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Fluorescent recognition of Fe^{3+} ion with photoinduced electron transfer (PET) sensor

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ABSTRACT

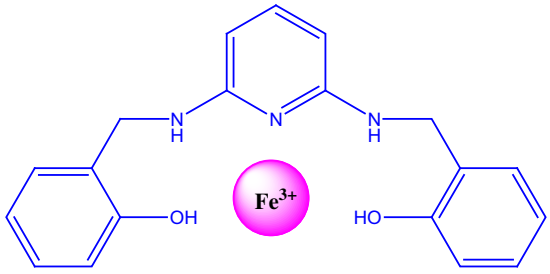
We synthesized a fluorescence receptor 2,2-(pyridine-2,6-diylbis(azanediyl))bis(methylene)diphenol (**2**) and report it as a photoinduced electron transfer (PET) cation sensor that is capable of indicating the presence of Fe^{3+} ion via a fluorescence signal. It was observed that fluorescence intensity changes and quenched. The association constant (K_a) of receptor (**2**) with Fe^{3+} ions was calculated from Benesi–Hildebrand and Scatchard Plot at 1.60 and $1.30 \times 10^4\ M^{-1}$ respectively.

1. Introduction

In recent years the development of fluorescent receptors for the detection of cation is the attractive research area in the field of supramolecular chemistry because of it's use in material science and biology [1–3]. The development of molecular systems capable of signaling different guest molecules or ions have been energetic areas of modern research [4–6]. One of the most frequently utilized design processes for fluorescence signaling system is the development of three-component system with a fluorophore-spacer-receptor architecture containing components that are selected such that the communication between the fluorophore and receptor leads to quenching of the fluorescence of the system. In the presence of a guest, the communication between the terminal moieties, which is responsible for fluorescence quenching, gets cut off, thus leading to the recovery of the fluorescence of the system. This is commonly referred to as 'off–on' fluorescence signaling of a guest. Interestingly, the off–on fluorescence signaling systems for the transition-metal ions, which are well-known for their fluorescence quenching abilities, have been developed only very recently [7,8].

Among the transition metal ions Fe^{3+} is having much importance as it plays a crucial role in the growth and progress of living systems. For example numerous enzymes use iron as a catalyst for oxygen metabolism, electron transfer as well as DNA and RNA synthesis. Both its lack and excess in the body can cause serious diseases [9–12].

In the present letter, we prepared a simple and efficient fluorogenic receptor **2** containing the two amine and two hydroxyl fragment (Scheme 1). It was found that the compound selectively complexes with Fe^{3+} ions in semi aqueous solution, resulting in a significant fluorescence quenching.



2. Experimental

2.1. Reagents

All spectroscopy grade chemicals and solvents were used without further purification. The fluorescence and UV–visible spectra were recorded on Fluoromax-4 Spectrofluorometer and Shimadzu UV-24500 with 5 nm slit of fluorescence and the nitrate salt of metal were used in the letter. Ultrapure water with a Millipore Purification System (Milli-Q water) was used throughout the analytical experiments. ^1H NMR spectra were recorded on a Varian NMR mercury system 300 spectrometer operating at 300 MHz in DMSO-d_6.

2.2. Sample preparation

A stock solution of probe **2** (1.00×10^{-3} M) in CH_3OH/H_2O (70:30, v/v) solution was prepared (probe **2** is freely soluble in

