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PII:	S1387-7003(19)30076-0
DOI:	https://doi.org/10.1016/j.inoche.2019.02.020
Reference:	INOCHE 7278
To appear in:	Inorganic Chemistry Communications
Received date:	20 January 2019
Revised date:	7 February 2019
Accepted date:	15 February 2019

Please cite this article as: V. Venkatesan, S. Kumar R, S.K.A. Kumar, et al., Highly selective turn-on fluorogenic chemosensor for Zn2+ based on chelation enhanced fluorescence, Inorganic Chemistry Communications, https://doi.org/10.1016/j.inoche.2019.02.020

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Highly Selective Turn-On Fluorogenic Chemosensor for Zn²⁺ Based on Chelation Enhanced Fluorescence

Vetriarasu Venkatesan^a, Selva Kumar R^a, S.K. Ashok Kumar^{a,*} and Suban K. Sahoo^b

^a Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore-632014, Tamil Nadu, India. E-mail: ashok312002@gmail.com

^b Department of Applied Chemistry, S. V. National Institute Technology, Surat-395007, Gujarat, India.

ABSTRACT

A new fluorescent zinc sensor was constructed based on cyclic and noncyclic Schiff's bases obtained by the condensation of 4-(diethylamino) salicylaldehyde and 2-aminobenzenethio under two different catalytic conditions such as sulphuric acid yields 2-(benzo[d]thiazol-2-yl)-5-(diethylamino) phenol (**L**₁) and acetic acid gives to (E)-5-(diethylamino)-2-(((2-mercaptophenyl)imino)methyl) phenol (**L**₂). Both **L**₁ and **L**₂ alone exhibits a week fluorescence in CH₃OH/H₂O (1:1, v/v) due to excited state intra-molecular change transfer (ESIPT) process but upon interaction with Zn²⁺ shows strong fluorescence due to chelation-enhanced fluorescence (CHEF) but with selected metal ions, there is no such a fluorescence change was observed. The Job's plot and B-H plot studies were revealed the formation of 2:1 and 1:1 stoichiometry with an estimated association constant (K_a) of 4.9 x 10⁴ M⁻¹ and 2.1 x 10⁴ M⁻¹ with **L**₁ and **L**₂ respectively. The detection limit of both sensors **L**₁ and **L**₂ were found to be 6.7 x 10⁻⁸ M and 3.6 x10⁻⁷ M respectively. The fluorescence reversibility study was done by adding Zn²⁺ and EDTA sequentially to **L**₁ and **L**₂. Both sensors were successfully used for the determination of Zn²⁺ in the different water samples and pharmaceutical multivitamins tablet. The sensing mechanism was studied by ¹H NMR, ESI-mass analysis as well as theoretical calculations using the Gaussian 09 program.

Keywords: Schiff's base, Fluorescence, CHEF, Zinc, DFT study.

1. Introduction

Zinc is the second richest transition metal in the human body after iron, and it plays important role in numerous biological processes including regulation of enzymes, a physical cofactor in metalloproteins, neural signal transmission and gene expression [1–4]. But higher levels of Zn^{2+} in humans have been concerned in neurodegenerative syndromes [5–7]. In recent years, the development of fluorescent sensors for Zn^{2+} has become a very active area in the field of chemical biology [8,9]. Recognition of metal ions such as Zn^{2+} , Cd^{2+} , and Hg^{2+} is still a challenge since those metals do not show spectroscopic and magnetic responses due to its d^{10} system [10]. So, optical sensors based on ion-induced fluorescence changes afford an optimal choice to detect Zn^{2+} in biological and environmental samples due to their high simplicity, sensitivity, selectivity, response time and low detection limit [11–13]. Accordingly, an organic molecules possessing a Schiff's base structure has potential applications in the development of colorimetric chemosensors and fluorogenic sensors used for environmental sample monitoring [14–16].

It has been observed from the literature, molecules having α -hydroxy imine-based structure provides week fluorescence with large Stokes shift are due to the excited state intramolecular proton transfer (ESIPT) phenomenon [17–25]. These molecules when it interacts with zinc ions form a complex either 1:1 or 1:2 ratio, which restricts the ESIPT process and increases the chelation-enhanced fluorescence (CHEF) process [26-30]. Schiff bases are known to form stable complexes with transition metal ions and act as ion carriers. The key features of Schiff bases are providing a proper geometry, cavity control of host-gest complexation, modulation of its lipophilicity leads to outstanding selectivity, sensitivity and stability for a specific ion. Besides, the presences of N, O, and S as donor atoms are well known to form a strong complex with transition metal ions and such systems are used as a ion carrier in making fluorogenic sensors for Zn²⁺ [29–33]. In this work, we have a designed and synthesized ligands L₁ and L₂ using 4diethylamino salicylaldehyde and 2-amino thiophenol in methanol to undergo a simple condensation reaction to get new Schiff's bases. The designed ligands in this work are already synthesized and used for biological applications [34,35] but there is no report on the fluorogenic sensor for Zn²⁺ estimation.

