



SYNTHESIS AND CHARACTERIZATION OF A NOVEL PYRIMIDINE BASED SCHIFF BASE CHEMOSENSOR FOR THE DETECTION OF Al⁺³ IN ORGANO-AQUEOUS MEDIUM

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Abstract:

A pyrimidine derived Schiff base chemosensor 2,6-bis-[(4,6-dimethyl-pyrimidin-2-yl)-hydrazonomethyl]-4-methyl-phenol (**HL**) has been synthesized [synthesized by single step condensation of 2,6-diformyl-4-methyl-phenol and (4,6-dimethyl-pyrimidin-2-yl)-hydrazine in methanol] and characterized for the detection of Al⁺³ ion in organo-aqueous medium. Compound **HL** is pale yellow coloured solid, weak fluorescent compound that offered potentiality for chemical sensing of Al⁺³. Investigation of the fluorescence behaviour of the compound has been carried out in DMSO: H₂O (8:2, v/v) solution under biologically relevant conditions in HEPES buffer (pH= 7.4) displayed a significant response to Al⁺³ as a result of chelation-induced enhanced fluorescence (CHEF). Rigorous experimental studies conclude that title compound (**HL**) is analytically potential to detect Al⁺³ in solution with high sensitivity and selectivity having a detection limit of 2.4239 μM.

Keywords: Pyrimidine derived Schiff base compound, Al⁺³ chemosensor, chelation-induced enhanced fluorescence.

1. Introduction:

After oxygen and silicon, aluminium is the third most abundant (8.3% wt.) element in the earth's crust. Al⁺³ is widely utilized in our day-to-day life in the form of food packaging, water purification, pharmaceuticals, cooking utensils [1-3] etc. The solubility of Al⁺³ compounds at lower pH may cause leaching that in turn enhances the concentration of Al⁺³ that became fatal to plant growth and its ultimate effect is the environmental acidification [4, 5]. Excessive accumulation of Al⁺³ in human being may cause Alzheimer's disease, osteoporosis and intoxication in haemodialysis patients [6]. The world Health Organization (WHO) has recommended the maximum permissible limit of Al⁺³ concentration to be 7.41 mM in drinking water [7]. Currently available Al⁺³ detection methods such as, graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry [8] are comparatively complex, time consuming and as well as expensive also. Henceforth the detection of Al⁺³ is highly appreciated due to its crucial impact on environment and human health. Small molecule chemosensors based on fluorescence

spectroscopy have provided chemists an alternative promising tool which selectively detects Al^{+3} with high sensitivity, easy visualization and rapid response. Therefore, considerable effort is being invested to develop the Al^{+3} specific chemosensors that would facilitate chemists from both fundamental and application levels and encourages them to design new and more potent systems eliminating the limitations [9-14].

Pyrimidine derivatives are widely known as biomolecules and hence potent physiologically. Exploration of structural investigations regarding transition metal complexes derived from pyrimidine-based Schiff base ligands are more frequent, but their sensing potentiality still remain less explored. In this article the synthesis and characterization of a new pyrimidine derived novel chemosensing system, 2,6-bis-[(4,6-dimethyl-pyrimidin-2-yl)-hydrazonomethyl]-4-methyl-phenol (**HL**, as shown in scheme) as a probe for selective detection of Al^{+3} with high efficacy has been reported. The crystallographic and theoretical characterization of synthesized complex is currently under the investigation.

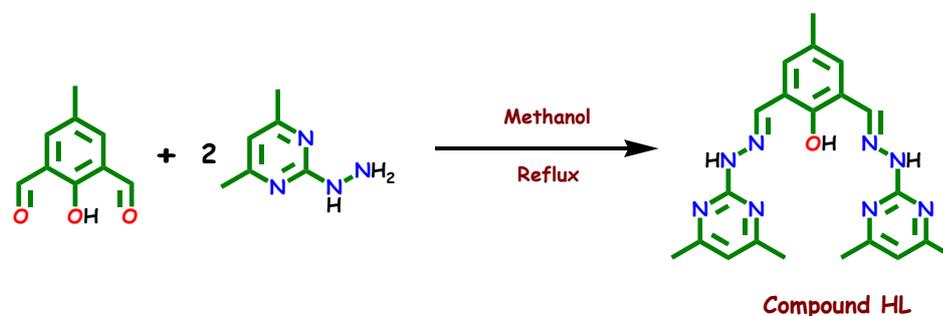
2. Materials and Physical measurements:

