subjects of an increasing societal demand as well as responsible for neurodegenerative disorders.

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Graphical Abstract
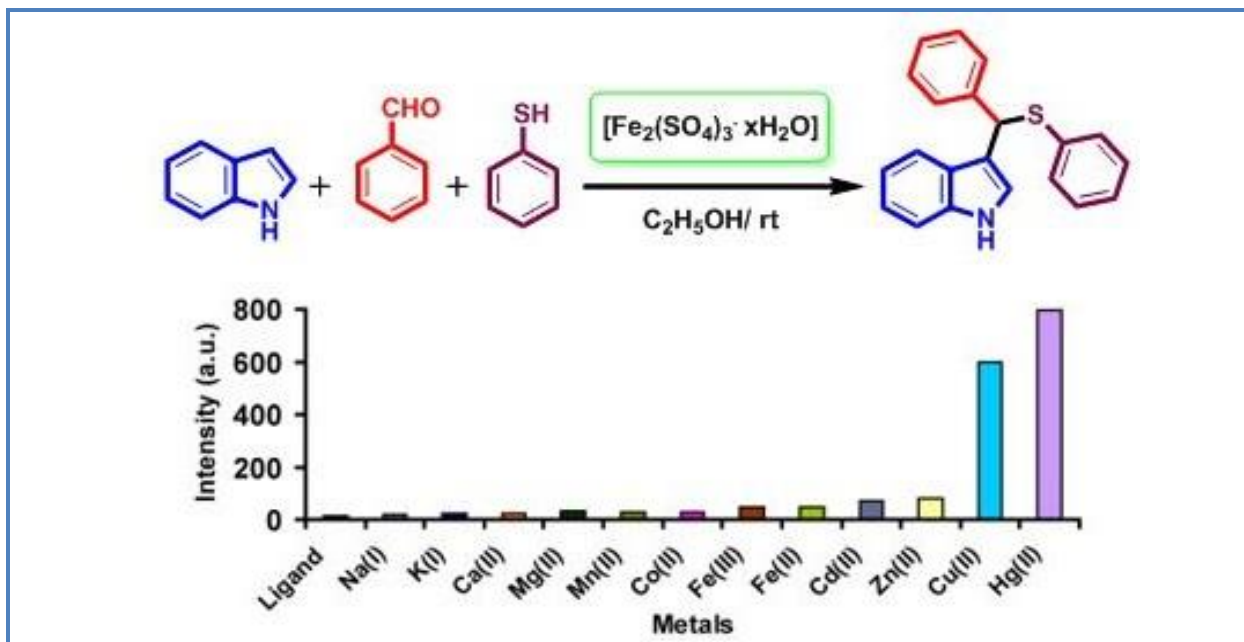
Introduction

Heavy and transition-metal ions' recognition is of great concern for environmental or biological applications. Among them, Hg^{2+} and Cu^{2+} sensing have been addressed by several research groups [1–6]. Mercury ions are highly toxic and in contaminated natural water it is considered as a major source of human exposure [7, 8]. Once the mercury finds its way inside the human body as organic or inorganic form, it results in kidney dysfunction, damaging liver, brain and central nervous system, enormous cognitive and motion disorders and minamata disease [9]. Also copper has been considered to have both beneficial [10, 11] as well as toxic effects [12, 13]. Copper is the second and the most abundant trace element in human physiology and other biological systems [14]. However, in excess, it is a significant metal pollutant and in deficiency causes neurological disorders (Menkes disease, wilson disease and alzheimer's disease) and mis-metalate other metal binding sites [15]. Moreover, free copper which has potential ability to generate reactive oxygen species by catalysing the fenton type reactions can break down the DNA [16]. The prescribed value of maximum contaminant level (MCL) for Hg^{2+} and Cu^{2+} by U.S. environmental protection agency's (EPA) in drinking water are 10 nm and 1.3 mg per litre. Therefore, rapid and facile Hg^{2+} and Cu^{2+} detection with suitable and sensitive fluorescent probes is the subject of an increasing societal demand [17–22]. Also, most of the sensor probes for mercury and copper ions that have been developed show a turn-off response [23, 24]. However, a sensitive and selective synthetic receptor with a turn on response remains a challenging task. Generally, to design such types of probes, internal charge

transfer (ICT) is disrupted from donor by blocking the photo induced electron transfer (PET) within the molecule. As a result of chelation enhanced fluorescence (CHEF) a fluorescent species is obtained [25]. Since the indole based chromophores are reported to be sensitive towards changes in the local perturbation and environment [26], the changes in the form of signals can be well utilized to establish a chemosensor for a particular analyte.