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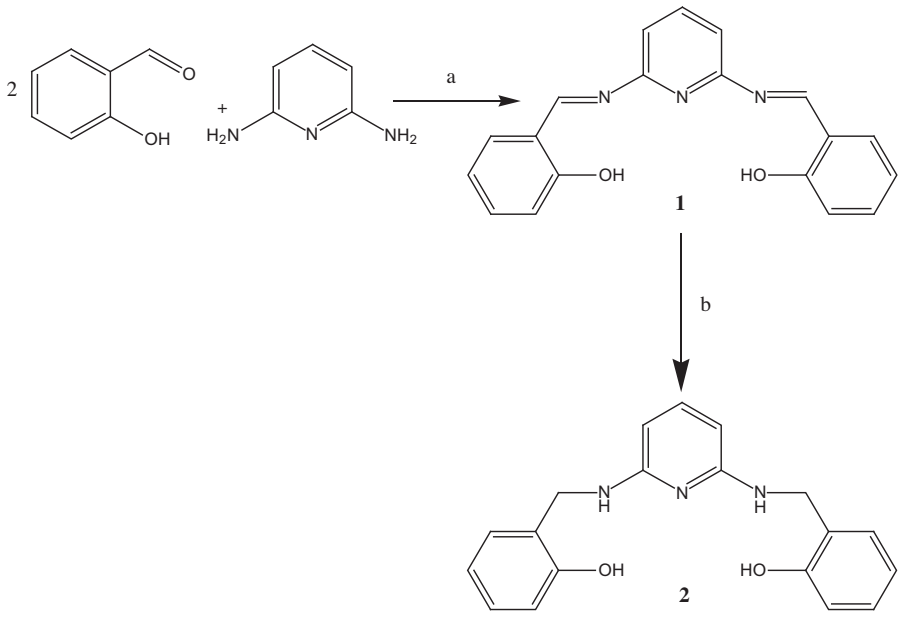

Scheme 1. Synthesis of receptor **2** (a) CH_3OH, 3 h (b) $NaBH_4$, CH_3OH, 0–5 °C and 1.5 h.

MeOH, DMF and DMSO at 25 °C) and the corresponding working solutions ($c = 1.00 \times 10^{-5}$ M) were simply prepared by diluting with CH_3OH/H_2O (70:30, v/v). All stock and working solutions were prepared in ultrapure water and spectroscopic grade CH_3OH. Stock solution of cation (1.00×10^{-2} M) was prepared with CH_3OH/H_2O (70:30, v/v) solution and the corresponding working solutions ($c = 1.00 \times 10^{-4}$ M) were simply prepared by diluting with CH_3OH/H_2O (70:30, v/v).

2.3. Fluorescence analysis

The fluorescence titration experiments were carried out with a Fluoromax-4 spectrofluorometer in CH_3OH/H_2O (70:30, v/v) solvent system at room temperature (298 K) with the aim of determining the association constant (K_a) for receptor **2**–cation in this solvent system. These titration experiments were accomplished through a stepwise addition of metal salt solutions (0.02 ml, 1.00×10^{-4} M, guest) to a solution of receptor **2** (2 ml, 1.00×10^{-5} M) in CH_3OH/H_2O (70:30, v/v) in the cell. The fluorescence intensity was recorded at $\lambda_{ex}/\lambda_{em} = 314/380$ nm alongside a reagent blank. The excitation and emission slits were both set to 5.0 nm.

2.4. Synthesis of receptor *2*

Compound **1** was synthesized by reacting one mole of 2,6-diaminopyridine (1.09 g, 1.00 mM) with two moles of 2-hydroxy benzaldehyde (2.40 g, 2.00 mM) in methanol, with stirring for 3 h at an ambient temperature. Compound **1** was obtained with excellent yield and having appearance of orange coloured powder. Solubility in methanol, 85% yield, mp >250 °C were observed. Further receptor **2** was obtained from compound **1** by reduction under $NaBH_4$ in methanol with good yield.

Name: 2,2-(pyridine-2,6-diylbis(azanediyl))bis(methylene) diphenol (**2**), yield 76% (white powder) solubility in methanol, mp ⩾250 °C. IR (KBr, cm^{-1}): υ = 621, 648, 719, 754,746, 871, 970, 3064, 3273, 3560 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, ppm) δ = 3.33 (s, 4H, Ar-CH_2), 4.258 (s, 2H, NH), 5.22 (s, 2H, Ar-OH), 6.12 (d, J = 7.2 Hz, 2H, Py-H), 6.55–7.06 (m, 8H, Ar-H), 7.23 (T, J = 7.2 Hz, 1H, Py-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 37.8, 98.5, 114.5, 122.6, 127.0, 129.3, 143.7, 153.0, 159.25. MS (EI): m/z $C_{19}H_{19}N_3O_2$ = 321.37; found 321.00.

2.5. Synthesis of receptor *2*·Fe^{3+} complex

2·Fe^{3+} complex was synthesized by reaction of one mole of receptor **2** (0.64 g, 0.20 mM) with one moles of $FeCl_3$ (0.32 g, 0.20 mM) in MeOH 50 ml reflux with stirring for 3 h. The precipitation was collect at room temperature and dried in vacuum. Further it was washed with water then ethanol followed by petroleum ether. Yield-82%, MS (ESI): m/z requires $C_{19}H_{17}N_3O_2Fe$: 375.28, found 375.07.

