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RECOGNITION OF Fe³⁺ BY A NEW AZINE-BASED FLUORESCENT "TURN-OFF" CHEMOSENSOR AND ITS BINDING MODE ANALYSIS USING DFT

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Graphical Abstract



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Abstract

A highly responsible Fe^{3+} , azine chemosensor (EBHMM) was successfully designed and synthesized. The structure of the synthesized azine was confirmed by FT-IR, UV-Vis, ¹H and ¹³C{¹H} NMR spectroscopic techniques and high resolution mass spectrometry. The azine shows sensitive and selective fluorescence "turn-off" towards Fe^{3+} ion over other metal ions in 1:1 DMF-H₂O (5µM, pH=7.4) solution. The fluorescence quenching on the addition of Fe^{3+} ion at various pH clearly indicate that the azine can be used for the quantitative determination of Fe^{3+} . The binding of Fe^{3+} with chemosensor was analyzed by confirming the formation of metal-ligand complex using HRMS-ESI spectrometry. The binding stoichiometry (1:1) was calculated using Stern-Volmer method and the binding constant 1.0034 M⁻¹ was calculated using

Benesi-Hildebrand plot. The exact binding sites of the chemosensor with Fe^{3+} ion is theoretically explained with Fukui Function using DFT study in B3LYP function with B3LYP/6-311++G (d,p) level of basis set. The "turn-off" fluorescence through photo induced electron transfer (PET) process is discussed and confirmed with the results of Fukui Function and Frontier molecular orbital analysis.

Keyword: Azine Chemosensor, Fluorescence, Fe³⁺, DFT, Fukui Function.

1. Introduction

In recent years, researchers have focused on organic fluorescent chemical probes, sensitive and selective for the metal ion recognition such as Cu^{2+} , Zn^{2+} , Pb^{2+} and Hg^{2+} , among others [1-9]. The improvement of fluorescent chemical probes for detecting the metal ion is an encouraging field because of their essential role in environmental, medical and biological

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applications [10]. Among the transition metal ions, Fe³⁺ ion has dynamic roles in the environment as well as in many biological processes [11]. Over sufficiency of iron is causing several diseases such as, kidney failure, liver damage, cancer, anemia, Huntington's, Alzheimer's and Parkinson's diseases [12, 13]. Furthermore, the development of chemosensor that can detect Fe^{3+} is very important to prevent and solve health and environmental problems induced by Fe³⁺. The literature survey revealed that there are few Fe³⁺ selective fluorescent probes with detection limit ranges between 3.38 x 10^{-3} and 1.63 x 10^{-7} M [14-19]. Among them, fluorescent probes of azines are having very attractive ligating ability with metal ions. Recently, azine derivatives have shown wide range of applications in various areas such as material and analytical, environmental, biomedical, catalysis and supramolecular chemistry [20-25]. The N-N linked diimines of azine moieties are very tolerant to hydrolysis and are having good ligating ability. The -C=N- moiety of azine shows potential detection of transition and post transition metal ions in the chemosensor study [26-28]. There are several methods available for detecting iron in a particular system [29-31]. Comparing with other methods, fluorescence technique is most versatile one to detect metal ions due its sensitivity, simplicity, instantaneous response, low-cost and capable of specific recognition of particular ions [32]. We reported the synthesis and characterization of an azine and its application as chemosensor for detecting Fe³⁺ ion. The results obtained from experimental and DFT studies provide information on the structural and photophysical properties of fluorescent compound and helps in elucidating the fundamental aspects concerning the sensing mechanisms.

2. Experimental

2.1. Materials

Hydrazine, 4-hydroxy-3-methoxybenzaldehyde and glyoxal were purchased from Merck chemical Co. and used without any further purification. Dimethylsulfoxide (DMSO), tetrahydrofuran (THF), dimethylformamide (DMF), ethanol, methanol, acetone, acetonitrile, ethyl acetate, heptane, toluene, *n*-hexane and chloroform were obtained from Merck, India and used after suitable distillations [33].

