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Azobenzene chemosensor based on nitrogen chelator for the detection of Cu (II) ion in aqueous medium

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ABSTRACT

A bis-pyridine unit containing azobenzene chemosensor for Cu^{2+} ion is designed and synthesized with good yield. The structure of the receptor **I** is determined by FT-IR, ^{1}H NMR, ^{13}C NMR, ESI-MS and single crystal XRD. The receptor **I** is constructed on the basis of internal charge transfer (ICT) mechanism with bis-pyridine unit acting as the binding part for Cu^{2+} ion. In solution, the proposed receptor **I** produces a cation induced 120 nm blue shift for Cu^{2+} ion from 457 nm to 337 nm with remarkable colour change from red to colourless. Whereas no significant colour change is observed upon addition of other metal ions in aqueous HEPES buffer (pH 7.0). Moreover, spectroscopic studies confirm the formation of 1: 1 stoichiometry between the receptor **I** and Cu^{2+} ion with an association constant of ca. 1.45×10^6 M^{-1}. The receptor **I** is highly specific to Cu^{2+} ions in aqueous solution attributed to the rational design of the molecular structure.

The development of naked eye chemosensor for sensing environmentally or biologically important metal ions has gained considerable field of research in recent years [1–5]. Copper is a vital nutrient in all living systems, from microorganism to human, and performs diverse array of biological functions and regulatory mechanisms to maintain a copper balance in our body [6,7]. The design and synthesis of specific ligands and receptors for metal ions are an emerging field of research. Various methods like atomic absorption spectroscopy, inductively coupled atomic emission spectroscopy are widely used for the detection of metal ions at lower concentration. However, these methods require sophisticated instruments and also are a time consuming process [8,9]. In order to overcome these difficulties a wide range of chemosensor techniques have been developed [10–12]. Among them, colorimetric technique has some advantages over the others [13]. It can be conveniently examined by naked eye, rather than by using sophisticated and expensive analytical instruments. A great effort has been invested to establish naked-eye diagnostic tools by easily fabricating simple compounds for sensing of metal ions [14,15].

Numerous receptors have been reported for selective detection of Cu^{2+} ion [16], but a very few reports are available for detection of Cu^{2+} ions by visible colorimetric sensors [17]. Recently, we have reported on the naked eye chemosensor based on azo chromophore for selectively sensing of Cu^{2+} ion in the aqueous solution [18]. However, the chemosensor has some disadvantages like weak association constant with Cu^{2+} ions and poor crystallization behavior for crystallographic studies. To overcome these issues, we have now designed and synthesized a new ligand for Cu^{2+} ion based on azobenzene which has pyridine moieties for interaction with cation and azobenzene as signaling unit. The bis-pyridine unit is introduced in receptor **I** ascribed to its good binding affinity for transition metal ions.

Herein, we report the synthesis, characterization and metal ion binding properties of 4-((4-nitrophenyl)diazenyl)-N,N-bis(pyridin-2-ylmethyl)aniline which comprises azobenzene as signaling unit, and a receptor composed of aromatic nitrogen and two pyridine unit.

The general synthetic route for azobenzene based receptor **I** is exhibited in Scheme 1. The precursor N, N-bis(pyridin-2-ylmethyl)aniline was prepared from the corresponding aniline by N-alkylation with 2-chloromethylpyridine hydrochloride catalyzed by potassium carbonate in acetonitrile. When nitro aniline was diazotized with $NaNO_2$/HCl in 0 °C to form diazonium salt, further it was electrophilically substituted with N,N-bis(pyridin-2-ylmethyl)aniline in the presence of sodium acetate in ethanol to form a desired receptor **I** with 90% yield. The purity of receptor **I** was confirmed by ^{1}H NMR, ^{13}C NMR, and ESI-MS spectra. (See ESI). A single crystal of receptor **I** was grown in acetone and a unique structure was obtained from X-ray analysis as indicated in Fig. 1. The CCDC reference number of receptor **I** is 923504.

