

In the light of potential applications [1-4] and in objectives of the present work, La(III), Ce(III), Pr(III), Nd(III) and Sm(III) complexes with 2-acetylpyridine benzoylhydrazone (APBH) are synthesized characterized based on physico-chemical techniques and spectral methods viz., IR, UV-Visible spectroscopy. Electrochemical properties of all the complexes are studied by cyclic voltammetry. Binding interactions of metal complexes with calf-thymus DNA are carried out using absorption spectroscopy. DNA cleavage activities of the complexes are investigated using gel electrophoresis experiments. Synthesis and characterization of APBH (Fig 6.1) are given in Chapter 2. The ligand is synthesized by using corresponding precursors.

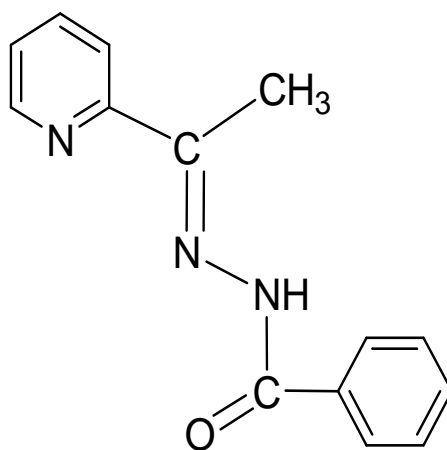


Fig 6.1: The structure of APBH

a. Physico-chemical properties

Syntheses of lanthanide(III) complexes are given in Chapter-2 (Section 2 iii). All the complexes are stable at room temperature, non-hygroscopic, soluble in DMF and DMSO. The analytical data are consistent with the proposed molecular formulae of complexes. Physical properties viz., colour of the complex, melting points and yields of the complexes are given in **Table 6.1**.

b. Conductivity measurements

All the complexes are freely soluble in dimethylformamide (DMF), hence the solutions of these metal complexes were prepared in DMF to perform conductivity measurements. Conductivity values of the solutions are measured at room temperature. The molar conductivity values ($12-19 \Omega^{-1}\text{cm}^2 \text{mol}^{-1}$) for the complexes suggest that these are non-electrolytes [5].

Table 6.1. Colors, analytical data, molar conductivities and melting points of the APBH ligand and its Lanthanide(III) complexes.

Compound	Colour	M.P °C	Yield %	Found (cal %)			Λ_m^*
				C	H	N	
APBH	White	103-105	73	70.16 (70.28)	5.34 (5.48)	17.66 (17.56)	...
[La(APBH) ₂ (NO ₃) ₃]	White	230-232	68	41.92 (41.86)	3.11 (3.26)	16.73 (15.69)	12
[Ce(APBH) ₂ (NO ₃) ₃]	Yellow	210-212	69	41.65 (41.79)	3.06 (3.26)	15.51 (15.67)	13
[Pr(APBH) ₂ (NO ₃) ₃]	Light Green	222-224	71	41.81 (41.75)	3.15 (3.25)	15.46 (15.65)	16
[Nd(APBH) ₂ (NO ₃) ₃]	Light Pink	232-234	70	41.73 (41.58)	3.36 (3.24)	15.64 (15.59)	15
[Sm(APBH) ₂ (NO ₃) ₃]	Light white	217-219	63	42.51 (41.27)	3.14 (3.22)	15.62 (15.47)	19

* ($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$)

c. Electronic Spectra

Lanthanide complexes exhibit various colours due to the redistribution of electrons in the partially filled f-orbitals and referred to as f-f transitions. In absence of the ligands around the metal, the energy of all seven f-orbitals of a lanthanide metal ion are equal and are degenerate. The presence of ligands will result in splitting in the energy levels of these orbitals. In metal complexes some orbitals will interact more strongly than others. The exact form of interaction and energies of f-orbitals depend on the arrangements of ligands around the metal ions. In UV-visible, near IR region of electromagnetic radiation, the transition associated with electronic energy levels of the compound under investigation can be identified. The electronic spectral data of lanthanide complexes are recorded in dimethylformamide (DMF).