2.2. Synthesis of 4-(hydrazonomethyl)-2-methoxyphenol (PHM)

Equimolar quantities of 4-hydroxy-3-methoxybenzaldehyde and hydrazine were dissolved separately in minimum volume of ethanol. The reaction mixture was stirred at room temperature for 4 h (Scheme 1). After checking the completion of the reaction with TLC, the reaction mixture was poured into ice cold water. The yellowish white coloured precipitate

formed was filtered and dried. (Yield:82%, M.P: 96⁰C). ^{*I}H NMR (DMSO-d6):* δ ppm 9.17 (s,-OH), 8.57 (s, -CH=N-), 7.62 (s, Ar-CH), 7.45(d, Ar-CH), 7.10(d, Ar-CH), 6.41 (s, -NH₂), 3.83 (s,-OCH₃).</sup>

¹³*C NMR* (*DMSO-d6*): *δ* ppm 161.17 (C7), 150.31(C1), 140.20(C2), 123.99(C4), 115.94(C5), 110.43(C6), 108.37 (C3) and 55.96 (-OCH₃). *m/z* (Found): 166.0806.

2.3. Synthesisof 4, 4-((ethane-1, 2-diylidene)bis(hydrazine-2,1-diylidene))bis (methanylylidene)bis(methanylylidene))bis(2-methoxyphenol) (EBHMM)

In to the ethanolic solution of 4-(hydrazonomethyl)-2-methoxyphenol (0.02 mol), 0.01 ole of glyoxal was added slowly with constant stirring. After refluxing the reaction mixture for 6h (Scheme 2), it was poured in to ice cold water. The deep yellow coloured product obtained was filtered, washed thoroughly with distilled water, and recrystallized with ethanol. (Yield: 78%, M.P: 184 0 C). ¹*H NMR (DMSO-d6):* δ ppm 9.86 (s, -OH), 8.59 (s, Ar-CH=N-), 8.36 (-CH=N), 7.49 (s, Ar-CH), 7.35 (d, Ar-CH), 6.91 (d, Ar-CH), 3.84(s, -OCH₃).

¹³*C NMR* (*DMSO-d6*): *δ* ppm 164.12(C7), 158.66(C1), 152.58(C8), 148.32(C2), 125.27(C4), 116.17(C5), 111.32(C6), 110.71(C3) and 56.11(-OCH₃). *m/z* (Found): 354.9497.

2.4. Characterization Techniques

FT-IR spectrum of EBHMM was recorded in the region of 4000-400 cm⁻¹ using KBr pellet with Perkin Elmer 8000 FT-IR spectrophotometer. The ¹H and ¹³C{¹H} NMR spectra were recorded with Bruker AV400 MHZ spectrometer by using DMSO-d6 as a deuterated solvent. The UV absorption spectra were recorded using Shimadzu double beam UV-240 spectrophotometer. The mass spectra were recorded with PerkinElmer GC-MS spectrometer.

2.5. Theoretical study

The quantum theoretical Fukui Function calculations of EBHMM was carried out using DFT, B3LYP/6-311+ +G (d,p) level of basis set in the Gaussian 09 package. The exact active sites of EBHMM for chemosensor activity was well identified using Fukui Function calculations.

2.6. Optical properties

The UV-visible spectra of various concentrated solutions of EBHMM in DMF were recorded between of 200 and 800nm. Fluorescence measurements were carried out with Jasco FP-8200 spectrofluorimeter using quartz cuvettes of 1cm path length. The absorption and emission spectra of the compound in 1:1 DMF/H₂O solution were recorded at 25 ± 1^{0} C in at pH = 7.4. The solutions of Cu²⁺, Fe²⁺, Fe³⁺, K⁺, La³⁺ as chlorides and Ag⁺, Al³⁺, Ba²⁺,Bi²⁺ Pb²⁺, Cr³⁺, Ce³⁺, Cd²⁺, Hg²⁺, Li⁺ Zn²⁺, Zr²⁺, Co²⁺, Ni²⁺, Mn²⁺, Mg²⁺, Ca.²⁺, Na⁺ as nitrate were prepared using 1:1 DMF/H₂O mixture. The binding studies of the compound was carried out by incorporating these metal ion solutions into 5µM 1:1 DMF/H₂O solution of EBHMM at pH 7.4.

