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# SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND DNA BINDING STUDIES OF Fe(II) COMPLEXES OF NICOTINOYLHYDRAZONES

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## ABSTRACT

A series of nicotinoyl hydrazones and their Fe(II) complexes have been synthesized and characterized based on elemental analysis, molar conductivity, infrared and electronic spectroscopy. The spectral data showed that the ligand behaved as a monobasic tridentate and was bonded to the metal ions *via* the carbonyl oxygen atom in the ketonic form, the azomethine nitrogen atom and the deprotonated phenolic hydroxyl oxygen. Electrochemical behaviour of these complexes is investigated by cyclic voltammetry. The DNA binding constants $K_b$ of the complexes are determined systematically with spectrophotometric titrations by using Calf Thymus DNA (CT-DNA). The complexes show better binding activity.

**KEY WORDS:** Nicotinoyl hydrogens, Iron complexes, Cyclic voltammetry, DNA binding.

## INTRODUCTION

There has been significance importance in studying hydrazones and their metal complexes owing to their application as antifungal[1–3] antibacterial[1–4] anticonvulsant[5] anti-inflammatory[3] antimalarial[6] analgesic[7] antiplatelets[8] antituberculosis[9] anticancer activities.[10]

Recently, the interaction of metal complexes with nucleic acids has attracted much attention because of their potential use as drugs, regulators of gene expression and tools for molecular biology. In general, two most common non-covalent binding modes for such systems are the groove binding and intercalating modes. Moreover, many therapeutic agents, particularly anticancer drugs, are known to bind DNA via either of these modes and the possibility of gene modulation by specific sequence binding of small molecules to DNA has also been explored.[11-13] In our previous reports, we have demonstrated that the complexes display better DNA binding activity.[14-17]

In the light of the above and in continuation of our ongoing research work, here in, we report synthesis, spectral characterization and DNA binding activity of Iron(II) complexes with a series of three NH ligands. Three ligands viz. 2,5-dihydroxy benzaldehyde nicotinoyl hydrazone(DBNH), 2,5-dihydroxy acetophenone nicotinoylhydrazone(DANH) and 2,5-dihydroxy benzophenone nicotinoyl hydrazone(DBPNH) are synthesized and characterized.

## EXPERIMENTAL

**Materials and methods:** Nicotinic acid hydrazide, 2,5-dihydroxybenzaldehyde, 2,5-dihydroxyacetophenone and 2,5-dihydroxybenzophenone were purchased from Sigma-Aldrich. All other chemicals were of AR grade and used as provided. The solvents used for the synthesis were distilled before use. Calf –Thymus DNA (CT-DNA) was purchased from Genio Bio labs, Bangalore, India. Elemental analyses were carried out on a Heraeus Vario EL III Carlo Erba 1108 instrument. Molar conductivity measurements at 298 ± 2K in dry and purified DMF were carried out using a ELICO CM model 162 conductivity meter. The electronic spectra were recorded in DMF with a UV lamda50 (Perkin-Elmer) spectrophotometer. IR spectra were recorded in the range 4000–400 $cm^{-1}$ with a Perkin-Elmer spectrum100 spectrometer on KBr discs. Cyclic voltammetric measurements were taken on a CH instruments assembly equipped with an X–Y recorder. Measurements were taken on degassed ($N_2$ bubbling for 5 min) solutions ($10^{-3}$ M) containing 0.1 M $Bu_4NPF_6$ as the supporting electrolyte. The three-electrode system consisted of glassy carbon (working), platinum wire (auxiliary) and Ag/AgCl (reference) electrodes.

### Preparation of Ligands

Ligands were prepared by reacting nicotinic acid hydrazide with carbonyl compounds. A methanolic solutions of nicotinylhydrazide (5mmol), carbonyl compound (5mmol) were mixed in a 100-ml round bottom flask. Two drops of HCl were added to the reaction mixture and refluxed for 3-6 hours. On cooling the reaction mixture to room temperature, yellow coloured crystalline products were separated. The products were collected, washed with methanol and few drops of hexane and dried in vacuum. General structure of ligands is shown in Fig. 1.

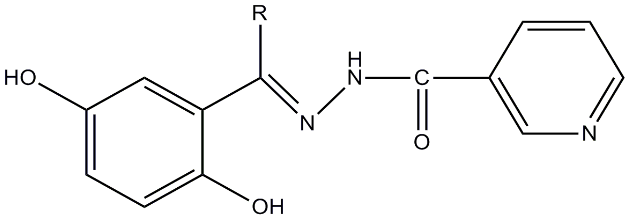

**Fig 1: General structure of ligand**

R= H, 2,5- Dihydroxy benzaldehyde nicotinoylhydrazone(DBNH),
R= $CH_3$, 2,5- Dihydroxy acetophenone nicotinoylhydrazone (DANH),
R= $C_6H_5$, 2,5- Dihydroxy benzophenone nicotinoylhydrazone (DBPNH).