Unless otherwise stated, all reagents were purchased from commercial sources and used without further purification. Solvents were dried by standard methods. 2,6-diformyl-4-methyl-phenol [15] and (4,6-dimethyl-pyrimidin-2-yl)-hydrazine [16] were prepared by literature methods. All reagents and chemicals were purchased from Sigma and used without further purification. Solvents used for spectroscopic studies were purified and dried by standard procedures before use. The HEPES buffer solution of pH 7.4 were prepared using a solid powder of pure HEPES (MW 238.3 g/mol) and then adjusted by a pH meter (Systronics digital pH meter, Model 335).

Elemental analyses (carbon, hydrogen and nitrogen) were carried out with a Perkin-Elmer 2400 Series-II CHN analyzer. ESI mass spectra were obtained from a Water HRMS model XEVO-G2QTOF#YCA351 spectrometer. 1H and ^{13}C -NMR spectra were recorded in d_6 -DMSO with TMS as the internal standard on a Bruker, AV 300 Supercon Digital NMR system. Fourier transform infrared (FT-IR) spectra were recorded from a PerkinElmer LX-1 FT-IR spectrophotometer (KBr disk, 4000–400 cm^{-1}). UV-vis absorption spectra were obtained using UV-1700 Pharma Spec UV-vis spectrophotometer (SHIMADZU). Fluorescence emission spectra were carried out in a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer. The fluorescence spectra were recorded using a PerkinElmer spectrofluorometer model LS55. For the titration experiment, I have used the cations, e.g., (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Ag^+ and Au^{3+}) in the form of their chloride salts and the nitrate salt of Al^{3+} .

2.1. Synthesis of the compound 2,6-bis-[(4,6-dimethyl-pyrimidin-2-yl)-hydrazonomethyl]-4-methyl-phenol (HL):

2,6-diformyl-4-methyl-phenol was synthesized starting from p-cresol following a published procedure [15]. A methanolic solution (20 mL) of (4,6-dimethyl-pyrimidin-2-yl)-hydrazine [16] (0.276 g, 2 mmol) was added dropwise to the methanolic solution (15 mL) of 2,6-diformyl-4-methyl-phenol (0.164 g, 1 mmol) with constant stirring (Scheme 1). The stirring was continued for 30 minutes and the mixture was refluxed for 5 h at water bath temperature and cooled to room temperature. Excess methanol was removed using a rotary evaporator to obtain a pale yellow microcrystalline solid. The solid was filtered off, washed thoroughly with cold methanol and dried in a vacuum over fused $CaCl_2$. Yield: (0.313 g, 77%). M.P: 237°C (decomp.). Anal. calc. for $C_{21}H_{24}N_8O$: C, 62.37; H, 5.94; N, 27.72. Found: C, 62.13; H, 5.77; N, 27.57%. MS (m/z) 404 (M^+ , 100%). 1H -NMR (300 MHz, DMSO- d_6): δ (ppm), 2.33 (12H, s), 2.34 (3H, s), 4.02 (2H, brs), 5.39 (1H, m), 6.74 (2H, s), 7.76 (2H, s), 8.54 (2H, s); ^{13}C NMR (300 MHz, DMSO- d_6): δ (ppm), 21.3, 24.6, 111.0, 118.6, 128.6, 133.2, 143.7, 158.1, 160.3, 166.5; Selected FT-IR data (KBr, cm^{-1}): ν_{NH} , 3323; $\nu_{CO/CN}$, 1657(s), 1589(s), ν_{N-N} , 1051(s); ν_{pym} , 1021(s).



scheme 1: synthetic scheme for the synthesis of the compound (**HL**)