3. Results and discussion

3.1. Solubility

The solubility of EBHMM was tested by dissolving 0.1 mg of it in 1ml of a series of solvents such as, THF, DMSO, DMF, ethanol, methanol, $CHCl_3$, CH_2Cl_2 and ethyl acetate, heptane, toluene, *n*-hexane and acetone at room temperature. The compound is soluble in all the above mentioned solvents except in *n*-hexane, toluene and acetone.

3.2 Spectral Characterization

The structures of PHM and EBHMM are confirmed by IR, ¹H, ¹³C{¹H}NMR, and mass spectrometry techniques and their spectra are given in SF1 to SF7. The IR spectrum of EBHMM showing –OH stretching vibration at 3469.31. PHM showing a broad band at 3483.59

cm⁻¹ due to the presence of one of the $-NH_2$ stretching vibration along with -OH stretching vibration. The other $-NH_2$ stretching vibration band of PHM appears at 3362.68 cm⁻¹ and the bands are absent in EBHMM. The bands at 2941.56-2933.20 cm⁻¹ is attributed to the -CH stretching vibrations of aromatic hydrogens. The -C=N- stretching vibration bands are appearing at around 1589-1892 cm⁻¹[34-37]

.In ¹H NMR spectra, the methoxy protons of both the compounds are appearing as a singlet in the up field region of around 3.84ppm and the phenolic –OH proton signals are appearing as a broad singlet at 9.87-9.17ppm. The two set of signals of azomethine protons of EBHMM (Hd & He) are appearing as singlet at 8.59 and 8.36ppm respectively and the shifting of Hd proton to downfield is due to the deshielding effects of benzene ring [38]. Similarly, the aromatic protons of both compounds shows two doublet and one singlet signal between 7.45 - 6.91ppm and 7.62 - 7.49ppm respectively. The shift at 6.41ppm is attributed to $-NH_2$ proton of PHM and the absence of such shift in EBHMM confirms the formation of expected structure.

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In the ¹³C{¹H} NMR spectra, the carbon atoms of different chemical environments are appearing at their respective chemical shift values. The appearance of an additional signal at 158.80ppm indicates the formation of azine moiety. The high resolution mass spectra of PHM and EBHMM respectively are giving their m/z values at 166.0806 and 354.9479, confirming the formation of expected structures.

3.3. UV-Vis spectrum of EBHMM

The absorption spectrum of EBHMM was recorded with various concentrated solutions (2, 4, 20 and 40µl in 1:1 DMF/H₂O) and is shown in SF. 8. The λ max appeared at 377nm is attributed to n- π * transition of imine moiety of sensor [39]. The intensity of the spectrum increases gradually with increase in concentration of EBHMM.

By observing the intensity of the spectra at various concentrations, the suitable concentration for the fluorescence study was explored. From the spectrum, the optical band gap (SF. 9) of the compound was calculated by Tauc method and is found to be 2.9 eV. This band gap value was compared with the theoretical band gap value and used for fukui function calculation to find the exact nucleophilic attack site in the molecule. For the fluorescence study the excitation wavelength was fixed as λ max 377nm and the emission was observed at a higher wavelength of 423nm. The combined spectra of absorption and emission are shown in the SF. 10.

