

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

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**Abstract** A Schiff-base fluorescent probe – N, N'-bis(salicylidene) trans 1, 2 – diaminocyclohexane (**H<sub>2</sub>L**) was synthesized and evaluated as a chemoselective  $\text{Zn}^{2+}$  sensor. Upon treatment with  $\text{Zn}^{2+}$ , the complexation of **H<sub>2</sub>L** with  $\text{Zn}^{2+}$  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  $\text{Zn}^{2+}$  from  $\text{Cd}^{2+}$ . 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  $\text{Zn}^{2+}$  metabolism. Therefore, estimation of  $\text{Zn}^{2+}$  is very important in neurobiology. However, there is a great need for developing  $\text{Zn}^{2+}$  selective sensors that can distinguish  $\text{Zn}^{2+}$  from other transition metal ions especially  $\text{Cd}^{2+}$ , 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<sub>2</sub>L**) by one step condensation of salicylaldehyde and 1, 2 – diaminocyclohexane in absolute ethanol (Scheme 1) and characterized by FT-IR, <sup>1</sup>H and <sup>13</sup>C NMR and elemental analysis. Photophysical behaviors of **H<sub>2</sub>L** 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<sup>2+</sup> 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<sup>2+</sup> sensors have difficulty in distinguishing Zn<sup>2+</sup> and Cd<sup>2+</sup>, 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<sup>2+</sup>, while there was no enhancement in presence of other metal ions. In particular, it was able to distinguish Zn<sup>2+</sup> from Cd<sup>2+</sup>.

## Materials and Experimental Method

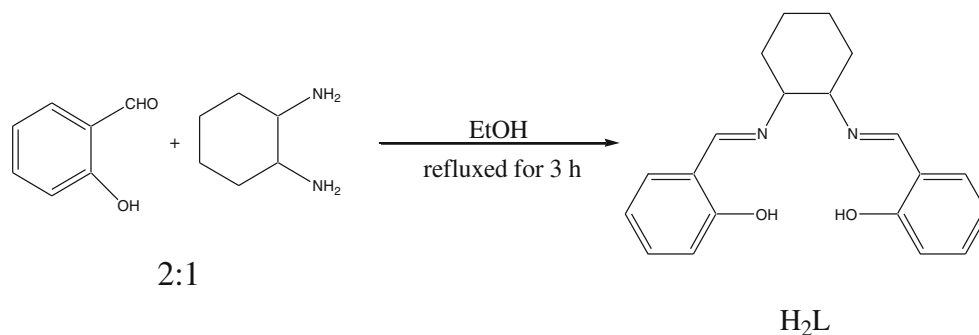
### Materials

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<sub>2</sub>L**)

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<sub>2</sub>L**) in 78 % yield. m.p. 147 °C; FT-IR ( $\nu_{\max}$ , cm<sup>-1</sup>, KBr): 3410( $\nu_{\text{OH}}$ ), 2985( $\nu_{\text{as}}(\text{C-H})$ ), 2862( $\nu_{\text{s}}(\text{C-H})$ ), 1614( $\nu(\text{C=N})$ ), 1562( $\nu(\text{C=C})$ ), 1277( $\nu(\text{C-O})$ ), 1151( $\nu(\text{C-N})$ ), 758( $\delta(\text{C-H})$ ); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS,  $\delta$ , 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,  $J=4.0$ Hz, H-2, H-2'); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>, TMS,  $\delta$ , ppm): 23.81, 28.95, 72.50, 115.89, 122.99, 131.12, 159.86, 162.15; Anal. Calcd. for C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub> (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<sub>2</sub>L**)



## 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  $\text{cm}^{-1}$ .  $^1\text{H}$  and  $^{13}\text{C}$  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 ( $\phi_f$ ) were calculated by comparing the total fluorescence intensity under the whole fluorescence spectral range with that of a standard ( $\phi_f=0.546$ , quinine sulfate in 1 M sulfuric acid) using the following equation as described before [27].