2. Experimental section

2.1. Materials and methods

Metal nitrates, chloride salts, and solvents were purchased from SD fine and Merck chemicals (AR Grade). 2-aminobenzenethiol and 4-(diethylamino)-2-hydroxybenzaldehyde were purchased from Sigma Aldrich. The absorption spectra were recorded on a JASCO V-730, UV-visible spectrophotometer using a 1 cm quartz cell. Fluorescence measurements were carried out on a JASCO FP-8200 fluorescence spectrophotometer equipped with a xenon lamp and instrumental parameters were controlled by JASCO Spectra Manager 2. Mass spectra of the prepared compounds were recorded on a Perkin-Elmer Clarus 680 GC and Clarus 600 MS. NMR spectra were recorded using a 400 MHz Advance Bruker NMR spectrometer. ¹H chemical shift was recorded in ppm downfield from tetramethylsilane (TMS) as an internal standard. Fluorescence lifetime analysis was performed using a HORIBA Jobin-Yvon instrument. The geometry of L₁, L₂, and its Zn²⁺ complexes was optimized by density functional theory (DFT) using the Gaussian 09 program[36]. And the Becke-3-Lee-Yang-Parr (B3LYP) exchange-correlation functional method with the standard basis set 6-31G(d,p) for C, H, N, O and S atoms and the LANL2DZ effective core potential for Zn²⁺ atom[9].

2.2 Synthesis and characterization of L1 and L2

The ligand L_1 was synthesized slightly modified from reported procedure [34,37] by reacting 2aminobenzenethiol (0.125g, 1 eq) and 4-(Diethylamino) salicylaldehyde (0.19g, 1 eq) were taken in 20 mL of methanol in the presence of catalytic amount of sulphuric and contents were allowed to react at room temperature for an overnight. After completing the reaction, the precipitate was filtered, washed by methanol several times and kept for air drying, after vacuum drying offers a yellow solid with a yield of 90%. Whereas L_2 was prepared by reacting 2-aminobenzenethiol (0.5g, 1 eq) and 4-(diethylamino)-2-hydroxybenzaldehyde (0.77g, 1 eq) in 20 mL methanol solution in the presence of few drops of acetic acid as a catalyst and reaction mixture was stirred at room temperature under N₂ atmosphere for overnight. The progress of the reaction was monitored through by TLC. The product was filtered and washed with methanol offer the yellow solid with a yield of 85%.



Scheme 1. Reagents and conditions to synthesize L1 and L2.

L1: Molecular formula C₁₇H₁₈N₂OS, ¹H NMR (400MHz, CDCl₃) 1.94-2.23 (t, 6H), 3.37-3.43 (q, 4H), 6.27 (d, 2H), 7.422 (s, 1H), 7.45-7.47 (d, 2H), 7.79-7.78 (m, 2H), 12.56 (s, 1H), ¹³C NMR (100MHz, CDCl₃) 12.72, 44.58, 97.91, 104.16, 121.03, 121.23, 124.16, 126.25, 129.87, 131.92, 151.34, 152.26, 159.82, 169.60. HR-MS: 298.1820.

L₂: Molecular formula C₁₇H₂₀N₂OS, ¹H NMR (400MHz, CDCl₃) 1.142 (t, 6H), 3.34-3.41 (q, 4H), 6.13 (s, 1H), 6.37 (S, 1H), 7.19-7.30 (t, 1H), 7.39-7.42 (t, 1H), 7.44-7.49 (m, 3H), 8.78 (s, 1H), 13.04 (s, 1H). ¹³C NMR (100MHz, CDCl₃) 12.73, 44.69, 97.78, 103.9, 109.18, 117.05, 126.16, 126.27, 126.96, 131.22, 134.09, 146.83, 152.04, 160.79, 163.48. HR-MS: 300.2263.

2.3 Preparation of pharmaceutical samples for Zn²⁺ analysis

Zinc-containing commercially available multivitamin pharmaceutical samples contain 1mg of zinc in 1mg tablet and 1.25 ml syrup form were quantitatively taken in a beaker and digested with 5mL of HNO₃ on a sand bath till its dryness then added 5 ml of 20% of H₂SO₄ and continued the heating till the volume was reduced to 1.5 mL then cooled the contents to room temperature [35]. Ammonium hydroxide solution was added to neutralize the solution. Then the solution was filtrated and transferred to a 10 ml standard flask further solution was diluted using MeOH/H₂O (1:1, v/v) and the total amount of zinc is 1.5 mM. A proper dilution was made to bring the zinc concentration to 10 μ M during the analysis.

3. Results and discussion

The synthesized benzothiazole based ligand L_1 containing an acidic hydroxy group at *o*-position and N, N'-diethyl group at *p*-position with respect to the -N=C moiety. An essential condition for

ESIPT is the presence of H- bond between donor (-OH and NH₂) and proton acceptor (=N- and – C=O) groups in close vicinity to each other in a molecule. Whereas in the case of ligand L₂ containing one more acidic thiol group at *o*'-position. The location of these groups in such that there is an intra-molecular hydrogen bonding. This leads to the excited state intramolecular proton transfer (ESIPT) and thus it shows week emission band[34]. According to Pearson's HASB concept[38], L₁ and L₂ act as a soft donor while Zn²⁺ act Lewis acid and host-guest formation between L₁ and L₂ with Zn²⁺ are characterized by optical spectroscopy, in which ligand L₁ can act as bidentate and ligand L₂ can at as tridentate coordination with a metal center. Hence, L₁ and L₂ are selected as a suitable sensing material for Zn²⁺ by the spectrofluorometric method.