In continuation of our research for the development of chemosensors [27], we have successfully synthesized 3-(phenyl(phenylthio)methyl)-1*H*-indole [28], as shown in Scheme 1, which was exploited as a dual chemosensor for Hg^{2+} and Cu^{2+} metal ions in CH_3CN/H_2O (9:1) media.
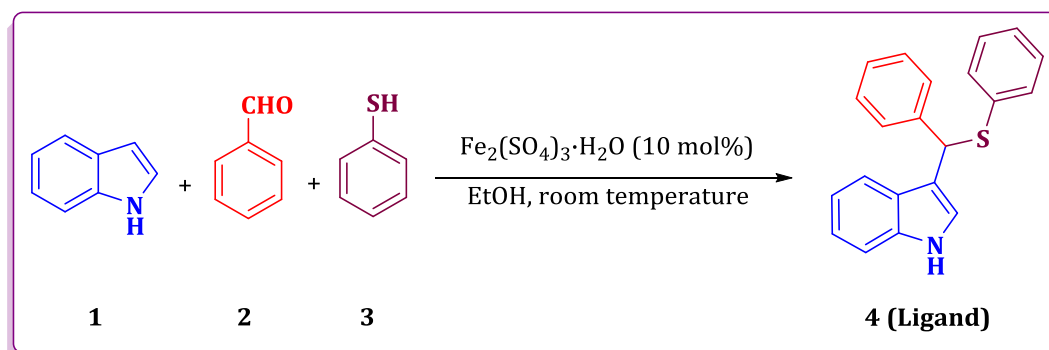
Scheme 1. Synthesis of 3-(phenyl(phenylthio)methyl)-1*H*-indole

Experimental

Matreials and methods

All the reagents and solvents were purchased from Aldrich Chemicals (India), Merck (India) or Ranbaxy (India) and were used as received. Milli-Q water was used in all the experiments. The solutions of metal ions from NaCl, KCl, $CaCl_2.2H_2O$, $MgCl_2$, $Zn(ClO_4)_2.6H_2O$, $Mn(ClO_4)_2.H_2O$, $Co(ClO_4)_2.6H_2O$, $Fe(ClO_4)_3.H_2O$, $Fe(ClO_4)_2.H_2O$, $Cd(ClO_4)_2.H_2O$, $CuCl_2.2H_2O$, $HgCl_2$ were prepared in deionized water. IR spectra were recorded on Perkin-Elmer 281 IR spectrophotometer using KBr pellets method in which a homogeneous mixture of sample (Pinch off) and some KBr (Oven dried) were mixed and ground up to a fine powder using the mortar and pistil. Finally, pellet was made by sandwich onto the pistil in the hydraulic press. ^{1}H and ^{13}C NMR spectra were recorded on varian 400 spectrometer TMS as internal reference; chemical shifts (δ scale) are reported in parts per million (ppm). ^{1}H NMR spectra are reported in the order: multiplicity, coupling constant (*J* value) in hertz (Hz) and no of protons; signals were characterized as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet). Elemental analyses were carried out using Perkin-Elmer 2400 Series II CHNS/O analyzer at the department of chemistry, indian institute of technology guwahati. Column chromatographic separations were performed using merck silica gel (60-120 mesh). The absorbance

and fluorescence were measured using UV-Vis spectrophotometer (Perkin elmer lambda 25 UV-visible spectrophotometer) and flourimeter (VARIAN Cary eclipse fluorescence spectrophotometer) in aqueous solution respectively.