**Preparation of complexes**

To a methanolic solution of ligand (5mmol), the aqueous solution of metal salt ($FeCl_2 . 4H_2O$) was added. The resulting solution was refluxed with stirring for one hour and then kept at room temperature and then filtered washed with methanol and dried in vacuo. The analytical data of all the complexes are given in Table 1. The $ES^+I$ mass spectrum of $Fe(DBNH)_2$complex are shown in Fig. 2.

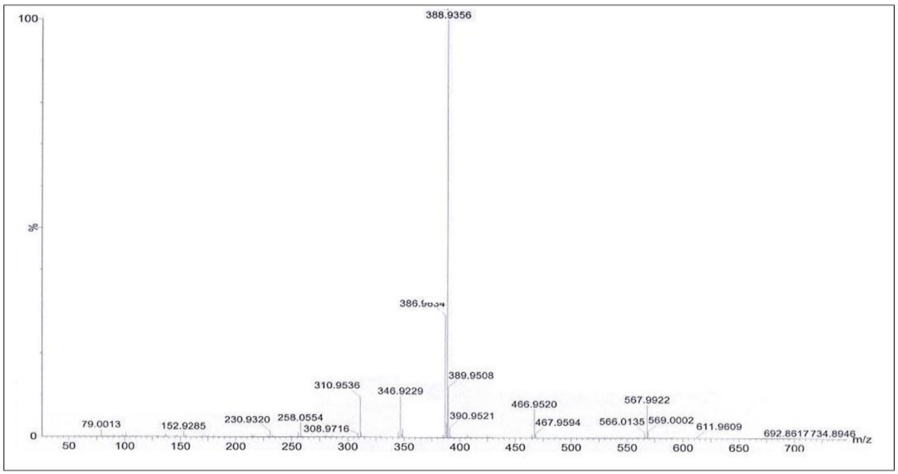

**Fig 2: $ES^+I$ Mass spectrum of $Fe(DBNH)_2$**

**DNA binding experiments:** The interaction of the complexes with DNA was studied in tris-buffer medium. Solution of calf thymus DNA (CT-DNA) in (50mM NaCl/5 mM Tris-HCl; pH =7.0) buffer medium gave absorbance ratio at 260 nm and 280 nm of 1.85, indicating that the DNA was sufficiently free of proteins.[18] The DNA concentration per nucleotide was determined by absorption coefficient (6600 $dm^3$ $mol^{-1}$ $cm^{-1}$) at 260 nm.[19] Stock solutions stored at 4ºC were used after no more than four days. The electronic spectra of metal complexes were monitored in the absence and presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration $2x10^{-5}M$ and varying nucleic acid concentration. Absorption spectra were recorded after each successive addition of DNA solution. The intrinsic binding constant ($K_b$) was calculated by the equation, $[DNA]/\varepsilon_a-\varepsilon_f = [DNA]/\varepsilon_a-\varepsilon_f + 1/K_b (\varepsilon_a-\varepsilon_f)$, where [DNA] is the molar concentration of DNA in base pairs , $\varepsilon_a$, $\varepsilon_b$, $\varepsilon_f$ are apparent extinction coefficient($A_{obs}/[M]$), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively. A plot of [DNA] / $(\varepsilon_a-\varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_a-\varepsilon_f) x K_b$ is the ratio of the intercept.

## RESULTS AND DISCUSSION

**Elemental analysis, molar conductivity measurements:** The analytical data for the complexes with some physical properties are summarized in Table -1. The metal complexes are presented in the way of colored powders. All the complexes are soluble in DMF. From the analytical data of the complexes result a stoichiometry 1:2 (metal: ligand) for all the complex combinations (Table 1). The recorded conductance for $10^{-3}$ molar DMF solutions of the complexes indicates that all complexes are non- conducting due to their neutrality.

