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

B. Moksharagni and K. Hussain Reddy*

Department of Chemistry, Sri Krishnadevaraya University, Anantapuramu - 515003, Andhra Pradesh, India.

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Correspondence to Author:

Dr. K. Hussain Reddy

Professor,
Department of Chemistry,
Sri Krishnadevaraya University,
Anantapuramu - 515003, Andhra
Pradesh, India.

E-mail: khussainreddy@yahoo.co.in


ABSTRACT: The lanthanide (III) complexes of general formula of $[Ln(FPNH)_2(NO_3)](NO_3)_2 \cdot nH_2O$ (where, Ln = La, Ce, Pr, Nd, Sm; FPNH = 2-formylpyridine nicotinoylhydrazone, n = 0, 2 and 5 for La/Ce/Pr, Sm and Nd complexes, respectively) were synthesized under mild reaction conditions with excellent yields. The complexes were characterized based on elemental analysis, molar conductivity, and infrared spectroscopy. Molar conductivity data suggest that the complexes are 1:2 electrolytes. IR spectral data suggest that FPNH acts as neutral tridentate ligand. Spectral data of complexes suggest that the ligand binds metal ion through pyridine nitrogen, azomethine-nitrogen and amido-oxygen donor atoms. Electrochemical behaviour of metal complexes was investigated by using cyclic voltammetry. The complexes undergo quasi-reversible one electron reduction. The binding interaction of complexes with CT-DNA was investigated using absorption spectrophotometry. Based on spectral changes, groove binding of complexes to DNA is suggested.

INTRODUCTION: Investigations of lanthanide complexes with polydentate ligands have received considerable attention because of their potential applications in various fields¹⁻⁶. Hence, investigation of newer lanthanide complexes is a potential field of research in inorganic chemistry. The chemistry of lanthanide complexes is of interest owing to their applications in biology and Medicine. Herein we report synthesis, spectral characterization and DNA binding properties of lanthanide (III) complexes of 2-formylpyridine nicotinoylhydrazone for the first time.

Experimental: Lanthanide nitrates, 2-formylpyridine and nicotinic acid hydrazide, purchased from Aldrich chemicals were used without further purification. Lanthanide salts were stored in desiccators to prevent hydration. CT DNA was purchased from Genie Biolabs, Bangalore, India.

Synthesis of 2-formylpyridine nicotinoyl hydrazone (FPNH): A 5 mmol of 2-formylpyridine (0.56 mL) dissolved in 20 mL of methanol was added to a methanolic solution of nicotinic acid hydrazide (0.685g, 5 mmol). The contents were taken in a round bottom flask stirred for 20 min. A yellow colored product was formed. It was collected by filtration, washed with a few drops of ethanol and dried in vacuum. Yield of the product was 85%, mp: 162-164 °C.

General procedure for the synthesis of lanthanide complexes: To the ligand (0.480g, 2 mmol) dissolved in 20mL of methanol, lanthanum

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(III) nitrate hexahydrate $[\text{Ln}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}]$ (1mmol) dissolved in 10mL of methanol was added. The reaction mixture (taken in 100mL round bottom flask) was heated under reflux on water bath for 2 - 4 hr. Upon cooling solid substance was formed. It was collected by filtration, washed with 10mL of hexane and dried in vacuum. Physicochemical and analytical data of lanthanide complexes are included in **Table 1**.

Physical measurements: The elemental analyses were performed using a Perkin-Elmer 2400 CHNS elemental analyzer. The molar conductance of the complexes in DMF (10^{-3} M) solution was measured at 28 °C with a Systronic model 303 direct-reading conductivity bridge. The electronic spectra were recorded in DMF with a Perkin Elmer UV Lambda - 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 electrodes, Ag/AgCl reference electrode, glassy carbon working electrode and platinum counter electrode. Nitrogen gas was purged and measurements were made on the degassed (N_2 bubbling for 5 min) complex solution in DMF (10^{-3} M) containing 0.1M tetrabutyl ammonium 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 (0.5 mM NaCl/5 mM 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 20×10^{-6} M and varying the nucleic acid concentration ($0-17.4 \times 10^{-4}$ M). The ratio of $r = [\text{complex}]/[\text{DNA}]$ values vary from 8.84 to 1.10. Absorption titration experiments were performed by maintaining the metal complex concentration constant while gradually increasing the concentration of CT-DNA within 0-80 μM .

RESULTS AND DISCUSSION: The ligand, FPNH was used for the preparation of lanthanide metal complexes. The ligand was characterized based on IR, NMR and Mass Spectral data.