Synthesis of ligand

The catalyst, hydrated ferric sulfate (0.042 g, 0.1 mmol) was added to a stirred mixture of indole (0.117g, 1 mmol) and benzaldehyde (1 mmol) in 3 mL of ethanol at room temperature. Subsequently, benzenethiol (1.2 mmol) was added into it and stirring was further continued. After completion of the reaction during almost 12 hours as monitored by TLC, ethanol was removed in rotary evaporator and the crude residue was extracted with dichloromethane (2×15 mL). The organic layer was washed with water followed by brine solution. The organic extract was dried over anhydrous sodium sulfate and it was concentrated in a rotary evaporator. Finally, the crude residue was passed through a silica-gel column to obtain the desired pure product in 24% yield [28].

General UV-vis and fluorescence spectra measurements

CH_3CN/H_2O (9:1) solution of ligand (33 μm solution) was placed in the quartz cell and the UV-vis as well as fluorescence spectra were recorded for increasing portions of metal salts up to 50 μm. The experiments were performed at room temperature and changes were monitored and recorded carefully.

Spectral data

*3-(Phenyl(phenylthio)methyl)-1H-indole (**4**)*

Dark red semi-solid, R_f = 0.40, IR (KBr) (ν_{max} / cm^{-1}): 3414, 3049, 2917, 1613, 1454, 1333, 1176, 1090, 1023, 806, 740, and 697. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.68 (s, 1H), 6.84 (s, 1H), 6.94-7.08 (m, 6H), 7.10 (s, 1H), 7.15 (t, J = 8.4 Hz, 4H), 7.34 (d, J = 7.6 Hz, 2H), 7.51 (d, J = 8.0 Hz, 1H), 7.68 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$): δ 49.9, 111.5, 116.3, 119.8 (2C), 122.5, 124.1, 126.4, 126.5, 127.3, 128.5 (2C), 128.6 (2C), 128.9 (2C), 130.5 (2C), 136.7, 136.8, 141.4. Anal Calcd for $C_{21}H_{17}NS$: C, 79.96, H, 5.43; N, 4.44; Found: C, 79.82; H, 5.37; N, 4.35.

Results and discussion

In early reports, indole based chemosensors [29, 30] have been utilized in the detection of anions utilizing indole N−H bond [31]. However, introduction of electron donating groups into an indole based skeleton may decrease the affinity towards anions [32] and indole based skeleton may be utilized for the selective detection of positively charged species such as metal ions.

