

**SYNTHESIS, SPECTRAL CHARACTERIZATION AND DNA BINDING PROPERTIES
OF LANTHANIDE(III) COMPLEXES WITH 2-
FORMYLPYRIDINEISONICOTINOYLHYDRAZONE**

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ABSTRACT

Lanthanide(III) complexes of general formula of $[Ln(FPINH)_2(NO_3)(NO_3)_2 \cdot nH_2O]$ (where, Ln = La, Ce, Pr, Nd, Sm, and FPINH = 2-formylpyridine isonicotinoylhydrazone) have been synthesized under mild reaction conditions with excellent yields. The new complexes have been characterized by elemental analysis, molar conductance and various spectral techniques such as FT-IR, UV-Vis, and ^1H-NMR . Electrolytic nature of complexes is investigated by conductivity studies. The coordination modes of ligands were determined using IR data. IR spectral data suggest that the ligands act as neutral trifunctional NNO-donor system. Electrochemical behaviour of metal complexes is investigated by using cyclic voltammetry. The complexes undergo quasi-reversible one electron reduction. Absorption titration studies revealed that these complexes are avid binders ($k_b = 10^5$) to calf-thymus DNA.

KEYWORDS: Lanthanide(III) complexes, 2-formylpyridineisonicotinoylhydrazone, spectral characterization, cyclic voltammetry and DNA binding.

INTRODUCTION

The synthesis of lanthanide complexes with variety of ligands has received considerable interest because of their potential applications in various fields.^[1-9] Hence, the synthesis of and characterization of newer lanthanide complexes is a potential field of research in Inorganic Chemistry. Though Sacconi reported^[10] lanthanide complexes of acetylacetonisonicotinoyl hydrazone in the year 1954. Literature review^[11-13] indicated that the study of such complexes is relatively less when compared with their transition metal complexes. Transition metal complexes of variety of isonicotinoyl hydrazones have been investigated. However investigations on lanthanide complexes of such ligands are relatively less. The chemistry of lanthanide complexes is of interest owing to their importance in biological and industrial processes. The bioactivity of heterocyclic hydrazones as well as their metal complexes is of interest, especially due to their pharmacological properties.^[14-16] Metal complexes of isonicotinoyl exhibit antitumour^[17] and antibacterial activity.^[18] There is also much interest in the development of artificial nucleases. Artificial metallonucleases require ligands which effectively deliver metal ions to the vicinity of DNA. Investigations on metal-DNA interactions^[19] have been an area of active research.^[20,21] Studies on chemical modification of nucleic acids with lanthanide complexes

are of great interest in the design of chemotherapeutic drugs, regulation of gene expression and design of tools for molecular biology.^[22]

In the light of the above and in continuation of our on going research work^[23,24], a series of functionalized isonicotinoyl hydrazones (Figure 1) have been synthesized and characterized. The design of such ligands is achieved by using corresponding precursors. A series of isonicotinoyl hydrazones have been synthesized by using aromatic carbonyl compounds and isoniazid.

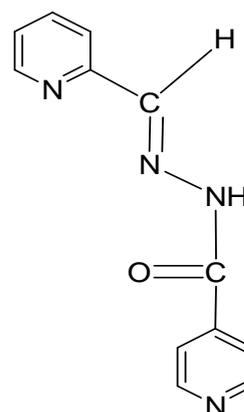


Figure 1: Structure of FPINH ligand.

Experimental

Precursors used in the synthesis of ligand (*viz.* 2-formylpyridine and isoniazid) and lanthanide nitrates were purchased from Aldrich chemicals and were used without further purification. Lanthanide salts were stored in desiccators to prevent hydration. CT DNA were purchased from Genie Biolabs, Bangalore, India.