3. Result and discussion

3.1. Synthesis of receptor *2*

Compound **1** was synthesized by reacting one mole of 1,2-diaminopyridine with two moles of 2-hydroxy benzaldehyde in methanol with stirring for 3 h. Compound **1** was obtained with quantitative yield. Receptor **2** was obtained from compound **1** by reduction under $NaBH_4$ with good yield (Scheme 1). The synthesized receptors were characterized by melting point, IR, ^1H NMR, ^{13}C NMR and mass spectroscopic methods. The spectral investigation gave consistent data of structure of both.

3.2. Fluorescence studies of receptors *2*

The solvent ratio CH_3OH/H_2O (70:30, v/v) respectively for receptor **2** was kept constant during the titrations. The fluorescence properties of receptors **2** was studied upon addition of various metal salts ($c = 1.00 \times 10^{-4}$ M) Ca^{2+}, Mg^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Bi^{3+} ions. The receptors **2** showed fluorescence emission at 380 nm upon excitation at 314 nm. Upon addition Fe^{3+} in receptor **2**, the intensity of emission band at 380 nm decreased along with the appearance of a new red-shifted emission band at 409 nm (Figure 1). The fluorescence was selectively and significantly red shifted and quenched in the presence of Fe^{3+} ions. There were no changes in the fluorescence emission of receptor **2** in the presence of other metal ions tested. The

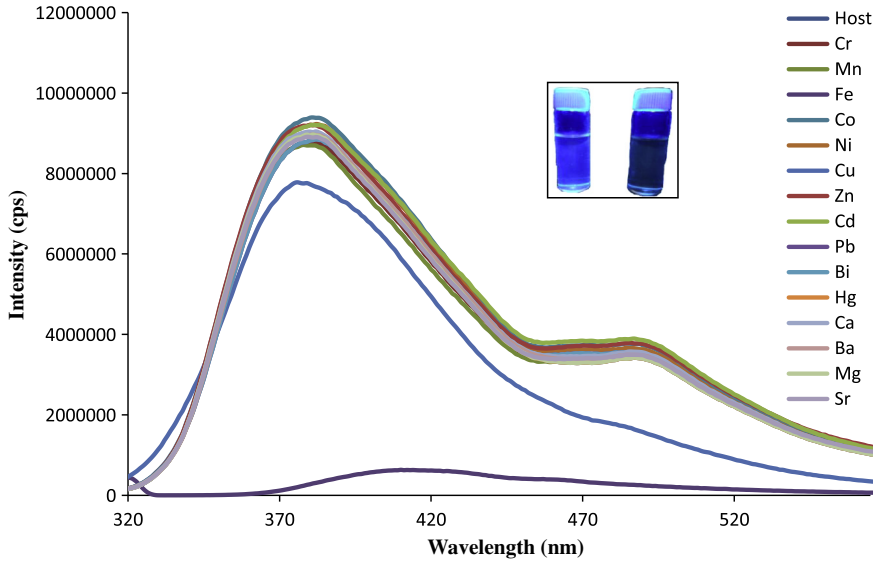

Figure 1. Fluorescent intensity (λ_{ex} = 314 nm) of receptor **2** upon the addition of a particular metal cation salt in CH_3OH/H_2O (70:30, v/v). Figure 1 inset corresponding fluorescent colour change in CH_3OH/H_2O (70:30, v/v) solution of **2** induced by Fe^{3+} (from left to right: **2** only and 1+ Fe^{3+}).