IR spectra: Bands at 3147, 1670, 1623, 1566, 991 and 753 cm^{-1} are respectively assigned to $\nu(\text{NH})$, $\nu(\text{C}=\text{O})$ (Amide I), $\nu(\text{C}=\text{N})$, $\nu(\text{Py ring})$, $\nu(\text{Py bending})$, and δ (Py ring oop bending) vibrations, respectively.

$^1\text{H-NMR}$ spectra: The spectrum was recorded in deuterated DMSO. δ 7.39-7.59 (multiplet 4H), δ 8.73-8.81 (multiplet 4H), δ 8.57 (singlet 1H), δ 11.01 (singlet 1 H), are respectively assigned to pyridine, nicotine, -NH and formyl proton.

GC-MS: The spectrum (**Fig. 1**) shows peak at (m/z) 240 corresponding to molecular ion peak of the ligand.

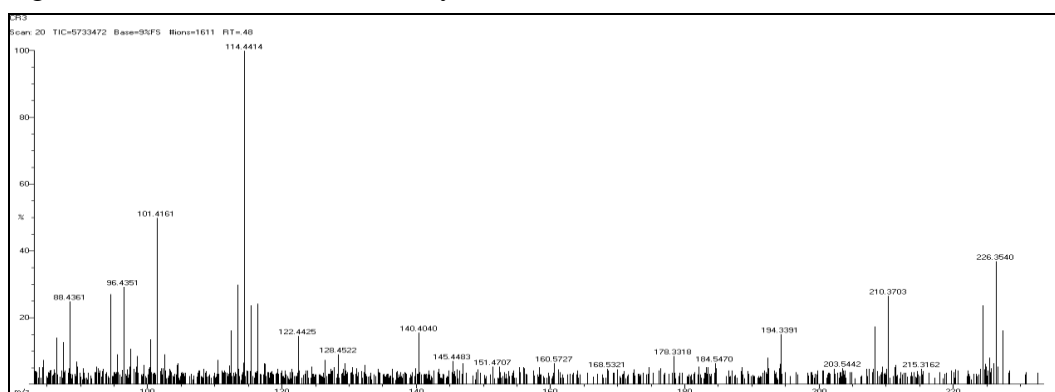
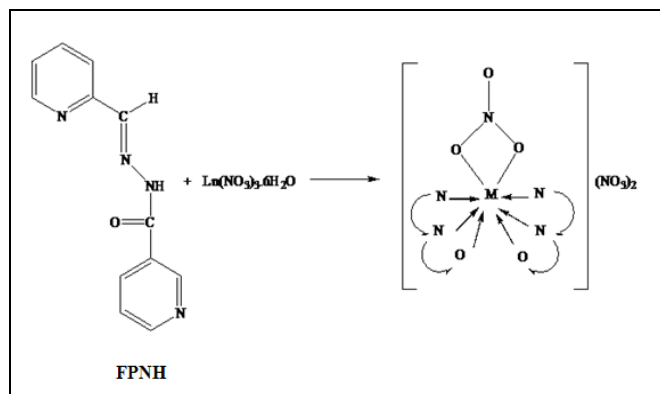


FIG. 1: GC MASS SPECTRUM OF FPNH

The complexes are obtained in good yields. The preparation of lanthanide complexes is given in **Scheme 1**. The complexes are stable at room temperature and non-hygroscopic and could be stored easily even at room temperature. The complexes are partially soluble in water, methanol, and ethanol and readily soluble in acetonitrile (CH_3CN), DMF and DMSO.



SCHEME 1: SYNTHESIS OF LANTHANIDE COMPLEXES WITH FPNH

Where, $M = \text{La, Ce, Pr, Nd, Sm}$;

The analytical data (**Table 1**) are consistent with the proposed molecular formulae $[\text{Ln}(\text{FPNH})_2(\text{NO}_3)(\text{NO}_3)_2]$ (where, $\text{Ln} = \text{La, Ce, Pr, Nd, Sm}$; $\text{FPNH} = 2\text{-formylpyridine nicotinoyl hydrazone}$), of complexes. The molar conductivity values ($87\text{--}132 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) for the complexes suggest that these are 1:2 electrolytes ⁷. The presence of nitrate in the complexes was confirmed by brown ring test ⁸.

Electronic spectra: The electronic spectra of ligand and their corresponding lanthanide (III) complexes are recorded in DMF. Typical electronic spectra (a) FPNH ligand (b) $[\text{La}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$ complex are given in **Fig. 2**. In the electronic spectra of complexes a broad peak is observed in the high energy region at $33230\text{--}33560 \text{cm}^{-1}$ assigned to $\pi - \pi^*$ transition. A strong peak is observed in the low energy region ($26690\text{--}27180 \text{cm}^{-1}$) is assigned to charge transfer transition. Electronic spectral data are given in **Table 2**.

