Solvent Controlled Colorimetric And Fluorometric Detection of Fe^{2+} and Cu^{2+} Ions By Naphthaldimine-Glucofuranose Conjugate

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Research Article

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Abstract

The sensing properties of naphthaldimine-glucofuranose conjugates **1** and **2** towards metal ions were investigated by ^1H NMR titration, FTIR, absorbance, and fluorescence spectroscopic methods. The absorbance and fluorescence studies indicated that compound **1** formed coordination with Fe^{2+} and Cu^{2+} ions in DMSO through color changes yellow to brown and colorless, respectively. The Job's plots using absorbance data showed metal-ligand binding ratio is 1:1 for both cases. The formation of **1**- Fe^{2+} and **1**- Cu^{2+} complexes have been analyzed by absorption and emission spectroscopy, high-resolution mass spectrometry (HRMS) data, FTIR, ^1H NMR titration experiment, and DFT calculations. The detection limits of naphthaldimine sugar conjugate **1** towards $\text{Fe}^{2+}/\text{Cu}^{2+}$ were calculated from UV-vis and fluorescence data according to the standard method. The sugar-naphthaldimine conjugate **2** has been used to establish the binding mode of **1** with Fe^{2+} or Cu^{2+} ions in DMSO.

Introduction

The design and synthesis of optical sensors to recognize biologically essential metal ions such as Cu^{2+} , Fe^{2+} , Fe^{3+} , Mn^{2+} , Zn^{2+} , Ca^{2+} , Na^+ , and K^+ have gained much attention in supramolecular chemistry.^[1] Among all the above metal ions, Fe^{2+} has drawn considerable attention because of its beneficial or threatening influences on the living system and environment.^[2] It plays a crucial role in the biochemical process at the cellular level, such as oxygen metabolism, electron transfer in enzymatic reaction, and DNA-RNA synthesis.^[3] Therefore, the presence of iron in the human body is also essential for normal growth. Its deficiency is also responsible for several diseases like anaemia, diabetes, lung disorder, liver and kidney damages.^[4–5] At the same time, the over-concentration of Fe^{2+} ions causes hemochromatosis, Alzheimer and Huntington's diseases.^[6–7] On the other hand, copper is the most abundant metal available in the human body and takes part in many biological processes, enzyme-catalyzed reactions, and transcriptional events.^[8] The minimum amount of copper absorption is required in our body to maintain the everyday activity of the brain, nervous systems for the normal growth and development of a living organism.^[9] It also helps to keep the formation of collagen, elastin, and hemoglobin level in the living body.^[10] Despite its importance, Cu^{2+} is highly bioaccumulative, causing the interruption of cellular balance and serious neurodegenerative diseases like Parkinson.^[11] Alzheimer,^[12] prion,^[13] Menkes, and Wilson's diseases by the formation of reactive oxygen species.^[14–16]

Owing to the significant importance of iron and copper, they are also acted as remarkable environmental pollutants due to widespread uses.^[17–19] In view of the above discussion, it is important to efficiently monitor the presence of Fe^{2+} and Cu^{2+} ions. As a result, the development of sensors for the detection of Fe^{2+} and Cu^{2+} ions has gained immense interest for researchers.^[20–23] The colorimetric method for Fe^{2+} and Cu^{2+} ions detection is a more attractive approach because their presence can be determined by the naked eye, rather than using sophisticated types of equipment, trained operators, and complicated procedures for sample preparations.^[24–27]

Therefore, the development of cheap new simple colorimetric sensors for naked-eye detection of Fe^{2+} and Cu^{2+} in the visible region without any instrument is in strong demand in recent years. Moreover, simultaneous detection of Fe^{2+} and Cu^{2+} through a naked-eye noticeable color change is highly important because Fe^{2+} and Cu^{2+} ions can act as fluorescent quenchers due to their paramagnetic nature.^[28–31] By considering the real application of the sensor, the development of a versatile chemosensor for the detection of different metal ions with the change of the tested media appears to be principally appealing because of its expediency in practical application. It was extracted from the literature that different types of sensors have been developed for selective differentiation of Pb^{2+} and Hg^{2+} ,^[32] Cu^{2+} and Hg^{2+} ,^[33–34] Mg^{2+} and Zn^{2+} ,^[35] Au^{3+} and Hg^{2+} ,^[36] Zn^{2+} and Cu^{2+} ,^[37] and Cd^{2+} , Hg^{2+} and Pb^{2+} ^[38] by changing of tested media. These studies informed that solvent has a major role in the selection of metal ions by a sensor.