3.4. Selectivity studies of EBHMM to the metal ions

The fluorescence emission of EBHMM with and without metal ion was recorded to find the selective and sensitive detection of metal ions by observing fluorescence emission intensity changes. The selectivity of the probe EBHMM was evaluated in DMF/H₂O (1:1, EBHMM=5 μ M, pH=7.4) solution with different metal ions such as Ag⁺, Al³⁺, Ba²⁺,Bi³⁺, Pb²⁺, Cu²⁺, Cr³⁺, Ce³⁺, Cd²⁺, Hg²⁺, La³⁺,Li⁺ Zn²⁺, Zr²⁺Co²⁺, Ni²⁺, Mn²⁺, Mg²⁺, Ca²⁺, Fe²⁺, Fe³⁺, Na⁺ and K⁺. In the selectivity study, Fe³⁺, La³⁺ and Fe²⁺ have shown significant change in the fluorescence emission, while the other metal ions under same concentrations (100 equiv.) and identical conditions does not. Among these three metal ions, Fe³⁺ has shown greater intensity variation in the selectivity fluorescence spectrum. It shows that there is an effective quenching "On Off" behavior with Fe³⁺ ion through energy or electron transfer processes which eventually leads to reverse photo induced electron transfer process. Additionally, the quenching behavior may be due to the unfilled d subshell and paramagnetic property of Fe³⁺ ion [40]. The hydrated Fe³⁺ ion in the solution is highly feasible to form Fe(OH)₃ at pH above 7 [41]. The interaction of $Fe(OH)_3$ with the probe is confirmed by ¹H NMR titration and mass spectrometry. The HRMS-ESI *m/z* value of the probe on binding is:[EBHMM+H⁺]= 462.3699. The spectra of ¹H NMR titration and mass analysis are shown in SF.11 and SF. 12. The selectivity of the probe with a series metal ions is shown in Figure 1.

3.5. Interference studies

The investigation of possible interferences of other metal ions (100 equiv.) with the sensor EBHMM-Fe³⁺ ion complex were carried out with the help of competitive complexation analysis. The results show that there is no significant change in the intensity of azine-Fe³⁺ ion on addition of other metal ions in to the solution. The extent of intensity variation due to the interference of other metal ions (Chloride of Cu²⁺, Fe²⁺, Fe³⁺, K⁺, La³⁺ and nitrate of Ag⁺, Al³⁺, Ba²⁺,Bi²⁺ Pb²⁺, Cr³⁺, Cd²⁺, Hg²⁺, Li⁺ Zn²⁺, Zr²⁺, Co²⁺, Ni²⁺, Mn²⁺, Mg²⁺, Ca.²⁺, Na⁺) is shown in Figure 2. It is concluded from the results that the sensor EBHMM can specifically detect Fe³⁺ ion without the interference of potentially competing metal ions.

3.6. Stern–Volmer and Benesi–Hildebrand plot

In the fluorescence study, the sensor exhibits strong fluorescence at 423nm in DMF/H₂O (1:1, EBHMM=5 μ M, pH=7.4) solution. A fluorescence titration was carried with different concentrations Fe³⁺ ion of FeCl₃ from 0 to 100 equiv. to inspect the sensing ability of EBHMM. The fluorescence intensity gradually decreases with the increase in concentration up to 80 equiv. of Fe³⁺, after that there is no much change in fluorescence intensity. The quenching of fluorescence intensity with various concentration of Fe³⁺ is shown in Figure 3. Further, the stoichiometry of EBHMM with Fe³⁺ is confirmed by the Stern–Volmer and Benesi–Hildebrand plot. The fluorescence quenching of EBHMM with different concentration of Fe³⁺ ion was analyzed with Stern-Volmer plot (Figure 4). The Stern-Volmer equation is,

$I_0/I = 1 + K_{SV}[Q]$

Where, I_0 and I are the fluorescence intensities of the probe in absence and presence of the quencher (Fe³⁺) respectively. [Q] is the concentration of the quencher and K_{sv} is Stern-Volmer quenching constant. The Stern-Volmer plot between I_0/I and [Q] gives a linear line indicating that there is a dynamic and dominant diffusion process taken place upon the addition of Fe³⁺ ion in to the sensor solution. The correlation coefficient value calculated from Stern-Volmer linear plot is (R²=0.97532) shows that there is a good linearity and it can be used for the

qualitative determination of Fe^{3+} ion both in environmental and biological systems. The limit of detection (LOD) was calculated using the formula,

$LOD = 3.3\sigma/k$

Where, σ is the y-intercept of the regression line and k is the slope of the calibration graph [42]. The estimated value of LOD is 7.7×10^{-8} M.

The Benesi–Hildebrand plot (Figure 5) is used to determine the binding constant of Fe^{3+} ion with the sensor EBHMM. Binding constant (Ka) calculated from the slope of the plot is 1.0034 M⁻¹.