Synthesis of 2-Formyl pyridine isonicotinoylhydrazone (FPINH)

A 5 mmol of 2-formylpyridine (0.55 ml) dissolved in 20ml of methanol were added to a methanolic solution of 5 mmol of isonicotinic acid hydrazide (0.68gms). The contents were taken in a round bottom flask stirred for 20

mins. A white colored product was formed. It was collected by filtration, washed with a few drops of ethanol and dried in vacuo. Yield of the product is 85%, M.P-158-160^oc, elemental analysis: C-63.71(63.42); H-4.42 (4.81); N: 24.77(24.98); IR spectra; 3294, 1668, 1542 cm⁻¹ are assigned to $\nu(\text{NH})$, $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ stretching vibrations respectively. ¹H-NMR spectra (DMSO solvent): δ (7.69-8.01) (multiplet 4H), δ (8.69) (multiplet 4H), δ (8.15) (singlet 1H), δ (12.25) (singlet 1 H), are respectively assigned to pyridine, isonicotine, -NH and aldehydic proton. GC-MS spectrum of FPINH shows molecular ion peaks at (*m/z*) 226. Mass spectrum of FPINH ligand is given in Figure 2.

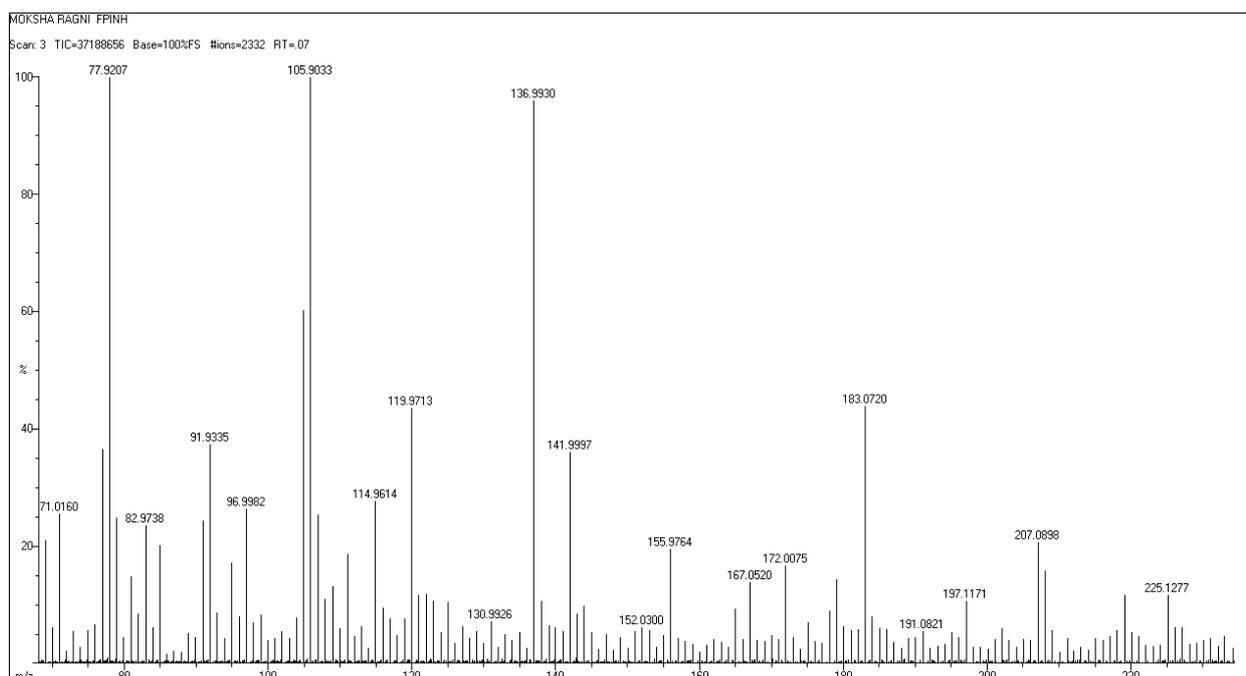


Figure 2: Mass spectrum of FPINH Ligand.