2.2. Synthesis of complex $[\text{Al}(\text{L})(\text{H}_2\text{O})](\text{NO}_3)_2$ (**1**):

The ligand (**HL**) (0.404g, 1mmol) was dissolved in 15 ml of hot methanol. $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (0.374g, 1mmol) was added to this solution and the mixture was stirred for 3 hours at ambient temperature. The resulting mixture was then filtered and kept undisturbed for crystallization at room temperature. After two weeks, a yellow-coloured microcrystalline compound (**1**) was found but X-ray diffraction quality single crystals were not obtained. Yield: 73%. Anal. calc. for $\text{C}_{21}\text{H}_{25}\text{AlN}_{10}\text{O}_8$: C, 44.05; H, 4.37; N, 24.47. Found: C, 43.93; H, 4.21; N, 24.33. MS (m/z) 448 (M^+ , 100%). Selected FT-IR data (KBr, cm^{-1}): $\nu_{\text{NH}/\text{H}_2\text{O}}$, 3400, 3383; $\nu_{\text{CO}/\text{CN}}$, 1627(s), 1539(s), $\nu_{\text{N-N}}$, 1084(s); ν_{pym} , 1013(s).

3. Result and Discussions:

The spectroscopic properties of the probe **HL** were investigated by monitoring absorption and fluorescence changes in presence of several metal ions, such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Al^{3+} , Ag^+ and Au^{3+} in DMSO: H_2O (8:2, v/v, 10 mM HEPES buffer, pH 7.4) solution at room temperature. As illustrated in figure 1, the free probe **HL** displayed a maximal absorption peak at 371 nm which corresponds to π - π^* transition from the conjugated moiety of **HL**. However, upon gradual addition of Al^{3+} ion to the solution of **HL**, the initial absorption band at 371 nm decreased and a simultaneous increase at 420 nm with a red shift of 49 nm was observed, accompanied by a naked eye colour change from almost colourless to light yellow (figure 1a, inset image). The clear isosbestic point at 395 nm undoubtedly indicates the formation of Al^{3+} complex in the binary mixture (figure 1a). Again, chemosensor **HL** did not exhibit any noteworthy changes in absorption spectra with the addition of other competitive metal ions (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Ag^+ and Au^{3+}) signifying the high selectivity of this probe (figure 1b).

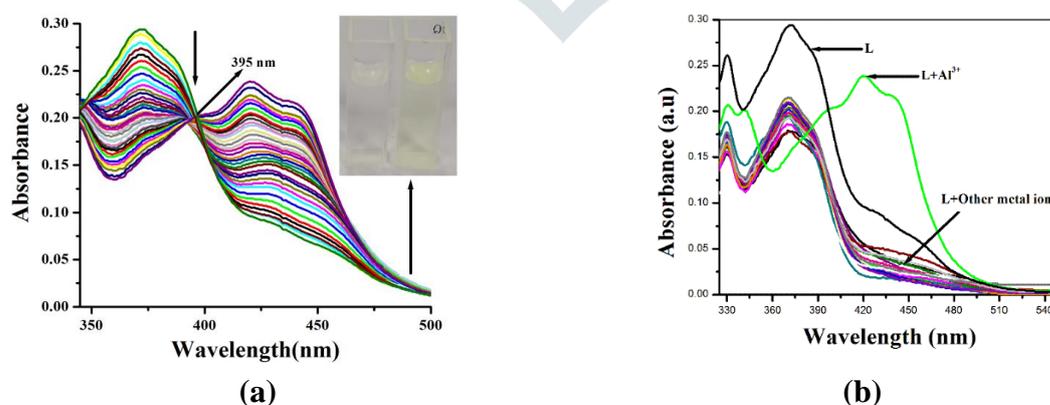


Fig.1 (a) UV-vis spectra of **HL** ($2 \times 10^{-5}\text{M}$) in the presence of increasing amount of $[\text{Al}^{3+}]$ in DMSO/ H_2O (8:2 v/v) HEPES buffer (pH = 7.4) solution. (b) Absorption spectra of **HL** in presence of different metal ions (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Ag^+ , Au^{3+} and Al^{3+}).

As shown in figure 2 in absence of Al^{3+} ion, probe **L** showed a very weak emission band centered at 501 nm ($\lambda_{\text{ext}} = 395$ nm) in DMSO/ H_2O (8:2, v/v, 10 mM HEPES buffer, pH = 7.4). This weak emission of probe **L** is due to photoinduced electron transfer (PET) from the lone pair of imine nitrogen and excited state intramolecular proton transfer (ESIPT) from the -OH group to the imine nitrogen [16]. However, on treatment with Al^{3+} ions, a gradual enhancement in fluorescence (10 folds) along with a blue shift of 11 nm having highest intensity at 490 nm and a colour change from dark to greenish blue under UV light (figure 2, inset image).