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

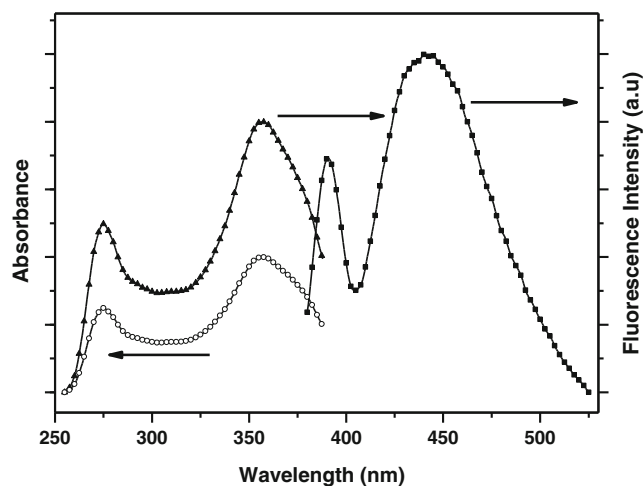
where  $F$  is the total fluorescence intensity under whole fluorescence spectral curve,  $A^i$  and  $A^s$  is the optical density of the sample and standard, respectively and  $n^i$  is the refractive index of the solvent at 293 K.

## Results and Discussion

### Steady-State Spectral Properties of $\text{H}_2\text{L}$ in Pure Solvents

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

The absorption spectra of  $\text{H}_2\text{L}$  were obtained in different homogeneous solvents giving two different peak positions at 278 nm and 350 nm. The large value of molar extinction coefficient ( $\epsilon \sim 1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ ) 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



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

double bond. These absorption spectra were coincident with the excitation spectra. The fluorescence emission spectra for  $\text{H}_2\text{L}$  shows main peak at 430 nm with a shoulder at around 375 nm upon excitation at 350 nm. The steady state spectral properties of  $\text{H}_2\text{L}$  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 ( $\phi_f$ ) values are fairly low and could be explained by nonradiative decay transitions to the low-lying  $n\pi^*$  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 ( $\sim 375$  nm) can be attributed due to the spontaneous emission.

### Metal Ions Titration

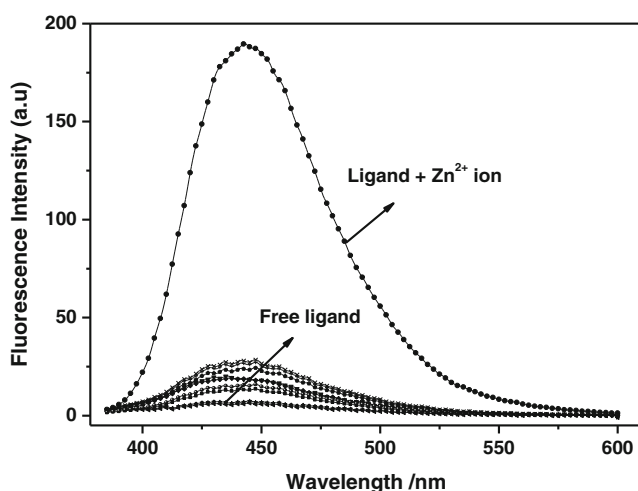
In order to explore metal ion binding and sensing ability of  $\text{H}_2\text{L}$  towards different metal ions, several titration followed by fluorescence emission were performed in ethanol solution at room temperature. Figure 2 demonstrates the changes in the fluorescence emission spectra of  $\text{H}_2\text{L}$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

**Table 1** Steady state spectral properties of ligand (**H<sub>2</sub>L**) in different homogeneous solvents

| Solvents              | $\lambda_{\text{abs}}^{\text{a}}$ (nm) | $\lambda_{\text{fl}}^{\text{b}}$ (nm) | $\Delta\nu_{\text{SS}}^{\text{c}}$ ( $\text{cm}^{-1}$ ) | $\Phi_{\text{F}}^{\text{d}}$ ( $10^{-3}$ ) |
|-----------------------|----------------------------------------|---------------------------------------|---------------------------------------------------------|--------------------------------------------|
| 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                                                    |                                            |