TABLE 1: ANALYTICAL AND PHYSICO-CHEMICAL PROPERTIES OF APINH LIGAND AND ITS LANTHANIDE(III) COMPLEXES

Complex	Colour (Yield %)	M. Wt.	M.P* (°C)	Analysis Found (calc. (%))			Λ_M^a
				C	H	N	
$[\text{La}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	White (78)	776	198-200	37.11 (38.10)	2.57 (2.60)	19.84 (18.50)	92
$[\text{Ce}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	Orange (72)	778	200-202	37.01 (38.10)	2.57 (2.90)	18.79 (19.90)	132
$[\text{Nd}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	Gray (68)	782	196-198	36.82 (35.70)	4.70 (4.60)	19.69 (18.70)	104
$[\text{Pr}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	Green (70)	778	188-190	37.01(38.10)	2.57 (2.60)	18.79 (19.80)	87
$[\text{Sm}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	Yellow (81)	788	198-200	36.54 (35.60)	2.53 (2.50)	19.54 (18.62)	90

* Decomposes, † Units: $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ in DMF.

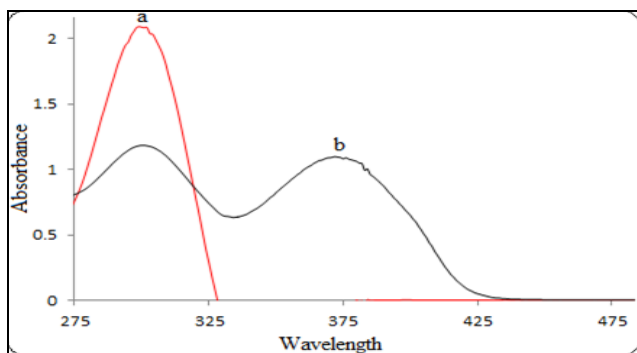


FIG. 2: ELECTRONIC SPECTRA OF (A) FPNH LIGAND AND (B) $[\text{SM}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$ COMPLEX

The weak f-f transitions occurring in the region due to lanthanide ion in the complexes are obscured by the intense ligand bands. Also the ligand band 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 ($M \rightarrow L$) charge transfer band ⁹.

TABLE 2: ELECTRONIC SPECTRAL DATA (CM-1) OF LANTHANIDE (III) COMPLEXES IN SOLUTION STATE*

Complex	λ_{max} nm(cm^{-1})	ϵ^\dagger	Band assignment
FPNH (ligand)	300(33340)	2150	$\pi - \pi^*$
$[\text{La}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	301(33230)	1070	$\pi - \pi^*$
	372(26890)	1060	CT
$[\text{Ce}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	300(33340)	1120	$\pi - \pi^*$
	373 (26810)	900	CT
$[\text{Nd}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	298 (33560)	1260	$\pi - \pi^*$
	370 (27030)	1520	CT
$[\text{Pr}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	300 (33340)	800	$\pi - \pi^*$
	368 (27180)	820	CT
$[\text{Sm}(\text{FPNH})_2(\text{NO}_3)](\text{NO}_3)_2$	300 (33340)	1180	$\pi - \pi^*$
	372 (26690)	1090	CT

* Spectra of the complexes were recorded in DMF solvent.

† Molar absorptivity Units, $\text{L. mol}^{-1} \text{cm}^{-1}$.

Infrared spectra: IR spectrum of FPNH is compared with its lanthanide complexes to determine donor atoms of ligand. IR spectral data are given **Table 3**. The IR spectrum of the ligand has several prominent peaks due to $\nu_{\text{N-H}}$, $\nu_{\text{C=O}}$ and $\nu_{\text{C=N}}$ stretching modes, respectively. In the spectra of complexes, $\nu_{\text{N-H}}$ and $\nu_{\text{C=O}}$ bands are present

suggesting that the hydrazone acts as neutral ligand. The $\nu_{\text{C=N}}$ 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.