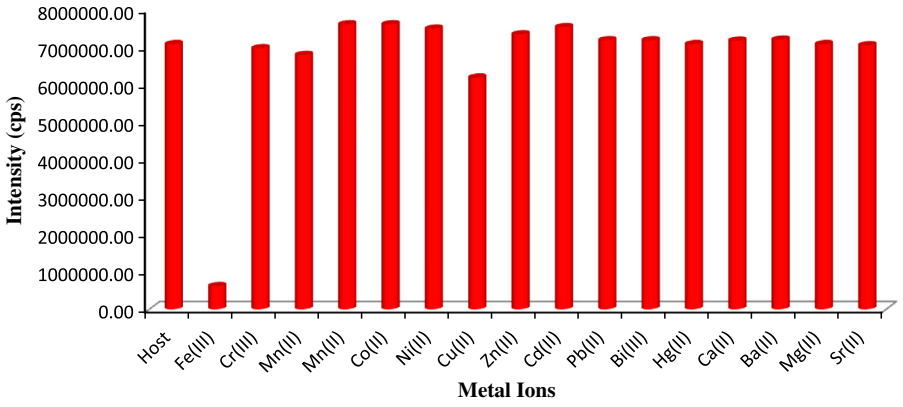

Figure 2a. Fluorescence ratiometric response of chemosensor **2** ($c = 1.00 \times 10^{-5}$ M) upon the addition of a particular metal salt ($c = 1.00 \times 10^{-4}$ M) in CH_3OH/H_2O (70:30, v/v).

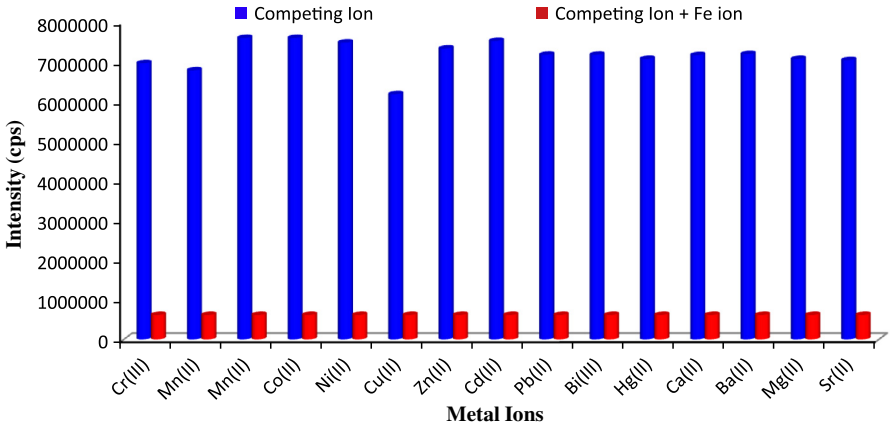

Figure 2b. Interference of metals at the time of Fe^{3+} ion detection.

fluorescence of receptor **2** is quenched and red shifted upon addition of Fe^{3+} due to the thermodynamically favored PET between the pyridine nitrogen and the excited chromophore. Increasing the oxidation potential of the pyridine nitrogen due to cation binding prevents this PET and regenerates the fluorescence of the chromophore.

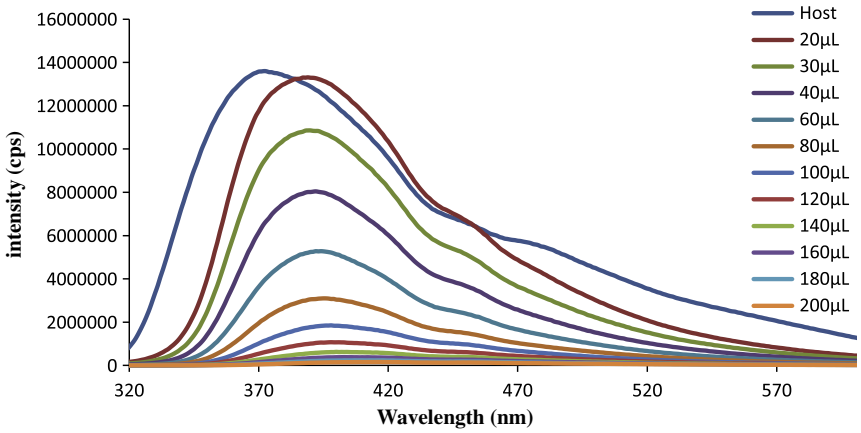

Figure 3a. Changes in fluorescence spectrum of probe **2** ($c = 1.00 \times 10^{-5}$ M) after the addition of Fe^{3+} salt of ($c = 1.00 \times 10^{-4}$ M) in CH_3OH/H_2O (70:30, v/v).

