Synthesis and characterization of \( p \)-dimethylaminobenzaldehyde benzoylthiourea and study towards selective and sensitive fluorescent sensor for detection of iron (III) cation in aqueous solution.

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Abstract. Benzoyl thiourea derivatives ligand backbones contain Oxygen (O), Nitrogen (N), and Suphur (S) donor atoms can react with transition metal ions and form stable metal complexes. A new ligand was synthesized by the reaction of benzoyl isothiocyanate with diethylenetriamine and characterized by using Elemental Analysis (EA), Infrared (IR) spectroscopy and proton Nuclear Magnetic Resonance (\(^1\)H NMR) spectroscopy. The monofunctionalize ligand were synthesized using 1:1 condensation of \( p \)-dimethylaminobenzaldehyde with diethylenetriamine and confirmed by disappearance of carbonyl group, C=O at 1656 cm\(^{-1}\) with the appearance of peak C=N in the range 1633 - 1638 cm\(^{-1}\) in the IR spectrum. This monofunctionalize ligand was synthesis by using Schiff base technique. Benzoyl thiourea derivatives, HN are synthesis from the 1:1 condensation of benzoyl isothiocyanates with monofunctionalized ligand. HN ligand was verified by the presence of peaks \( \nu \)(N-H), \( \nu \)(C=O), \( \nu \)(C=N) \( \nu \)(C-N) and \( \nu \)(C=S) at 3317 - 3336 cm\(^{-1}\), 1612 - 1660 cm\(^{-1}\), 1550 -1589 cm\(^{-1}\), 1234-1366 cm\(^{-1}\) and 709-767 cm\(^{-1}\) respectively while \(^1\)H NMR show peaks of alkane (CH\(_2\)), benzene (Ar-H), CONH, CSNH at 3.75, 6.73 – 7.33, 8.19, and 8.25 respectively. The total percent composition of C, H, N, O, and S using Elemental Analysis for HN, C\(_2\)H\(_2\)N\(_2\)OS found was 85.23%. For application, further study on selectivity and sensitivity of HN ligand was conducted on several metal ions. The fluorescent emission spectroscopy shown that HN is a selective fluorescent sensor for Fe\(^{3+}\) and Cu\(^{2+}\) ions but not for other metal ions such as Co\(^{2+}\), Ni\(^{2+}\), Mn\(^{2+}\), Cr\(^{3+}\) and Zn\(^{2+}\). However, HN ligand shows more sensitive towards Fe\(^{3+}\) ions than Cu\(^{2+}\) ions which provide rapid detection of Fe\(^{3+}\) ions at concentrations as low as 4.5 x 10\(^{-7}\) M than 2.9 x 10\(^{-6}\) M of Cu\(^{2+}\) ions. As conclusion, HN ligand was expected to be useful as efficient chemical sensor for detection of Fe\(^{3+}\) ion.