**Table -1: Physico-chemical properties of metal complexes**

| Complex | Melting Point ºC | Elemental analysis found(cal.)% | | | $\Lambda_M{}^a$ |
|---|---|---|---|---|---|
| | | Carbon | Hydrogen | Nitrogen | |
| $Fe(DBNH)_2$ | 275-276 | 53.25(54.92) | 3.11 (3.52) | 14.51(14.78) | 12.4 |
| $Fe(DANH)_2$ | >300 | 56.97(56.37) | 3.24(4.02) | 13.10 (14.09) | 14.8 |
| $Fe(DBPNH)_2$ | >300 | 62.18(63.33) | 3.06( 3.88) | 12.02(11.66) | 9.5 |

**Electronic spectra:** The electronic spectra of the complexes are recorded in DMF. The significant bands obtained from electronic spectral data are presented in Table -2. A sharp band appears at 32745-33112 cm$^{-1}$ and maybe assigned to $n \rightarrow \pi^*$ of the azomethine and carbonyl group.[20,21] A medium intense band is located at 24154-25252 cm$^{-1}$ which corresponds to charge transfer spectra caused by ligand to the metal ion.[22,23] A weak band is observed in the region of 17895-19920 cm$^{-1}$ is due to d-d transition which is assigned to the $^5T_{2g} \rightarrow {}^5E_g$ transition in octahedral field.

The electronic spectrum of complex Fe(DANH)$_2$ is shown in Fig. 3.

**Table – 2: Electronic Spectral data(cm$^{-1}$) of Iron(II) complexes**

| Complex | n-$\pi^*$ ransition | CT transition | d-d transition |
|---|---|---|---|
| Fe(DBNH)$_2$ | 32786 | 24875 | 17895 |
| Fe(DANH)$_2$ | 33112 | 24154 | 18286 |
| Fe(DBPNH)$_2$ | 32745 | 25252 | 19920 |

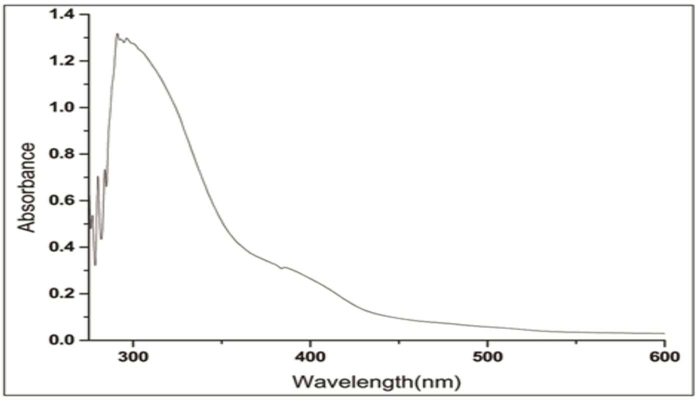

**Fig 3**: **The Electronic spectrum of Fe(DANH)$_2$**

**Infrared Spectra**

In order to study the bonding mode of ligand to metal in the complexes, IR spectrum of the free ligand was compared with the spectra of the metal complexes. The structurally significant IR bands for free hydrazone ligand s and their complexes have been reported in Table -3. The broad band due to phenolic –OH appeared in the region at 3253-3415 cm$^{-1}$ in the ligands was disappeared in the complexes.[24,25] The H-bonded –OH groups have been replaced by the metal ion. This is strongly supported by the observation of υ(C-O) stretching Vibrations of ligands in the region of 1231-1248 cm$^{-1}$ undergoes shift to higher wave number at 1242-1258 cm$^{-1}$ in complexes. These observations support the formation of M–O bonds via deprotonation.[26] This is further evidenced by the appearance of a band in the complexes in the region 506-517 cm$^{-1}$ assigned as υ(M–O) bands.[27,28] For all the complexes the azomethine ν(C=N) band undergoes a remarkable shift towards higher wavenumbers(1569-1592 cm$^{-1}$) as a consequence of coordination of the nitrogen atom to the metal ion and it is further confirmed by the appearance of new band at 406-418 cm$^{-1}$ (lower wave number region) due to υ(M–N).

Based on physico-chemical and spectral data (Mass spectra; Fig. 2) a general structure (Fig. 4) is tentatively assigned for iron(II) complexes of nicotinoylhydrazones.