Preparation of lanthanide [La(III), Ce(III), Pr(III), Nd(III) and Sm(III)] complexes

An ethanolic solution of (20 ml) of FPINH(0.452 g, 2 mmol) was added slowly to a methanolic solution (10 ml) of the lanthanide(III) nitrate hexahydrate (1 mmol) in a clean 100-ml round bottom flask and the contents were heated under reflux on water bath for 2-4 hrs. The reaction solution was allowed to stand at 25^oC. After few days, crystals were isolated from the solution. Crystals suitable for X-ray structure determination were not obtained even after several attempts.

Physical measurements

The elemental analyses were performed using a Perkin-Elmer 2400 CHNS elemental analyzer. The molar conductance of the complexes in DMF (10⁻³M) solution was measured at 28^oC with a Systronic model 303 direct-reading conductivity bridge. The magnetic measurements were recorded at room temperature at various field strengths using Lakeshore VSM 7410 magnetometer. The electronic spectra were recorded in DMF with a

Perkin Elmer UV Lamda -50 spectrophotometer. FT-IR spectra in KBR disc were recorded in the range 4000-400 cm⁻¹ with a Perkin Elmer spectrum 100 spectrometer. The cyclic voltammetry was performed with a CH instruments 660C electrochemical analyzer and a conventional three electrode, Ag/AgCl reference electrode, glassy carbon working electrode and platinum counter electrode. Nitrogen gas was purged and measurements were made on the degassed (N₂ bubbling for 5 min) complex solution in DMF (10⁻³M) containing 0.1M tetrabutylammonium hexafluorophosphate (TBAHP) as the supporting electrolyte.

DNA binding experiments

Interaction of complexes with calf thymus DNA was studied by electronic absorption spectroscopy. The interaction of the complexes with DNA was carried out in tris-buffer. Solution of calf thymus-DNA (CT-DNA) in (0.5mM NaCl/5mM Tris-HCl; pH = 7.0) buffer gave absorbance ratio at 260 nm and 280 nm of 1.89 indicating that the DNA was sufficiently free of proteins.

The DNA concentration per nucleotide was determined by absorption coefficient ($6600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) at 260 nm. Stock solutions stored at 4°C were used after no more than four days. The electronic spectra of metal complexes in aqueous solutions were monitored in the absence and presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration $20 \times 10^{-6} \text{ M}$ and varying the nucleic acid concentration ($0 - 14.9 \times 10^{-6} \text{ M}$). The ratio of $r = [\text{complex}]/[\text{DNA}]$ values vary from 11.52 to 1.28. Absorption titration experiments were performed by maintaining the metal complex concentration constant while gradually increasing the concentration of CT-DNA within $0-80 \mu\text{M}$.

RESULTS AND DISCUSSION

The ligand, 2-formylpyridine isonicotinoylhydrazone (FPINH) is synthesized and characterized based on IR, NMR and mass spectral data (see experimental section). The complexes are obtained in good yields. The complexes are stable at room temperature, non-hygroscopic, partially soluble in water, methanol, ethanol and readily soluble in acetonitrile (CH_3CN), DMF and DMSO. Our several attempts to obtain diffraction quality crystal for the complexes using

different combination of solvents were not successful. The analytical data are consistent with the proposed molecular formulae of complexes. The molar conductivity values ($104-150 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$) for the complexes suggest that these are 1:2 electrolytes.^[25] The room temperature magnetic hysteresis curves of Ce(III), Nd(III), Sm(III) and Pr(III) complexes exhibit characteristic patterns of paramagnetic component. The positive slope of the curve clearly indicates paramagnetic nature of the complexes.^[26] The lanthanum(III) complex, $[\text{La}(\text{FPINH})_2(\text{NO}_3)] (\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ is diamagnetic and other tripositive lanthanide complexes are paramagnetic. The observed magnetic moment values are less than theoretical values predicted for the trivalent lanthanide ions. This fact suggests that the 4f electrons/ 4f-orbitals do not participate in the bond formation of the metal to ligand.^[27]

Electronic spectra

The electronic spectra of ligand and their corresponding lanthanide(III) complexes are recorded in DMF. Typical electronic spectra (a) FPINH ligand (b) $[\text{La}(\text{FPINH})_2(\text{NO}_3)] (\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ complex are given in Figure 2.