3.1 Sensing ability of L1 and L2 towards cations

A preliminary investigation was performed using the spectrophotometric method in solution phase using solvent media MeOH/H₂O (1:1, v/v). The recognition ability of L₁ and L₂ (5×10⁻⁵M) were investigated in the presence and absence of different metal ions such as Ag⁺, Cr³⁺, Co²⁺, Ni²⁺, Ca²⁺, Zn²⁺, Cu²⁺, Hg²⁺, Cd²⁺, Mg²⁺, Al³⁺, Pb²⁺, Fe³⁺ and In³⁺ (5x10⁻⁴ M) by recording the UV-visible spectra (**Fig. 1a**). Alone L₁ showed two absorption bands appearing at 380 nm and 425 nm while L₂ showed only one absorption band at 380 nm. Upon interacting L₁ with different metal ions, the L₁ peak appeared at 380 nm undergo hypochromic effect and peak appeared at 425 nm undergo hyperchromic effect. But in the case of L₂ on interacting with some transition metal ions exhibits a red-shift while and other metal ions exhibit a hypochromic effect (**Fig. 1b**). Overall from this study, the quantitative estimation of metal ions using L₁ and L₂ is not possible due to an overall of spectral bands or at moderate concentration possible interference from each other.



Fig 1. UV-Vis spectral responses of (a) L_1 and (b) L_2 with various metal ions in MeOH/H₂O (1:1, v/v) media

In order to know fluorescence ability of ligands and its complexes, the initially visual test was conducted by exposing the prepared solutions of L₁ and L₂ with selected metal ions (10 eq.) in MeOH/H₂O (1:1, v/v) using wavelength 365 nm in UV-chamber. The results reveal that only solution containing Zn^{2+} exhibits fluorescence phenomenon visible to the necked eye and this evidence used for spectrofluorometric characterization of L1 and L2 with intention as a fluorogenic sensor for Zn^{2+} (Fig. 2 a,b). In order to know the fluorogenic behavior of L₁ and L₂ with selected metal ions, the fluorescence spectra were recorded by excitation at 380 nm. The studies revealed that only L_1 and L_2 with Zn^{2+} exhibits a strong fluorescence enhancement at 440 nm due to strong chelation-enhanced fluorescence (CHEF) (Fig. 2 c,d) [26]. The week fluorescence of L₁ and L₂ can be explained by the ESIPT process [34]. In the case of L_1 -Zn²⁺ complex shows 5-fold enhanced emission exhibits at 435 nm and similarly in L_2 -Zn²⁺ complex shows 4-fold enhanced emission intensity at 440 nm. Enhancement of fluorescence of ligands and its Zn²⁺ complexes may be attributed to the strong binding of Zn^{2+} that would impose rigidity and hence decrease the nonradioactive decay of the excited state of ligand. The transition metal ions with close shell d-orbitals, such as Zn^{2+} do not introduce low-energy metal-centered or charge-separated excited state into the molecule, so energy and electron-transfer process cannot take place[39]. The fluorescence response time and L_2 with Zn^{2+} are found to be 30 and 60s respectively (Fig. 7S).



Fig 2. Image taken in UV light chamber and emission spectral responses of (a) L_1 (b) L_2 (c) L_1 and (d) L_2 with various metal ions (2x10⁻⁴M) in MeOH/H₂O (1:1, v/v) media.

The steady changes in fluorescence response of L_1 and L_2 with the addition of Zn^{2+} were studied by spectrofluorimetric titration. Incremental addition of Zn^{2+} (1 to 50 µM) into L_1 and L_2 leads to study the change in emission intensity (**Fig 3a,b**). The limit of detection (LOD) and limit of quantification (LOQ) of Zn^{2+} using L_1 and L_2 were calculated based on the calibration curve obtained from the fluorescence titration. According to IUPAC method, the LOD and LOQ can be calculated by using 3σ /slope and 10σ /slope respectively, where the slope is obtained from the calibration curve (**Fig 3c,d**) and σ is the standard deviation of the emission intensity of L_1 and L_2 [40]. Results show that by using L_1 and L_2 , the LOD of Zn^{2+} down to 6.67 x 10⁻⁸ M and 3.56 x 10^{-7} M respectively while LOQ found to be 2.23 x 10^{-7} M and 1.2 x 10^{-6} M respectively.



Fig 3. Emission spectral titration of probes (a) L_1 (2x10⁻⁵M), (b) L_2 (2x10⁻⁵M) with Zn²⁺ (2x10⁻⁴M) in MeOH/H₂O (1:1, v/v) media. The inserted figure represents a calibration curve of Zn²⁺ using (c) L_1 and (d) L_2 .

The binding stoichiometry of ligands (L_1 and L_2) with Zn^{2+} was determined by Job's plot method. In this method, both ligands (L_1 and L_2) and Zn^{2+} taken in a different mole ratio in MeOH/H₂O (1:1, v/v) media and recorded their fluorescence spectra (**Figure 4a,b**) [41]. It reveals that the maximum fluorescence was noticed at 0.35 and the 0.5 mole-fraction which confirms the formation of 2:1 and 1:1 ligand to Zn^{2+} complex with L_1 and L_2 respectively. The binding constant of both ligands with Zn^{2+} was estimated using the Benesi Hildebrand (B-H) plot[42]. From the fluorescence titration data, the binding constant was calculated using the equation (i) for the L_1 and L_2 with Zn^{2+} complex (**Fig. 4c,d**).

$$\frac{1}{(F-F_0)} = \left(\frac{1}{(F-F_s)} + \frac{1}{(K(F-F_0)[Zn^{2+}])}\right) - - -(i)$$