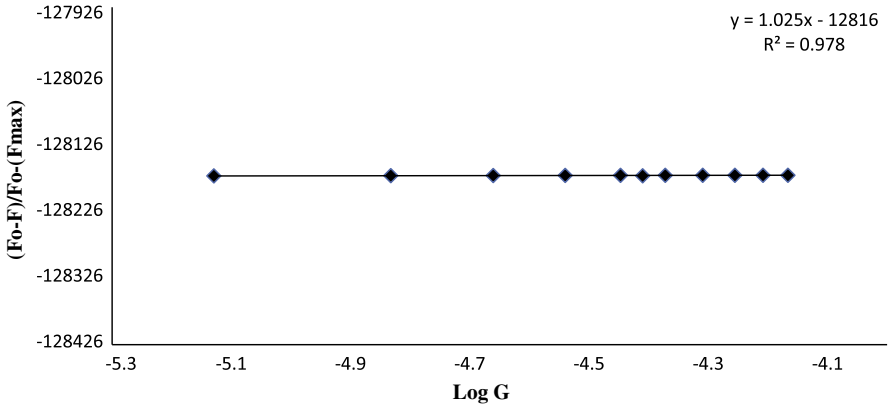

Figure 3b. Normalized response of fluorescence signal to changing Fe^{3+} concentrations in the CH_3OH/H_2O (70:30, v/v) solution (receptor **2** $c = 1.00 \times 10^{-5}$ M and Fe^{3+} salt of $c = 1.00 \times 10^{-4}$ M).

Fluorescence ratiometric response of receptor **2** and interference toward the surveyed metal ions is shown in Figures 2a and 2b. The results show a highly selective response of receptor **2** towards Fe^{3+} ions as compared to the other metal ions such as transition and heavy-metals.

We also determined the fluorescence quantum yields (Φ) of **2** and **2**·Fe^{3+}. The quantum yield of receptor **2** is high (0.36) and **2**·Fe^{3+} is low (0.02). It is noted that, upon complexation with iron metal, the corresponding quantum yield dramatically decrease, which is consistent with the fluorescence spectra feature. These observations suggest that the receptor **2** is highly selective and sensitive towards Fe^{3+} ion [13].

The binding pattern of receptor **2** explores the importance of pyridine nitrogen and hydroxyl group in cation binding. The pyridine nitrogen and hydroxyl groups are considered as good binding sites and play an effective role in binding of Fe^{3+} ions. Therefore, it appears that the core functionality required for receptor **2** to efficiently bind with Fe^{3+} upon excitation is a pyridine nitrogen and hydroxyl linkage. However, due to binding of Fe^{3+} ion in the gap, the fluorophore interaction is modulated.

Figure 3a shows the emission spectra of receptor **2** on addition of Fe^{3+} on the various concentrations. The fluorescence intensity of receptor **2** at 380 nm was shifted up to 409 nm ($\Delta\lambda = 29$ nm) after addition of Fe^{3+} ion. The emission maximum 409 nm was decreases with increase in concentration of Fe^{3+}. The titration experiment is plan for showing the linear response between receptor **2** and changing concentrations of Fe^{3+} cation. A linear relationship between concentrations was observed with the fluorescence intensity and correlation coefficient is high. Normalized response of fluorescence signal to changing concentration of Fe^{3+} cation in CH_3OH/H_2O (70:30, v/v) solution is shown in Figure 3b. Receptor **2** can be used for selective recognition of Fe^{3+} ions in quite a wide range of iron concentrations, and it can detect as little as 0.50 μM of Fe^{3+} ion concentration [14].

3.3. Calculation of association constant for receptor **2**

On the basis of Benesi–Hildebrand Plot [15] Eq. (1) and Scatchard Eq. (2) [16] methodologies, association constant (K_a) was calculated by following equation (K_a).

$$1/(F - F_0) = 1/(F_\infty - F_0)K_a[G] + 1/(F_\infty F_0) \quad (1)$$

$$(F - F_0)/[G] = (F_\infty - F_0)K_a - (F - F_0)K_a \quad (2)$$

Applying the above equations for receptor **2,** graph obtained is illustrated in Figures 4a and 4b. Where F_0 represents the fluorescence intensity in the absence of guest ion, F represents the fluorescence intensity in presence of guest ion, F_∞ represents fluorescence intensity after titration and $[G]$ is the concentration of guest. The association constant (K_a) of receptor (**2**) with Fe^{3+} ions was calculated from Benesi–Hildebrand and Scatchard Plot at 1.60 and 1.30×10^4 M^{-1} respectively.