3.7. Effect of Time

The effect of time during the detection of Fe^{3+} ion utilizing the sensor EBHMM was analyzed with DMF/H₂O solution (1:1, EBHMM=5µM, pH=7.4). The sensor shows high fluorescence intensity at 423nm in the absence of Fe³⁺ ion in the solution. Upon the addition of Fe³⁺ ion into the sensor solution, the fluorescence intensity was gradually decreased and attained saturation in 4 minutes. This instantaneous quenching of intensity is a spot response of EBHMM towards the detection of Fe³⁺ ion. Therefore, it can be used for the detection of Fe³⁺ ion real time analysis [43]. The time response of azine with Fe³⁺ is shown in the SF. 13.

3.8. Effect of pH

The fluorescence emission of EBHMM at different pH (from 1 to 12) both in presence and absence of Fe³⁺ ion was investigated in (DMF/H₂O1:1, EBHMM=5 μ M) solution to get the response of EBHMM in the detection of Fe³⁺. When the pH of the solution increased the equilibrium between phenolic and phenoxide is shifted to phenoxide formation. The formation of phenoxide ion reduces the energy gap between HOMO and LUMO by increasing the conjugation of π electrons. Therefore, less energy is required for the electron transition and the intensity of fluorescence emission is gradually increasing [44].The quenching and enhancing of fluorescence at different pH indicates the capability of EBHMM to choose a suitable pH for the detection of Fe³⁺ ion. The effect of pH on fluorescence emission is illustrated in the SF. 14.

3.9. Reversibility of the chemosensor

The reversible capability is an important requirement of a chemosensor for the detection of ions for several cycles. The reversibility of EBHMM with Fe^{3+} ion was investigated in 1:1 DMF/H₂O solution (EBHMM=5µM, pH=7.4) by the addition of ethylene diamine tetraacetate (EDTA). The fluorescence intensity was increasing gradually by the

addition of EDTA (10 to 100 equiv.) into the solution of EBHMM-Fe³⁺. The entire quenching was nullified by the addition of EDTA in the EBHMM -Fe³⁺ solution. The reversibility of EBHMM with Fe³⁺ ion on the addition of EDTA is clearly shown in Figure 6.

4. Computational Method

4.1. Optimized molecular Structure

The exact binding mode of EBHMM with Fe^{3+} ion was found with the help of DFT study. The structure of EBHMM was theoretically studied with DFT. The optimized structure is in trans form due to steric effect and is depicted in the SF. 15.

4.2. Frontier molecular orbital analysis

The analysis of wave function ascertains that the electron absorption corresponding to transition from the ground state to the first excited state via one-electron excitation from HOMO to LUMO. So, both HOMO and LUMO are taking part in the chemical reaction. HOMO energy characterizes the capability of electron donating and LUMO characterizes the capability of electron accepting [45]. The energies of HOMO and LUMO were calculated with B3LYP/6-311++G (d, p) level and is shown in SF. 16, which shows the distribution and energy levels of the HOMO and LUMO orbitals. In the HOMO and LUMO of EBHMM the electron densities are localized on all the atoms except the methoxy group (E_{HOMO}=-5.8097eV & E_{LUMO} =-2.5598eV). The value of energy separation between the HOMO and LUMO is 3.2499eV. In the contour map, the lines with red colors are regarded as the negative phase and the lines with green colors are the positive phase. The contour of HOMO-LUMO diagram is in accordance with the frontier orbital. The frontier orbital gap helps to characterize the optical polarizability chemical hardness-softness and chemical reactivity of a molecule [46]. Small H_{OMO}-L_{UMO} energy gap means, the compound has low excitation energy with low chemical hardness and good stability. The molecules having small energy gap are known as soft, and molecules having large energy gap are known as hard molecules. The soft molecules are more polarizable than the hard ones, because they need small energy for excitation. The hardness value of a molecule can be determined by the formula [47],

$\eta = 1/2(I - A)$

Where, E_{HOMO} and E_{LUMO} are the energies of HOMO and LUMO molecular orbitals. The value of η of azine is -1.62495 (a.u), indicates that the compound has low chemical hardness [48] and less resistance to charge transfer. The result clearly illustrated that the metal ions can easily make bond with azine molecule.

