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A Sensitive Schiff-Base Fluorescent Chemosensor for the Selective Detection of Zn^{2+}

Nayan Roy · Harun A. R. Pramanik · Pradip C. Paul · Sanjoy T. Singh

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Abstract A Schiff-base fluorescent probe – N, N′-bis(salicylidene) trans 1, 2 – diaminocyclohexane **(H_2L)** was synthesized and evaluated as a chemoselective Zn^{2+} sensor. Upon treatment with Zn^{2+}, the complexation of **H_2L** with Zn^{2+} resulted in a bathochromic shift with a pronounced enhancement in the fluorescence intensity in ethanol solution. Moreover, other common alkali, alkaline earth and transition metal ions failed to induce response or minimal spectral changes. Notably, this chemosensor could distinguish clearly Zn^{2+} from Cd^{2+}. The stoichiometric ratio and association constant were evaluated using Benesi – Hildebrand relation giving 1:1 stoichiometry. This further corroborated 1:1 complex formation based on Job's plot analyses.

Keywords Chemosensor · Zn(II) ion · Schiff base · Fluorescence · Selectivity · Association constant

Introduction

In recent years, more and more attentions have been drawn to devise ingenious fluorescent sensors capable of selective recognition and effectively detecting the presence of alkali, alkaline earth and transition metal ions because of their importance in biological systems as well as to environmental concerned [1, 2]. However, among different chemosensors, fluorescence-based ones present many advantages as fluorescence measurements are usually very sensitive, low cost, easily performed and versatile, offering subnanometer spatial resolution with submicron visualisation and submillisecond temporal resolution [3, 4].

Nowadays, among the different analyte, special interest is devoted to develop chemosensors for transition metal ions: usually they represent an environmental concern when present in uncontrolled amounts, but at the same time some of them such as iron, zinc, copper and cobalt are present as essential elements in biological systems. Zinc is the second most abundant transition metal ion in the human body after iron, and plays a myriad of roles in numerous cellular functions such as regulation of gene expression, apoptosis, co-factors in metalloenzyme catalysis and neurotransmission in biological systems [5, 6]. Many server neurological diseases, including Alzheimer's disease, cerebral ischemia and epilepsy [7–9] are associated with the disorder of Zn^{2+} metabolism. Therefore, estimation of Zn^{2+} is very important in neurobiology. However, there is a great need for developing Zn^{2+} selective sensors that can distinguish Zn^{2+} from other transition metal ions especially Cd^{2+}, because they have very similar chemical properties often respond together with similar spectral changes.

The nitrogen atom of azomethine C=N double bond in Schiff base also exhibits a strong affinity for transition metal ions. Therefore, the Schiff base are known to be good ligand for metal ions [10, 11] and used to develop chemosensors. A number of the Schiff base metal complexes have antitumor properties [12], antioxidative activities [13], and attractive electronic and photophysical properties [14]. In addition, Schiff base derivatives incorporating a fluorescent moiety are appealing tools for optical sensing of metal ions. Tetradentate ligands such as salen- or pyridine-type symmetrical Schiff bases are capable of forming complexes with certain metal ions which can exhibit unusual coordination, high thermodynamic stability, good fluorescent properties and biological activities [15]. However, controllable synthesis of these compounds is a great challenge because many factors

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may affect their self-assembly, in which the coordination geometry of the complex depends upon the chemical structure of ligand chosen, the coordination geometry preferred by metal, pH dependent, metal-to-ligand ratio, reaction temperature, solvent system [16]. Nowadays, designing and synthesis of fluorescent sensors with high selectivity and sensitivity to metal ions is an important and vibrant field. Having much availability of commercial metal ions sensors, chemists still continue endeavouring to design new ones to improve their sensitivity, selectivity and reliability in order to satisfy various needs that are due to the wide existence of metal ions in organisms and its extensive significance. Many excellent metal ions sensors has been contributed significantly based on quinoline, anthracene, BODIPY, benzoxazole and fluorescein as fluorophore [17, 18], but some of the reported synthesis methods are always too complicated. In this paper, we have designed and synthesized a Schiff-base fluorescent probe – N, N$^{/}$-bis(salicylidene) trans 1, 2 – diaminocyclohexane **(H_2L)** by one step condensation of salicylaldehyde and 1, 2 – diaminocyclohexane in absolute ethanol (Scheme 1) and characterized by FT-IR, ^{1}H and ^{13}C NMR and elemental analysis. Photophysical behaviors of **H_2L** were studied in different homogeneous solvents and in presence of different metal ions, focussing the attention on their absorption and emission properties using steady-state absorption and fluorescence spectroscopy.

Till now, a variety of fluorescent sensors for Zn^{2+} have been developed with successful applications [19–26]. However, many of these sensors suffer from interference of some heavy metal ions and transition metal ions. In addition, some available Zn^{2+} sensors have difficulty in distinguishing Zn^{2+} and Cd^{2+}, because they have very similar chemical properties often respond together with similar spectral changes including change in fluorescence intensity and the wavelength shift. Herein, we have observed its prominent fluorescence enhancement in the presence of Zn^{2+}, while there was no enhancement in presence of other metal ions. In particular, it was able to distinguish Zn^{2+} from Cd^{2+}.