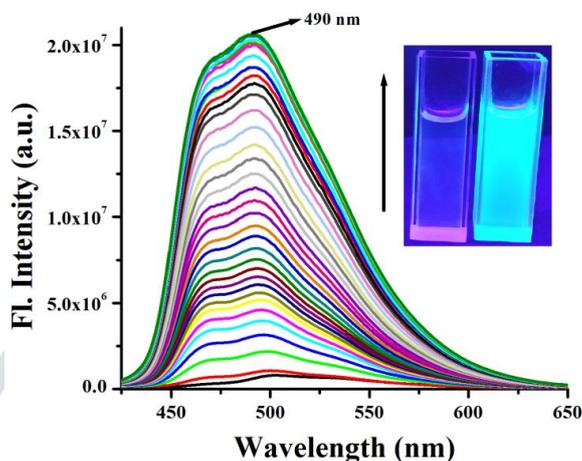
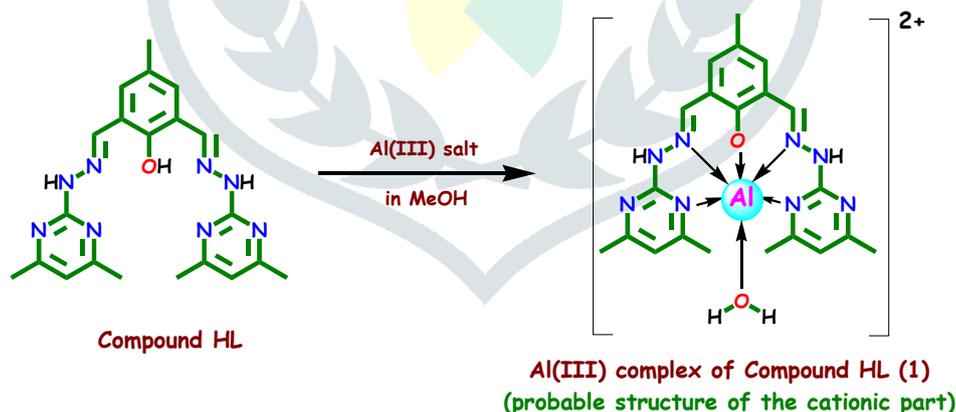


Fig. 2 Changes in fluorescence spectra of **HL** (2×10^{-5} M) in the presence of increasing amount of Al^{3+} (5×10^{-4} M) in DMSO/ H_2O (8:2 v/v) HEPES buffer (pH = 7.4) solution. Inset: Fluorescence emission intensity of '**HL**' at 490 nm under UV light.

This large fluorescence 'turn-on' in presence of Al^{3+} ions might be attributed to the formation of complex between the probe **L** and Al^{3+} through the coordination of oxygen and nitrogen atoms (probable structure is shown in scheme 2) that inhibited both the ESIPT and PET processes as well as C=N isomerization resulting in a distinctive chelation enhanced fluorescence (CHEF) effect.



scheme 2: schematic representation of probable 1:1 metal to ligand composition

To establish the selectivity and sensitivity of **HL** towards Al^{3+} I have assessed its fluorescence behaviour in the presence of a series of comparable analytes namely Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Ag^+ and Au^{3+} beside Al^{3+} in DMSO: H_2O (8:2, v/v, 10 mM HEPES buffer, pH 7.4) solution as shown in figure 3.

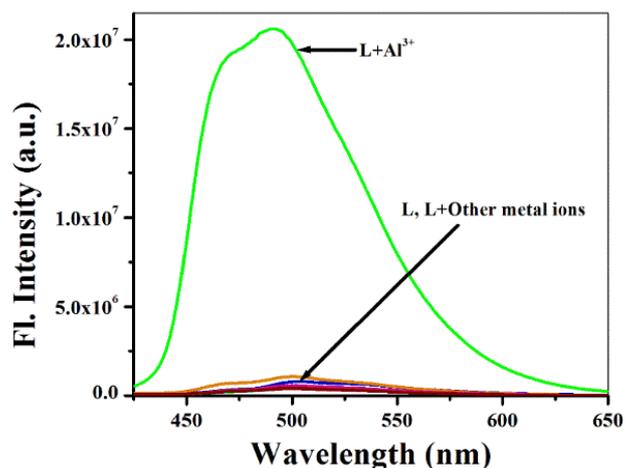


Fig. 3 Fluorescence response of **HL** (2×10^{-5} M) to different metal ions in DMSO/H₂O (8:2 v/v) HEPES buffer (pH = 7.4) solution ($\lambda_{\text{ext}} = 395$ nm)