Mechanism of sensing

To study the mechanism of "Turn off" fluorescence on binding Fe^{3+} with azine probe is proved by carrying out DFT studies of azine probe and $Fe(OH)_3$. The HOMO-LUMO energy diagram of the probe and metal ion ($Fe(OH)_3$ are depicted in SF.17. In general, the quencher (metal ion) will act as electron acceptor in photoinduced electron transfer (PET) process when its LUMO is lower than the probe. Since the LUMO of Fe^{+3} is lower than the azine probe they involve in PET and is responsible for Turn off fluorescence [49-52]. The values HOMO-LUMO are given in the Table 3.

4.3. Molecular electrostatic potential [MEP] and Contour map

The different values of the electrostatic potential are symbolized by different colors at the MEP surface. The red and blue colours represent the regions of most negative (electrophilic reactivity) and most positive (nucleophilic reactivity) sites. Other colours in the map indicate zero electrostatic potential sites of the molecule and the contour map shows that the electron flow is maximum around the azine moiety than the other atoms. Due to high electron density, the nitrogen atom is more prone to electrophilic attack. The three dimension charge distributions of the azine MEP and the contour map are clearly shown in the SF.18. Furthermore, the exact active site for electrophilic and nucleophilic attack on the molecule can be explained using global and local descriptor.

4.4. Global descriptors

The global descriptors were calculated from the energy gap values of HOMO-LUMO. By using this energy gap value, Koopmans's theorem can be used to determine the global descriptors of electronegativity (χ), electrophilicity index (ω), global softness (S), chemical potential (μ) and global hardness (η) of the molecule [53]. Here, A and I indicates the HOMO and LUMO energy values.

$$\chi = -(I+A)/2$$

 $\mu = -\chi = (I+A)/2$
 $\eta = 1/2(I - A)$
 $= \mu^2/2\eta$
 $S = 1/2\eta$.

N max=- μ/η

The global descriptors of the molecule calculated using the above equations are depicted in Table 1.

Electrophilicity index measures the energy stabilization when the system gains additional electronic charge from surroundings [54]. The higher value of electrophilicity index indicates the greater electrophilic behaviour of the system as well as lower chemical potential [55]. The low value of global electrophilicity index and higher value of chemical potential of EBHMM, clearly indicate strong nucleophilic behavior of the azine sensor.

4.5. Local descriptors

Fukui function is one of the widely used local reactivity descriptors for chemical reactivity and site selectivity. Fukui functions (f_{k+} , f_{k-} , f_{k0}) were calculated using B3LYP/6-311+ +G (d,p) and the following equations based on the research work of Yang and Mortier [56].

$$\begin{split} f_k^{\ +} &= [q(N+1)\text{-}q(N)] - \text{For nucleophilic attack} \\ f_k^{\ -} &= [q(N)\text{-}q(N\text{-}1)] - \text{For electrophilic attack} \\ f_k^{\ 0} &= 1/2[q(N+1)\text{-}q(N\text{-}1)] - \text{For radical attack} \end{split}$$

Where N, N-1 and N+1 are total electrons present in neutral, cationic and anionic state on the molecule respectively.

Local softness

Local softness $(S_k^+, S_k^- \text{ and } S_k^0)$ and local electrophilic index $(\omega_k^+, \omega_k^- \text{ and } \omega_k^0)$ are also used to describe the reactivity of atoms in molecule. Here +, - and 0 indicate nucleophilic, electrophilic and radical attacking sites and their values are presented in Table 2.

The atoms N17 and N18 are having high electrophilicity index value respectively 0.9817 and 1.1524 than the other atoms. It is confirmed that N17 and N18 atoms have more electronegativity and are more prone to electrophilic attack [57] in the chemosensor study. The results of local descriptor confirmed the fluorescence quenching of probe due to PET by involving N17 and N18 atoms. The possible mode of binding between azine and Fe³⁺ ion is shown in Figure 7. The non-involvement of -OH in binding is verified by ¹H NMR titration. The spectra don't show any decrease in intensity of -OH signal during the titration. Therefore, the binding of Fe³⁺ with azine probe occur only with N17 and N18 atoms and not with phenolic groups.