Materials and Experimental Method

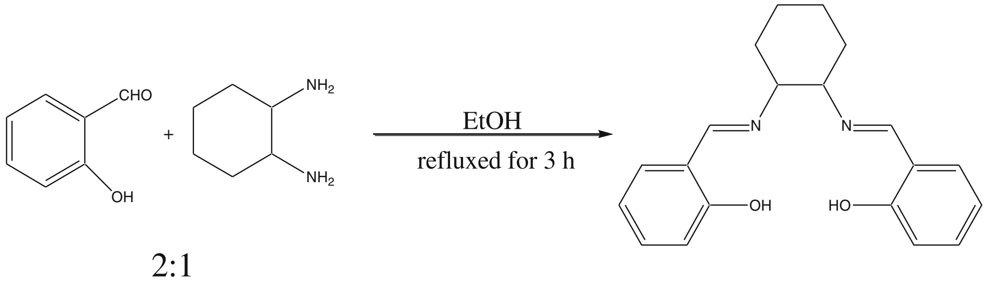
Materials

Salicylaldehyde and 1, 2 – diaminocyclohexane were obtained from Aldrich Chemical Company. All the spectroscopic grade solvents used were obtained from Sisco Research Laboratory (SRL) Pvt. Ltd and in some cases, from Aldrich Chemical Company. Chemical reagents were obtained from Lancaster as well as S.D. Fine Chemical Ltd and used without further purification. All experimental solutions of varying pH were made with buffer (Qualigen). The analytical grade type – II water used in the measurements was obtained from Elix10 water purification system (Millipore India Pvt. Ltd.). All experiments were carried out at room temperature (293 K) unless mentioned otherwise.

Synthesis and Characterization of N, N$^{/}$-bis(salicylidene) Trans 1,2 – Diaminocyclohexane (H_2L)

A portion of salicylaldehyde (1.22 g, 10 mmol) and 1, 2 – diaminocyclohexane (0.58 g, 5 mmol) were separately dissolved in absolute ethanol and combined together to get yellow colour solution. The solution was stirred under reflux conditions for 3 h in presence of 2–3 drops of acetic acid, and precipitate was filtrated, washed with cold absolute ethanol three times, then recrystallized with ethanol/chloroform (1/3, v/v) to get yellow microcrystal (**H_2L**) in 78 % yield. m.p. 147 °C; FT-IR (ν_{max}, cm^{-1}, KBr): 3410(νOH), 2985(ν_{as}(C-H), 2862(ν_s(C-H),), 1614(νC=N), 1562(νC=C), 1277(ν(C-O)), 1151(ν(C-N)), 758(δ(C-H)); ^{1}H NMR (400 MHz, $CDCl_3$, TMS, δ, ppm): 13.1 (br s, H-5, H-5$^{/}$), 8.6 (s, H-6, H-6$^{/}$), 7.2–7.5 (m, H-1, H-1$^{/}$, H-3, H-3$^{/}$, H-7, H-7$^{/}$, H-8, H-8$^{/}$), 7.0 (d, *J*=8.2 Hz, H-4, H-4$^{/}$), 6.8 (t, *J*=4.0Hz, H-2, H-2$^{/}$); ^{13}C NMR (400 MHz, $CDCl_3$, TMS, δ, ppm): 23.81, 28.95, 72.50, 115.89, 122.99, 131.12, 159.86, 162.15; Anal. Calcd. for $C_{20}H_{16}N_2O_2$ (316.20): C, 75.96 %; H, 5.06 %; N, 8.85 %. Found: C, 76.01 %; H, 5.00 %; N, 8.92 %.

Scheme 1 Reaction scheme for the synthesis of N, N$^{/}$-bis(salicylidene) trans 1,2-diaminocyclohexane (**H_2L**)



H_2L

Physical Measurements

Elemental analyses were carried out using PE2400 elemental analyser. The infra-red spectra were recorded on a PerkinElmer L 120-000A spectrometer with KBr pellets in the range 4000–400 cm^{-1}. ^{1}H and ^{13}C Nuclear magnetic resonance spectra were recorded on Bruker DPX- 400 MHz spectrometer and chemical shifts are expressed in ppm using tetramethylsilane as internal standard.

Spectrophotometric and Spectrofluorimetric Measurements

Steady-state absorption spectra were recorded on a Shimadzu UV – 1601PC absorption spectrophotometer. Fluorescence spectra were obtained in a PerkinElmer LS 45 spectrofluorimeter and all the spectra were corrected for the instrument response function. Quartz cuvettes of 10 mm optical path length received from PerkinElmer, USA (part no. B0831009) and Hellma, Germany (type 111-QS) were used for measuring absorption and fluorescence spectra, respectively. In both fluorescence emission and excitation spectra measurements, 5 nm bandpass was used in the excitation and emission side. The linearity of the fluorescence emission versus concentration was checked in the concentration range used (10^{-5} to 10^{-6} M). Fluorescence quantum yields (φ_f) were calculated by comparing the total fluorescence intensity under the whole fluorescence spectral range with that of a standard (φ_f=0.546, quinine sulfate in 1 M sulfuric acid) using the following equation as described before [27].

$$\phi_f^i = \phi_f^s \cdot \frac{F^i}{F^s} \cdot \frac{1-10^{-A^s}}{1-10^{-A^i}} \cdot \left(\frac{n^i}{n^s}\right)^2 \qquad (1)$$

where F is the total fluorescence intensity under whole fluorescence spectral curve, A^i and A^s is the optical density of the sample and standard, respectively and η^i is the refractive index of the solvent at 293 K.

Results and Discussion

Steady-State Spectral Properties of H$_2$L in Pure Solvents

The absorption, emission and excitation spectra of **H$_2$L** in absolute ethanol solution at 293 K are reported in Fig. 1.