Previously, we have reported that naphthaldimine-based glucofuranose derivative **1** could selectively and sensitively detect the Cu^{2+} ions in the acetonitrile/water (1/4, v/v) system whereas, sugar conjugate **2** was used as a model compound to establish the sensing mechanism of **1** with Cu^{2+} ions (Fig. 1).^[39] Presence of several -OH groups and oxygen atoms in a sugar moiety is suitable to behave as a good sensor for cations sensing^[40–41] and thus, it is realistic to expect that sensor **1** exhibits different selectivity towards metal ions by changing the testing media. Herein, we revealed that naphthaldimine-glucofuranose conjugate **1** showed simultaneous colorimetric and fluorometric selectivity towards Fe^{2+} and Cu^{2+} ions in DMSO. It is worth mentioning that this is the second example of a sugar-modified solvent-controlled sensor for the detection of metal ions.^[34]

Experimental Section

2.1. Materials and general methods

Commercially available analytical grade reagents and solvents were used for synthesis purposes. Thin Layer Chromatography (TLC) was carried out using Merck silica gel 60 (F_{254}) coated aluminum sheets, and the progress of reactions was visualized in UV-vis and fluorescence lamp or by charring the plate after immersion in 5% H_2SO_4 -MeOH solution. Purification of compounds was carried out by column chromatography using silica gel (230–400 mesh). Double focusing high-resolution instrument was used for high-resolution mass spectra (HRMS) of compounds and complexes. For the spectral studies, HPLC grade solvents and double distilled water were used. Absorption spectra were recorded at room temperature by using a V-730 spectrophotometer (JASCO, Japan). FP-8200 spectrophotometer (JASCO, Japan) was used to take fluorescence spectra in DMSO. The salts of NaNO_3 , KNO_3 , LiCl , AgNO_3 , $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CdCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Hg}(\text{OAc})_2$, $\text{Pb}(\text{OAc})_2 \cdot 3\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, FeCl_3 , and MnCl_2 were used to prepare the initial solutions of different metal ions in doubled distilled water. All the experimental works were executed at room temperature unless otherwise stated.

2.2. UV-visible and fluorescence experiments

For UV-vis and fluorescence experiments, solutions of sensor **1** (95 μM) and metal ions (100 mM) were prepared in DMSO and double-distilled water, respectively. The absorption and emission spectra were recorded after the addition of 2 equiv. of metal ions to the 1 mL solution of sensor **1**. The fluorescence spectra were recorded by using medium sensitivity with excitation and emission slits at 5 nm at room temperature.

2.3. Colorimetric experiments

The colorimetric nature of **1** (95 μM) in DMSO was monitored before and after the addition of 2 equiv. (3.8 μL) of each metal ions viz Na^+ , K^+ , Sr^{2+} , Li^+ , Ag^+ , Ca^{2+} , Au^{3+} , Fe^{3+} , Cu^{2+} , Fe^{2+} , Cd^{2+} , Ni^{2+} , Mg^{2+} , Hg^{2+} , Co^{2+} , Pb^{2+} , Zn^{2+} , Mn^{2+} and Al^{3+} separately to the sensor **1** (1 mL). We observed that the solution color of **1** was not affected effectively after the addition of all cations except Fe^{2+} or Cu^{2+} ions. In the existence of Fe^{2+} and Cu^{2+} , the yellow solution of **1** changes to brown (for Fe^{2+}) and colorless (for Cu^{2+}).

2.4. Competitive experiments

A stock solution of **1** (12.9534 mM) was prepared in HPLC grade DMSO and diluted to 95 μM using DMSO. Stock solutions (100 mM) of different cations such as Hg^{2+} , Co^{2+} , Mn^{2+} , Na^+ , Mg^{2+} , K^+ , Li^+ , Cd^{2+} , Cu^{2+} , Ag^+ and Ni^{2+} in distilled water were prepared. First, 1.9 μL (2 equiv.) of Fe^{2+} or Cu^{2+} solution was mixed with 1 mL solution of **1** (95 μM), then absorbance spectra were taken at room temperature. Thereafter, 1.9 μL (2 equiv.) of each metal solution was added to the abovementioned mixed solution, and then absorbance spectra were recorded again.

2.5. Binding stoichiometry calculations

Job's plot experiments were conducted in order to obtain the metal-ligand binding ratios for both complexes. A series of solutions containing **1** (95 μM) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ or $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ions (95 μM each) were prepared separately in DMSO. Then, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, and 0 mL of sensor **1** solutions were taken in vials separately and then added 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mL solutions of Fe^{2+} or Cu^{2+} systematically to get constant volume 1 mL of each vial. After a few seconds of mixing, UV-vis spectra were recorded at room temperature. The binding stoichiometry was calculated by plotting the molar fraction of Cu^{2+} or Fe^{2+} ($X_{\text{Cu}^{2+} \text{ or } \text{Fe}^{2+}}$) versus $\Delta A \times (X_{\text{Cu}^{2+} \text{ or } \text{Fe}^{2+}})^{[42]}$.