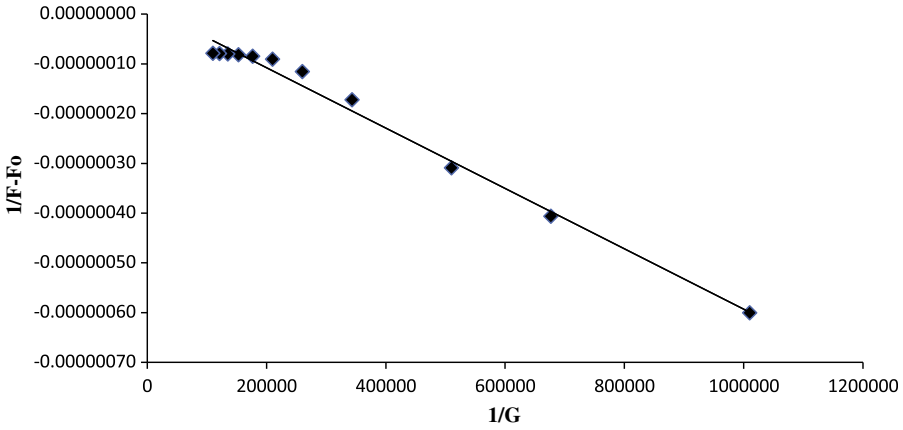

Figure 4a. Benesi–Hildebrand Plot receptor **2** (adjusted equation: $1/F - F_0 = -6E - 13 + 1E - 081/[G]$, $R = 0.98$) at the K_a value $1.60 \times 10^4\ M^{-1}$.

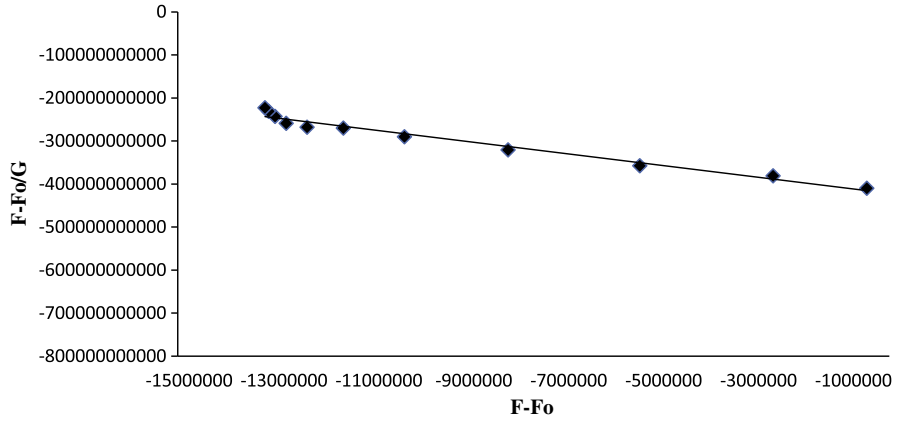

Figure 4b. Scatchard Plot for receptor 2 (adjusted equation: $F - F_0/[G] = -13530x + 4E + 11$, $R = 0.97$) at the K_a value $1.30 \times 10^4\ M^{-1}$.

3.4. The Stern–Volmer quenching constant of **2**·Fe^{3+}

The quenching can be mathematically expressed by the Stern–Volmer Eq. (3), which allows for calculating quenching constants [17].

$$(F_0/F) = (1 + k_q\tau_0)[Q] = (1 + K_{sv})[Q] \quad (3)$$

Where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, k_q is the bimolecular quenching constant, τ_0 is the lifetime of the fluorescence in the absence of the quencher [Q] is the concentration of the quencher, and K_{sv} is the Stern–Volmer quenching constant. In the presence of a quencher (Lns), the fluorescence intensity is reduced from F_0 to F. The ratio (F_0/F) is directly proportional to the quencher concentration [Q].