1. Introduction

Nowadays, the development of chemosensors for selectivity and sensitivity quantification of environmentally and biologically for heavy and transition metal ions, has attracted a high attention [1]. They usually represent an environmental concern when present in uncontrolled amounts, but at the same time some of them such as iron, zinc, manganese, copper and cobalt are present as essential elements in biological systems. Among biologically important metal ions, iron is the most abundant transition metal ion present in the human body. As one of the most essential trace elements in
biological systems, Fe$^{3+}$ performs a major role in many biochemical processes. Fe$^{3+}$ ion is an important and essential for proper functioning of all living cells and acts as a cofactor in many enzymatic reactions to human as well as in specialized transport and storage of proteins. The deficiency of Fe$^{3+}$ causes anaemia, liver damage, diabetes, hemochromatosis, Parkinson’s disease and cancer [2]. However, iron overload is a clinical condition in which there is an excess of iron levels in the organism typically due to abnormally high iron absorption from the diet, periodical blood transfusions or wrong use of iron by the organism as result of the blocking action of determined cytokines [3]. If continued in time, high levels of Fe$^{3+}$ within the body have been associated with increasing incidence of certain cancers and dysfunction of certain organs, such as heart, pancreas and liver. In recent years, several fluorescent sensors have been developed for the detection of transition and heavy metal ions, such as dimethylaminocinnamaldehyde-aminothiourea (DA) for detecting Ag$^+$, Hg$^{2+}$ and Cu$^{2+}$ [4], p-dimethylaminobenzaldehyde thiosemicarbazone for detecting Hg$^{2+}$ [5], rhodamine derivatives (RBS) for detecting Cu$^{2+}$ and F$^-$ [6] and also salicylaldimine based receptor sensing of multi metal ions and anions [7]. Meanwhile, various sensors for Fe$^{3+}$ have been reported [8][9][10]. However, to the best of our knowledge, the static quenching mechanism for aminothiourea-based chemosensor to monitor trace levels of iron ions is rarely reported. Fluorescence-based analytical methods are widely used for detection of metal ions in an early stage because they are sensitive, simple and less expensive. In this study, the fluorescent sensor for sensitive determination of iron is reported. In design of fluorescent sensors for these ions, aminothiourea is a preferred receptor because of its strong affinity [4]. Scheme 1 below shows the reaction of synthesis of ligand HN step by step.

![Scheme 1 Synthesis of ligand HN](image-url)
1.1. Apparatus and reagents

The inorganic salts Fe(Cl$_2$)$_3$.6H$_2$O, Mn(SO$_4$)$_2$, Cr(SO$_4$)$_2$.6H$_2$O, Co(Cl$_2$)$_2$.6H$_2$O, Ni(NO$_3$)$_2$.6H$_2$O, Cu(NO$_3$)$_2$.3H$_2$O and Zn(Cl$_2$)$_2$ were purchased from Sigma Aldrich. All solvents and other reagents were of analytical grade. $^1$H NMR spectra are recorded on a Bruker Avance III 300 Spectrometer at room temperature. $^1$H chemical shifts are reported in ppm downfield from tetramethylsilane (TMS, δ scale with the solvent resonances as internal standards). Melting points were measured using BÜCHI Melting Point B-545 and the elemental analyses were conducted using CHNS Analyzer Flash EA 1112 series. The infrared spectra were performed using FTIR Perkin Elmer 100 Spectrophotometer in the spectral range of 4500-600 cm$^{-1}$.

1.2. General Procedure for Fluorescence spectrometer

The fluorescence spectra were recorded on Luminescence Spectrometer LS 55 equipped with quartz cuvettes of 1 cm path length. The excitation and emission slit widths were 10 nm. Stock solutions for analysis were prepared (0.1 M for Ligand HN (Acetonitrile/H$_2$O, 9:1 (v/v), pH = 7.0) immediately before the experiments. The fluorescence titration was performed with a series of 5 × 10$^{-5}$ M solutions of HN containing various equivalents of Fe$^{3+}$ ions. Binding studies were confirmed by job plot. The association constant (Ka) were calculated by the linear Benesi-Hildebrand equation from fluorescence (Eq. (1)) study by,

$$\frac{1}{F-F_0} = \frac{1}{(F_{\text{min}}-F_0)} + \frac{1}{K(F_{\text{min}}-F_0)} \times \frac{1}{[M^{n+}]} \quad (1)$$

Where $F$, $F_0$ and $F_{\text{min}}$ are the emission intensities, respectively in the absence and infinite concentration of the metal ion (Fe$^{3+}$).