5. Conclusion

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Fluorescently spot chemosensor responded azine EBHMM was synthesized and confirmed by using FT-IR, UV-Vis, ¹H, ¹³C{¹H}NMR spectroscopic techniques and mass spectrometry. In chemosensor study, EBHMM selectively gives very spot response towards Fe^{3+} ion and is confirmed by HRMS-ESI spectrum. A good linearity was observed in Stern-Volmer plot of EBHMM with Fe^{3+} between 15 and 85µM concentration. The variation of fluorescence intensities indicate the capability of azine towards the detection of Fe^{3+} ion in different pH.

The concentration depended quenching and reversibility of the probe with EDTA clearly indicating the strong binding between azine and Fe^{3+} ion. No change in the intensity of phenolic proton in ¹H NMR titration indicate that it is not coordinated with Fe^{3+} ion. The binding sites of molecule with metal ion was theoretically calculated by using DFT study in B3LYP functional with B3LYP/6-311+ +G (d,p) level of basis set. ESP and Contour analysis map clearly indicates the electron rich and electron flow mostly around the azine in the molecule. Global and Local descriptors are strongly gives the evidence that molecule has high nucleophilic behavior and the atoms N17 and N18 atoms are more prone to electrophilic attack in the chemosensor study due to high electrophilicity index. The results of Fukui Function and the Frontier molecular orbital analysis strongly supported the quenching mechanism occurred by the electron transition from the probe to metal through the N17 and N18 atoms.

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Table 1: Global descriptors values of EBHMM

I= - HOMO	A= -LUMO	$\chi = -(I{+}A)/2$	μ= - χ	η= 1/2(I - A)	S=1/2n	ω=μ²/2η	N_{max} =- μ/η
-5.8097	-2.5598	4.1847	-4.1847	-1.6249	-0.8123	-14.2281	-2.5753