The absorption spectra of **H$_2$L** were obtained in different homogeneous solvents giving two different peak positions at 278 nm and 350 nm. The large value of molar extinction coefficient (ε ~1.2×10^5 M^{-1} cm^{-1}) indicates the absorption to be originated due to $\pi\pi^*$ transition of the benzene ring. However, the band at 278 nm is attributed to azomethine C=N double bond. These absorption spectra were coincident with the excitation spectra. The fluorescence emission spectra for **H$_2$L** shows main peak at 430 nm with a shoulder at around 375 nm upon excitation at 350 nm. The steady state spectral properties of **H$_2$L** in pure solvent were tabulated in Table 1. It is interesting to note that emission spectra does not show any appreciable change in different solvent except very prominent peak at around 375 nm and little broadening in the less polar protic solvents like acetonitrile, 1,4-dioxane and toluene. The fluorescence quantum yield (φ_f) values are fairly low and could be explained by nonradiative decay transitions to the low-lying nπ^* states of the nitrogen atoms [28]. The origin of these fluorescence bands can be attributed to specific species undergoing different excited state photophysics as mentioned below. Due to the present of azomethine nitrogen atom in the free Schiff base ligand, the phenolic OH group make it possible for intramolecularly hydrogen bonded OH group. So, this intramolecular hydrogen bond between the azomethine and phenolic hydrogen can undergo intramolecular proton translocation upon excitation. So, the largely Stokes shifted fluorescence is believed to be originated from intramolecular hydrogen bonded structure and due to isomerisation of the C=N double bond which usually resulted in weak fluorescence emission. The fluorescence in the blue side of the spectrum (~375 nm) can be attributed due to the spontaneous emission.

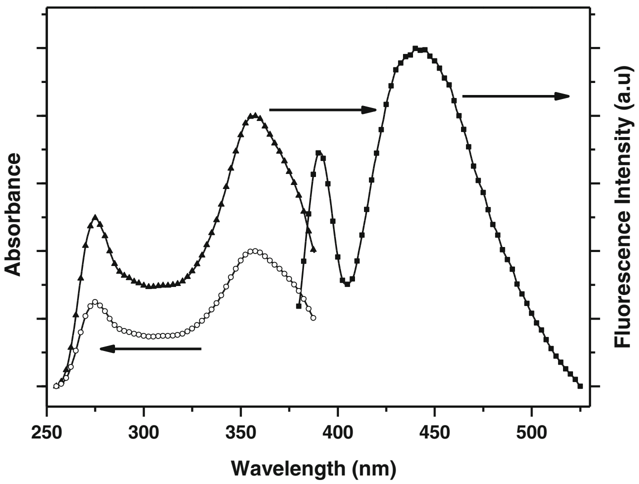


Fig. 1 Steady state absorption (*open circles*), fluorescence emission (*solid squares*, λ_{exc}=350 nm) and fluorescence excitation (*solid triangles*, λ_{emm}=430 nm) spectra of ligand (**H$_2$L**) in absolute ethanol

Metal Ions Titration

In order to explore metal ion binding and sensing ability of **H$_2$L** towards different metal ions, several titration followed by fluorescence emission were performed in ethanol solution at room temperature. Figure 2 demonstrates the changes in the fluorescence emission spectra of **H$_2$L** (2 μM) in ethanol in presence of different metal ions. The fluorescence maxima

Table 1 Steady state spectral properties of ligand (**H_2L**) in different homogeneous solvents

Solvents	λ_{abs} ^{a} (nm)	λ_{fl} ^{b} (nm)	$\Delta\nu_{SS}$ ^{c} (cm^{-1})	φ_F ^{d} (10^{-3})
Ethanol	268	375	1742	3.6
	332	432	6972	
Methanol	273	376	1904	2.8
	350	430	5315	
Acetonitrile	273	388	2475	3.4
	354	434	5207	
1,4-Dioxane	276	376	1975	2.6
	350	428	5206	
N,N-dimethylformamide	274	370	1062	2.4
	356	415	3993	
Toluene	295	376	1813	3.2
	352	428	5044	
Dimethylsulfoxide	273	380	1308	2.8
	362	414	3469	
Chloroform	278	372	440	5.6
	355	425	3792	
Ethyl acetate	267	376	1975	4.4
	350	428	5206	
Tetrahydrofuran	268	375	1742	4.8
	352	424	4824	

[a] Absorption maxima

[b] Fluorescence maxima

[c] Stokes shift ($\Delta\nu_{SS}$) calculated based on low-energy absorption band

[d] Fluorescence quantum yield (φ_F) error limit ±5 %

peak of **H_2L** (2 μM) appeared at 430 nm upon excitation at 350 nm. As soon as Zn^{2+} (30 μM) were added at room temperature, fluorescence maxima give red shift at 445 nm with large increased in fluorescence intensity (Fig. 2). However, in presence of other different metal ions like alkali (Na^+, K^+), alkaline earth (Ca^{2+}, Mg^{2+}, Sr^{2+}) and transition metal ions (Cd^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Ba^{2+} and Fe^{3+}) **H_2L** showed either no change in the fluorescence peak and very negligible amount of changes occurs in the fluorescence intensity (Fig. 2). So, there was no appreciable change in the fluorescence emission behavior of **H_2L** with other metal ions. Again, fluorescence intensity profile changes were also reported in the histogram (Fig. 3) which clearly indicates high selectivity of Zn^{2+}. Importantly, **H_2L** can distinguish Zn^{2+} from Cd^{2+}, whereas the discrimination of Zn^{2+} from Cd^{2+} is well known to be a major obstacle.