As shown in figure 3, probe **HL** demonstrated strong fluorescence response towards Al^{3+} at 490 nm, while the other cations [Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Ag^+ and Au^{3+}] did not cause any notable emission spectral changes. In order to further evaluate the practical capability of probe **L** as Al^{3+} -selective fluorescent chemosensor, I conducted competitive experiments on addition of Al^{3+} ions to the solution of **L** in presence of excess equivalent of other individual competitive metal ions. The increase in fluorescence intensity induced by mixing Al^{3+} ions indicating stable complexation between **HL** and Al^{3+} than other miscellaneous cations. The above results indicate the high selectivity of probe **HL** for Al^{3+} over other cations in DMSO solution and its limit of detection (LOD) [17] for Al^{3+} was determined. UV-vis spectral titration provided a good linear fit ($R^2=0.984$) with the Al^{3+} based on which the detection limit [19, 20] for Al^{3+} was calculated to be $2.4239 \mu\text{M}$ [the slope (S) = 9×10^{11} , standard deviation ($S_b1 = 727180.50286$)].

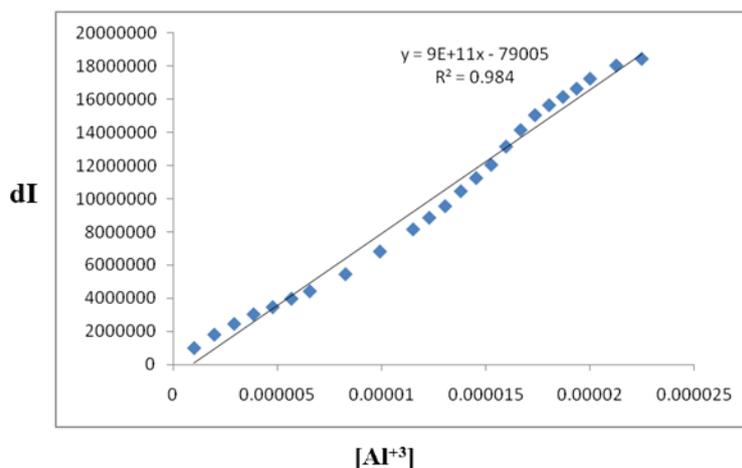


Fig. 4: Calibration curve for fluorescence titration of **HL** with Al^{3+} . From the graph the limit of detection (LOD) = 2.4239×10^{-6} M = $2.4239 \mu\text{M}$.

The probe to Al^{3+} ratio was investigated using Job's plot and the result suggested the formation of a 1:1 stoichiometry of the complex (figure 5).

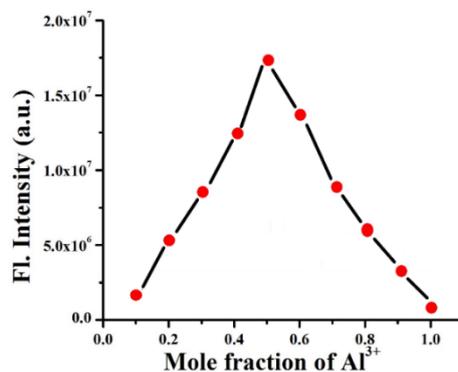


Fig. 5 Fluorescence Job's plot for **HL** with Al^{3+} in DMSO/ H_2O solution (8:2, v/v, 10 mM HEPES buffer, pH 7.4).

To attain such type of stoichiometry imine, pyrimidine nitrogen and phenolic oxygen atoms are the most likely binding sites for Al^{3+} ions (scheme 2). The association constant (K_a) of **HL** for Al^{3+} was calculated from the Benesi–Hildebrand equation [18] on the basis of fluorometric titration as $2.45 \times 10^3 \text{ M}^{-1}$ (figure 6) which suggests a strong binding of probe towards Al^{3+} ions. The selectivity of chemosensor **HL** towards different metal cations were examined under identical working conditions.