Electronic spectrum of the free ligand (APBH) in UV region shows an intense band at 295 nm and weaker band at 365 nm which are assigned to the $\pi-\pi^*$ and $n-\pi^*$ transition respectively. These are slight by shifted to higher or lower energy levels in absorption spectra of the lanthanide complexes. In visible region the electronic spectrum of Pr(III), Nd(III), Sm(III) complexes show several important f-f spectral bands, shown in Fig. 6.2. The electronic spectra of the lanthanide complexes in the visible region exhibit red shift of all the f-f spectral bands relative to the corresponding Ln(III) aquo ion, shown in Fig. 6.3. The shifts have been attributed by Jorgenson to the effect of crystal field upon interelectronic repulsion between the 4f electrons and is related to covalence in the metal-ligand bond. The values of the bonding parameters are given in Table 6.2. The positive and negative values of δ and $b^{1/2}$ for a complex correspond to covalent and ionic characters, respectively. The

nephelauxetic ratio (β) is less than unity. The positive values of Sinha's parameter (δ) and the bonding parameter ($b^{1/2}$) suggest the occurrence of some covalent character in the metal– ligand bond [6, 7]. the small ($\delta\%$) values of the complexes indicate weak covalent bonding in the complexes. the small $b^{1/2}$ values suggest a small participation of 4f orbitals in bonding.

Nephelauxetic ratio (β) :

$$\beta = \frac{v_c}{v_f}$$

(where v_c & v_f = wave numbers of f-f transition in spectra of metal complex and free metal ion in solvent)

Bonding Parameter ($b^{1/2}$) :

$$b^{1/2} = \left[\frac{1}{2}(1-\beta) \right]^{1/2}$$

Sinha's Covalency parameter ($\delta\%$)

$$\delta = \frac{1-\beta}{\beta} \times 100$$

Covalency angular overlap parameter (η):