Evidently:

$$K_{sv} = kq \cdot \tau_0 \quad (4)$$

$$(F_0/F) = (1 + K_{sv})[Q] \quad (5)$$

According to Eq. (5), a plot of F_0/F versus [Q] shows a linear graph (Figure 5) with an intercept of **2** and a slope of K_{sv} indicating that fluorescence quenching is dynamic in nature in the linear part. In order to elucidate the static or dynamic quenching without measurement of fluorescence lifetime, the absorption spectra were measured carefully to distinguish static and dynamic quenching. Dynamic quenching only affects the excited states of the fluorophores, and thus no change in the absorption spectra is predicted. It was found that the measured spectra were not influenced by the metal ions in the presence of metal ions.

3.5. Stoichiometry of complexations

The binding stoichiometry was found by the continuous variation method (Job's plot) [18]. Figure 6 shows the Job plot of the fluorescence intensity of free receptor **2** and the intensity of the system with the molar fraction of the host $\{[H]/([H] + [G])\}$ for a series of solutions, in which the total concentration of host and guest was constant, with the molar fraction of host continuously varying. The results showed that the complex **2**·Fe^{3+} formed between **2** and Fe^{3+} is with 1:1 stoichiometry. This data was further confirmed by mass spectroscopy analysis. ESI-MS data showed the formation 1:1 complex between two deprotonated ligand (receptor **2**) and MS (ESI): m/z requires $C_{19}H_{17}N_3O_2Fe$: 375.28, found 375.07.

3.6. UV–Vis absorption spectral studies

The absorption spectrum of probe **2** with Fe^{3+} ion is shown in Figure 7. Receptor **2** exhibit maximum peaks at 209, 251, 280 and 314 nm in CH_3OH/H_2O (70:30, v/v). The longer wavelength band at 314 nm may be assigned to transitions associated with phenol ring. The addition of Fe^{3+} ion into receptor **2**, there had no change in receptor **2**. This is fact that the UV–vis spectra of **2**

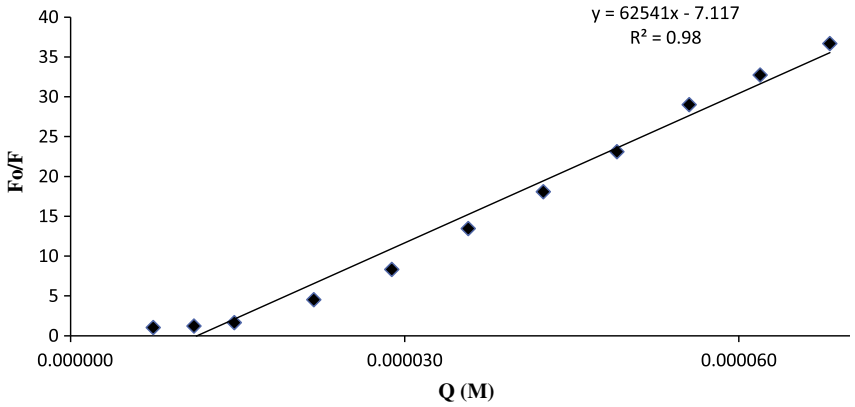

Figure 5. The Stern–Volmer plot of the titrations of probe **2** with different concentrations of Fe^{3+} metal salt show normalized graph with regression 0.98.

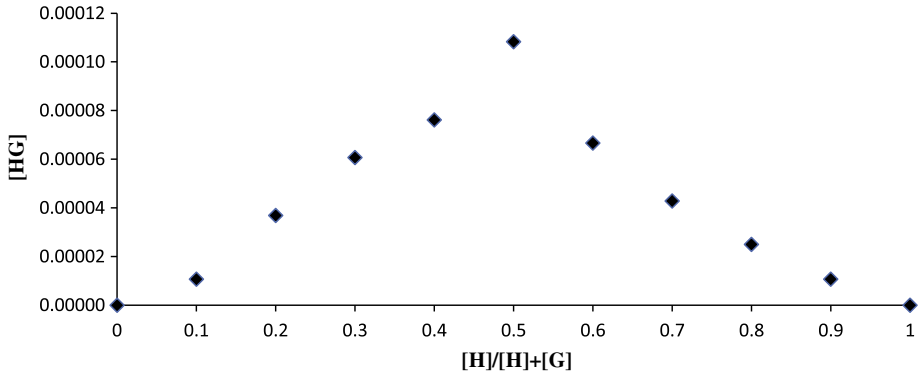

Figure 6. 1:1 Stoichiometry of the host guest relationship realized from the Job's plot for receptor **2**.