2.6. Limit of detection (LOD) and binding constant (K_a) measurements

The UV-vis and fluorescence titration data were used to calculate the limit of detection ($\text{LOD} = 3\sigma/k$) and binding constant values of **1** for both the ions. The absorbance/fluorescence versus function of the concentration of Fe^{2+} or Cu^{2+} was plotted to calculate LOD values. The blank (without Fe^{2+} or Cu^{2+} ions) was measured 10 times, and then the standard deviation (σ) of the blank was calculated accordingly. The slope (k) was obtained from the calibration plot between ion concentration and absorbance/emission

intensities. The binding constant (K_a) of **1** for detection of Fe^{2+} or Cu^{2+} , the inverse of absorbance/fluorescence was plotted with the inverse of concentration of Fe^{2+} or Cu^{2+} ions.

2.7. ^1H NMR titration

We have taken four NMR tubes and poured 400 μL solution of sensor **1** (1.08 mM) in each tube. The NMR spectrum of the compound was taken before the addition of Fe^{2+} ions. Thereafter, 1.5 μL (0.1 equiv.), 3 μL (0.2 equiv.), 7.5 μL (0.5 equiv.) and 15 μL (1 equiv.) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (24.9 mM) were added separately to the four different NMR tubes, and spectra were recorded.

Results And Discussion

3.1 Synthesis of Naphthaldimine-glucofuranose conjugates **1** and **2**

In our previous report, naphthaldimine-glucofuranose conjugate **1** was used for colorimetric detection of Cu^{2+} ions in the acetonitrile/water system (1/4, v/v), and compound **2** was utilized to establish the binding mode of **1** with Cu^{2+} ions.^[39] Taking into account the solvent effect for the selection of metal ions, we have synthesized and characterized sugar-naphthaldimine derivatives **1** and **2** as per the reported method for the detection of metal ions in the DMSO system. The recognition properties of **1** and **2** towards metal ions were investigated by absorbance and fluorescence spectroscopic measurements, FTIR, ^1H NMR titration, and mass spectrometry.

3.2 Solvent selectivity

The absorption property of **1** (95 μM) was initially investigated in different solvents such as DMSO, DMSO- H_2O , EtOH, and CH_3CN in order to understand the solvent effects on photophysical properties (Fig. 2). Compound **1** showed better solubility and high absorbance intensity in DMSO among the other solvents systems. As shown in Fig. 2, sugar-conjugate **1** exhibited three distinct absorption peaks at 305 nm, 401 nm, and 420 nm in both polar protic or aprotic solvents. Three distinct absorption peaks at 420 nm, 401 nm, and 305 nm corresponding to π - π^* transition of aromatic naphthalene moiety and n - π^* transition of $>\text{C}=\text{N}$ group.^[43] The absorption band at 420 nm may be attributed to the intramolecular charge transfer (ICT) transition from imine nitrogen to corresponding naphthalene moiety.^[44] The selective recognition ability of **1** towards metal ions was investigated in various solvents systems such as ethanol, acetonitrile, DMSO- H_2O and DMSO (Supporting information, Fig.S1-S4). The results informed that sensor **1** could be used to recognize the Fe^{2+} and Cu^{2+} ions in DMSO (Figs. S4/4a) only.

3.3 Colorimetric studies for metal ions

The colorimetric experiments were performed after the addition of 2 equiv. of metal ions *viz.* Na^+ , K^+ , Sr^{2+} , Li^+ , Ag^+ , Ca^{2+} , Au^{3+} , Fe^{3+} , Cu^{2+} , Fe^{2+} , Cd^{2+} , Ni^{2+} , Mg^{2+} , Hg^{2+} , Co^{2+} , Pb^{2+} , Zn^{2+} , Mn^{2+} and Al^{3+} to the DMSO solutions of **1** (95 μM) and **2** (95 μM). The solution colors of **1** in the presence and absence of metal ions

are represented in Fig. 3. We observed that the yellow color of sensor **1** was not affected after the addition of 2 equiv. of various metal ions except for Fe^{2+} and Cu^{2+} ions in DMSO. Sensor **1** exhibited significant naked-eye detectable color change from yellow to brown in the presence of Fe^{2+} ions. In the respective UV-vis spectrum, the absorbance intensity of **1** at 401 nm and 420 nm was decreased in the presence of Fe^{2+} ions. The Fe^{2+} induced color change from yellow to brown was further verified by a blank color test experiment (Supporting information, Fig.S5). In this experiment, the same amount of Fe^{2+} ions (3.8 μL) was added to DMSO (2 mL, in the absence of sensor **1**). We noticed that the DMSO solution (absence of sensor **1**) did not turn brown in the presence of Fe^{2+} ions. This result indicates that the color change from yellow to brown is due to the interaction between sensor **1** and Fe^{2+} ions. Similarly, the Cu^{2+} induced color change from yellow to colorless could also be noticed by the naked eye. The visual color changes in the presence of Fe^{2+} and Cu^{2+} were supported by their individual UV-vis absorbance curve responses^[39,45–46] and changes in absorption in the region at 340 nm to 360 nm. Under similar experimental conditions, no remarkable color change was noticed for **2** (95 μM) in the presence of all metal ions, including Fe^{2+} and Cu^{2+} ions (Supporting information, Fig.S6). The color change experiment was also indicated that sensor **2** was unable to form the appropriate metal-binding core in DMSO. The above phenomena indicated that chelation occurred between sensor **1** and Fe^{2+} or Cu^{2+} ions in DMSO solvent.