Where F is the fluorescence intensity of ligand (L_1 and L_2), F_0 is fluorescence intensity with the incremental addition of Zn^{2+} and Fs is fluorescence intensity at saturation point. The plot of 1 / [F – F₀] versus 1/ [Zn^{2+}] is linear (R^2 of L_1 = 0.9989 and L_2 = 0.9987) and the binding constant (K_a) was found to be 4.92 x 10⁴ M⁻¹ and 2.048 x 10⁴ M⁻¹ for L_1 - Zn^{2+} and L_2 - Zn^{2+} respectively. Further, the stability of L_1/L_2 with Zn^{2+} complex in the presence and absence of common interfering metal ions such as Ca²⁺, Cd^{2+,} and Pb²⁺ were studied for 6 h by recording the emission intensity at



intervals of 30 min. Results showed that both L_1 and L_2 complex with Zn^{2+} is stable for 100 min, beyond 100 min incremental decrease in intensity was observed (**Fig. 8S**).

Fig 4. Job's plots and BH plots of (a) and (c) L_1 - Zn^{2+} (b) and (d) L_2 - Zn^{2+}

3.2 pH studies

The influence of pH on fluorescence response of L₁, L₂, and their Zn²⁺ complexes was carried out between pH 1-11 using appropriate buffer solutions (**Fig. 6a**). The intensity of free ligands (L₁ and L₂) did not get altered from pH 4.5-10 But the intensity decreases below pH 3.0 due to protonation of hydroxyl functional groups present in the ligands which reduce the ESIPT process. The same study was extended in the presence of Zn²⁺, results reveal that emission intensity remains unaltered in the pH range 4-9 and 5.5-10 with respect to L₁-Zn²⁺ and L₂-Zn²⁺ which represents the working range of the ligands. However, the intensity decreases below the pH 4 with L₁-Zn²⁺ and pH 5 with

L₂-Zn²⁺ respectively the decrease in intensity in the acidic region is due to protonation of a nitrogen atom, which restricts the ESIPT phenomenon. In case of L₂-Zn²⁺ intensity remains the same 5.5-10 whereas the intensity decreases in below pH 5 while above it starts to increases (**Fig. 6b**). The decrease in intensity in acidic region (1.0-4.0) due to protonation of a nitrogen atom which restricts ESIPT phenomenon. The increasing fluorescence intensity of L₂ and L₂-Zn²⁺ complex in basic pH (> 9) may be due to deprotonation at hydroxy (-OH) and thiol (-SH) leads to intra-molecular charge transfer (ICT) process. But in the case of L₁-Zn²⁺ at higher pH (>10) there is a decrease in the intensity could be due to the formation of Zn(OH)₂[43].



Fig 6. Effect of pH on (a) L_1 and L_1 -Zn²⁺ (b) L_2 and L_2 -Zn²⁺

3.3 Interference studies

The specificity of L₁ and L₂ as a fluorogenic sensor for the detection of Zn²⁺ in the presence of various competing cations was explored in CH₃OH/H₂O (1:1, v/v) (**Fig. 7 a,b**). For competitive studies, L₁ and L₂ (50 μ M) were treated with 10 eq of interfering metal ions such as (Ag⁺, Cr³⁺, Co²⁺, Ni²⁺, Ca²⁺, Cu²⁺, Hg²⁺, Cd²⁺, Mg²⁺, Al³⁺, Pb²⁺, Fe^{3+,} and In³⁺) then each solution was excited at wavelength maximum and emission intensity measured at 440 nm and 435 nm respectively. Results show that both L₁ and L₂ exhibits good selectivity with Zn²⁺ ions.



Fig 7. Bar diagram representing fluorescence intensity of (a) L_1 , L_1 - Zn^{2+} and L_1 - Zn^{2+} (b) L_2 , L_2 - Zn^{2+} and L_2 - Zn^{2+} with various competing metal ions in MeOH/H₂O (1:1, v/v) media excited at λ_{ex} =380 nm.

3.4 Reversibility studies

To check the dynamic response of the L_1 - Zn^{2+} complex, sequentially L_1 contacted with Zn^{2+} and EDTA in CH₃OH/H₂O (1:1 v/v) media were performed. Due to the high stability constant of EDTA- Zn^{2+} complex, binding reversibility L_1 - Zn^{2+} complex with EDTA was observed. A similar method was adopted for L_2 - Zn^{2+} complex. The purpose of this test to show how quickly L_1 and L_2 can be recoverable from its complex using EDTA (Fig. 8 a,b).