	Natural atomic charges (eV)		Fukui functions(eV)		Local softness(eV)		Local electrophilicity index(eV)					
Atoms	qN+1	qN0	qN-1	fk+	fk0	fk-	Sk+	Sk0	Sk-	ω+	ω0	ω-
1C	-0.263	-0.251	-0.239	-0.012	-0.012	-0.012	0.0097	0.0097	0.0097	0.1707	0.1707	0.17073
2C	0.293	0.314	0.311	-0.021	-0.009	0.003	0.0170	0.0073	-0.0024	0.2987	0.1280	-0.0426
3C	0.014	-0.021	-0.05	0.035	0.032	0.029	-0.0284	-0.0260	-0.0235	-0.4979	-0.4553	-0.4126
4C	-1.101	-1.125	-1.159	0.024	0.029	0.034	-0.0195	-0.0235	-0.0276	-0.3414	-0.4126	-0.4837
5C	0.876	0.832	0.796	0.044	0.040	0.036	-0.0357	-0.0325	-0.0292	-0.6260	-0.5691	-0.5122
6C	0.728	0.656	0.606	0.072	0.061	0.05	-0.0585	-0.0495	-0.0406	-1.0244	-0.8679	-0.7114
7C	-0.872	-0.881	-0.948	0.009	0.038	0.067	-0.0073	-0.0308	-0.0544	-0.1280	-0.5406	-0.9532
8C	0.201	0.161	0.163	0.04	0.019	-0.002	-0.0325	-0.0154	0.0016	-0.5691	-0.2703	0.0284
9C	1.083	1.048	1.088	0.035	-0.002	-0.040	-0.0284	0.0020	0.0324	-0.4979	0.0355	0.5691
10C	-0.223	-0.213	-0.154	-0.01	-0.034	-0.059	0.0081	0.0280	0.0479	0.1422	0.4908	0.8394
11C	0.139	0.086	0.039	0.053	0.05	0.047	-0.0430	-0.0406	-0.0381	-0.7540	-0.7114	-0.6687
12C	0.047	0.006	-0.02	0.041	0.033	0.026	-0.0333	-0.0272	-0.0211	-0.5833	-0.4766	-0.3699
13C	-0.627	-0.637	-0.707	0.01	0.04	0.07	-0.0081	-0.0325	-0.0568	-0.1422	-0.5691	-0.9959
14C	-0.535	-0.521	-0.666	-0.014	0.065	0.145	0.0113	-0.0532	-0.1178	0.1991	-0.9319	-2.0630
15N	0.116	0.060	0.074	0.056	0.021	-0.014	-0.0455	-0.0170	0.0113	-0.7967	-0.2987	0.1991
16N	0.085	0.021	0.048	0.064	0.018	-0.027	-0.0520	-0.0150	0.0219	-0.9106	-0.2632	0.3841
17N	-0.217	-0.251	-0.32	0.034	0.051	0.069	-0.0276	-0.0418	-0.0560	-0.4837	-0.7327	0.9817
18N	-0.195	-0.22	-0.301	0.025	0.053	0.081	-0.0203	-0.0430	-0.0658	-0.3557	-0.7540	1.1524
19C	0.002	0	-0.041	0.002	0.021	0.041	-0.0016	-0.0174	-0.0333	-0.0284	-0.3059	-0.5833
20C	0.003	-0.002	-0.05	0.005	0.026	0.048	-0.0040	-0.0215	-0.0390	-0.07114	-0.37705	-0.6829
210	-0.491	-0.535	-0.56	0.044	0.034	0.025	-0.0357	-0.0280	-0.0203	-0.62604	-0.49087	0.3557
220	-0.364	-0.371	-0.375	0.007	0.005	0.004	-0.0056	-0.0044	-0.0032	-0.0996	-0.07825	0.0569
23C	0.184	-0.176	-0.17	0.36	0.177	-0.006	-0.2924	-0.1438	0.0048	-5.12214	-2.51839	0.0853
240	-0.449	-0.508	-0.541	0.059	0.046	0.033	-0.0479	-0.0373	-0.0268	-0.83946	-0.6545	-0.4695
250	-0.34	-0.360	-0.372	0.02	0.016	0.012	-0.0162	-0.0130	-0.0097	-0.28456	-0.22765	-0.1707
26C	-0.108	-0.111	-0.117	0.003	0.004	0.006	-0.0020	-0.0036	-0.0048	-0.04268	-0.06403	-0.0853

Table 2: Local descriptors values of EBHMM

Compound	I=-Homo	A=-Lumo	Band Gap(eV)	MO.Number
EBHMM	-5.8097	-2.5598	3.2499	93(U),94(O)
Fe(OH) ₃	-6.8395	-6.6582	0.1812	27(O),28(U)

Table 3: HOMO LUMO and band gap value of ligand and metal

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Figure 1. Selective responses of EBHMM in DMF-H₂O solution (1:1, EBHMM=5μM, pH=7.4) in the presence of various metal ions (100 equiv. λex=423 nm)







Figure 3. Fluorescence titration spectrum of EBHMM (1:1, EBHMM=5µM, pH=7.4) in the presence of different concentrations of Fe³⁺ (0–100 equiv.) excited at 423 nm



Figure 4. Stern–Volmer linearity plot of EBHMM with [Fe³⁺]



(1:1) binding stoichiometry



Figure 6. Reversibility of EBHMM - Fe³⁺ with EDTA



Figure 7. Proposed binding mode of Fe³⁺ with EBHMM

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Scheme 2: Synthesis of 4,4-((ethane-1,2-diylidene)bis(hydrazine-2,1diylidene))bis(methanylylidene) bis(methanylylidene)) bis(2-methoxyphenol) (EBHMM)

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Highlights

- > Azine based chemosensor was successfully designed and synthesized.
- > The sensor showed "turn-off" responses for Fe^{3+} ion over other metal ions.
- > The sensor is sensitive and selective to Fe^{+3} ion at different concentrations and various pH.
- The binding sites of the molecule with metal ion was theoretically obtained using DFT study in B3LYP functional with B3LYP/6-311+ +G (d,p) level of basis set.

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ANALYSIS USING DFT

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