Furthermore, a metal ion selectivity study was performed for **H_2L** under same experimental conditions. The fluorescence intensity of **H_2L** (~2 μM) was unaffected upon addition of an excess amount of different metal ions. Further, tolerance of the fluorescence intensity due to Zn^{2+} (30 μM) in the presence of 50 times an excess of various metal ions like

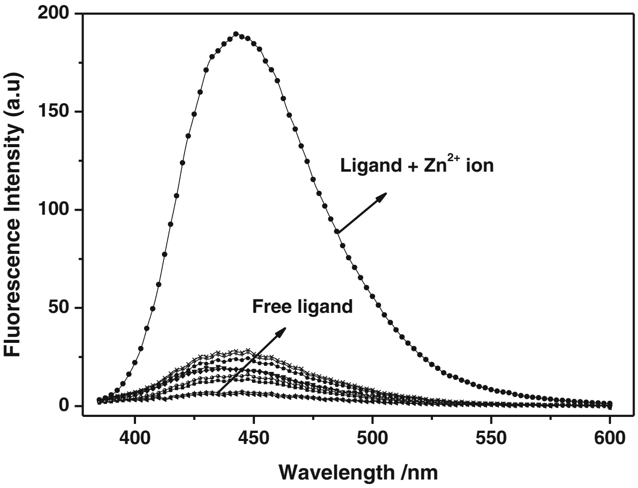


Fig. 2 Fluorescence spectra of **H_2L** in absence and presence of 30 μM concentration of different metal ions at room temperature. Excitation was done at λ_{exc}=350 nm

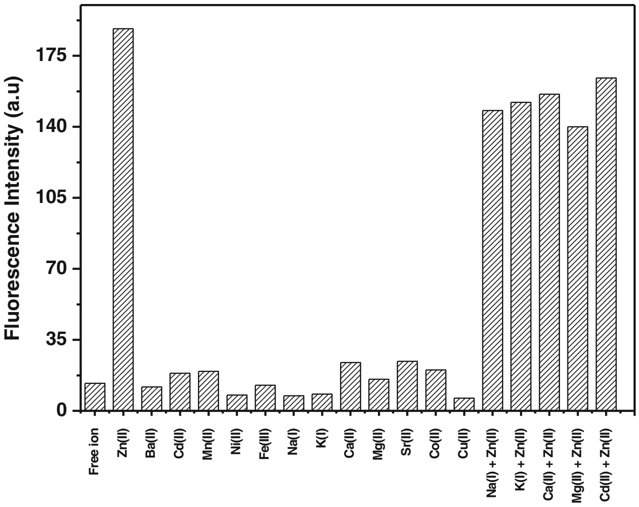


Fig. 3 Fluorescence intensity profile changes of **H_2L** at λ_{exc}=350 nm in absence and presence of 30 μM concentration of various metal ions at room temperature. Fluorescence intensity changes that occurs upon subsequent addition of Zn^{2+} (30 μM) in presence of different metal ions were also reported

Na^+, K^+, Cd^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ni^{2+}, Ba^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+} and Fe^{3+} has been successfully examined and verified as shown in Fig. 3, through competition experiments, with no significance changes on the fluorescence intensities. In contrast, the fluorescence emission intensity was found out to be reduced when it was mixed to the solution containing transition metal ions like Co^{2+} and Ni^{2+} which can be explain due to the fact that transition metal ions containing unoccupied molecular d-orbital quench the fluorescence of fluorophore [29]. So, all competitive metal ions had no obvious interference with the detection of the Zn^{2+}, which also indicates that, $\mathbf{H_2L}$ – Zn^{2+} complex was hardly affected by these coexistent metal ions, indicating high selectivity of $\mathbf{H_2L}$ towards Zn^{2+}.

Influence of Zn^{2+} Concentration

In an attempt to evaluate the influence of Zn^{2+} concentration, the fluorescence properties of $\mathbf{H_2L}$ were studied in ethanol solution with excitation at 350 nm and the fluorescence emission intensity was monitored at 430 nm. Here, the fluorescence intensity of $\mathbf{H_2L}$ was very weak which can be explain due to isomerization of C=N double bond. The C=N isomerization is the predominant decay process of the excited states for compounds with an unbridged C=N structure so that those compounds were very weak fluorescent or nonfluorescent. However, with gradual addition of Zn^{2+} concentration (saturated at~45 μM) to that of $\mathbf{H_2L}$, the fluorescence intensity yield increases with a red-shift of emission peak from 430 nm to 445 nm, which can be explain due to prevention of isomerization by metal ions binding (Fig. 4). The enhancement of fluorescence was attributed to the introduction of Zn^{2+} and consequently occurrence of the strong complexation with $\mathbf{H_2L}$, resulting in decreased non-radiative decay of the excited state and increased radiative decay.

However, this effect was not observed in presence of other metal ions like Na^+, K^+, Cd^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ni^{2+}, Ba^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+} and Fe^{3+} even with very high concentration (say~60 μM). The fluorescence quantum yields were also calculated both in the free ligand as well as in presence of Zn^{2+}. Here, the fluorescence quantum yield increases drastically from 3.6×10^{-3} for $\mathbf{H_2L}$ compared to 1.8×10^{-1} for $\mathbf{H_2L}$ – Zn^{2+} complex.

The possibility of using fluorescent chemosensor $\mathbf{H_2L}$ for determining Zn^{2+} in various homogeneous solvents like N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), methanol, ethanol, ethylacetate, tetrahydrofuran (THF), acetonitrile (ACN), toluene, chloroform and 1, 4-dioxane were also investigated and reported in Fig. 5. Here, the fluorescence intensity enhanced largely in addition of Zn^{2+} to certain solvents like THF, acetonitrile, ethylacetate, toluene and chloroform. We also knew that THF, acetonitrile, toluene and chloroform are hard to be coordinated for the lack of easy-coordinated atom which indicates that no solvent molecule contributes to the coordination. However this experimental data may be due to inherent complicated properties of the solvent based on their different polarity.