Fig 8. Reversibility studies of (a) L_1 -Zn²⁺ (b) L_2 -Zn²⁺ with EDTA

3.5 Quantum yield and fluorescence lifetime analysis

The fluorescence properties of L₁, L₂ L₁- Zn^{2+} and L₂- Zn^{2+} were studied in methanol medium. When Zn^{2+} ions interacted with L₁ and L₂, the fluorescence intensity of the L₁- Zn^{2+} and L₂- Zn^{2+} were enhanced significantly. Fluorescence quantum yield (??_F) of L₁, L₂, L₁-Z n^{2+} and L₂-Z n^{2+} were studied in methanol solution and calculated by using comparative William's method [44] using quinine sulphate as a standard reference [45]. To calculate the quantum yield, initially record UV-Vis absorption and emission spectra of five different concentrations of standard reference (quinine sulphate) and sample (L₁, L_1 -Zn²⁺, L_2 and L_2 -Zn²⁺) were recorded. The integrated fluorescence intensity was plotted against absorbance of reference and sample to calculate the gradients of reference and sample (Fig. 9S). The quantum yield was calculated and tabulated in the Table 1. Further, fluorescence lifetime is an average time of the molecules taken from excited state to ground state of the emitting photon. Fluorescence lifetime parameter is important in fluorescence studies such as fluorescence resonance energy transfer and fluorescence-lifetime imaging. The fluorescence lifetime of L_1 , L_1 - Zn^{2+} , L_2 and L_2 - Zn^{2+} complex solution was excited at 370 nm using a standard excitation light emitting diode laser (LED) as source and emission was observed at 440 nm for L_1 and L_1 - Zn^{2+} and 435 nm for L_2 and L_2 - Zn^{2+} . (Fig. 5a, b). Table 1 shows that the calculated (k_r) value for the L₁-Zn²⁺ and L₂-Zn²⁺ is more compare to L₁ and L₂ and also the non-radioactive decay (k_{nr}) of the L₁-Zn²⁺ and L₂-Zn²⁺ is decreased compare to L₁ and L₂ shows that the complex emits a strong fluorescence even at a lower concentration of the complex. Table 1. Photophysical properties of ligands (L_1, L_2) and complexes $(L_1-Zn^{2+}, L_2-Zn^{2+})$.

Ligand	Quantum Yield (?? _F)	Lifetime (ns)	$\frac{K_r}{(x10^7 \text{ s}^{-1})}$	K_{nr} (x10 ⁷ s ⁻¹)
L1	0.033	1.68	1.99	57.6
L ₁ -Zn ²⁺	0.085	2.1	4.05	43.5
L ₂	0.023	1.16	2.0	84.2
L ₂ -Zn ²⁺	0.067	1.64	4.1	56.9



Fig 5. (a) Time-resolved fluorescence decay of (a) L_1 and L_1 - Zn^{2+} (b) of L_2 and L_2 - Zn^{2+} complex and prompt ($\lambda_{ex} = 370 \text{ nm}$)

3.6 Binding mechanism study

The binding mechanism of L_1 and L_2 with Zn^{2+} were studied by ¹H NMR and ESI-mass analysis. For this purpose, the ¹H NMR of alone L_1 and L_2 and their complexes with Zn^{2+} were recorded in DMOS-d₆ (Figure 9a,b,). Only those protons which are influenced during binding events are discussed. Accordingly, the ligand L₁ shows a peak at 12.56 ppm corresponds to –OH and aromatic protons in the range from 6.27 to 7.79 ppm while in the case of L₂, the peak appeared at 13.04 ppm indicates –OH, at 6.13ppm correspond to -SH and aromatic peaks are resonated in the range of 6.39 ppm to 8.78 ppm. When L_1 and L_2 are binding with Zn^{2+} then ⁻OH peak is disappeared and other aromatic peaks are experiencing shielding effect which confirms the direct involvement of -OH and -C=N- in the binding process with Zn^{2+} . In the case of L_2-Zn^{2+} , both the ⁻OH and ⁻SH peaks are disappeared and the other aromatic peaks (7-7.5 and 6.39 ppm) are experienced the shielding effect which confirms the direct involvement of –OH, -SH and nitrogen of –C=N- in the binding process with Zn²⁺. Overall the ¹H NMR spectral change of free ligand and its Zn²⁺ complex supports the complexation mechanism proposed. Further, the ESI mass spectra of L_1 - Zn^{2+} and L_2 - \mathbf{Zn}^{2+} complex was studied. Accordingly, the mass spectra of $\mathbf{L_1}$ - \mathbf{Zn}^{2+} shows a mass peak at 660.8450 which corresponds to involvement of two ligands and one zinc $(2L_1+1Z_n)$ with a calculated mass of 660.1701 whereas in the case of L_2 -Zn²⁺ shows a major mass peak at 423.8832



which corresponds to one ligand, one zinc and one nitrate $(1L_2+1Z_n+1NO_3)$ with the calculated mass of 423.0218.

Fig 9. ¹H NMR and ESI-MS spectra of (a) L₁, L₁-Zn²⁺ (b) L₂, L₂-Zn²⁺. (c) L₁-Zn²⁺ and (d) L₂-Zn²⁺

3.7 Theoretical studies

To support experimental photophysical properties of L_1 and L_2 towards Zn^{2+} were calculated by using density functional theory (DFT) using Gaussian 09 computational code. The geometries of optimization of L_1 and L_2 and their complex towards Zn^{2+} were carried out using a B3LYP/6-31G** basis set in the gas phase. Accordingly, the optimized structure of L_1 shows slightly nonplanar due to the strain created by the five-member and six-member rings present in amino thiophenol and salicylic aldehyde while in L_2 , L_1 - Zn^{2+} and L_2 - Zn^{2+} exhibits a planar geometry (**Fig. 10 a,b**). The optimized geometry shows that in L_1 - Zn^{2+} system, the Zn^{2+} is at center surrounded by 2L1 ligands while in case of L_2 - Zn^{2+} complex due to the formation of semi cavity