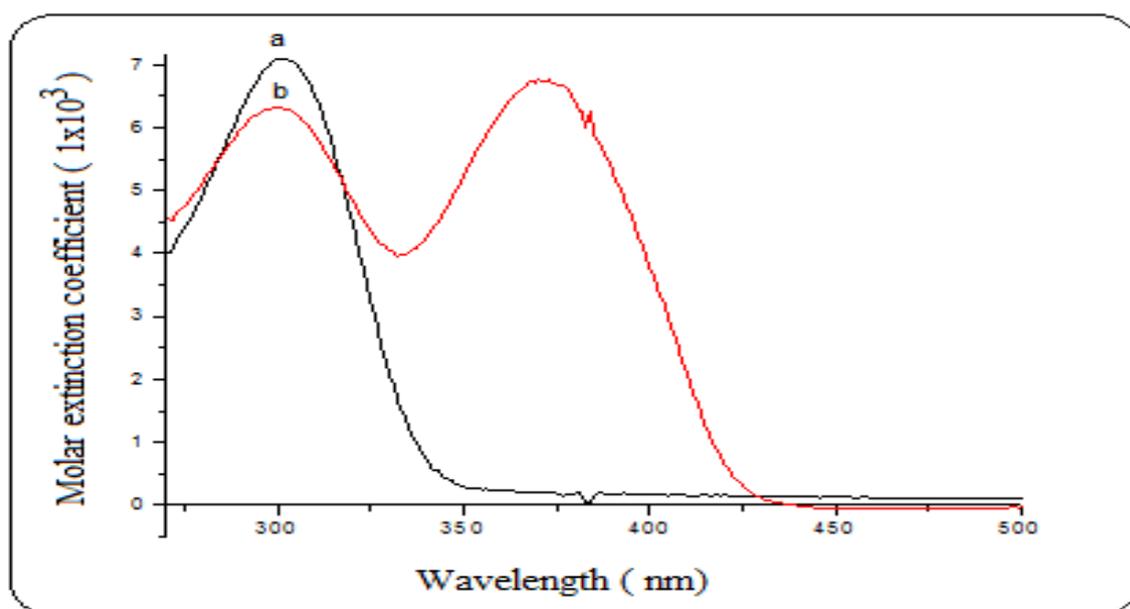


Figure 2: Electronic spectra of (a) FPINH ligand. (b) $[\text{La}(\text{FPINH})_2(\text{NO}_3)] (\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ complex.

In the electronic spectra of complexes a broad peak is observed in the high energy region at 33340 cm^{-1} assigned to $\pi \rightarrow \pi^*$ transition. A strong peak is observed in the low energy region ($26,600 - 26,960 \text{ cm}^{-1}$) of electronic spectra of complexes. It is assigned to charge transfer transition. The intense CT band observed in $26,600- 26,960 \text{ cm}^{-1}$ region. Thus the weak f-f transitions occurring in the region due to lanthanide ion in the complexes are obscured in the intense ligand bands. Also the ligand bands remains largely unaffected on complexation and insensitive to the lanthanide ion. No

absorption band due to f-f transition of lanthanide(III) ions could be located in the visible region in the spectra of complexes. This is probably due to the fact that the f-f bands are weak and obscured by the intense charge transfer bands.^[27]

Infrared spectra

IR spectra of ligands are compared with those of metal complexes to determine donor atoms of ligand. IR spectral data are given Table 1.

Table 1: Infrared Spectral data (cm⁻¹) for the FPINH ligand and its lanthanide (III) complexes.