3.4 UV-vis spectroscopy studies for metal ions

The absorption behavior of **1** (95 μM) toward a wide range of 2 equiv. of metal ions in DMSO was investigated before and after the addition of various metal ions such as Na^+ , K^+ , Li^+ , Cu^{2+} , Fe^{2+} , Cd^{2+} , Ni^{2+} , Mg^{2+} , Hg^{2+} , Co^{2+} , Pb^{2+} , Zn^{2+} , Mn^{2+} , Al^{3+} , and Fe^{3+} (Supporting information, Fig. S4). We noticed that the absorbance of sugar -OH free naphthaldimine-glucofuranose conjugate **1** was not affected in the presence of the abovementioned metal ions except Fe^{2+} and Cu^{2+} ions in DMSO (Fig. 4a). In the presence of 2 equiv. of Fe^{2+} ions, the intensity of absorption bands at 401 nm and 420 nm were decreased evidently. Whereas in the presence of Cu^{2+} ions, the absorption bands at 420 nm and 401 nm were blue-shifted from 420 nm to 398 nm and 401 to 382 nm with a remarkable decrease in absorption intensity. These observations indicate that there are some interactions between compound **1** and Fe^{2+} or Cu^{2+} ions in DMSO. The selectivity of sensor **1** towards Fe^{2+} or Cu^{2+} ions is also understood in terms of relative absorption intensity at 420 nm of receptor **1** (95 μM) in DMSO before and after the addition of the various metal ions (Fig. 4b). The sugar -OH protected naphthaldimine-glucofuranose conjugate **2** did not exhibit specific selectivity towards metal ions in DMSO through spectral change (Fig. S7). This phenomenon was observed due to the protection of sugar hydroxyl groups. The protected sugar -OH group could not generate a suitable metal binding pocket. The absorption natures of **1** and **2** with the presence of metal ions indicate that the -OH free naphthaldimine-glucofuranose conjugate **1** can be used as a sensor for the detection of Fe^{2+} and Cu^{2+} ions in DMSO.

UV-vis absorption titrations of **1** (95 μM) were conducted with increasing concentrations of both ions to get better insight into compound **1** as a sensor for Fe^{2+} and Cu^{2+} ions. Upon the addition of increasing

concentration of Fe^{2+} or Cu^{2+} to the solution of sensor **1**, the changes in absorption spectra of **1** are presented in Fig. 5. As shown in Fig. 5a, upon addition of 0–9 equiv. of Fe^{2+} ions, the intensity of absorption bands at 420 nm and 401 nm decreased remarkably with the appearance of a new peak at 335 nm. It was also observed the formation of two clear isosbestic points at 376 nm and 434 nm with increasing concentration of Fe^{2+} (0–9 equiv.). Upon gradual addition of Cu^{2+} ions (0–9 equiv.) to the solution of **1**, the intensity of absorption peaks at 420 nm and 401 nm decreased with the shifting of peaks from 420 nm to 398 nm and 401 to 382 nm. In the case of Cu^{2+} , only one clear isosbestic point at 389 nm was generated with an increasing concentration of Cu^{2+} ions. Further addition of Fe^{2+} or Cu^{2+} ions (more than 9 equiv.) to the solution of **1**, no change in absorption spectrum was observed. The above discussion indicates that the formation of a complex between sensor **1** and Fe^{2+} or Cu^{2+} in DMSO.