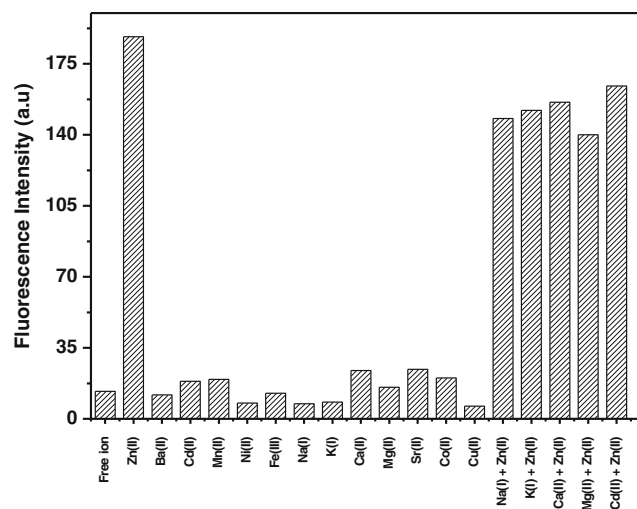
<sup>a</sup> Absorption maxima<sup>b</sup> Fluorescence maxima<sup>c</sup> Stokes shift ( $\Delta\nu_{\text{SS}}$ ) calculated based on low-energy absorption band<sup>d</sup> Fluorescence quantum yield ( $\Phi_{\text{F}}$ ) error limit  $\pm 5\%$ 

peak of **H<sub>2</sub>L** (2  $\mu\text{M}$ ) appeared at 430 nm upon excitation at 350 nm. As soon as  $\text{Zn}^{2+}$  (30  $\mu\text{M}$ ) 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 ( $\text{Na}^+$ ,  $\text{K}^+$ ), alkaline earth ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Sr}^{2+}$ ) and transition metal ions ( $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ba}^{2+}$  and  $\text{Fe}^{3+}$ ) **H<sub>2</sub>L** 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<sub>2</sub>L** with other metal ions. Again, fluorescence intensity profile changes were also

**Fig. 2** Fluorescence spectra of **H<sub>2</sub>L** in absence and presence of 30  $\mu\text{M}$  concentration of different metal ions at room temperature. Excitation was done at  $\lambda_{\text{exc}}=350$  nm

reported in the histogram (Fig. 3) which clearly indicates high selectivity of  $\text{Zn}^{2+}$ . Importantly, **H<sub>2</sub>L** can distinguish  $\text{Zn}^{2+}$  from  $\text{Cd}^{2+}$ , whereas the discrimination of  $\text{Zn}^{2+}$  from  $\text{Cd}^{2+}$  is well known to be a major obstacle.

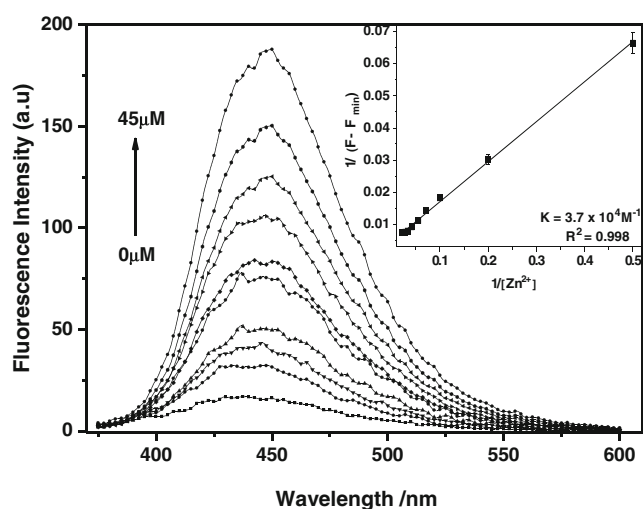
Furthermore, a metal ion selectivity study was performed for **H<sub>2</sub>L** under same experimental conditions. The fluorescence intensity of **H<sub>2</sub>L** ( $\sim 2$   $\mu\text{M}$ ) was unaffected upon addition of an excess amount of different metal ions. Further, tolerance of the fluorescence intensity due to  $\text{Zn}^{2+}$  (30  $\mu\text{M}$ ) in the presence of 50 times an excess of various metal ions like

**Fig. 3** Fluorescence intensity profile changes of **H<sub>2</sub>L** at  $\lambda_{\text{exc}}=350$  nm in absence and presence of 30  $\mu\text{M}$  concentration of various metal ions at room temperature. Fluorescence intensity changes that occurs upon subsequent addition of  $\text{Zn}^{2+}$  (30  $\mu\text{M}$ ) in presence of different metal ions were also reported

$\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Fe}^{3+}$  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  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  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  $\text{Zn}^{2+}$ , which also indicates that,  $\text{H}_2\text{L} - \text{Zn}^{2+}$  complex was hardly affected by these coexistent metal ions, indicating high selectivity of  $\text{H}_2\text{L}$  towards  $\text{Zn}^{2+}$ .