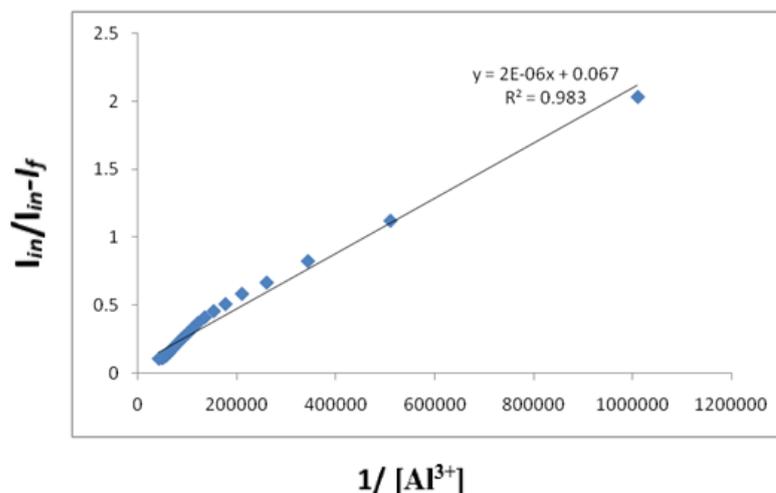


Fig. 6: Benesi-Hildebrand plot obtained from the Fluorescence (emission calculated from 490 nm) studies. Binding constant ($K_a = 2.45 \times 10^3 \text{ M}^{-1}$) curve of sensor **HL** with Al^{3+} determined by fluorescence method.

The excitation of the probe in the solid state at 395 nm exhibits high-intense greenish yellow fluorescence (λ_{em} , 542 nm) (Fig. 7).

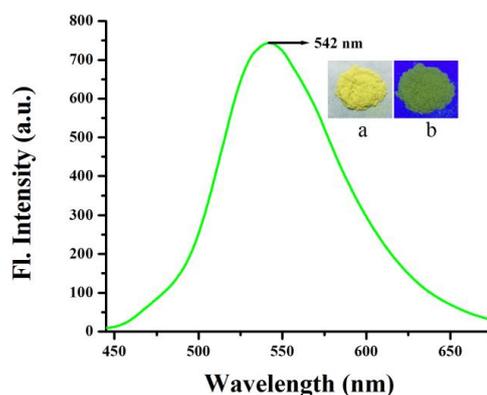


Fig. 7: Solid-state fluorescence spectrum of **HL** on excitation by 395 nm; inset: images of chemosensor **HL** (a) under normal light and (b) under UV (λ , 365 nm) light.

4. Conclusion:

A new Al^{3+} chemosensor derived from pyrimidine-based Schiff base 2,6-bis-[(4,6-dimethylpyrimidin-2-yl)-hydrazonomethyl]-4-methyl-phenol (**HL**) has been synthesized and characterized successfully. Free **HL** displayed a maximum absorption peak at 371 nm that corresponds to $\pi-\pi^*$ transition from the conjugated moiety of the compound. The large fluorescence ‘turn-on’ behaviour in presence of Al^{3+} ions in a HEPES buffer (DMSO: H_2O =8:2 v/v) might be ascribed due to the formation of 1:1 complex between the probe **L** and Al^{3+} through the coordination that inhibited both the excited state intramolecular proton transfer (ESIPT) and photoinduced electron transfer (PET) processes as well as a distinctive chelation enhanced fluorescence (CHEF) effect. The experimental results indicate the high selectivity of probe **L** for Al^{3+} over other cations [Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Ag^+ and Au^{3+}] in DMSO solution and its limit of detection for Al^{3+} was determined to be 2.4239 μM . The association constant (K_a) of **L** for Al^{3+} was calculated from the Benesi–Hildebrand equation on the basis of fluorometric titration as $2.45 \times 10^3 \text{ M}^{-1}$ that signifies a strong binding towards Al^{3+} ions. All experimental results demonstrate that **HL** offers an easy to operate sensing system for selective detection of Al^{3+} with naked eye detect ability.

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