$$\eta = \frac{1-\beta^{1/2}}{\beta^{1/2}}$$

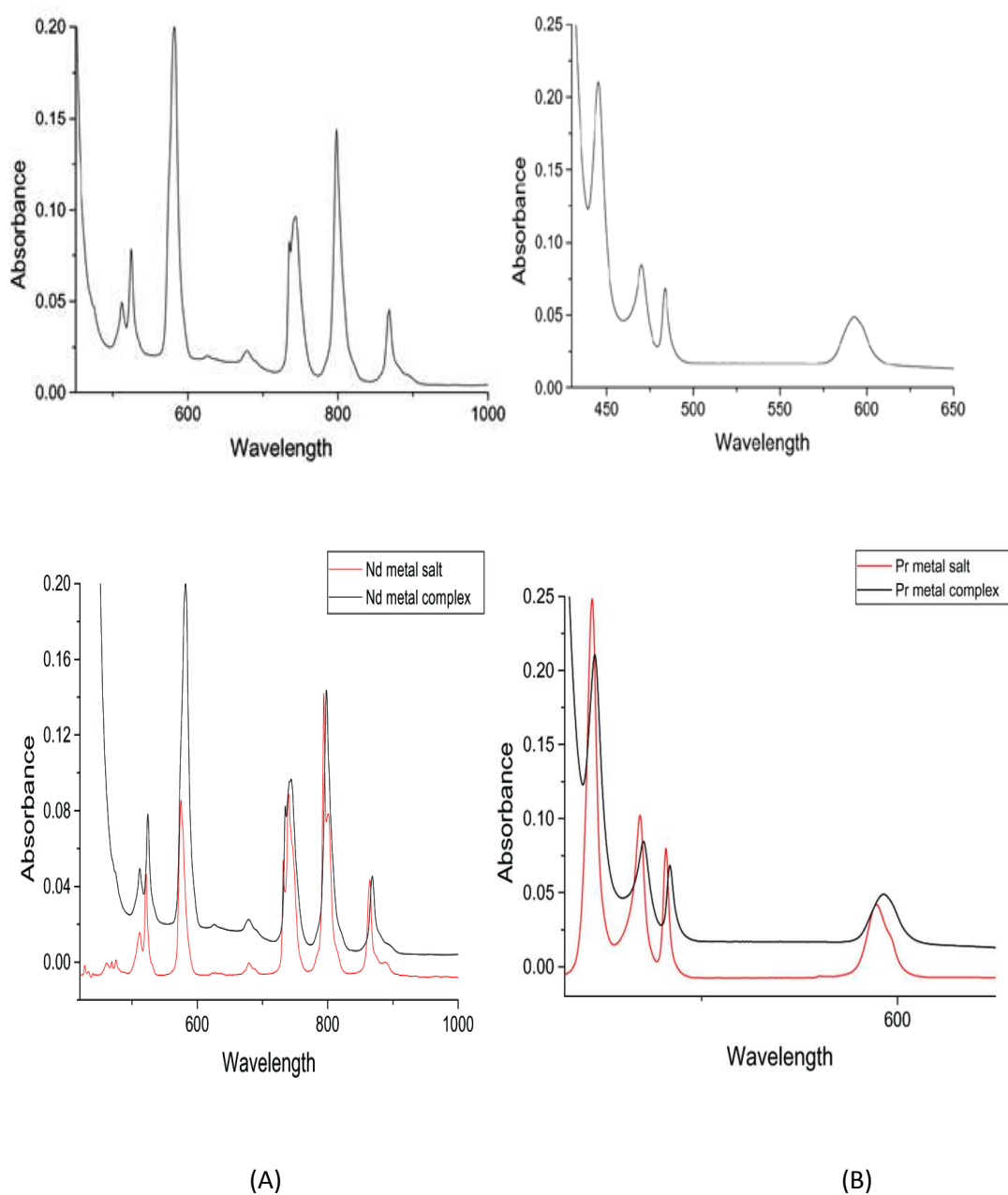


Fig 6.3: Electronic absorption spectra of (A) $\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (red) & $[\text{Nd}(\text{APBH})_2(\text{NO}_3)_3]$ (black) and (B) $\text{Pr}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (red) & $[\text{Pr}(\text{APBH})_2(\text{NO}_3)_3]$ (black) in the visible region

Table 3.2

Electronic spectral data of the lanthanide complexes and related bonding parameter

complexes	Frequency (cm ⁻¹)		Red shift (cm ⁻¹)	Assignment	Covalent Parameter
	Lanthanide Aqua ion	Lanthanide complexes			
[Pr(APBH) ₂ (NO ₃) ₃]	16,977	16,863	116	³ H ₄ → ¹ D ₂	β =0.9875 δ% =1.26
	20,746	20,661	86	³ H ₄ → ³ P ₀	
	21,367	21,276	93	³ H ₄ → ³ P ₁	b ^{1/2} =0.079
	22,522	22,471	51	³ H ₄ → ³ P ₂	η =0.00633
[Nd(APBH) ₂ (NO ₃) ₃]	11,560	11,520	41	⁴ I _{9/2} → ⁴ F _{3/2}	β =0.9957
	12,594	12,531	63	⁴ I _{9/2} → ⁴ F _{5/2}	δ % =0.43
	13,513	13,495	20	⁴ I _{9/2} → ⁴ F _{7/2} , ³ S _{3/2}	b ^{1/2} =0.046
	17,391	17,182	209	⁴ I _{9/2} → ⁴ G _{5/2} , ² G _{7/2}	η =0.0022
	19,120	19,083	37	⁴ I _{9/2} → ⁴ G _{7/2}	
	19,569	19,531	38	⁴ I _{9/2} → ⁴ G _{9/2}	
[Sm(APBH) ₂ (NO ₃) ₃]	24,038	23,980	58	⁶ H _{5/2} → ⁶ P _{5/2}	β =0.9989
	22,675	22,650	25	⁶ H _{5/2} → ⁴ G _{9/2}	δ% =0.11
	21,598	21598	4	⁶ H _{5/2} → ⁴ I _{13/2}	b ^{1/2} = 0.023
	20,876	20,876	6	⁶ H _{5/2} → ⁴ I _{11/2}	η = 0.0006
	20,040	20,000	38	⁶ H _{5/2} → ⁶ G _{7/2}	