TABLE 3: INFRARED SPECTRAL DATA (CM⁻¹) FOR THE FPNH LIGAND AND ITS LANTHANIDE (III) COMPLEXES

Complex	$\nu(\text{N-H})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{NO}_3)$				
				ν_1	ν_2	ν_3	ν_4	$\nu_1-\nu_4$
FPNH (ligand)	3147	1670	1566	-	-	-	-	-
[La(FPNH) ₂ (NO ₃)](NO ₃) ₂	3071	1629	1552	1417	1089	818	1230	187
[Ce(FPNH) ₂ (NO ₃)](NO ₃) ₂	3077	1629	1560	1418	1089	819	1230	188
[Nd(FPNH) ₂ (NO ₃)](NO ₃) ₂	3066	1627	1564	1438	1088	824	1229	209
[Pr(FPNH) ₂ (NO ₃)](NO ₃) ₂	3181	1638	1557	1440	1086	822	1227	213
[Sm(FPNH) ₂ (NO ₃)](NO ₃) ₂	3061	1609	1561	1468	1086	824	1223	245

The IR spectrum of the complexes demonstrates 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 1417- 1468 (ν_1) and 1086 - 1089 (ν_4) cm⁻¹. The frequency separation $\Delta\nu$ ($\nu_1 - \nu_4$) increases as the coordination of nitrate group increases from monodentate to bidentate and/or bridging. The magnitude of $\Delta\nu$ is used to establish the type of nitrate coordination. In the present complexes the $\Delta\nu$ is about 180 cm⁻¹ and is typical of bidentate bonding of nitrate. The other vibrational band is observed around 1380 cm⁻¹ in IR spectra of complexes indicating the presence of ionic nitrate¹⁰. (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 region are assigned to ν (Ln-O) and ν (Ln-N) vibration respectively.

Based on molar conductivity, magnetic moments, electronic spectral data, a tentative and general structure for the complexes is given in **Fig. 3**.

Electrochemical studies: Redox behavior of the lanthanide (III) complexes has been investigated by cyclic voltammetry in DMF using 0.1M tetrabutyl ammonium hexafluorophosphate (TBAHEP) as supporting electrolyte. The cyclic voltammetric profile of [Sm(FPNH)₂(NO₃)](NO₃)₂ complex is shown **Fig. 4**.

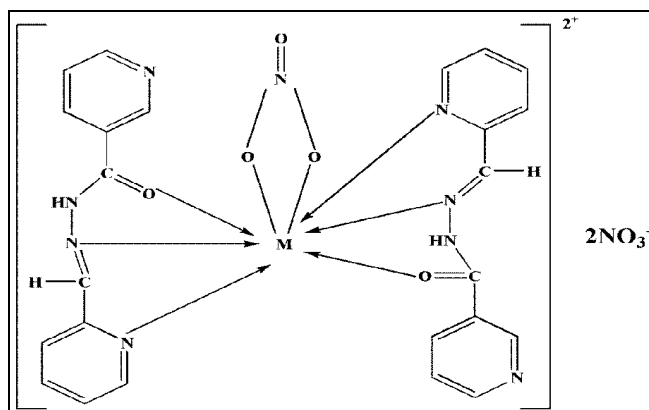


FIG. 3: GENERAL STRUCTURE FOR LANTHANIDE (III) COMPLEXES. (M = LA, CE, PR, ND AND SM)

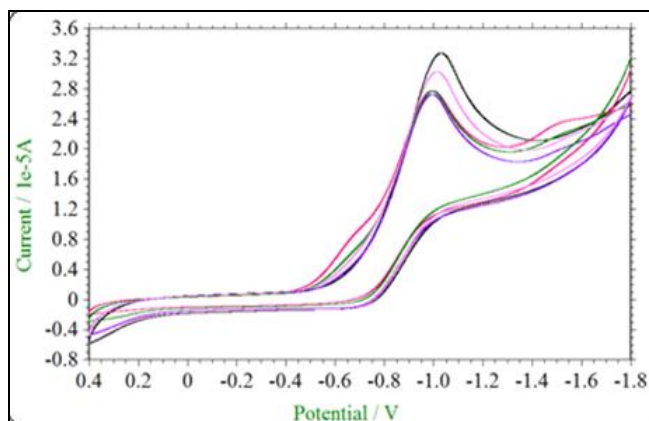


FIG. 4: CYCLICVOLTAMMETRIC PROFILE OF [SM(FPNH)₂(NO₃)](NO₃)₂ COMPLEX AT DIFFERENT SCAN RATES 25, 50, 75, 100 MV. S⁻¹

The electrochemical data of complexes are presented in **Table 5**. Repeated scans at various scan rates suggest the presence of stable redox species in solution. $E_{1/2}$ values are incorporated in **Table 4**.