The binding stoichiometries of the **1**- Fe^{2+} and **1**- Cu^{2+} complexes were calculated from Job's plots to establish the complex formation mechanism of receptor **1** with Fe^{2+} or Cu^{2+} (Fig. 6).^[39, 43] The mole fraction of Fe^{2+} or Cu^{2+} was plotted against $\Delta A \times X_{\text{Fe}^{2+} \text{ or } \text{Cu}^{2+}}^{2+}$ at maximum absorption (420 nm) under a constant total volume of 1 mL. The maximum absorption changes of sensor **1** for Fe^{2+} and Cu^{2+} ions were seen when the mole fractions were found to be 0.49 and 0.5, respectively. These results indicate that the formation of 1:1 (host/guest) complexes between sensor **1** with Fe^{2+} and Cu^{2+} ions. To further support the formation of 1:1 complex, ESI mass spectra of **1** + Fe^{2+} and **1** + Cu^{2+} were analyzed. The mass spectrum of the **1** + Fe^{2+} complex showed a peak at m/z 518.1239, corresponding to the $[\text{1} + \text{Fe}^{2+} - \text{H}]^+$ (Supporting information, Fig. S8). Similarly, we have seen a peak at m/z 525.1262 in the mass spectrum of **1** + Cu^{2+} complex (Supporting information, Fig. S9), which is assigned to the corresponding of **1** + $\text{Cu}^{2+} - \text{H}]^+$. The mass spectrum of both complexes indicated that the formation of 1:1 complex after deprotonation. It was assumed that the formation of **1** + Fe^{2+} or **1** + Cu^{2+} complex after deprotonation of aromatic hydroxyl group due to its high acidic nature.

The binding constant (K_a) values of **1** for detection of Fe^{2+} and Cu^{2+} ions were calculated from the Benesi-Hildebrand equation by using absorbance titrations data.^[44] To calculate the binding constant (K_a) value, we have plotted $1/(A_0 - A)$ against $1/[\text{Fe}^{2+}]$ or $1/[\text{Cu}^{2+}]$ (Fig. 7). First, the absorbance data were linearly fitted according to the Benesi-Hildebrand equation. Finally, the K_a values **1**- Fe^{2+} and **1**- Cu^{2+} complexes were calculated using the slope and intercept of the linear line. The calculated K_a values of **1**- Fe^{2+} and **1**- Cu^{2+} complexes were found to be $9.4 \times 10^3 \text{ M}^{-1}$ and $1.4 \times 10^4 \text{ M}^{-1}$, respectively (Fig. 7a/b). Furthermore, the limit of detection values of **1** for Fe^{2+} and Cu^{2+} ions were calculated from $3\text{SD}/k$ (SD is the standard deviation of the blank and k in the slope of the calibration curve).^[44] The LOD values for Fe^{2+} and Cu^{2+} were found to be 3.58 μM and 3.49 μM , respectively (Fig. 7c/d).

The interference experiments were performed to examine **1** as an ion-specific sensor for Fe^{2+} and Cu^{2+} ions in the presence of other competing metal ions such as Na^+ , K^+ , Li^+ , Ag^+ , Cu^{2+} , Fe^{2+} , Cd^{2+} , Ni^{2+} , Mg^{2+} , Hg^{2+} , Co^{2+} , and Mn^{2+} (Fig. 8). Sensor **1** was treated with other metal ions (2 equiv.) in the presence of

Fe^{2+} or Cu^{2+} ions (2 equiv.). The absorbance intensity of **1** at 420 nm in the presence of Fe^{2+} or Cu^{2+} (green bar) and after the addition of other metal ions (pink bar) are represented in fig. 8. As shown in fig. 8, the absorption intensity in the presence of Fe^{2+} or Cu^{2+} ions was not interfered with by other metal ions. The change of absorbance of the **1**- Fe^{2+} complex with the addition of Cu^{2+} indicated that the replacement of Fe^{2+} from the **1**- Fe^{2+} complex in the presence of Cu^{2+} ions. Thus, it is notable that naphthaldimine-glucofuranose conjugate **1** can be used as a Fe^{2+} and Cu^{2+} selective fluorescent sensor in the presence of other competing metal ions.

In order to investigate the nature of the sensor (reversible or irreversible) with Fe^{2+} and Cu^{2+} ions, two separate reversibility experiments were performed with the presence of Na_2EDTA in DMSO (Fig. 9). As shown in Fig. 9a/b, the absorbance intensity at 401 nm and 420 nm was reduced after the addition of 2 equiv. of Fe^{2+} or Cu^{2+} ions, which was regenerated after the addition of 2 equiv. Na_2EDTA to the mixed solution of **1** and Fe^{2+} or Cu^{2+} . This result was explained by the strong interaction of Fe^{2+} or Cu^{2+} with EDTA than with sensor **1**. Further addition of $\text{Fe}^{2+}/\text{Cu}^{2+}$ in the same cuvette, the absorbance intensity was decreased effectively. With this observation, we have also performed a naked eye reversible experiment with Na_2EDTA (Fig. 9c/d). The brown color solution of the **1** + Fe^{2+} complex was turned to yellow in the presence of the sodium salt of EDTA. Similarly, the colorless solution of the **1**- Cu^{2+} complex was changed to yellow in the presence of EDTA solution. Spectral changes experiments were repeated many times without loss of much absorbance sensitivity. These observations indicated that the interaction of **1** with Fe^{2+} or Cu^{2+} ions is reversible in nature, and sensor **1** can be used as a colorimetric reusable and recyclable sensor.