d. Infrared Spectra

The FT-IR spectra of complexes in the region 4000-400 cm^{-1} are analysed in comparison with that of the spectrum of metal free APBH. The characteristic IR peaks of APBH and its lanthanide complexes are given in Table 6.3. IR spectra of the five complexes are strikingly similar in relative positions and intensities of the peaks, which suggest a close structural relationship among the compounds. The IR spectrum of the free ligand shows strong band at 1675 cm^{-1} , which is attributable to stretching vibrations of the carbonyl group ($\nu(\text{C}=\text{O})$). The vibrational band at 1576 cm^{-1} can be assigned to the $\nu(\text{C}=\text{N})$ of azomethine. In the IR spectra of their lanthanide(III) complexes, the $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ bands are shifted by 33–55, 06–16 cm^{-1} . The shifts of the $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ vibrations towards lower wave numbers on complexation indicate participation of the carbonyl oxygen and azomethine nitrogen in coordination to the metal ion [8]. The vibrational band at 3176 cm^{-1} can be assigned to the $\nu(\text{N}-\text{H})$ for the free ligand. The $\nu(\text{N}-\text{H})$ band is observed in the range 3188-3235 cm^{-1} in the spectra of complexes. The observance of $\nu(\text{C}=\text{O})$ and $\nu(\text{N}-\text{H})$ bands in the IR spectra of complexes indicate that the ligand acts as neutral tridentate ligand. The pyridine ring in-plane deformation mode observed at 629 cm^{-1} in the spectrum of APBH. The band is shifted to higher wave numbers (630-632 cm^{-1}) in the spectra of lanthanide complexes indicating coordination of the pyridine nitrogen [8] to form chelate ring structure.

The absorption bands assigned to the coordinated nitrate groups (C_{2v}) are observed at about 1476-1474 cm^{-1} (ν_1), 1295-1297 cm^{-1} (ν_4), 1024-1026 cm^{-1} (ν_2) and 816-818 cm^{-1} (ν_3) for the nitrate complexes. The frequency separation [$\Delta\nu = \nu_1 - \nu_4$]

between the asymmetric and symmetric stretching of this group can be used in the distinction between these binding states. The separation of the two bands (ν_1 and ν_4) is in the range $169\text{--}179\text{ cm}^{-1}$, which shows the presence of bidentate nitrates [9, 10]. The vibrational band is absent at 1384 cm^{-1} in IR spectra of complexes indicating the absence of ionic nitrate (D_{3h} symmetry), which is in agreement with the results of the conductivity experiments. The new bands in $416\text{--}423$ and $541\text{--}546\text{ cm}^{-1}$ regions are assigned to $\nu(\text{Ln-O})$ and $\nu(\text{Ln-N})$ vibration respectively. The IR spectrum of $[\text{Sm}(\text{APBH})_2(\text{NO}_3)_3]$ complex is shown in Fig 6.4