created by the L₂. Hence, Zn^{2+} is tied up at the center of N, S and O groups of L₂. The interaction energy ($E_{int}=E_{complex} - E_{receptor} - E_{analyte}$) of the complex formed between the L1 and Zn²⁺ is -2528.9 kcal/mol while L_2 with Zn^{2+} is -1225.9 kcal/mol. It shows that L_1 exhibits more negative energy compare to L_2 due to stable complexation occurred between L_1 and Zn^{2+} . Theoretical results are well correlated with association constant values of L_1 - Zn^{2+} (4.92 x 10⁴ M⁻¹) and L_2 - Zn^{2+} (2.048 x 10⁴ M⁻¹) estimated by spectrofluorometric. The Mullikan's population and NBO charge analysis show that electron populations are more with L_1 - Zn^{2+} and L_2 - Zn^{2+} compared to individual L_1 and L₂ (Table 1S). The bond length between L₁ towards Zn^{2+} shows N- Zn^{2+} is 2.084 Å and O- Zn^{2+} is 1.958 Å while the bond length between L₂ towards Zn^{2+} shows N- Zn^{2+} is 2.033 Å, O- Zn^{2+} is 1.897 Å and S-Zn²⁺ is 2.274 Å. The frontier molecular orbitals (FMOs) studies show that the L1, L1- Zn^{2+} , L₂ and L₂- Zn^{2+} were analyzed (Fig. 10S and 11S). HOMO of L₁ is predominantly present on amino thiophenol group while LUMO is on salicylic aldehyde group however in the case of L1- Zn^{2+} , it is opposite. In the case of L₂, both HOMO and LUMO present all over the L₂ but in L₂- Zn^{2+} , LUMO is maximum appeared over ligand and HOMO is located over Zn^{2+} . These observations show there is a ligand to metal charge transfer is observed. Besides, both the L1 and L₂ shows a decrease in the energy band gap (ΔE) of L₁ and L₂ after interacting with Zn²⁺.



Fig 10. DFT computed optimized structures of (a) L_1 (b) L_1 - Zn^{2+} (c) L_2 (d) L_2 - Zn^{2+} using B3LYP method.

3.8 Analytical application

The analytical utility of L_1 and L_2 were assisted by determination of Zn^{2+} in different water samples and a pharmaceutical multivitamin tablet. The results of various water sample analysis and multivitamin sample analysis shown in **Table 2S**. All results were derived from triplicate measurements with standard error. The recovery percentage is greater than 98 % which shows proposed sensor L_1 and L_2 can be utilized for the Zn^{2+} analysis in real samples. Also, the present sensor was compared with reported literature shown in **Table 3S** [1,6,12,13,46] and It concludes that the present sensor highly useful

4. Conclusion

In conclusion, we have synthesized and characterized L_1 and L_2 by reacting 4-(diethylamino) salicylaldehyde with 2-aminobenzenethio under different catalytic conditions. Both ligands L_1 and L_2 show week fluorescence upon the complexation with Zn^{2+} , the increase in fluorescence is due to CHEF. The spectrofluorometric studies reveal that L_1 and L_2 forms 2:1 and 1:1 ligand to metal complex with Zn^{2+} with an estimated association constant of 4.9 x10⁴ M⁻¹ and 2.1 x 10⁴ M⁻¹ and LOD of Zn^{2+} was found to be 6.7 x 10⁻⁸ M and 3.6 x10⁻⁷ M respectively. The present sensor was used to estimate Zn^{2+} present in various water samples as well as in the pharmaceutical samples. The sensing mechanism was studied by ¹H NMR, ESI-mass analysis and DFT theoretical studies.

Conflicts of interest

There are no conflicts to declare

Acknowledgments

Authors are grateful to Department of Science and Technology, GOI for supporting the work through the project grant (EMR/2017/000816) and thankful to DST-VIT-FIST for NMR and Vellore Institute of Technology, Vellore for other research facilities.

References

- W. Cao, X.-J. Zheng, J.-P. Sun, W.-T. Wong, D.-C. Fang, J.-X. Zhang, L.-P. Jin, A highly selective chemosensor for Al (III) and Zn (II) and its coordination with metal ions, Inorg. Chem. 53 (2014) 3012–3021.
- [2] Z. Xu, J. Yoon, D.R. Spring, Fluorescent chemosensors for Zn²⁺, Chem. Soc. Rev. 39 (2010) 1996-2006.
- [3] Y. Ma, F. Wang, S. Kambam, X. Chen, A quinoline-based fluorescent chemosensor for distinguishing cadmium from zinc ions using cysteine as an auxiliary reagent, Sensors Actuators B Chem. 188 (2013) 1116–1122.
- [4] H. Liu, Y. Dong, B. Zhang, F. Liu, C. Tan, Y. Tan, Y. Jiang, An efficient quinoline-based fluorescence sensor for zinc (II) and its application in live-cell imaging, Sensors Actuators B Chem. 234 (2016) 616–624.
- [5] M. Shyamal, P. Mazumdar, S. Maity, S. Samanta, G.P. Sahoo, A. Misra, Highly selective turn-on fluorogenic chemosensor for robust quantification of Zn (II) based on aggregation induced emission enhancement feature, ACS Sensors. 1 (2016) 739–747.
- [6] Y. Li, K. Li, J. He, A "turn-on" fluorescent chemosensor for the detection of Zn (II) in aqueous solution at neutral pH and its application in live cells imaging, Talanta. 153 (2016) 381–385.
- [7] C. Gao, H. Zhu, M. Zhang, T. Tan, J. Chen, H. Qiu, A new highly Zn²⁺-selective and "off– on" fluorescent chemosensor based on the pyrene group, Anal. Methods. 7 (2015) 8172– 8176.
- [8] G.K. Walkup, B. Imperiali, Fluorescent chemosensors for divalent zinc based on zinc finger domains. Enhanced oxidative stability, metal binding affinity, and structural and functional characterization, J. Am. Chem. Soc. 119 (1997) 3443–3450.
- [9] R. Selva Kumar, S.K. Ashok Kumar, K. Vijayakrishna, A. Sivaramakrishna, P. Paira, C.V.S.B. Rao, N. Sivaraman, S.K. Sahoo, Bipyridine bisphosphonate-based fluorescent optical sensor and optode for selective detection of Zn²⁺ ions and its applications, New J. Chem. 42 (2018) 8494–8502.
- [10] T. Mandal, A. Hossain, A. Dhara, A. Al Masum, S. Konar, S.K. Manna, S.K. Seth, S. Pathak, S. Mukhopadhyay, Terpyridine derivatives as "turn-on" fluorescence chemosensors for the selective and sensitive detection of Zn²⁺ ions in solution and in live cells, Photochem.