1.3. Synthesis of 1-Benzoyl-3-(2-{2-[4-dimethylamino-benzylidene)-amino]-ethylamino}-ethyl)-thiourea, HN.

A solution of benzoyl chloride (10 mmol; 1.2 ml) in 10 ml acetonitrile is added to a solution of ammonium thiocyanate (10 mmol; 0.80 g) in 40 ml acetonitrile under stirring for 30 minutes at room temperature. The white precipitate NH$_4$Cl is will bestow and filtered off. Diethylene triamine (50 mmol; 5.4 ml) is dissolved in 20 ml of acetonitrile and added of 4-dimethylaminobezaldehyde (50 mmol; 7.46 g) in 25 ml of acetonitrile. The reaction mixture is refluxed for 6 h at 100°C. Then, the acetonitrile is vaporized from the mixture by using rotary evaporator. The oily solution is dried to surrounding for an hour until it becomes solid. The percentage yield and melting point of the monofunctional symmetrical triamine are calculated and recorded. The benzoyl isothiocyanate (10 mmol) in 50 ml of acetonitrile is added dropwise to a round-bottomed flask containing the monofunctional ligand. Then, the mixture is refluxed for 24 hours at 100°C. On cooling, water is added to furnish as solid. The product is recrystallized twice. 

HN: Yield 66%; dark orange solid, m.p 119 °C. IR (cm$^{-1}$): ν(C=O) 1660, ν(N-H) 3336, ν(C=N) 1550, ν(C=S) 1064. $^1$H NMR (600 MHz, Acetone-δ$_6$) δ 8.19 (s, 1H), 8.19 (s, 1H), 6.73-7.33 (dd, 6H), 2.75 (s, 6H), 2.06 (s, 1H). Anal. Calc. for C$_{21}$H$_{27}$N$_3$OS: C, 63.45; H, 6.85; N, 17.62; S, 8.06. Found: C, 60.01; H, 5.31; N, 17.54; S, 8.06.
2. Results and Discussions

2.1. Sensing in the presence of competing ions.

Ligand **HN** was applied as chemosensor by using Fluorescence Emission Spectroscopy (FES) to observe several parameters such as selectivity, sensitivity, stoichiometry and binding mode studies and also the competition.

2.1.1. Selectivity

For an excellent chemosensor, high selectivity is a matter of necessity. Among the various metal ions, the fluorescence emission spectra showed a noteworthy high selectivity to Fe$^{3+}$ and Cu$^{2+}$ with respect to the fluorescence quenching. Other metal ions developed no distinct significant fluorescence intensity changes. Related metal ions, including Fe$^{3+}$, Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Cr$^{3+}$ and Zn$^{2+}$, were used to evaluate the metal ion binding properties of **HN** in ACN/ H$_2$O (10:1, v:v, pH 7.0) solution by fluorescence spectroscopy (Fig. 1).

![Fluorescence emission spectra of HN in the presence of various metal ions in ACN/H$_2$O (10:1, v:v, pH 7.0) solution at room temperature.](image)

**Figure 1.** Fluorescence emission spectra of **HN** in the presence of various metal ions in ACN/H$_2$O (10:1, v:v, pH 7.0) solution at room temperature.

2.1.2. Sensitivity

![Fluorescence intensity vs. concentration plot for Fe$^{3+}$](image)

(a)  

For an excellent chemosensor, high selectivity is a matter of necessity. Among the various metal ions, the fluorescence emission spectra showed a noteworthy high selectivity to Fe$^{3+}$ and Cu$^{2+}$ with respect to the fluorescence quenching. Other metal ions developed no distinct significant fluorescence intensity changes. Related metal ions, including Fe$^{3+}$, Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Cr$^{3+}$ and Zn$^{2+}$, were used to evaluate the metal ion binding properties of **HN** in ACN/ H$_2$O (10:1, v:v, pH 7.0) solution by fluorescence spectroscopy (Fig. 1).
HN can detect both Fe$^{3+}$ ion and Cu$^{2+}$ ion but HN more sensitive sensor towards Fe$^{3+}$ ion than Cu$^{2+}$ ion. Figure 2 shows the results of fluorescence titration of HN upon the gradual addition of Fe$^{3+}$ (Fig. 2.(a)) and Cu$^{2+}$ (Fig 2.(b)) in ACN/H$_2$O solution under excitation at $\lambda = 561$ nm. A linear relationship was observed to exist between the relative fluorescent intensity of HN and the concentration of Fe$^{3+}$ in the range of $5 \times 10^{-7}$ M - $7.5 \times 10^{-7}$ M with a detection limit of $4.5 \times 10^{-7}$ M while the concentration of Cu$^{2+}$ in the range of $1 \times 10^{-5}$ M - $4 \times 10^{-5}$ M with a detection limit of $2.9 \times 10^{-6}$ M. According to the U.S. Environmental Protection Agency (EPA), the maximum content of iron in drinking water is ~5.357 µM. Therefore, detection of trace irons becomes very important in the chemical research[11].