3.5 Fluorescence studies of **1** for metal ions

Further, the sensing property of **1** towards Fe^{2+} and Cu^{2+} was confirmed by fluorescence spectroscopy ($\lambda_{\text{ex}} = 410 \text{ nm}$). The metal ions recognition nature of **1** in DMSO was established by change of emission spectra of **1** (95 μM) before and after the addition of 2 equiv. of various metal ions *viz.* Na^+ , K^+ , Sr^{2+} , Li^+ , Ag^+ , Ca^{2+} , Au^{3+} , Fe^{3+} , Cu^{2+} , Fe^{2+} , Cd^{2+} , Ni^{2+} , Mg^{2+} , Hg^{2+} , Co^{2+} , Pb^{2+} , Zn^{2+} , Mn^{2+} and Al^{3+} (Fig. 10). As shown in Fig. 10, the effective fluorescence quenching of **1** was observed in the presence of Fe^{2+} and Cu^{2+} ions into the solution of **1**. The fluorescence of **1** was not affected in the presence of other metal ions (Fig. 10a). The quenching efficiencies of **1** at 466 nm for Fe^{2+} and Cu^{2+} were calculated to be 62% and 73%, respectively. The fluorescence quenching of **1** with the addition of Fe^{2+} and Cu^{2+} suggesting that the formation of the complex between **1** and Fe^{2+} or Cu^{2+} ions. The significant fluorescence quenching may have occurred by the excitation energy transfer from the ligand to the metal d-orbital and/or ligand to the metal charge transfer process (LMCT).^[47] Further, to understand the interactions of **2** towards all metal ions, we investigated the fluorescence changes of sugar -OH protected compound **2** upon the addition of all metal ions, including Fe^{2+} and Cu^{2+} ions. The fluorescence changes are depicted in Fig. 10b. As mentioned in Fig. 10b, the quenched fluorescence was observed in the presence of various metal ions, including Fe^{2+} or Cu^{2+} ions. This experiment indicated that naphthaldimine-glucofuranose

conjugate **2** has no specific selectivity towards metal ions in DMSO. The above results and discussion indicate that sensor **1** can be utilized as a selective fluorescent sensor for Fe^{2+} and Cu^{2+} ions over other common metal ions. This result showed good agreement with the results obtained from absorbance and colorimetric experiments. Sensor **1** shows high selectivity and sensitivity towards Fe^{2+} and Cu^{2+} ions due to the formation of the appropriate metal-binding core by imine N and two hydroxyl groups.

The fluorescence titration experiments of sensor **1** (95 μM) with Fe^{2+} and Cu^{2+} ions were carried out with increasing concentrations of both ions in DMSO (Fig. 11). On successive addition of Fe^{2+} or Cu^{2+} ions to the solution of **1**, the fluorescence intensity gradually decreases. The quenching efficiencies of sensor **1** calculated to be 66% and 82% in the presence of Fe^{2+} (10 equiv.) and Cu^{2+} (3 equiv.), respectively. The continuous decreasing of fluorescence intensity with increasing concentration of Fe^{2+} or Cu^{2+} ions indicated the formation of **1**- Fe^{2+} or **1**- Cu^{2+} complex.

The binding constant (K_a) values of **1** for Fe^{2+} and Cu^{2+} in DMSO were calculated from fluorescence data and observed to be $1.156 \times 10^5 \text{ M}^{-1}$ and $2.025 \times 10^5 \text{ M}^{-1}$, respectively (fig. 12a/b). Further, fluorometric detection limits of **1** for detection of Fe^{2+} and Cu^{2+} ions were calculated to be 0.4846 μM and 0.099 μM respectively (fig 12c/d). The detection limits and binding constant values of sensor **1** towards $\text{Fe}^{2+}/\text{Cu}^{2+}$ were calculated and given in Table 1. The obtained LOD values from UV-vis and fluorescence titration data are well supported by earlier reported sensors. Finally, the calculated LOD values of naphthaldimine-glucofuranose conjugate **1** for recognition of Fe^{2+} and Cu^{2+} ions were compared with other reported sensors (Table 1).

Table 1
The limit of detection and binding constant values of reported sensors and naphthaldimine sugar conjugate **1** with Fe²⁺ and Cu²⁺ ions.