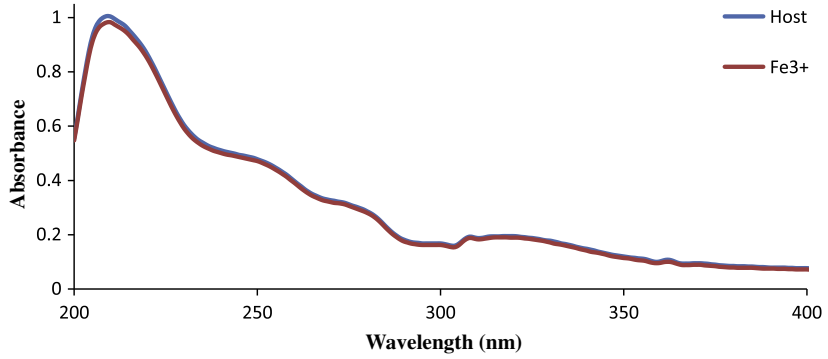

Figure 7. Changes in absorbance spectrum of probe **2** ($c = 1.00 \times 10^{-5}$ M) upon the addition of Fe^{3+} metal ($c = 1.00 \times 10^{-4}$ M) in CH_3OH/H_2O (70:30, v/v).

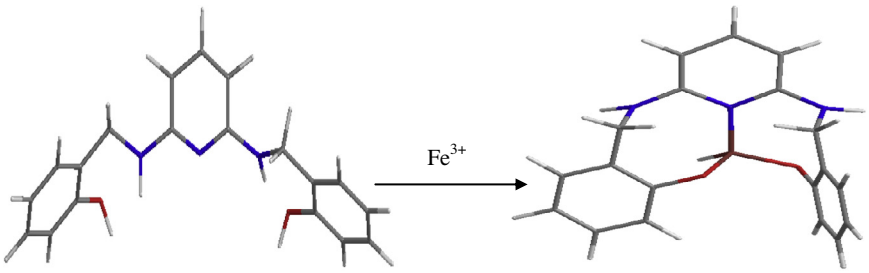

Figure 8. Proposed binding mode and mechanism of sensing of probe **2** with Fe^{3+} ions.

before and after the addition of 10 equiv of Fe^{2+} remain relatively unchanged illustrated that it did not bound with Fe^{3+} in ground state and receptor **2** need to be excited for binding [9].

3.7. Naked eye detection under UV irradiation

In addition, the selective recognition of receptor **2** with Fe^{3+} ion gives a remarkable fluorescent colour quenched which can be visualized by the naked eye under UV irradiation. Visual detection studies of receptor **2** ($c = 1.00 \times 10^{-5}$ M) were conducted with all cations. The receptor **2** in mixed solutions of CH_3OH/H_2O (70:30, v/v) was appeared to be fluorescent at room temperature. With the addition of these ions (10 equiv) in receptor **2**, only Fe^{3+} ion cause's spectacular fluorescent quenched shown in the Figure 1 inset.

3.8. Proposed binding mode and mechanism of sensing

From the fluorescence spectra, a possible binding model of probe **2** with Fe^{3+} ion was proposed which is shown in Figure 8. The pyridine nitrogen and hydroxyl are capable to bind cation. The Fe^{3+} could be bound with phenolic OH via deprotonation and pyridine nitrogen donates lone pair. The proposed binding mechanism was confirmed by mass spectroscopic method.

4. Conclusion

In summary, we have synthesized the selective and sensitive fluorescent chemical sensor that is, receptor **2** for the detection of Fe^{3+} ions in CH_3OH/H_2O (70:30, v/v) solvent system. The addition of Fe^{3+} gave rise to major fluorescent colour change, which can be easily seen by the naked eye under UV irradiation. Furthermore, our sensor is not affected by the common interference of other ions. The 1:1 stoichiometry of the host guest relationship was realized from the Job's plot and the association constant (K_a) of receptor (**2**) with Fe^{3+}ions was calculated from Benesi–Hildebrand and Scatchard Plot at 1.60 and $1.30 \times 10^4\ M^{-1}$ respectively.

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