Complex/ ligand	Stretching Frequencies				ν(NO ₃)				
	O-H	N-H	C=O	C=N	ν ₁	ν ₂	ν ₃	ν ₄	ν ₁ -ν ₄
FPINH Ligand	3397	3291	1664	1586	-	-	-	-	-
[La(FPINH) ₂ (NO ₃) ₂](NO ₃) ₂ ·2H ₂ O	3350	3184	1638	1568	1447	1299	817	1480	170
[Ce(FPINH) ₂ (NO ₃) ₂](NO ₃) ₂	3385	3084	1637	1570	1464	1042	852	1293	171
[Nd(FPINH) ₂ (NO ₃) ₂](NO ₃) ₂ ·1.5H ₂ O	3390	3086	1614	1572	1468	1087	828	1294	174
[Sm(FPINH) ₂ (NO ₃) ₂](NO ₃) ₂ ·2H ₂ O	3395	3035	1612	1581	1473	1087	848	1293	180
[Pr(FPINH) ₂ (NO ₃) ₂](NO ₃) ₂ ·3H ₂ O	3393	3093	1617	1572	1467	1043	851	1296	171

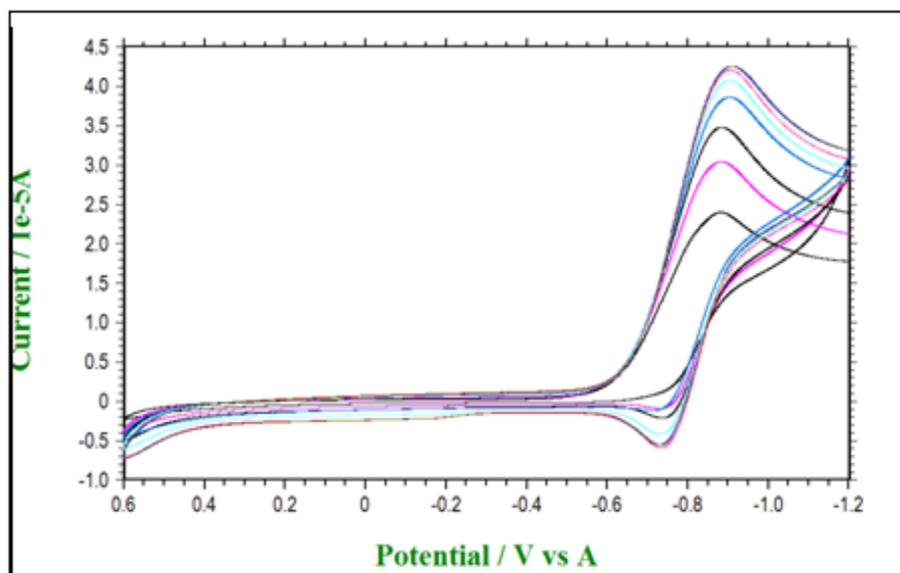
The IR spectra of the ligands have several prominent peaks due to ν_{N-H}, ν_{C=O} and ν_{C=N} stretching modes, respectively. In the spectra of complexes, ν_{N-H} and ν_{C=O} bands are present suggesting that the hydrazone acts as neutral ligand. The ν_{C=N} in the spectrum of free hydrazone is shifted to lower frequency in the spectra of complexes suggesting the involvement of azomethine nitrogen in chelation. IR data suggest that the hydrazone ligands act as neutral tridentate ligand in dinuclear complexes.

The IR spectra of the complexes demonstrate the presence of coordinated nitrate. The two strong bands are observed in complexes due to the presence of coordinated nitrates. The two strong bands associated with asymmetric and symmetric stretch of coordinated to NO₃⁻ (C_{2v}) group appear in the range 1447- 1473 (ν₁) and 1042- 1299(ν₄) cm⁻¹. The frequency separation Δν (ν₁-ν₄) increases as the coordination of nitrate group increases from monodentate to bidentate and/ or

bridging. The magnitude of Δν is used to establish the type of nitrate coordination. In the present complexes the Δν is about 180 cm⁻¹ and is typical of bidentate bonding of nitrate.^[28] The vibrational band is observed around 1380 cm⁻¹ in IR spectra of complexes indicating the presence of ionic nitrate. (D_{3h} symmetry, free NO₃⁻ ion) The data indicate that the complexes contain free ionic nitrate and coordinated bidentate nitrate groups. The new bands in 416 -418 and 522 - 524 cm⁻¹ regions are assigned to ν (Ln-O) and ν (Ln-N) vibration respectively.