Stoichiometry of Complexation

For determination of stoichiometry between $\mathbf{H_2L}$ and Zn^{2+}, Job's plot analyses were used. The method is that keeping total concentration of $\mathbf{H_2L}$ and Zn^{2+} at 10.0 mM and changing the molar ratio of Zn^{2+} from 0.1 to 0.9. From Fig. 6 when molar fraction of Zn^{2+} was 0.5, the fluorescence maxima at

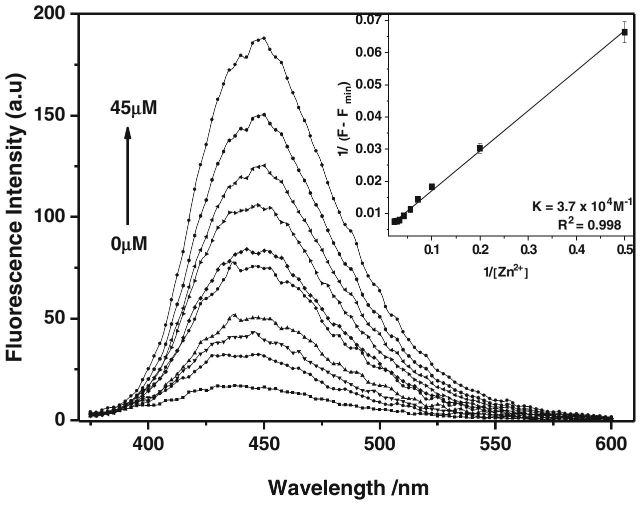


Fig. 4 Variation of fluorescence intensity of $\mathbf{H_2L}$ against concentration of Zn^{2+}. The concentrations of Zn^{2+} (μM) are: 0.0 (1), 2.0 (2), 5.0 (3), 10.0 (4), 18.0 (5), 24.0 (6), 30.0 (7), 35.0 (8), 40.0 (9), 45.0 (10). Inset shows the double reciprocal plot for 1:1 complex formation

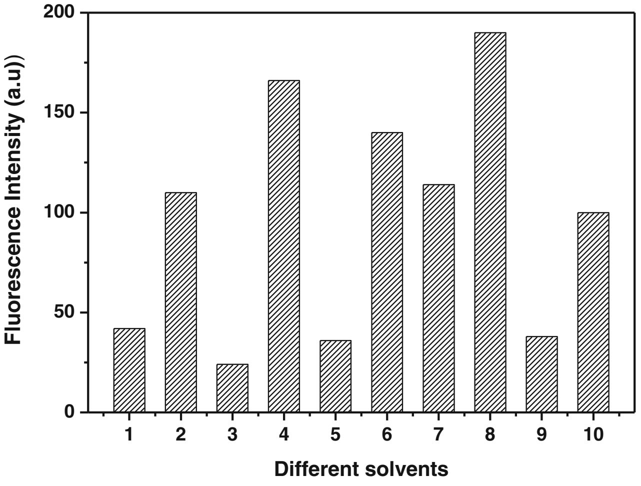


Fig. 5 Fluorescence intensity changes of $\mathbf{H_2L}$ in presence of Zn^{2+} (30 μM) in different solvents: **1** – methanol; **2** – ethanol; **3** – DMF; **4** – THF; **5** – 1, 4 – dioxane; **6** – ACN; **7** – ethylacetate; **8** – chloroform; **9** – DMSO; **10** – toluene at room temperature, respectively

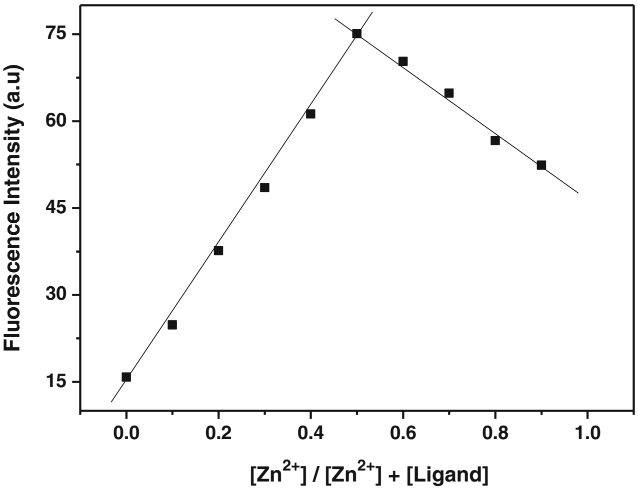


Fig. 6 Job's plot of $\mathbf{H_2L}$ with Zn^{2+} in ethanol solution

445 nm got to maximum, indicating that forming a 1:1 complex between $\mathbf{H_2L}$ and Zn^{2+}.

The stoichiometric ratio and apparent binding constant of ligand with Zn^{2+} was also determined using Benesi – Hildebrand relation as follows [30]

$$\frac{1}{F-F_0}=\frac{1}{F_\alpha-F_0}+\frac{1}{K(F_\alpha-F_0)}\times\frac{1}{[Zn^{2+}]} \tag{2}$$

where, F_0 and F are the fluorescence intensities in the absence and presence of Zn^{2+} respectively. F_α is the fluorescence intensity in the presence of excess amount of Zn^{2+}. Therefore, for 1:1 complex formation, the double reciprocal plot of $1/(F-F_0)$ against $1/[Zn^{2+}]$ should give a straight line; from the slope and intercept of which, the equilibrium constant (K) can be calculated. Inset of Fig. 4 shows the representative linear fitting using Eq. (2) and confirm 1:1 stoichiometry between $\mathbf{H_2L}$ and Zn^{2+}. The apparent K value obtained from the BH plot can be used as an initial guess in a non-linear regression (NLR) analysis to fit the fluorescence data directly using the following equation [31].

$$F=\frac{F_0+F_1K[Zn^{2+}]}{1+K[Zn^{2+}]} \tag{3}$$

where, $F_1=aF_0$, and a is the ratio of fluorescence quantum intensities of $\mathbf{H_2L}$ and $\mathbf{H_2L}$ – Zn^{2+}. The iterative NLR analysis based on Lavenberg – Marquardt algorithm was done using ORIGIN 8.0 (Microcal Inc.) software (Fig. 7). The association constant of $\mathbf{H_2L}$ with Zn^{2+} in ethanol solution was calculated to be 3.7×10^4 M^{-1}. Moreover, this further corroborated 1:1 complex formation based on Job's plot analyses.