The binding behavior of receptor **I** was investigated against different metal ions like Na^+, K^+, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Al^{3+} using UV–vis spectrophotometer. All the titration experiments were carried out at 7.2 pH (50 mM HEPES buffer). The experiments were carried out with miscible carrier solvent (CH_3CN/H_2O, 80/20 v/v) attributed to the weak solubility nature of receptor **I** in H_2O. The absorption spectra of receptor **I** (10×10^{-6} M) show a strong absorption band at 457 nm. The maximum absorbance at 457 nm corresponds to $n \rightarrow \pi^*$ transition of the azo group [19] of the receptor **I** in CH_3CN/H_2O due to the internal charge transfer event of the azo chromophore from donor to acceptor (push–pull effect) [20]. Fig. 2 depicts the UV–vis spectra

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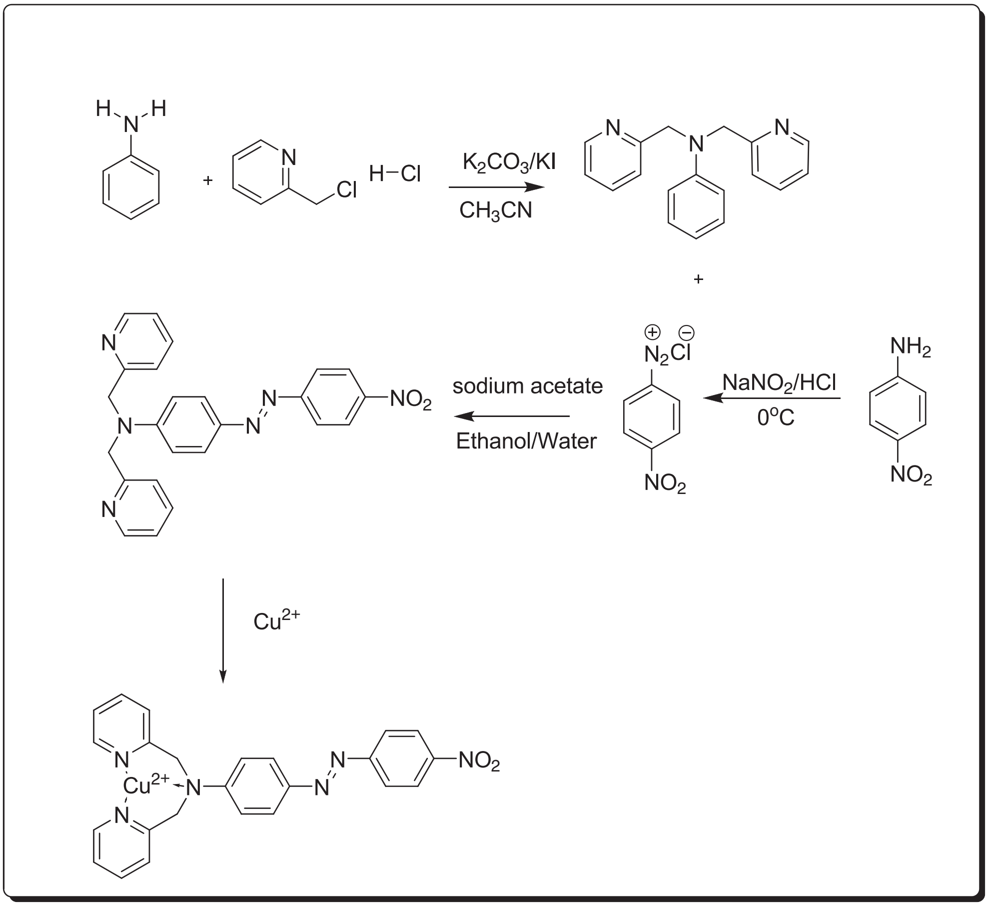

Scheme 1. Synthetic route of receptor **I**.