TABLE 4: CYCLIC VOLTAMETRIC DATA OF LANTHANIDE(III) COMPLEXES

Complex	Redox couple	E_{pc} V	E_{pa} V	ΔE (mV)	$E_{1/2}$	$\log K_c^a$	$-\Delta G^b$
FPNH (ligand)	-	-0.874	-	-	-	-	-
[La(FPNH) ₂ (NO ₃)](NO ₃) ₂	III/II	-1.057	-0.822	235	-0.939	0.1429	820
[Ce(FPNH) ₂ (NO ₃)](NO ₃) ₂	III/II	-1.019	-0.758	261	-0.888	0.128	739
[Nd(FPNH) ₂ (NO ₃)](NO ₃) ₂	III/II	-1.423	-1.289	134	-1.356	0.250	1439
[Pr(FPNH) ₂ (NO ₃)](NO ₃) ₂	III/II	-1.596	-1.322	274	-1.459	0.122	704
[Sm(FPNH) ₂ (NO ₃)](NO ₃) ₂	III/II	-0.995	-0.756	239	-0.875	0.140	807

^a $\log K_c = 0.434ZF/RT\Delta E_p$; ^b $\Delta G = -2.303RT \log K_c$

TABLE 5: ELECTRONIC ABSORPTION DATA UPON ADDITION OF CT-DNA TO THE COMPLEX

Complex	λ_{max} (nm)		$\Delta\lambda/nm$	H (%)	K_b (M ⁻¹)
	free	bound			
[La(FPNH) ₂ (NO ₃)](NO ₃) ₂	300	301	1	-13.5	6.68×10^5
[Ce(FPNH) ₂ (NO ₃)](NO ₃) ₂	300	301	1	-2.13	2.96×10^5
[Nd(FPNH) ₂ (NO ₃)](NO ₃) ₂	301	302	1	-13.5	1.12×10^5
[Pr(FPNH) ₂ (NO ₃)](NO ₃) ₂	348	350	2	-3.39	9.12×10^4
[Sm(FPNH) ₂ (NO ₃)](NO ₃) ₂	301	302	1	-1.07	4.80×10^5

It may be inferred that Ln(III) complexes undergo reduction to their respective Ln(II) complexes¹¹. The complexes have large separation (134- 274 mV) between anodic and cathodic peaks indicating quasi-reversible character. The $\log K_c$ and ΔG° values suggest that the complexes are stable in solution state.

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 [Ce(FPNH)₂(NO₃)](NO₃)₂ in the absence and in presence of CT-DNA are shown **Fig. 5**.

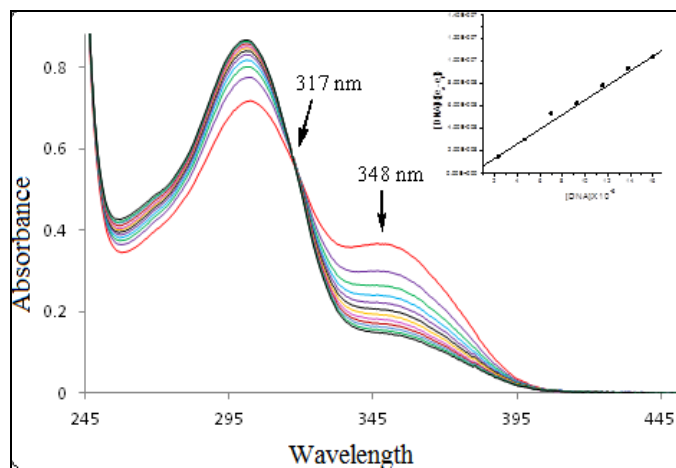


FIG. 5: ABSORPTION SPECTRA OF [PR(FPNH)₂(NO₃)](NO₃)₂ IN THE ABSENCE AND IN THE PRESENCE OF INCREASING CONCENTRATION OF CT-DN; INSET IS A PLOT OF $[DNA]/(\epsilon_a - \epsilon_f)$ VERSUS $[DNA]$

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

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

Where [DNA] is the concentration of DNA in base pairs, ϵ_a , ϵ_b and ϵ_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 $[DNA]/(\epsilon_a - \epsilon_f)$ versus [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 5**) suggest that the complexes bind DNA strongly. On addition of DNA, the absorbance of the complexes decreases (hyperchromism) and absorption maximum of all complexes is shifted to higher wavelength (bathochromism). A sharp isobestic point (317 nm) for the absorption titration curves (**Fig. 5**) suggests that there is only one mode of binding¹². Small bathochromic shift ($\Delta\lambda = 2$ nm) low binding constants (K_b) values and bulky nature of complexes suggest groove binding of complexes to DNA.

CONCLUSION: Lanthanide complexes of 2-formylpyridine nicotinic acid hydrazone have been synthesized and characterized based on physicochemical spectral data. A general structure is proposed for the complexes. DNA binding properties of lanthanide complexes are investigated using absorption spectroscopy.

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