Electrochemical studies

Redox behavior of the lanthanide(III) complexes has been investigated by cyclic voltammetry in DMF using 0.1M tetrabutylammonium hexafluorophosphate (TBAHEP) as supporting electrolyte. The cyclic voltammetric profile of [Nd(FPINH)₂(NO₃)₂](NO₃)₂·5H₂O complex is given in Figure 3.

**Figure 3: Cyclic voltammetric profile of [Nd(FPINH)₂(NO₃)₂](NO₃)₂·5H₂O complex at different Scan rates.**

Repeated scans at various scan rates suggest the presence of stable redox species in solution.

It may be inferred that Ln(III) complexes undergo reduction to their respective Ln(II) complexes. The complexes have large separation (154- 205 mv) between anodic and cathodic peaks indicating quasi-reversible character.

Based on molar conductivity, MS-EI mass spectra, IR spectral data, a general structure (Fig. 4) is proposed for the complexes in analogy with our previous observations.^[29]

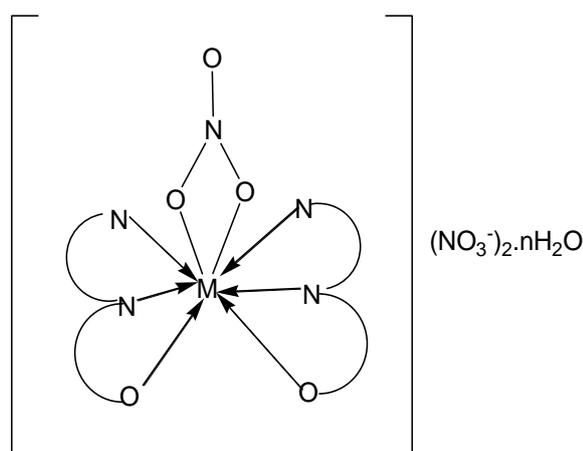


Figure 4: A general structure of lanthanide complexes.

Where, = An abbreviated structure of FPINH
 M = La(III), Ce(III), Pr(III), Nd(III) and Sm(III)

DNA binding studies

Electronic absorption spectroscopy is an effective method for examining the interaction of DNA with metal complexes. The binding interaction of complexes with CT-DNA was monitored by comparing their absorption spectra with and without CT-DNA. All the complexes exhibit an intense absorption band due to $\pi \rightarrow \pi^*$ transition. Absorption spectra of $[\text{Ce}(\text{FPINH})_2(\text{NO}_3)](\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in the absence and in presence of CT-DNA are shown Figure 5.