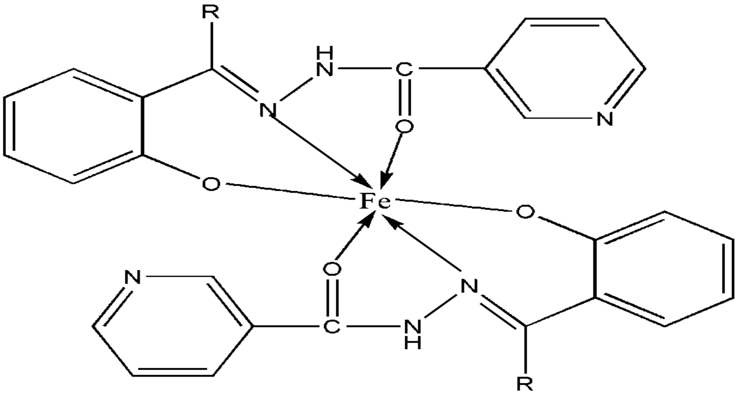

**Fig 4: A general structure for iron(II) complexes**

**Table -3: Important IR spectral bands of complexes & ligands**

| Compound | ν(O-H) $cm^{-1}$ | ν(NH) $cm^{-1}$ | ν(C=O) $cm^{-1}$ | ν(C=N) $cm^{-1}$ | ν(C-O) $cm^{-1}$ | ν (M-O) $cm^{-1}$ | ν (M-N ) $cm^{-1}$ |
|---|---|---|---|---|---|---|---|
| DBNH | 3253 | 3009 | 1674 | 1591 | 1248 | - | - |
| $Fe(DBNH)_2$ | - | 3004 | 1653 | 1569 | 1258 | 506 | 402 |
| DANH | 3324 | 3011 | 1665 | 1604 | 1231 | - | - |
| $Fe(DANH)_2$ | - | 3009 | 1638 | 1587 | 1242 | 524 | 418 |
| DBPNH | 3415 | 3005 | 1659 | 1605 | 1239 | - | - |
| $Fe(DBPNH)_2$ | - | 3006 | 1638 | 1592 | 1246 | 517 | 406 |

### Cyclic voltammetry

The redox behavior of the complexes has been investigated by cyclic voltammetry in DMF using 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. The cyclic voltammogram of $Fe(DBPNH)_2$ complex is given in Fig. 5. The electrochemical data of Iron(II) complexes are presented in Table -4. The data reveal that Iron(II) complexes have single cathodic wave, corresponding to one electron reduction Fe(II)→Fe(I). The reduction is reversible which occurs in the range -0.922 to -0.038 vs Ag/AgCl reference electrode.

The separation between cathodic and anodic peaks (ΔE = 139-243 mV) indicates quasi-reversible character. The potential difference $\Delta Ep = Ep_c - Ep_a$ in all the complexes exceeds the Nerstian requirement of 59/n mV (n = number of electrons involved in oxidation-reduction) which further suggests quasi-reversible character of the electron transfer reaction.

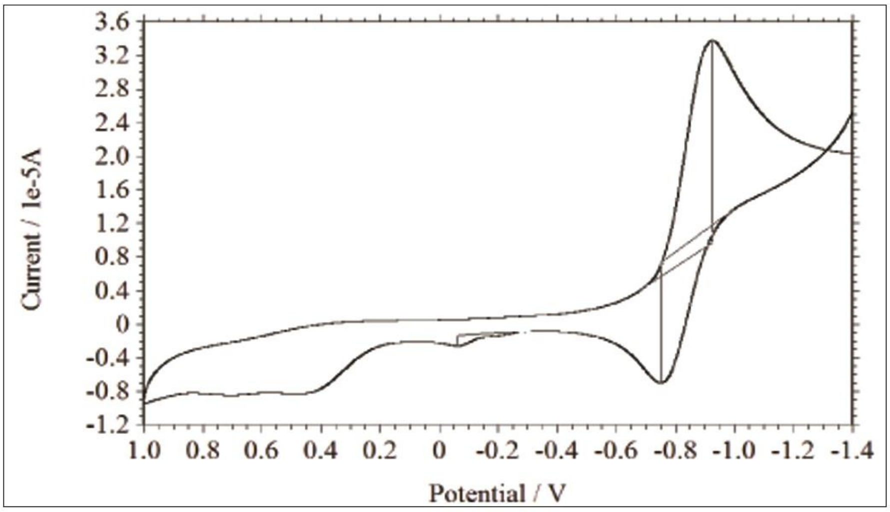

**Fig 5: Cyclic voltammogram of $Fe(DBPNH)_2$ at 100 $Vs^{-1}$**

**Table- 4: Cyclic voltammetric data of Iron(II) complexes**

| Complex | Redox couple | $E_{pc}V$ | $E_{pa}V$ | ΔE (mV) | $E_{1/2}$ | $logK_c^a$ | $-\Delta G^0$ |
|---|---|---|---|---|---|---|---|
| $Fe(DBNH)_2$ | II/I | -0.729 | -0.535 | 194 | -0.632 | 0.173 | 994 |
| $Fe(DANH)_2$ | II/I | -0.038 | -0.205 | 243 | 0.121 | 0.138 | 793 |
| $Fe(DBPNH)_2$ | II/I | -0.922 | -0.783 | 139 | -0.852 | 0.241 | 1387 |