#### Influence of $\text{Zn}^{2+}$ Concentration

In an attempt to evaluate the influence of  $\text{Zn}^{2+}$  concentration, the fluorescence properties of  $\text{H}_2\text{L}$  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  $\text{H}_2\text{L}$  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  $\text{Zn}^{2+}$  concentration (saturated at  $\sim 45 \mu\text{M}$ ) to that of  $\text{H}_2\text{L}$ , 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  $\text{Zn}^{2+}$  and



**Fig. 4** Variation of fluorescence intensity of  $\text{H}_2\text{L}$  against concentration of  $\text{Zn}^{2+}$ . The concentrations of  $\text{Zn}^{2+}$  ( $\mu\text{M}$ ) 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

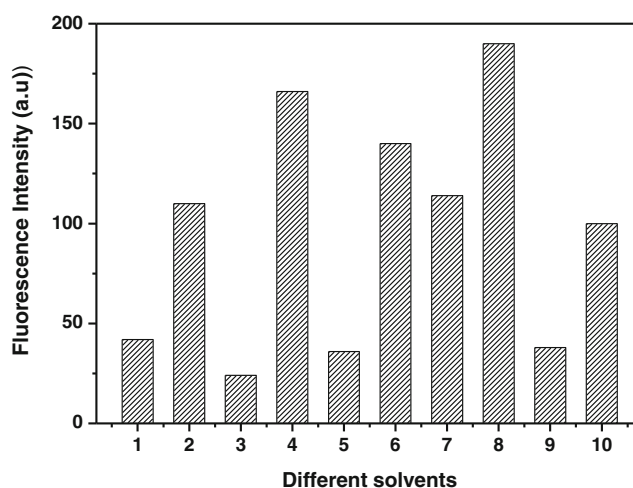
consequently occurrence of the strong complexation with  $\text{H}_2\text{L}$ , resulting in decreased non-radiative decay of the excited state and increased radiative decay.

However, this effect was not observed in presence of other metal ions like  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Fe}^{3+}$  even with very high concentration (say  $\sim 60 \mu\text{M}$ ). The fluorescence quantum yields were also calculated both in the free ligand as well as in presence of  $\text{Zn}^{2+}$ . Here, the fluorescence quantum yield increases drastically from  $3.6 \times 10^{-3}$  for  $\text{H}_2\text{L}$  compared to  $1.8 \times 10^{-1}$  for  $\text{H}_2\text{L} - \text{Zn}^{2+}$  complex.

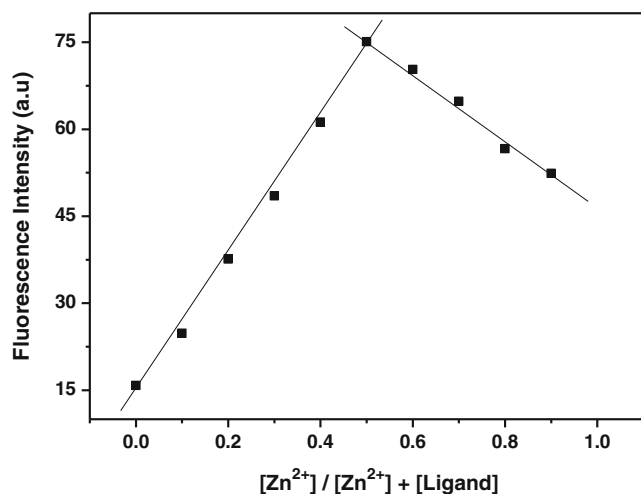
The possibility of using fluorescent chemosensor  $\text{H}_2\text{L}$  for determining  $\text{Zn}^{2+}$  in various homogeneous solvents like N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), methanol, ethanol, ethylacetate, tetrahydrofuran (THF), acetonitrile (ACN), toluene, chloroform and 1, 4-dioxane were also investigated and reported in Fig. 5. Here, the fluorescence intensity enhanced largely in addition of  $\text{Zn}^{2+}$  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  $\text{H}_2\text{L}$  and  $\text{Zn}^{2+}$ , Job's plot analyses were used. The method is that keeping total concentration of  $\text{H}_2\text{L}$  and  $\text{Zn}^{2+}$  at 10.0 mM and changing the molar ratio of  $\text{Zn}^{2+}$  from 0.1 to 0.9. From Fig. 6 when molar fraction of  $\text{Zn}^{2+}$  was 0.5, the fluorescence maxima at