Metal ions	LOD (μM)	Binding constant (M ⁻¹)	Method	Solvent	Ref.
Cu ²⁺	6.08		UV-vis	DMSO/H ₂ O (9:1, v/v)	[48]
	130	7.4 × 10 ¹	Fluorescence	CH ₃ OH	[49]
	9.37	3.3 × 10 ⁵		CH ₃ CN	[50]
	4.47	1.59 × 10 ⁷		DMSO/H ₂ O (1:1, v/v)	[51]
Fe ²⁺	7.78	4.86 × 10 ³		THF-Tris (4:1, v/v)	[52]
	14.5			CH ₃ CN/H ₂ O (2:1, v/v)	[53]
	85.1			HEPES/MeOH (9:1, v/v)	[54]
Fe ²⁺	14.4	3.3 × 10 ³	UV-vis	DMF-buffer (1:1, v/v)	[55]
Cu ²⁺	2.9	4.2 × 10 ³			
Fe ²⁺	2.99 × 10 ⁵	1.2 × 10 ⁶	Fluorescence	Aqueous solvent	[56]
Cu ²⁺	10.4	1.6 × 10 ⁶			
Cu ²⁺	0.23	9.7 × 10 ⁵	UV-vis	CH ₃ CN/H ₂ O (1:4, v/v)	[39]
Fe ²⁺	3.58	0.94 × 10 ⁴	UV-vis	DMSO	This work
	0.485	1.16 × 10 ⁵	Fluorescence		
Cu ²⁺	3.49	1.4 × 10 ⁴	UV-vis		
	0.099	2 × 10 ⁵	Fluorescence		

3.6. ¹H NMR titration and FTIR studies

To further clarify the binding mode of **1** with Fe²⁺ or Cu²⁺, a ¹H NMR titration experiment of **1** was conducted in the presence of Fe²⁺ ions in DMSO-d₆ (Fig. 13). We have used lower concentrations of iron (up to 1 equiv.) to avoid broadening the NMR peaks. As shown in Fig. 13, the intensity of sugar hydroxyl proton at 5.49 was decreased with increasing concentration of Fe²⁺ ions. These results suggested that sugar -OH is involved in the complex formation. The signal corresponding to aromatic -OH proton (> 10

ppm) did not find in the ^1H NMR spectrum of **1**. The imine proton at 8.97 ppm was shifted in the presence of 1 equiv. of Fe^{2+} ions indicated that the imine group was involved in the metal-binding process. The changes of NMR spectra in aromatic and aliphatic regions also confirmed that both aromatic group and sugar moiety were taken part in the binding mechanism.

The involvement of imine and -OH groups of **1** in recognition of Fe^{2+} and Cu^{2+} was further confirmed by the FT-IR spectra of **1**, **1-Fe** $^{2+}$ and **1-Cu** $^{2+}$ complexes (Supporting information, Fig. S10). The characteristic imine ($-\text{C}=\text{N}$) peak at 1652 cm^{-1} (Fig. S8a) shifted to 1659 cm^{-1} (Fig. S8b) and 1658 cm^{-1} (Fig. S8c) in the presence of Fe^{2+} and Cu^{2+} ions, respectively.^[43, 46] Similarly, the characteristic -OH peak appeared 3418 cm^{-1} for Fe^{2+} and 3407 cm^{-1} for Cu^{2+} instead of 3446 cm^{-1} in the complexes of **1-Fe** $^{2+}$ and **1-Cu** $^{2+}$.^[57] All these results strongly support the imine and -OH groups were involved in the binding event. Finally, (i) ^1H NMR titration of **1** with Fe^{2+} ions, (ii) Job's plot, and ESI-MS data of sensor with Fe^{2+} and Cu^{2+} ions (Supporting information, Fig. S8, and Fig. S9), (iii) UV-vis. and fluorescence measurements of compound **2** (Supporting information, Fig. S7 and Fig. 10b), FTIR studies (Supporting information, Fig. S10), we proposed that two hydroxyl groups and imine N atom of sensor **1** formed a tridentate complex with Fe^{2+} or Cu^{2+} ions in DMSO after deprotonation of aromatic -OH group (Fig. S8/9).

3.7. Theoretical calculations

The possible binding sites of ligand **1** for coordination with Fe^{2+} and Cu^{2+} were also investigated by density functional theory (DFT) calculations (Fig. 14). The energy minimized structures of **1-Fe** $^{2+}$ and **1-Cu** $^{2+}$ complexes in DMSO were optimized at the Becke-3-Lee-Yang-Parr (B3LYP)/ SDD theory level with the GAUSSIAN 09W package. Vibrational frequency analyses have been done to ensure that all the geometries belong to a minimum potential energy surface. As shown in fig. 14(a), the Fe^{2+} ion is coordinated with imine N and two hydroxyl groups of the sugar ligand **1**. A similar type of coordination was observed for Cu^{2+} ion with the ligand (Fig. 14b). The average bond distances of Fe-N, Fe-O (aromatic), and Fe-O (sugar) for **1-Fe** $^{2+}$ were calculated as $1.88\text{ \AA}$, $1.86\text{ \AA}$, and $1.98\text{ \AA}$, respectively. For the **1-Cu** $^{2+}$ complex, the calculated bond lengths of Cu-N, Cu-O (aromatic), and Cu-O(sugar) were found as $1.90\text{ \AA}$, $1.82\text{ \AA}$, and $1.92\text{ \AA}$, respectively.