Effect of pH

We also measured the fluorescence intensity of $\mathbf{H_2L}$ over a wide range of pH in absence and presence of Zn^{2+}. Over a wide range of pH, there was no obvious change in the fluorescence emission intensity of the ligand alone which indicates insensitivity to pH as shown in Fig. 8. However, in presence of Zn^{2+}, $\mathbf{H_2L}$ had a strong pH dependent even though it had a very weak fluorescence response to Zn^{2+} in the acidic environment because of protonation of the phenolic hydroxyl [32] leading to a weak coordination ability of Zn^{2+} [33]. However, satisfactory Zn^{2+} sensing abilities were exhibited when pH was increased from 8.0 to 11.5 (Fig. 8). Thus, $\mathbf{H_2L}$ indicates good fluorescence sensing ability to Zn^{2+} over a wide range of pH=8.0–11.5, giving almost constant emission intensity in the physiological conditions. These results indicate that $\mathbf{H_2L}$ can be employed as a selective fluorescent

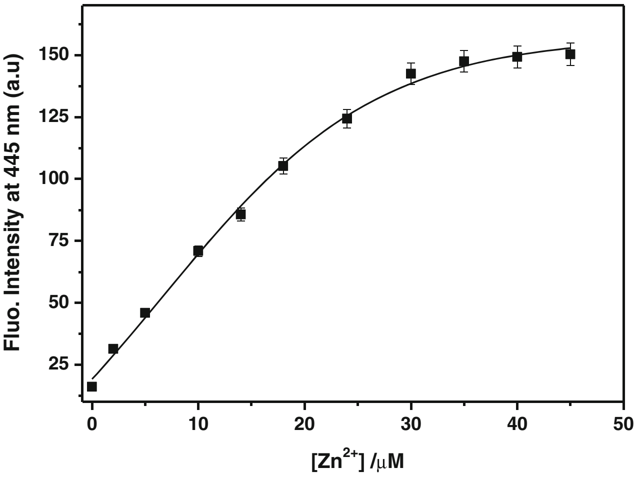


Fig. 7 Variation of fluorescence intensity of $\mathbf{H_2L}$ at λ_{exc}=350 nm against concentration of Zn^{2+} to determine the association constant

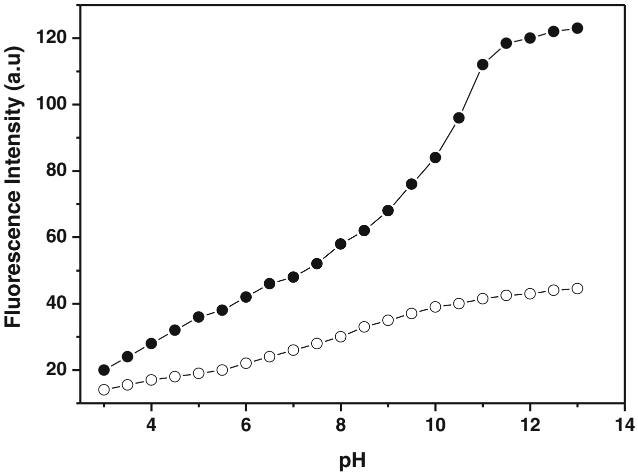


Fig. 8 Effect of pH on the fluorescence intensity of $\mathbf{H_2L}$ in absence (○) and presence (●) of Zn^{2+}

probe to recognize and distinguish Zn^{2+} in presence of other different metal ions.

Effect of Different Substituent on Fluorescence Intensity of H_2L

Metal-ligand complexes have been studied showing both the affect on the fluorescence emission wavelength and intensity of the ligand through metal coordination [34]. Moreover, the luminescent properties of Zn(II) complexes were reported to be determined by the organic ligand because of the electronic configuration of Zn^{2+} ($3d^{10}4s^0$) where the d-shell are completely filled which causes lack of intrinsic spectroscopic or magnetic signal [35]. Herein, the fluorescence behaviors of $\mathbf{H_2L}$ and complexes with different substituent of Zn^{2+} were studied in solution phase at room temperature. Here, the fluorescence emission spectra of complexes are very similar with $\mathbf{H_2L}$ except for the fluorescence emission intensity and maximum peak position, indicating that the fluorescence of complexes is L-based emission. Meanwhile, the fluorescence emission for the complexes were red-shifted compared to $\mathbf{H_2L}$ showing maximum emission wavelength at 430 nm for $\mathbf{H_2L}$ (**1**), whereas 445 nm, 447 nm and 448 nm for the complexes (**2–4**), respectively. Comparing the emission spectra of the corresponding $\mathbf{H_2L}$ varying degrees of red shifts has been observed in complexes, which are considered to mainly arise from the coordination of three Zn(II) centres to the ligand. The incorporation of Zn^{2+} effectively increases the conformational rigidity of the ligand (**1**) and enhanced fluorescence intensities of all three complexes (**2–4**), which shows that it is good candidate material in photochemical applications of these complexes [36]. Moreover, from Fig. 9, the different in fluorescence intensities of the complexes with different substituent's like NO_2, Cl and CH_3COO, can be explain due to bigger conformational rigidity for a 3D supramolecular network, as well as hydrogen bonds and π….π packing interactions [37].