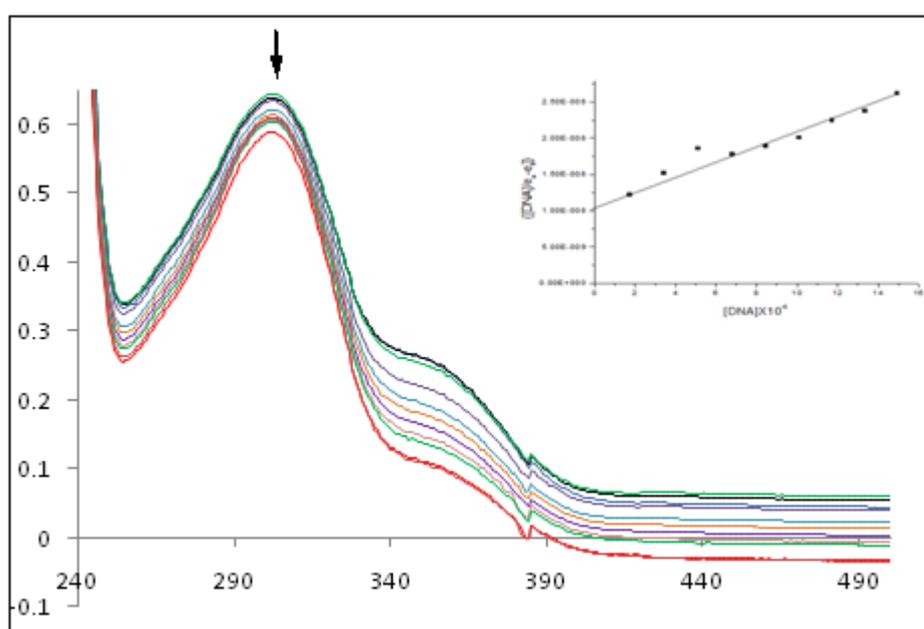


Figure 5: Absorption spectra of $[\text{Ce}(\text{FPINH})_2(\text{NO}_3)](\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in the absence and in the presence of increasing concentration of CT-DNA; the top most spectrum is recorded in the absence of DNA; Insert is a plot of $[\text{DNA}] / (\epsilon_a - \epsilon_f)$ versus $[\text{DNA}]$.

The intrinsic binding constants (K_b), were determined by using the equation,

$$[\text{DNA}] / (\epsilon_a - \epsilon_f) = [\text{DNA}] / (\epsilon_b - \epsilon_f) + 1 / K_b(\epsilon_b - \epsilon_f) \text{ -----(1)}$$

Where $[\text{DNA}]$ is the concentration of DNA in base pairs, ϵ_a , ϵ_b and ϵ_f are apparent extinction coefficient ($A_{\text{obs}}/[\text{M}]$),

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 $[\text{DNA}] / (\epsilon_a - \epsilon_f)$ versus $[\text{DNA}]$ gave a slope of $1/(\epsilon_b - \epsilon_f)$, and vertical intercept equal to $1/ K_b(\epsilon_b - \epsilon_f)$; K_b was calculated from these values. The binding constants (Table 2) for suggest that the complexes bind DNA very strongly.

Table 2: Electronic absorption data upon addition of CT-DNA to the complexes.

Complex	λ_{max} (nm)		$\Delta\lambda/\text{nm}$	H (%)	K_b (M^{-1})
	Free	Bound			
$[\text{La}(\text{FPINH})_2(\text{NO}_3)](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$	302	305	3	+ 4.34	4.35×10^5
$[\text{Ce}(\text{FPINH})_2(\text{NO}_3)](\text{NO}_3)_2$	302	303	1	+7.85	3.42×10^5
$[\text{Nd}(\text{FPINH})_2(\text{NO}_3)](\text{NO}_3)_2 \cdot 1.5\text{H}_2\text{O}$	304	305	1	+6.41	7.19×10^5
$[\text{Sm}(\text{FPINH})_2(\text{NO}_3)](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$	302	304	2	+8.16	8.0×10^5
$[\text{Pr}(\text{FPINH})_2(\text{NO}_3)](\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$	303	304	1	+8.79	11.4×10^5

On addition of DNA, the absorbance of the complexes decreases (hypochromism) and absorption maximum of all complexes is shifted to higher wavelength (bathochromism). These observations suggest that the complexes bind DNA through intercalation involving a strong π -stacking interaction of aromatic chromophore (pyridine/isonicotine moiety) between the and base pairs of DNA.

CONCLUSIONS

A new ligand FPINH is synthesized and characterized based on spectral data. La(III), Ce(III), Pr(III), Nd(III) and Sm(III) complexes of 2-formylpyridine isonicotinoylhydrazone (FPINH) have been synthesized and characterized. Physico-chemical and spectral studies reveal that the complexes have general formula $[M(FPINH)_2(NO_3)](NO_3)_2 \cdot nH_2O$ (where, M = La, Ce, Pr, Nd, and Sm). FPINH acts as neutral tridentate ligand and NO_3^- acts as bidentate ligand. Two FPINH ligands occupy six coordination sites, and one NO_3^- ligands another two coordination sites to form octa-coordinate mono nuclear complexes. Absorption titrations suggest that the complexes bind DNA through intercalation involving a strong π -stacking interaction of aromatic chromophore (pyridine/isonicotine moiety) between the base pairs of DNA.

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