### DNA binding experiments

The binding interactions of the complexes with CT-DNA were monitored by comparing their absorption spectra with and without CT-DNA. Absorption spectra of complexes and $Fe(DBPNH)_2$ in the absence and presence of CT-DNA are shown Fig. 6. The intrinsic binding constants of all the complexes are given in Table -5. With increasing DNA concentrations, the hypochromism of the band centered at 392–399 nm observed in the presence of DNA can be assigned to LMCT transitions in the absorption spectra of all complexes. The changes in the absorbance values with increasing amounts of CT-DNA were used to evaluate the intrinsic binding constant $K_b$. Intercalation of a complex to DNA generally results in large hypochromism and significant red shift of the absorption band, due to strong stacking interactions between the aromatic chromophore of the ligand and the base pairs of the DNA.[29] The intrinsic equilibrium DNA binding constants for all complexes (Table 5) indicates that strong stacking interactions between complexes and DNA base pairs.

Hypochromism results from the contraction of DNA in the helix axis as well as from the change in conformation on DNA while hyperchromism emerges from the damage of the double helix structure.[30] Hypochromism and bathochromic shift in the case of Iron(II) complexes of DBNH, DANH, DBPNH suggest that these complexes bind DNA through intercalation involving a strong π-stacking interaction between the aromatic chromophore and base pairs of DNA.[18]

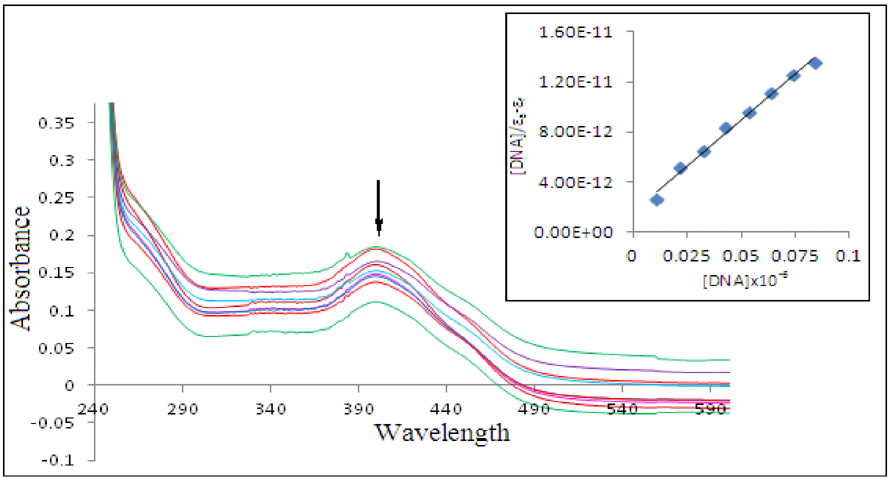

**Fig 6: Absorption spectra of Fe(DBPNH)$_2$ in the absence and in the presence of increasing concentration of CT-DNA; top most spectrum is recorded in the absence of DNA and below spectra on addition of 10 µl DNA each time; A plot of [DNA]/ ($\varepsilon_a$- $\varepsilon_f$) vs. [DNA] is shown in the inset**

**Table -5: Electronic absorption data upon addition of CT-DNA to the complexes**

| **Complex** | **$\lambda_{max}$ (nm)** | | **Δλ/nm** | **H%** | **$K_b$ ($M^{-1}$)** |
|---|---|---|---|---|---|
| | **Free** | **Bound** | | | |
| Fe(DBNH)$_2$ | 394 | 398 | 4 | +53.72 | $3.59 \times 10^5$ |
| Fe(DANH)$_2$ | 392 | 393 | 1 | +51.03 | $2.48 \times 10^5$ |
| Fe(DBPNH)$_2$ | 399 | 402 | 3 | +33.15 | $1.83 \times 10^5$ |

## CONCLUSIONS

Iron (II) complexes of a series of six nicotinoyl hydrazones have been synthesized and characterized based on various physicochemical and spectral techniques. These studies revealed that the complexes have general formula FeL$_2$ (where L = hydrazone). The hydrazones act as uni-negative tridentate ligand. Electronic spectral data suggest that the complexes have octahedral geometry. Absorption studies reveal that the complexes bind DNA via intercalation involving strong π-stacking interaction of aromatic moiety of complex between base pairs of DNA.

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