Photobiol. Sci. 17 (2018) 1068-1074.

- [11] S.W. Bae, E. Kim, I.-S. Shin, S.B. Park, J.-I. Hong, Fluorescent chemosensor for biological zinc ions, Supramol. Chem. 25 (2013) 2–6.
- [12] Y.W. Choi, J.J. Lee, C. Kim, A highly selective fluorescent chemosensor based on a quinoline derivative for zinc ions in pure water, RSC Adv. 5 (2015) 60796–60803.
- [13] Y. Xu, Y. Zhou, W. Ma, S. Wang, A fluorescent sensor for zinc detection and removal based on core-shell functionalized Fe3O4@ SiO₂ nanoparticles, J. Nanomater. 2013 (2013) 1-7.
- [14] Q. Feng, Y. Li, K. Li, J. Lu, J. Wang, P. Fan, D. Li, D. Wu, H. Hou, Fluorescent Chemosensor for Zinc Ion Detection with Significant Emission Color Change in Aqueous Solution Based on AIEgen, ChemistrySelect. 2 (2017) 3158–3162.
- [15] S. Arulmurugan, H.P. Kavitha, B.R. Venkatraman, Biological activities of Schiff base and its complexes: a review, Rasayan J Chem. 3 (2010) 385–410.
- [16] H. Schiff, Mittheilungen aus dem Universitätslaboratorium in Pisa: eine neue Reihe organischer Basen, Justus Liebigs Ann. Chem. 131 (1864) 118–119.
- [17] K. Das, N. Sarkar, A.K. Ghosh, D. Majumdar, D.N. Nath, K. Bhattacharyya, Excited-state intramolecular proton transfer in 2-(2-hydroxyphenyl) benzimidazole and-benzoxazole: effect of rotamerism and hydrogen bonding, J. Phys. Chem. 98 (1994) 9126–9132.
- [18] V.I. Minkin, A.D. Garnovskii, J. Elguero, A.R. Katritzky, O. V Denisko, Tautomerism of Heterocycles: Five-Membered Rings with Two or More Heteroatoms, Adv. Heterocycl. Chem. 76 (2000) P157–P323.
- [19] E.M. Kosower, D. Huppert, Excited state electron and proton transfers, Annu. Rev. Phys. Chem. 37 (1986) 127–156.
- [20] K.P. Ghiggino, A.D. Scully, I.H. Leaver, Effect of solvent on excited-state intramolecular proton transfer in benzotriazole photostabilizers, J. Phys. Chem. 90 (1986) 5089–5093.
- [21] A.O. Doroshenko, E.A. Posokhov, A.A. Verezubova, L.M. Ptyagina, Excited state intramolecular proton transfer reaction and luminescent properties of the ortho- hydroxy derivatives of 2, 5- diphenyl- 1, 3, 4- oxadiazole, J. Phys. Org. Chem. 13 (2000) 253–265.
- [22] S.S. Sun, Z. Wang, X.W. Wu, J.H. Zhang, C.J. Li, S.Y. Yin, L. Chen, M. Pan, C.Y. Su, ESIPT Modulated Emission of Lanthanide Complexes: Different Energy Transfer Pathway and Multi- Responses, Chem. Eur. J. 24 (2018) 10091–10098.
- [23] L. Chen, S.Y. Yin, M. Pan, K. Wu, H.P. Wang, Y.N. Fan, C.Y. Su, A naked eye colorimetric

sensor for alcohol vapor discrimination and amplified spontaneous emission (ASE) from a highly fluorescent excited-state intramolecular proton transfer (ESIPT) molecule, J. Mater. Chem. C. 4 (2016) 6962–6966.