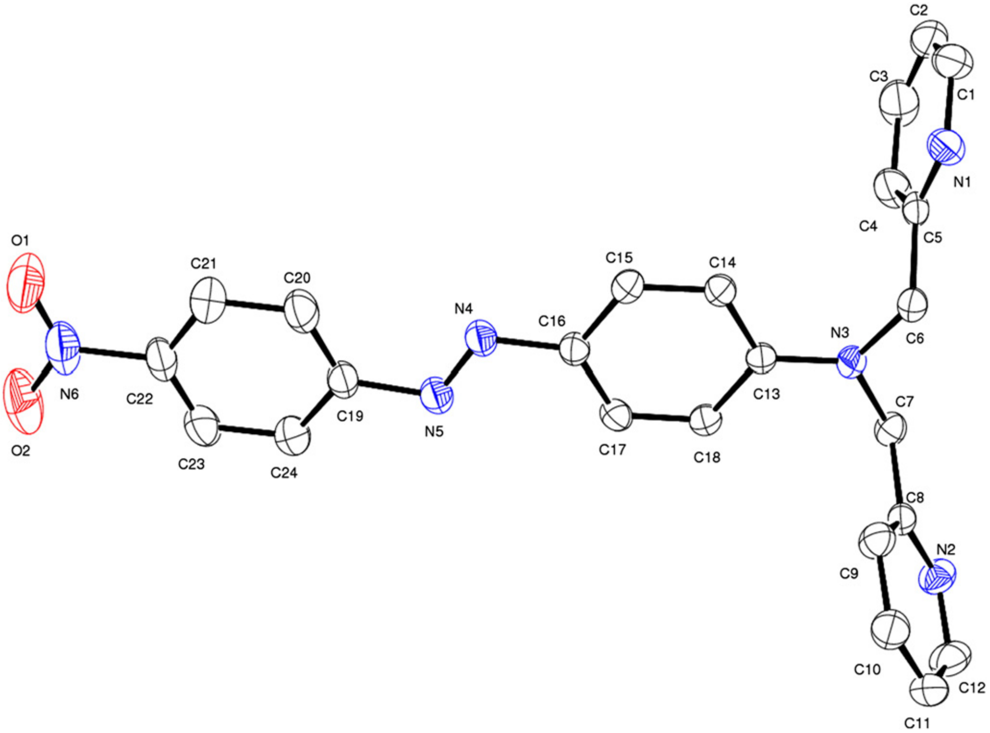

Fig. 1. ORTEP diagram of receptor **I** with thermal ellipsoids at the 50% probability level. Hydrogen atoms are omitted for clarity.

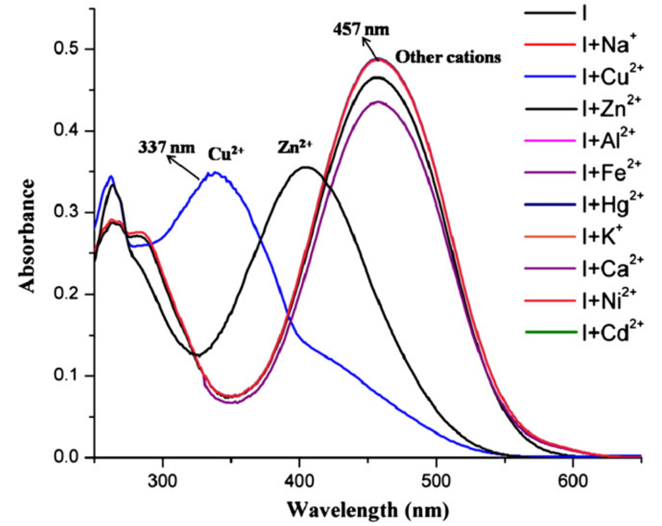

Fig. 2. UV–vis absorption spectra of receptor **I** [1×10^{-5} M in CH_3CN/H_2O (80:20, v/v)] in the presence of different cations (5 equiv) of Na^+, K^+, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Al^{3+} ions.

and visual colour changes of receptor **I** (see ESI, Fig. S6) in aqueous solutions upon addition of 5 equiv of various metal ions like Na^+, K^+, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, $Cd^{2}+$, Hg^{2+} and Al^{3+}. We have observed that remarkable blue shift appears from 457 to 337 nm upon addition of Cu^{2+} ions only, Zn^{2+} ion also shows some spectral shift from 457 to 403 nm, but there were no spectral shift observed with other metal ions.

The complexation behavior of receptor **I** with Cu^{2+} was investigated by UV–vis absorption spectra, in this experiment $Cu(ClO_4)_2$ as copper source, it was gradually added to a solution of receptor **I**. Addition of Cu^{2+} ions to the probe resulted in a blue-shift in absorption spectra and a gradual decrease in absorption intensity at 457 nm. A new absorption peak appears at 337 nm as shown in Fig. 3 and intensity increased gradually with increased Cu^{2+} concentration implying a typical ICT process [21], which generally occurs when receptor (Cu^{2+} chelating domain) integrates into chemosensor unit. In this receptor **I** molecule, the lone pair electron of nitrogen atom in aromatic amine group is perpendicularly coordinated to Cu^{2+} ion, which in turn co-operates with two nitrogen atoms on the dipicolyl amine moiety with three coordinated complex with Cu^{2+}. Therefore, the charge of the aromatic amine is transferred to the cation and the electron donating efficiency of the amine group (to the conjugated plane of the chromophore) is weakened. As a result, a Cu^{2+}-induced blue-shift was observed in the absorption spectra.