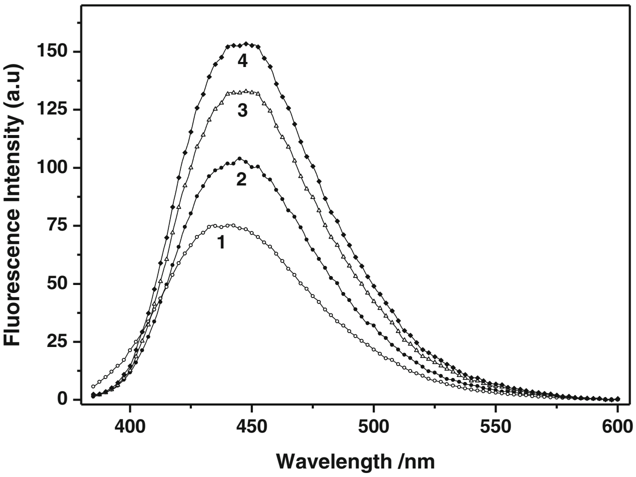


Fig. 9 Fluorescence emission spectra of $\mathbf{H_2L}$ in complexes with different metal ions substituents; ligand (**1** – free ion) and complexes (**2** – $Zn(CH_3COO)_2$, **3** – $ZnCl_2$ and **4** – $Zn(NO_2)_2$) in the solution phase at room temperature, respectively temperature, respectively

Conclusions

A Schiff-base fluorescent compound–N,N/-bis(salicylidene) trans 1, 2 – diaminocyclohexane **($\mathbf{H_2L}$)** was synthesized and evaluated as a chemoselective Zn^{2+} sensor. Addition of Zn^{2+} to ethanol solution of $\mathbf{H_2L}$ resulted in a red-shift with a pronounced enhancement in the fluorescence intensity while many other common alkali, alkaline earth and transition metal ions failed to induce response or minimal spectral changes. Most importantly, $\mathbf{H_2L}$ could differentiate Zn^{2+} from Cd^{2+}. Fluorescence studies on free Schiff base ligand **($\mathbf{H_2L}$)** and $\mathbf{H_2L}$ – Zn^{2+} complex reveal that the quantum yield strongly increases upon coordination. The stoichiometric ratio and association constant were evaluated using Benesi – Hildebrand relation giving 1:1 stoichiometry. This further corroborated 1:1 complex formation based on Job's plot analyses. Further, $\mathbf{H_2L}$ exhibits good fluorescence sensing ability to Zn^{2+} over a wide range of pH=8.0–11.5, giving almost constant emission intensity in the physiological conditions. The incorporation of Zn^{2+} effectively increases the conformational rigidity of the ligand and enhanced fluorescence intensities of the complex, which shows that it is good candidate material in photochemical applications of these complexes.

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References

1. Krämer R (1998) Fluorescent chemosensors for Cu^{2+} ions: fast, selective and highly sensitive. Angew Chem Int Ed 37:772–773
2. Uauy R, Olivares M, Gonzalez M (1998) Essentiality of copper in humans. Am J Clin Nutr 67:952–959
3. De Silva AP, Gunaratne HQN, Gunnlaugsson T, Huxley AJM, McCoy CP, Rademacher JT, Rice TE (1997) Signaling recognition events with fluorescent sensors and switches. Chem Rev 97:1515–1566
4. Czarnik AW (1992) Fluorescent chemosensors for ion and molecule recognition, 1st edn. ACS Washington, Washington DC
5. Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. Physiol Rev 73:79–118
6. De Silva JJRF, Williams RJP (2001) The biological chemistry of elements: The inorganic chemistry of life, 2nd edn. Oxford University Press, New York
7. Bush AI, Pettingell WH, Multhaup G, Paradis M, Vonsattel JP, Gusella JF, Beyreuther K, Masters CL, Tanzi RE (1994) Rapid induction of Alzheimer A beta amyloid formation by zinc. Science 265:1464–1467