Chemosensor activity of designed ligand (33 μm) towards various metals ions was checked in CH$_3$CN/H$_2$O (9:1) with an excitation wavelength at 280 nm. According to the Figure 1**a** fluorescence spectra of ligand with an excitation wavelength at 280 nm was almost unaffected and no fluorescence intensity enhancement was observed in the presence of various alkali as well as transition metal ions such as Na$^+$, K^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Fe^{3+}, Fe^{2+}, Cd^{2+}, Zn^{2+}, even at 50 μm concentration. However, fluorescence intensity enhancement of ligand with an emission band centered at 338 nm, as shown in Figure 1**b** and 1**c**, was observed in presence of increasing concentration of Hg^{2+} and Cu^{2+} ions and these fluorescence intensities enhancement were levelled off at 16 μm and 37 μm concentration of Hg^{2+} and Cu^{2+} ions respectively.
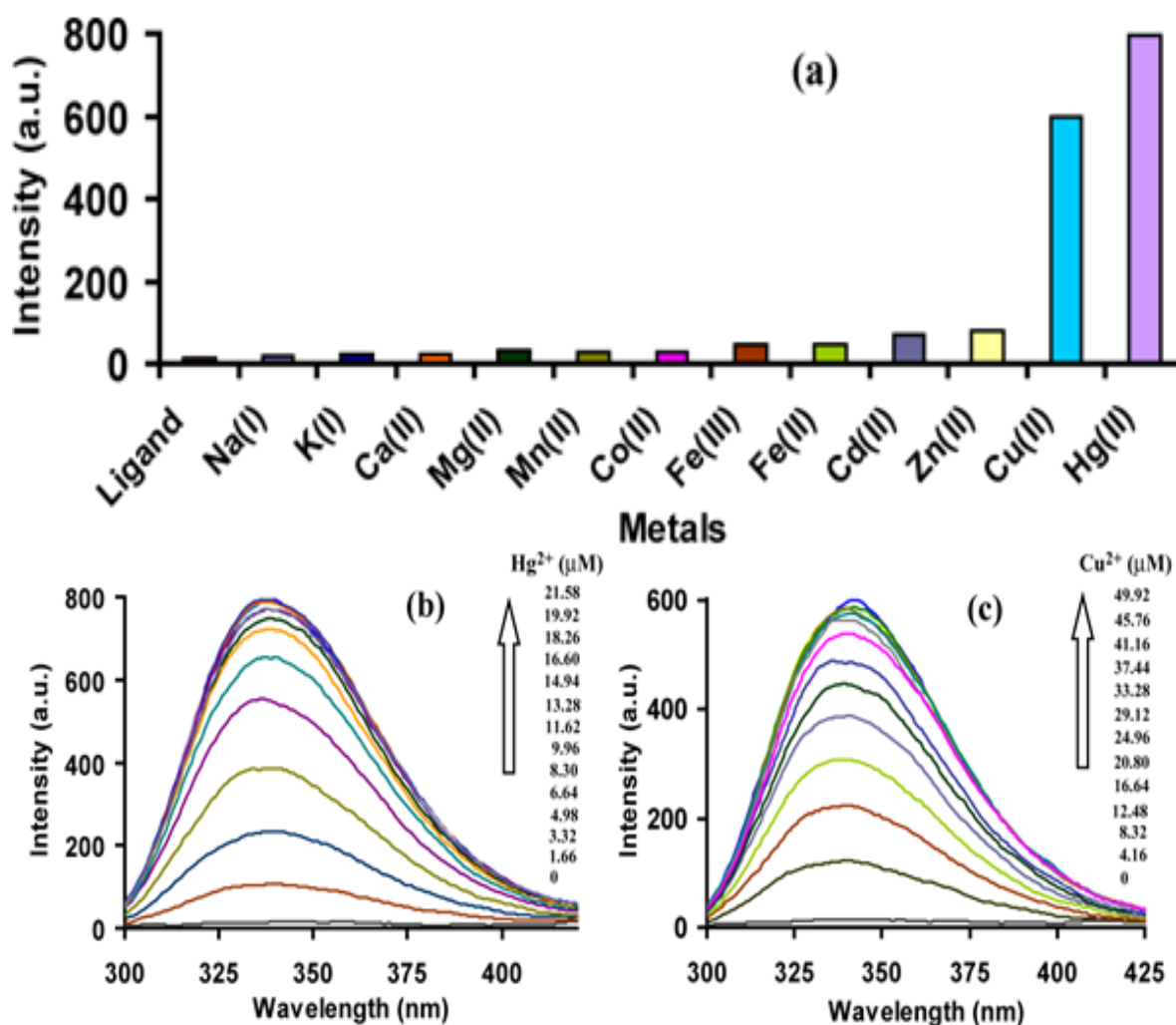

Figure 1. a) Changes in the fluorescence intensity of ligand (33 μm) in presence of various metals upto 50 μm concentration, b) Fluorescence intensity enhancement of ligand (33 μm) in presence of increasing concentration of Hg^{2+} ion, c) Fluorescence intensity enhancement of ligand in presence of increasing concentration of Cu^{2+} ion in CH$_3$CN/H$_2$O (9:1) solution

From the Figures 1**b** and 1**c** we observed that 16 μm concentration of Hg^{2+} was able to enhance the fluorescence of ligand by 57 fold whereas 37 μm of Cu^{2+} was able to enhance the original fluorescence intensity of ligand by 43 fold. Therefore, Hg^{2+} was found to be more efficient than Cu^{2+} towards fluorescence intensity enhancement of ligand. Furthermore, to examine whether designed ligand was able to detect Hg^{2+} and Cu^{2+} in a competitive environment of other metal ions, we prepared two separate solutions of metal ions, one with Hg^{2+} and other with Cu^{2+} ion. In our experiment, when these two solutions were added separately into the solution of ligand (33 μm), we observed the fluorescence intensity enhancement similar to the earlier observation. Therefore, it was concluded that the designed ligand was able to detect Hg^{2+} and Cu^{2+} ions even in a competitive environment of other metal ions. The better response of mercury towards fluorescence enhancement may be due to the high affinity of Hg^{2+} ion for soft sulfur donor. Moreover, fluorescence enhancement may be attributed to ceasing non-radiative channels through complex formation as the complex is rigid with respect to the free ligand. Therefore, flexible modes may be responsible for non-radiative processes.