The binding stoichiometry of chemosensor with Cu^{2+} ions was studied by Job's plot method [22]. The total concentration of chemosensor and Cu^{2+} was held constant while the mole fraction of Cu^{2+} ions was altered; the absorbance value was plotted against the mole fraction (Fig. 4). The maximum absorbance value occurred at mole fraction 0.5, indicating the formation of 1: 1 complex, consistent with the appearance of an isobestic point at 387 nm in the absorption spectrum (see Fig. 3). The receptor **I**–Cu^{2+} association constant was $K_a = 1.45 \times 10^6\ M^{-1}$ by using the Benesi–Hildebrand plot (see ESI, Fig. S8), using the following equation

$$1/\Delta A = 1/\Delta A_{sat} + 1/(\Delta A_{sat} K_a[\text{guest}])$$

Where ΔA is the absorption difference between the apparent metal complex and the free ligand and ΔA_{sat} is the absorption difference at saturation. The association constant value K_a was evaluated graphically by plotting 1/ΔA against 1/[metal ion]. In this study, the concentration of the ligand was kept constant (10^{-5} M), and the concentration of metal ions was kept in the range ([metal ion]/[ionophore]) = 0.1–2.0 eq. Plot gave a straight line, and the K_a value was obtained from the slope and intercept of this line.

The recognition behavior of the receptor was further confirmed by FT-IR spectroscopy, in which receptor **I** and its coordination with Cu^{2+} ion are presented in Fig. 5, it gives prominent sign of differentiation between the spectra of receptor **I** and its complex with Cu^{2+} in the region of 2500–1000 cm^{-1}. In the IR spectra of Fig. 5, the stretching frequency of C = N in the pyridine ring appears at 1593 cm^{-1}, when receptor **I** coordinated with Cu^{2+}, an electron transfer occurred from C = N to Cu^{2+}, which induces drastic shift in IR peaks of stretching vibrations of C = N to 1639 cm^{-1}. This supported that nitrogen atom of pyridine ligand is involved in coordination of Cu^{2+} ion with receptor **I**. Symmetric and asymmetric stretching frequency of the free perchlorate ion appears at 915 and 1050 cm^{-1} respectively [23].

Verifying the binding mode of the complex was further investigated by ^1H NMR spectroscopic method. We assigned all individual proton signals of receptor **I** and receptor **I**–Cu^{2+} complex. Upon addition of 0.5 equiv of Cu^{2+} to the solution of receptor **I**, some of the receptor **I** protons exhibited downfield shift and upfield shift (Fig. 6). Particularly, a huge downfield shift of aromatic proton in the pyridine ring is observed with coordination of Cu^{2+} ion from 8.62 ppm (**a**) to 8.92 ppm

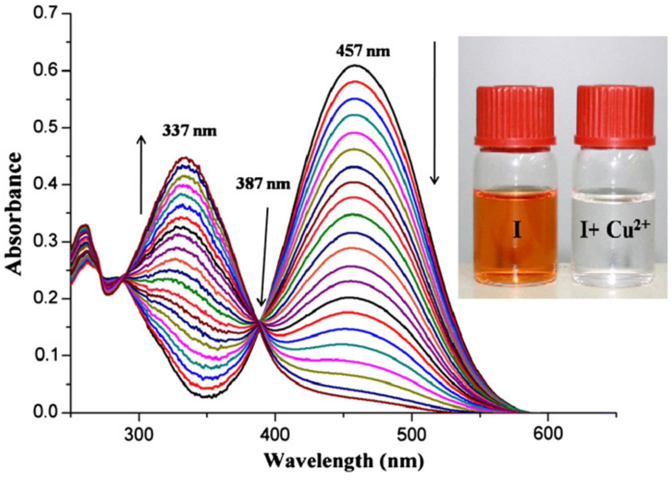

Fig. 3. UV–vis absorption spectra of receptor **I** with increasing concentration of Cu^{2+} (0- 100 μM) in (CH_3CN/H_2O, v/v) medium.

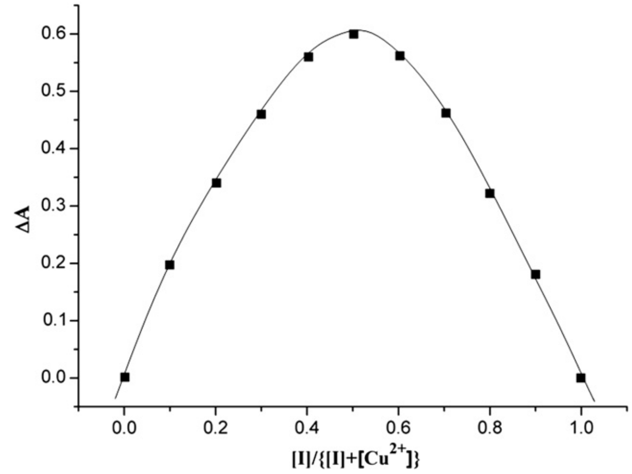

Fig. 4. Job plot of a 1:1 complex of receptor **I** and Cu^{2+} ion, where the absorption at 337 nm was plotted against the mole fraction of Cu^{2+} ions at an invariable total concentration of 10 μM in CH_3CN/H_2O (80:20, v/v).