Conclusions

The present findings provide a promising demonstration of the role of solvent on metal binding and selectivity. The naphthalaldimine sugar conjugate **1** was found to be an elegant tool for the colorimetric sensing of Fe^{2+} and Cu^{2+} over other competitive miscellaneous ions in DMSO. Moreover, Fe^{2+} and Cu^{2+} detection can also be envisioned by significant naked-eye observable color changes from yellow to brown and colorless for Fe^{2+} and Cu^{2+} ions, respectively. Further, the complexation of **1** with Fe^{2+} and Cu^{2+} was also confirmed by UV-visible and fluorescence experiments, ^1H NMR titration, FTIR studies, HRMS values of the complexes and DFT calculations. These results suggest that sensor **1** acts as an efficient sensor

for recognizing Fe^{2+} and Cu^{2+} ions for analytical applications. This work will provide immense interest for the analysis of metal ions with the changing of the solvent system by a colorimetric/fluorometric sensor.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Ethics approval/declarations

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Code availability

Not Applicable

Authors' contributions

ML and BD: Conceptualization (supported), investigation (supporting), and conducting experiments (lead). AKA: Conceptualization, Supervision, Project administration, Writing - review & editing, Funding acquisition. All authors read and approved the final manuscript.

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Figures

Figure 1

Naphthaldimine-glucofuranose conjugates 1 and 2.

Figure 2

UV-vis absorption study of sensor 1 (95 μ M) in different solvents such as DMSO, DMSO-H₂O, EtOH, and CH₃CN.

Figure 3

Observed colors after addition of different metal ions (2 equiv.) to the solution of 1.

Figure 4

(a) Absorption spectra of 1, 1 + Fe²⁺ (2 equiv.) and 1 + Cu²⁺ (2 equiv.) in DMSO. (b) Bar diagram represents the absorption intensity at 420 nm of receptor 1 (95 μM) before and after the addition of 2 equiv. of various metal ions.

Figure 5

UV-vis titration spectra of sensor 1 (95 μM) with increasing concentration of (a) Fe²⁺ ion (10 mM) and (b) Cu²⁺ ion (10 mM)

Figure 6

Stoichiometric calculations of 1 (95 μM) for (a) Fe^{2+} and (b) Cu^{2+} by Job's plots in DMSO using UV-vis spectroscopy.

Figure 7

Benesi-Hildebrand plot of (a) $1/\text{Fe}^{2+}$ and (b) $1/\text{Cu}^{2+}$ complex at 420 nm in DMSO. Calculated detection limits of 1 (95 μM) for (c) Fe^{2+} and (d) Cu^{2+} at 420 nm from UV-vis titration data.

Figure 8

Competitive absorbance of 1 (95 μ M in DMSO) at 420 nm (a) with the presence of 2 equiv. of Fe²⁺ (green bar) and then the addition of 2 equiv. of other metal ions to the same solution (pink bar). (b) with the presence of 2 equiv. of Cu²⁺ (green bar) and then addition of 2 equiv. of other metal ions to the same solution (pink bar).

Figure 9

Reversible absorption changes of 1 (95 μM) after the sequential addition of (a) Fe²⁺ (b) Cu²⁺ with Na₂EDTA in DMSO. Reversible colorimetric changes of 1 (95 μM) after the sequential addition of (c) Fe²⁺ (d) Cu²⁺ with Na₂EDTA in DMSO.

Figure 10

Fluorescence spectra of (a) 1 (95 μM) and (b) 2 (95 μM) in DMSO with the presence and absence of metal ions ($\lambda_{\text{ex}} = 410 \text{ nm}$).

Figure 11

Fluorescence changes of receptor 1 (95 μM) with continuous addition of (a) Fe^{2+} and (b) Cu^{2+} ions in DMSO.

Figure 12

Benesi-Hildebrand plot of (a) 1-Fe^{2+} and (b) 1-Cu^{2+} complex at 465 nm from fluorescence titration experiments in DMSO. Calculated detection limits of 1 (95 μM) for (c) Fe^{2+} and (d) Cu^{2+} ions in DMSO.

Figure 13

Partial ^1H NMR (400 MHz) spectra of **1** (1.08 mM) in d_6 -DMSO upon gradual addition of (i) 0 equiv., (ii) 0.1 equiv., (iii) 0.2 equiv., (iv) 0.5 equiv. (v) 1 equiv. of Fe^{2+} .

Figure 14

B3LYP/SDD Energy minimized structures of the (a) 1-Fe^{2+} and (b) 1-Cu^{2+} complexes by DFT calculations.

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