- [24] L. Chen, J.W. Ye, H.P. Wang, M. Pan, S.Y. Yin, Z.W. Wei, L.Y. Zhang, K. Wu, Y.N. Fan, C.-Y. Su, Ultrafast water sensing and thermal imaging by a metal-organic framework with switchable luminescence, Nat. Commun. 8 (2017) 15985.
- [25] M. Pan, W.M. Liao, S.Y. Yin, S.S. Sun, C.Y. Su, Single-phase white-light-emitting and photoluminescent color-tuning coordination assemblies, Chem. Rev. 118 (2018) 8889– 8935.
- [26] M.E. Huston, K.W. Haider, A.W. Czarnik, Chelation enhanced fluorescence in 9, 10-bis [[(2-(dimethylamino) ethyl) methylamino] methyl] anthracene, J. Am. Chem. Soc. 110 (1988) 4460–4462.
- [27] H.-H. Wang, Q. Gan, X.-J. Wang, L. Xue, S.-H. Liu, H. Jiang, A water-soluble, small molecular fluorescent sensor with femtomolar sensitivity for zinc ion, Org. Lett. 9 (2007) 4995–4998.
- [28] S. Huang, R.J. Clark, L. Zhu, Highly sensitive fluorescent probes for zinc ion based on triazolyl-containing tetradentate coordination motifs, Org. Lett. 9 (2007) 4999–5002.
- [29] L. Wang, W. Qin, W. Liu, A sensitive Schiff-base fluorescent indicator for the detection of Zn²⁺, Inorg. Chem. Commun. 13 (2010) 1122–1125.
- [30] C.Y. Lu, Y.W. Liu, P.J. Hung, C.F. Wan, A.T. Wu, A turn-on and reversible Schiff-base fluorescence sensor for Al³⁺ ion, Inorg. Chem. Commun. 35 (2013) 273–275.
- [31] V. Raju, R.S. Kumar, S.K.A. Kumar, Y. Tharakeswar, S.K. Sahoo, Development of highly selective chemosensor for chomium (III) estimation in aqueous environment, Inorg. Chem. Commun. 101 (2019) 74–80.
- [32] J. Yan, L. Fan, J. Qin, C. Li, Z. Yang, A novel and resumable Schiff-base fluorescent chemosensor for Zn (II), Tetrahedron Lett. 57 (2016) 2910–2914.
- [33] X. Bai, J. Yan, J. Qin, Z. Yang, A multi-ion fluorescent probe for Mg²⁺/Zn²⁺ based on a novel chromone-dendron Schiff base, Inorganica Chim. Acta. 474 (2018) 44–50.
- [34] V.S. Padalkar, A. Tathe, V.D. Gupta, V.S. Patil, K. Phatangare, N. Sekar, Synthesis and photo-physical characteristics of ESIPT inspired 2-substituted benzimidazole, benzoxazole and benzothiazole fluorescent derivatives, J. Fluoresc. 22 (2012) 311–322.

- [35] B.S. Kusmariya, A.P. Mishra, Experimental, theoretical and docking studies of 2-hydroxy Schiff base type compounds derived from 2-amino-4-chlorobenzenethiol, J. Mol. Struct. 1101 (2015) 176–188.
- [36] M. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, Ga. Petersson, Gaussian 09, revision a. 02, gaussian, Inc., Wallingford, CT. 200 (2009).
- [37] Y. Cheng, S. Pu, C. Hsu, C. Lai, P. Chou, Femtosecond Dynamics on 2- (2'- Hydroxy-4'- diethylaminophenyl) benzothiazole: Solvent Polarity in the Excited- State Proton Transfer, ChemPhysChem. 7 (2006) 1372–1381.
- [38] R.G. Pearson, Hard and soft acids and bases, J. Am. Chem. Soc. 85 (1963) 3533–3539.
- [39] P. Jiang, Z. Guo, Fluorescent detection of zinc in biological systems: recent development on the design of chemosensors and biosensors, Coord. Chem. Rev. 248 (2004) 205–229.
- [40] G.L. Long, J.D. Winefordner, Limit of Detection A Closer Look at the IUPAC Definition, Anal. Chem. 55 (1983) 712A–724A.
- [41] P. Job, Formation and stability of inorganic complexes in solution, Ann. Di Chim. Appl. 9 (1928) 113–203.
- [42] H.A. Benesi, J.H. Hildebrand, A Spectrophotometric Investigation of the Interaction of Iodine With Aromatic Hydrocarbons, J. Am. Chem. Soc. 71 (1949) 2703–2707.
- [43] S. Sinha, B. Chowdhury, P. Ghosh, A Highly sensitive ESIPT-based ratiometric fluorescence sensor for selective detection of Al³⁺, Inorg. Chem. 55 (2016) 9212–9220.
- [44] A.T.R. Williams, S.A. Winfield, J.N. Miller, Relative fluorescence quantum yields using a computer-controlled luminescence spectrometer, Analyst. 108 (1983) 1067–1071.
- [45] K.P. Divya, S. Sreejith, P. Ashokkumar, K. Yuzhan, Q. Peng, S.K. Maji, Y. Tong, H. Yu, Y. Zhao, P. Ramamurthy, A. Ajayaghosh, A ratiometric fluorescent molecular probe with enhanced two-photon response upon Zn²⁺ binding for in vitro and in vivo bioimaging, Chem. Sci. 5 (2014) 3469–3474.
- [46] G.J. Park, J.J. Lee, G.R. You, L. Nguyen, I. Noh, C. Kim, A dual chemosensor for Zn²⁺ and Co²⁺ in aqueous media and living cells: Experimental and theoretical studies, Sensors Actuators B Chem. 223 (2016) 509–519.



Highlights

- Developed an easy-to-synthesize cyclic and noncyclic Schiff's bases for the selective detection of Zn²⁺ by spectrofluorometric method.
- Both ESIPT and CHEF processes were explored as a key sensing mechanism.
- The LOD of the sensor is 6.7 x 10⁻⁸ M which is lower than the WHO guidelines in drinking water.
- The sensing mechanism was well supported by ¹H NMR, mass analysis and DFT.
- The receptor L can be recyclable after treatment with a EDTA

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