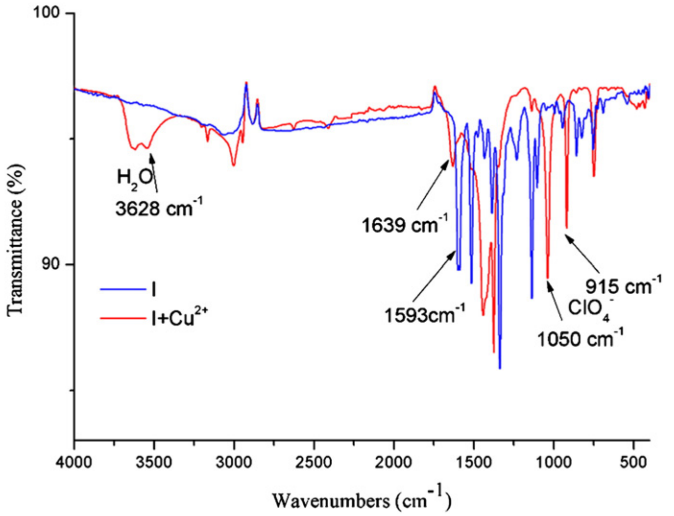

Fig. 5. FT-IR spectra of receptor **I** with Cu^{2+} ion.

(**a***) ascribed to the change in electron density of the pyridine ring in the chromophore. It can also be observed that when metal ion is coordinated with pyridine the electron density of the methylene proton upfield shift is altered from 4.96 ppm (**e**) to 4.58 ppm (**e***). The aniline nitrogen in the azo chromophore plays an important role in the chelating of metal ion. This can be further supported by ^{1}H NMR spectrum of receptor **I** with Cu^{2+}. Fig. 6 indicates that ortho proton of the aniline (**f**) in the receptor **I** resonates at 6.74 ppm without coordination of Cu^{2+}. When the Cu^{2+} is coordinated with receptor **I**, the ortho proton of aniline is drastically shifted to downfield and resonates at 8.16 ppm (**f***). It reveals that the overall electron density of the aniline group of the receptor is transferred to lower electron density of Cu^{2+} ions. Further addition of Cu^{2+} into receptor **I** solutions induces peak broadening in its NMR spectrum attributed to the paramagnetic nature of Cu^{2+} ions.

In conclusion, a new bis-pyridine containing azobenzene based receptors **I** has been designed and synthesized for selective detection of Cu^{2+} ions. Receptor **I** can be used as a visible chemosensor owing to the noticeable color change in the presence of Cu^{2+} ions. Receptor **I** could bind Cu^{2+} with 1:1 stoichiometric manner to induce large blue shift in the absorption spectra with a marked color change. Significantly, the selectivity of this receptor **I** for Cu^{2+} over other tested metal ions is extremely high.

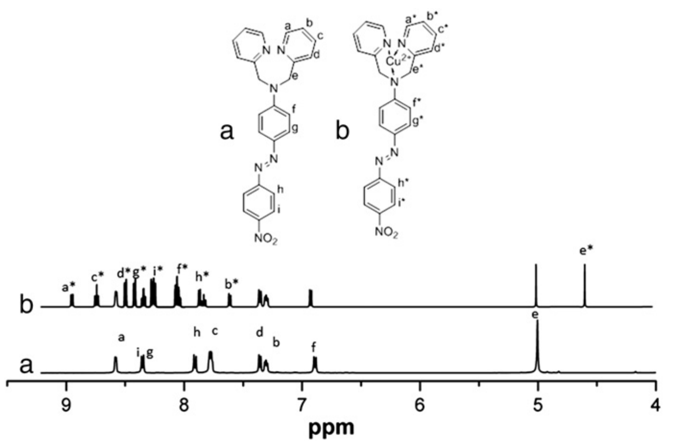

Fig. 6. Partial ^{1}H NMR spectra of receptor **I** in DMSO- *d*6: (a) receptor **I** only, (b) receptor **I** + 0.5 equiv of Cu^{2+}.

Acknowledgments

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Appendix A. Supplementary material

CCDC 923504 contains the supplementary crystallographic data for receptor **I**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Synthetic procedures, characterization data and additional spectroscopic data as supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2013.09.010.

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