Further binding ratios between ligand and metals were confirmed by Job's plot as shown in Figure 2**a** and 2**c**. whereas Stoichiometry obtained between ligand and mercury was 2:1 stoichiometry between ligand and copper was found 1:1. Therefore mercury may be quite efficient towards ceasing the non-radiative channels through complex formation than copper. Besides, binding constants values 0.07 and 0.05 M^{-1}, for Hg^{2+} and Cu^{2+} obtained by Benesi-Hildebrand curve as shown in Figure 2**b** and 2**d** were found in good agreement as stated above in Job's plot. It is worth mentioning that mercury is quite more efficient than copper towards complex formation with ligand.

In addition to PL titration, UV/Vis titration of ligand in the presence of Hg^{2+} and Cu^{2+} ions were also performed in CH_3CN/H_2O (9:1) solvent as shown in Figure 3**a** and 3**b**. For ligand three absorption bands centered at 220 nm, 278 nm and 288 nm were observed. In addition to increasing concentration of Hg^{2+} ion (0 to 50 μm) into the ligand (33 μm), absorption intensities at 220 nm, 278 nm and 288 nm gradually increased without any shift in absorption position. Whereas in the presence of increasing concentration of Cu^{2+} ion (0 to 50 μm), ligand absorption intensity at 278 and 288 nm were gradually decreased and the absorption intensity at 220 nm increased.