2.1.3. Stoichiometry and binding mode studies

Figure 3. Job’s plot for determining the binding stoichiometry in ACN/H$_2$O (10:1/ v:v, pH 7.0) solution, showing a 1:1 stoichiometry between HN and Fe$^{3+}$. The variation of which indicated that the stoichiometry of HN-Fe$^{3+}$ complex was 1:1. The variation of Job’s function ($F_{Job}$) at 561 nm was measured as a function of the molar ratio X ([Fe$^{3+}$]/([Fe$^{3+}$] + [chemosensor])).
Fe\(^{3+}\) ion was selected for further study in binding mode to the sensor HN. The stoichiometry formation of HN-Fe\(^{3+}\) complex was 1:1. To gain an insight into the stoichiometry of the HN-Fe\(^{3+}\) complex, the binding stoichiometry was determined by the Job plot. The Job’s function \(F_{\text{Job}}\) is calculated according to the equation \(F_{\text{Job}} = (1 - X) F_0 - F\). The plot of the luminescence versus the mole fraction of the added Fe\(^{3+}\) (Fig. 3) shows two parts that can be fitted by straight lines. The lines intersect at X equal 0.5. According to the Benesi-Hilderbrand equation \(1/(F_0 - F) = 1/(K_a(F_0 - F_{\min}) + 1/(F_0 - F_{\min}))\), where \(F_{\min}\) is the minimum fluorescence intensity at 561 nm in the presence of Fe\(^{3+}\)), the binding constant \(K_a\) is calculated to be 4.58 x 10\(^{5}\) M\(^{-1}\).

2.1.4. Competition of Fe\(^{3+}\) toward other metal ions

The competition experiment, which was carried out by adding Fe\(^{3+}\) to HN solution in the presence of other metal ions (Fig. 4), showed that the Fe\(^{3+}\)-induced fluorescence response was not interfered by commonly coexistence ions. The result suggested that probe HN showed a remarkable selectivity toward Fe\(^{3+}\) over other competitive ions. However, Cu\(^{2+}\) also showed a remarkable selectivity but not as sensitive as Fe\(^{3+}\) after we used all the metal ions in 5 x 10\(^{-6}\) M.

3. Conclusion

In conclusion, HN bearing thiourea moiety as the binding site and \(p\)-dimethylaminobenzaldehyde moiety as the signal group was designed and synthesized to apply as fluorescent sensor for Fe\(^{3+}\) ion. This sensor showed specific selectivity for Fe\(^{3+}\) in ACN/H\(_2\)O binary solutions indicated that the aminobenzaldehyde moiety acted as a signal group (fluorophore) and played a crucial role in the process of fluorescent recognition. Investigation of the binding constant mechanism indicated that the sensor HN recognized Fe\(^{3+}\) by forming a stable 1:1 HN- Fe\(^{3+}\) complex. The coexistence of other cations did not interfere with the Fe\(^{3+}\) recognition process unless for Cu\(^{2+}\). HN also sense the Cu\(^{2+}\) but not as sensitive as Fe\(^{3+}\). Moreover, the detection limit of the sensor HN toward Fe\(^{3+}\) was 4.5 x 10\(^{-7}\) M and 2.9 x 10\(^{-6}\) M toward Cu\(^{2+}\). This indicated that the sensor HN may be useful for preliminary detection of Fe\(^{3+}\) ion by simple solvent extraction in chemical and environmental applications.
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