**Fig. 5** Fluorescence intensity changes of  $\text{H}_2\text{L}$  in presence of  $\text{Zn}^{2+}$  ( $30 \mu\text{M}$ ) 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



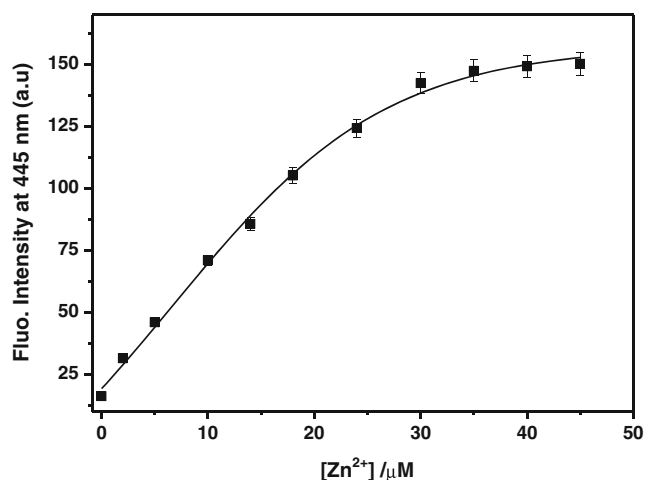
**Fig. 6** Job's plot of  $\text{H}_2\text{L}$  with  $\text{Zn}^{2+}$  in ethanol solution

445 nm got to maximum, indicating that forming a 1:1 complex between  $\text{H}_2\text{L}$  and  $\text{Zn}^{2+}$ .

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

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

where,  $F_0$  and  $F$  are the fluorescence intensities in the absence and presence of  $\text{Zn}^{2+}$  respectively.  $F_\alpha$  is the fluorescence intensity in the presence of excess amount of  $\text{Zn}^{2+}$ . Therefore, for 1:1 complex formation, the double reciprocal plot of  $1/(F-F_0)$  against  $1/[\text{Zn}^{2+}]$  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  $\text{H}_2\text{L}$



**Fig. 7** Variation of fluorescence intensity of  $\text{H}_2\text{L}$  at  $\lambda_{\text{exc}}=350$  nm against concentration of  $\text{Zn}^{2+}$  to determine the association constant

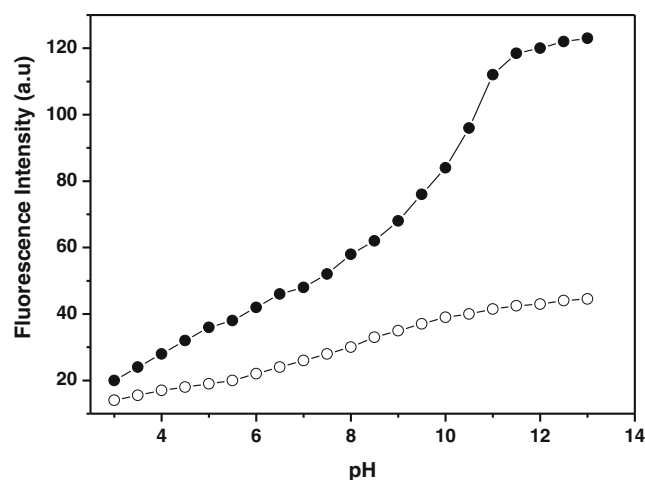
and  $\text{Zn}^{2+}$ . The apparent  $K$  value obtained from the BH plot can be used as an initial guess in a non-linear regression (NLR) analysis to fit the fluorescence data directly using the following equation [31].