Further to evaluate the limit of detection (LOD) of metals by our designed ligand, a linear relationship between maximum emission intensity at 338 nm and the concentration of metal ions was obtained as shown in Figure 4**a** and 4**b**. We observed that fluorescence intensity centered at 338 nm was increased linearly by increasing concentrations of Hg^{2+} from 0 to 8.3×10^{-6} M as shown in Figure 4**a** and Cu^{2+} from 0 to 20.8×10^{-6} M as shown in Figure 4**b**.
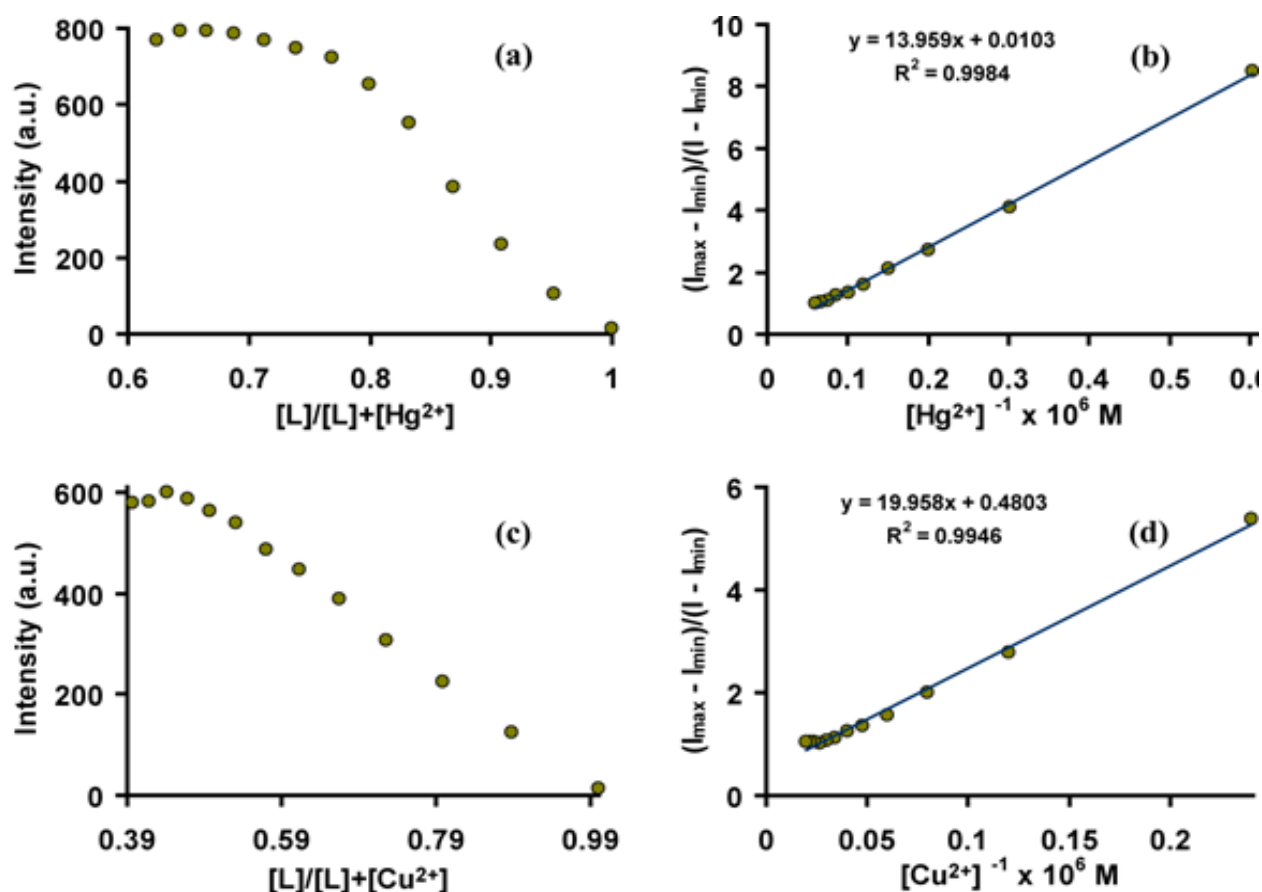
Figure 2. a) Job's plot depicting the binding ratio between ligand and Hg^{2+} ion, b) Benesi-Hildebrand curve for the evaluation of binding constant of ligand with Hg^{2+} ion, c) Job's plot depicting the binding ratio between ligand and Cu^{2+} ion, d) Benesi-Hildebrand curve for the evaluation of binding constant of ligand with Cu^{2+} ion
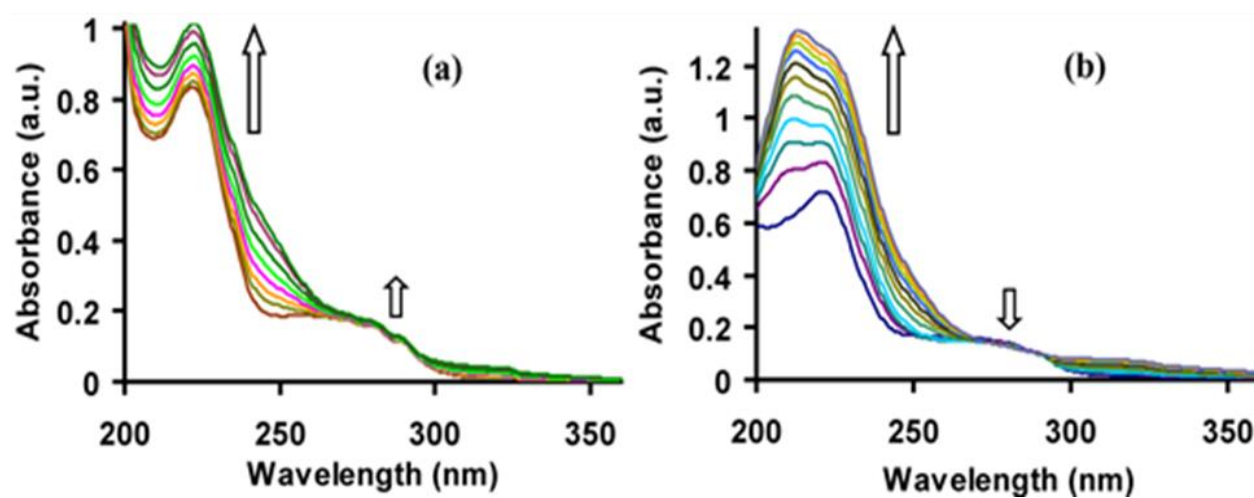
Figure 3. Changes in absorption spectra of ligand (33 μm) on addition of a) Hg^{2+} (0-50 μm), and b) Cu^{2+} (0-50 μm) in CH$_3$CN/H$_2$O (9:1) solution
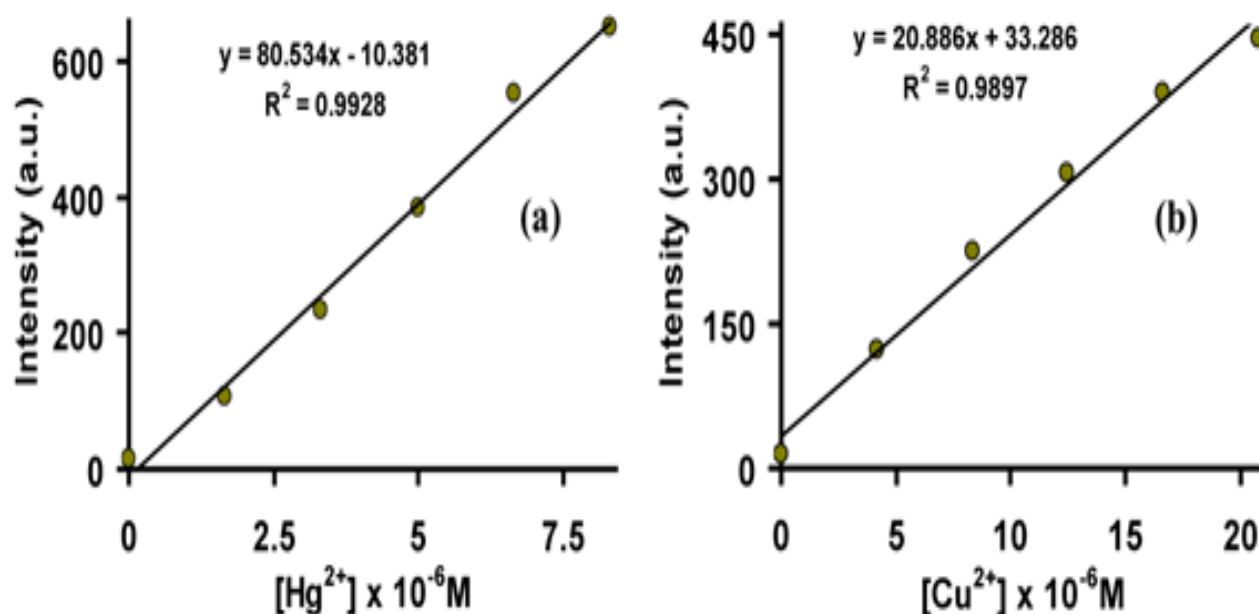
Figure 4. A linear relationship between maximum emission intensity at 338 nm and the concentrations of a) Hg^{2+} ion (0 to 8.3×10^{-6} M), and b) Cu^{2+} ion (20.8×10^{-6} M)