8. Koh JY, Suh SW, Gwag BJ, He YY, Hsu CY, Choi DW (1996) The role of zinc in selective neuronal death after transient global cerebral ischemia. Science 272:1013–1016
9. Walker CF, Black RE (2004) Zinc and the risk for infectious disease. Annu Rev Nutr 24:255–275
10. Salmon L, Thuéry P, Rivière E, Ephritikhine M (2006) Synthesis, structure and magnetic behavior of a series of trinuclear schiff base complexes of 5f (UIV, ThIV) and 3d (CuII, ZnII) ions. Inorg Chem 45:83–93
11. Epstein DM, Choudhary S, Churchill MR, Keil KM, Eliseev AV, Morrow JR (2001) Chloroform-soluble schiff-base Zn(II) or Cd(II) complexes from a dynamic combinatorial library. Inorg Chem 40: 1591–1596
12. Da Silveira VC, Luz JS, Oliveira CC, Graziani I, Ciriolo MR, Ferreira AM (2008) Double-strand DNA cleavage induced by oxindole-schiff base copper(II) complexes with potential antitumor activity. J Inorg Biochem 102:1090–1103
13. Li Y, Yang ZY (2009) DNA binding affinity and antioxidative activity of copper (II) and zinc (II) complexes with a novel hesperetin Schiff base ligand. Inorg Chim Acta 362:4823–4831
14. Kasselouri S, Garoufis A, Katehanakis A, Kalkanis G, Perlepes SP, Hadjiliadis N (1993) 1:1 metal complexes of 2-(2′-pyridyl) quinoxaline, a ligand unexpectedly formed by the reaction between 2-acetylpyridine and 1,2-phenylenediamine. Inorg Chim Acta 207: 255–258
15. Morris GA, Zhou H, Stern CL, Nguyen ST (2001) A general high-yield route to Bis(salicylaldimine) Zinc(II) complexes: application to the synthesis of pyridine-modified salen-type zinc(II) complexes. Inorg Chem 40:3222–3227
16. Chen SC, Zhang ZH, Huang KL, Chen Q, He MY, Cui JA, Li C, Liu Q, Du M (2008) Solvent-controlled assembly of manganese(II) tetrachloroterephthalates with 1D chain, 2D layer and 3D coordination architectures. Cryst Growth Des 8:3437–3445
17. Pearce DA, Jotterand N, Carrico IS, Imperiali B (2001) Derivatives of 8-Hydroxy-2-methylquinoline are powerful prototypes for zinc sensors in biological systems. J Am Chem Soc 123:5160–5161
18. Zhang Y, Guo XF, Si WX, Jia LH, Qian XH (2008) Ratiometric and water-soluble fluorescent zinc sensor of carboxamidoquinoline with an alkoxyethylamino chain as receptor. Org Lett 10:473–476
19. Wong BA, Friedle S, Lippard SJ (2009) Solution and fluorescence properties of symmetric dipicolylamine-containing dichlorofluorescein-based Zn^{2+} sensors. J Am Chem Soc 131:7142–7152
20. Tomat E, Nolan EM, Jaworski J, Lippard SJ (2008) Organelle-specific zinc detection using zinpyr-labeled fusion proteins in live cells. J Am Chem Soc 130:15776–15777
21. Hanaoka K, Kikuchi K, Kojima H, Urano Y, Nagano T (2004) Development of a zinc ion-selective luminescent lanthanide chemosensor for biological applications. J Am Chem Soc 126: 12470–12476
22. Qian F, Zhang C, Zhang Y, He W, Gao X, Hu P, Guo Z (2009) Visible light excitable Zn^{2+} fluorescent sensor derived from an intramolecular charge transfer fluorophore and its in vitro and in vivo application. J Am Chem Soc 131:1460–1468
23. Xu Z, Baek KH, Kim HN, Cui J, Qian X, Spring DR, Shin I, Yoon J (2010) Zn^{2+}-triggered amide tautomerization produces a highly Zn^{2+}-selective, cell-permeable and ratiometric fluorescent sensor. J Am Chem Soc 132:601–610
24. Xu Z, Yoon J, Spring DR (2010) Fluorescent chemosensors for Zn^{2+}. Chem Soc Rev 391:1996–2006
25. Choi JY, Kim D, Yoon J (2013) A highly selective “turn-on” fluorescent chemosensor based on hydroxy pyrene–hydrazone derivative for Zn^{2+}. Dyes Pigments 96:176–179
26. Guo Z, Kim GH, Shin I, Yoon J (2012) A cyanine-based fluorescent sensor for detecting endogenous zinc ions in live cells and organisms. Biomaterials 33:7818–7827
27. Singh TS, Mitra S, Chandra AK, Tamai N, Kar S (2008) A combined experimental and theoretical study on photoinduced intramolecular charge transfer in *trans*-ethyl *p*-(dimethylamino)cinamate. J Photochem Photobiol A Chem 197:295–305
28. Rettig W, Lapouyade R, Lakowicz JR (2002) Topics in fluorescence spectroscopy: Probe design and chemical sensing, 3rd edn. Plenum Press, New York, pp 109–149
29. Gunnlaugsson T, Lee TC, Parkesh R (2004) Highly selective fluorescent chemosensors for cadmium in water. Tetrahedron 60:11239–11249
30. Benesi HA, Hildebrand JH (1949) A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbon. J Am Chem Soc 71:2703–2707
31. Mitra S, Das R, Mukherjee S (1998) Intramolecular proton transfer in inclusion complexes of cyclodextrins: role of water and highly polar nonaqueous media. J Phys Chem B 102:3730–3735
32. Roy P, Dhara K, Manassero M, Ratha J, Banerjee P (2007) Selective fluorescence zinc ion sensing and binding behavior of 4-Methyl-2,6-bis(((phenylmethyl)imino)methyl) phenol: biological application. Inorg Chem 46:6405–6412
33. Liu Y, Zhang N, Chen Y, Wang L (2007) Fluorescence sensing and binding behavior of aminobenzenesulfonamido- quinolino-β-cyclodextrin to Zn^{2+}. Org Lett 9:315–318
34. Wu G, Wang XF, Okamura T, Sun WY, Ueyama N (2006) Syntheses, structures and photoluminescence properties of metal(II) halide complexes with pyridine-containing flexible tripodal ligands. Inorg Chem 45:8523–8532
35. Kotova OV, Eliseeva SV, Averjushkin AS, Lepnev LS, Vaschenko AA, Rogachev AY, Vitukhnovskii AG, Kuzmina NP (2008) Zinc(II) complexes with Schiff bases derived from ethylenediamine and salicylaldehyde: the synthesis and photoluminescent properties. Russ Chem Bull Int Ed 57:1880–1889
36. Majumder A, Rosair GM, Mallick A, Chattopadhyay N, Mitra S (2006) Synthesis, structures and fluorescence of nickel, zinc and cadmium complexes with the N, N, O-tridentate Schiff base N-2-pyridylmethylidene-2-hydroxy-phenylamine. Polyhedron 25:1753–1762
37. Kang B, Weng L, Liu H, Wu D, Huang L, Lu C, Cai J, Chen X, Lu JX (1990) Syntheses, structures and properties of vanadium, cobalt, and nickel compounds with 2-mercaptophenol. Inorg Chem 29:4873–4877