$$F = \frac{F_0 + F_1K[\text{Zn}^{2+}]}{1 + K[\text{Zn}^{2+}]} \quad (3)$$

where,  $F_1=aF_0$ , and  $a$  is the ratio of fluorescence quantum intensities of  $\text{H}_2\text{L}$  and  $\text{H}_2\text{L} - \text{Zn}^{2+}$ . The iterative NLR analysis based on Lavenberg – Marquardt algorithm was done using ORIGIN 8.0 (Microcal Inc.) software (Fig. 7). The association constant of  $\text{H}_2\text{L}$  with  $\text{Zn}^{2+}$  in ethanol solution was calculated to be  $3.7 \times 10^4 \text{ M}^{-1}$ . Moreover, this further corroborated 1:1 complex formation based on Job's plot analyses.

#### Effect of pH

We also measured the fluorescence intensity of  $\text{H}_2\text{L}$  over a wide range of pH in absence and presence of  $\text{Zn}^{2+}$ . 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  $\text{Zn}^{2+}$ ,  $\text{H}_2\text{L}$  had a strong pH dependent even though it had a very weak fluorescence response to  $\text{Zn}^{2+}$  in the acidic environment because of protonation of the phenolic hydroxyl [32] leading to a weak coordination ability of  $\text{Zn}^{2+}$  [33]. However, satisfactory  $\text{Zn}^{2+}$  sensing abilities were exhibited when pH was increased from 8.0 to 11.5 (Fig. 8). Thus,  $\text{H}_2\text{L}$  indicates good fluorescence sensing ability to  $\text{Zn}^{2+}$  over a wide range of pH=8.0–11.5, giving almost constant emission intensity in the physiological conditions. These results indicate that  $\text{H}_2\text{L}$  can be employed as a selective fluorescent

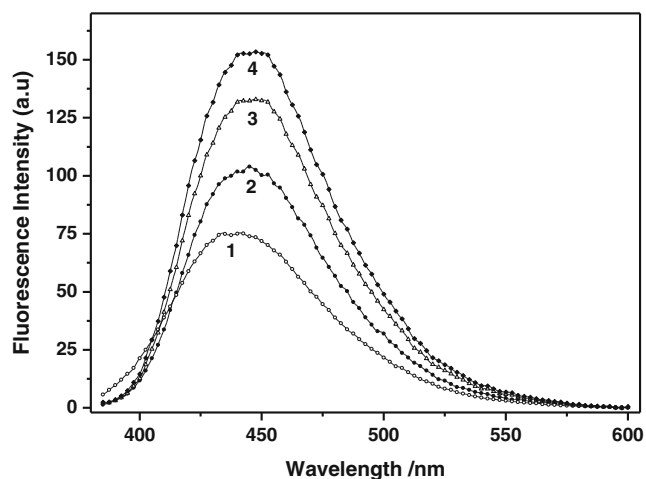


**Fig. 8** Effect of pH on the fluorescence intensity of  $\text{H}_2\text{L}$  in absence (○) and presence (●) of  $\text{Zn}^{2+}$

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  $H_2L$  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  $H_2L$  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  $H_2L$  showing maximum emission wavelength at 430 nm for  $H_2L$  (1), whereas 445 nm, 447 nm and 448 nm for the complexes (2–4), respectively. Comparing the emission spectra of the corresponding  $H_2L$  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



**Fig. 9** Fluorescence emission spectra of  $H_2L$  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

conformational rigidity for a 3D supramolecular network, as well as hydrogen bonds and  $\pi \dots \pi$  packing interactions [37].

#### Conclusions

A Schiff-base fluorescent compound– $N,N'$ -bis(salicylidene) trans 1, 2 – diaminocyclohexane ( $H_2L$ ) was synthesized and evaluated as a chemoselective  $Zn^{2+}$  sensor. Addition of  $Zn^{2+}$  to ethanol solution of  $H_2L$  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,  $H_2L$  could differentiate  $Zn^{2+}$  from  $Cd^{2+}$ . Fluorescence studies on free Schiff base ligand ( $H_2L$ ) and  $H_2L - Zn^{2+}$  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,  $H_2L$  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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