LOD was calculated using the equation $3\sigma/K$, where σ is the standard deviation and K is the slope of the plot. The calculated limit of detection for Hg^{2+} and Cu^{2+} were found to be 30 nm and 108 nm. LOD value obtained for Hg^{2+} is close to the prescribed MCL value, given by U.S. environmental protection agency's (EPA) in drinking water. The LOD of Cu^{2+} is much below the MCL values prescribed by EPA, thus demonstrating the efficiency of our designed ligand towards the chemosensor activity.

Conclusions

In summary, an indole derivative has been employed as "turn-on" fluorometric probe for dual detection of Hg^{2+} and Cu^{2+} ions that has environmental and clinical importance in our daily life with high detection limits of 30 nm (Hg^{2+}) and 108 nm (Cu^{2+}), respectively. From the Job's plot, stoichiometry was attained as 2:1 for ligand:Hg^{2+} and 1:1 for ligand:Cu^{2+}. Using Benesi-Hildebrand equation, the binding constants values were also calculated for Hg^{2+} (0.07 M^{-1}) and Cu^{2+} (0.05 M^{-1}) and found to be in good agreement which clarified the fact that mercury is more efficient than copper towards complex formation with ligand.

Acknowledgements

Disclosure statement

No potential conflict of interest was reported by the authors.

Supporting information

Additional supporting information related to this article can be found, in the online version, at http://www.ajgreenchem.com/article_57071.html.

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