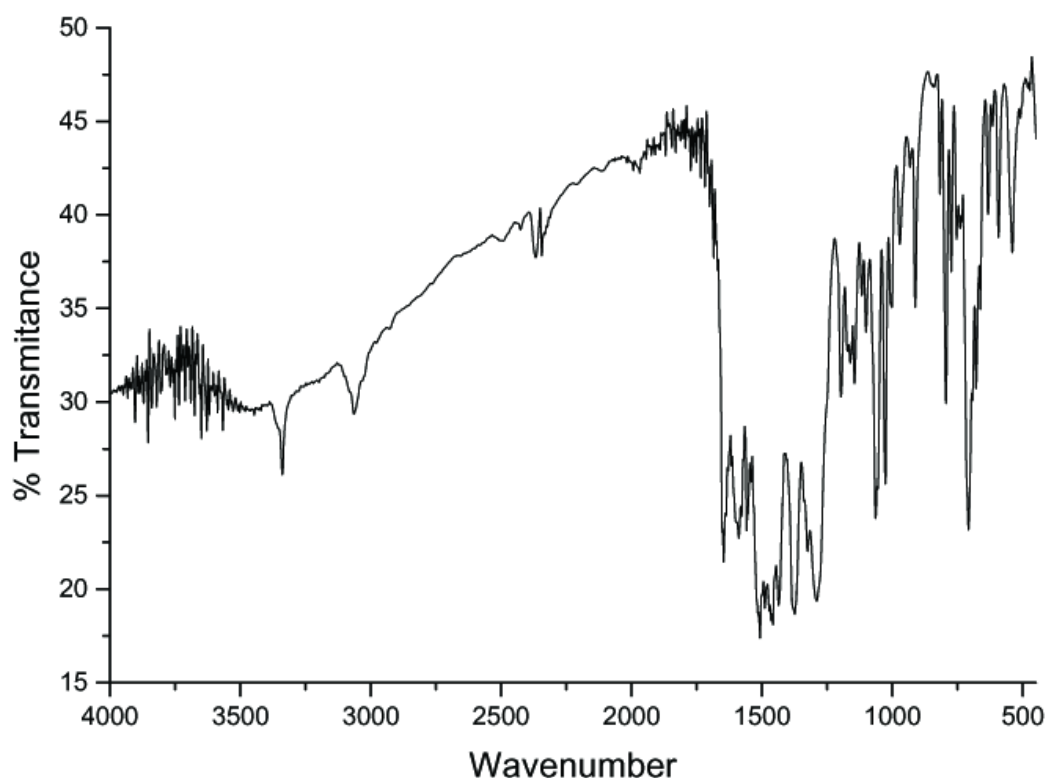


Fig 6.4: The IR spectrum of $[\text{Sm}(\text{APBH})_2(\text{NO}_3)_3]$ complex in KBr disc

Table 6.3. Infrared spectral data(cm^{-1}) for the APBH ligand and its lanthanide(III) complexes

Compound	$\nu(\text{N-H})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{NO}_3^-)$				
				$\nu_1(\text{NO}_3^-)$	$\nu_4(\text{NO}_3^-)$	$\nu_2(\text{NO}_3^-)$	$\nu_3(\text{NO}_3^-)$	$\nu_1-\nu_4$
APBH	3176	1675	1576
$[\text{La}(\text{APBH})_2(\text{NO}_3)_3]$	3235	1642	1570	1476	1297	1026	817	179
$[\text{Ce}(\text{APBH})_2(\text{NO}_3)_3]$	3199	1633	1560	1474	1295	1024	816	179
$[\text{Pr}(\text{APBH})_2(\text{NO}_3)_3]$	3202	1624	1565	1476	1307	1024	818	169
$[\text{Nd}(\text{APBH})_2(\text{NO}_3)_3]$	3188	1620	1564	1474	1296	1024	817	178
$[\text{Sm}(\text{APBH})_2(\text{NO}_3)_3]$	3201	1624	1563	1474	1296	1025	817	178

e. Thermal analysis

Thermogravimetric (TG) and differential thermal analysis of BPBH ligand and its lanthanide complexes are carried out in the temperature range, from ambient temperature up to 800 °C under a nitrogen flow with heating rate 10°C/min. All the Ln(III) complexes showed similar thermal decomposition. The TGA curve of La(III) complex undergoes two-stage changes. All Ln(III) complexes are thermally stable up to 212 °C indicating absence of lattice or coordinated water and solvent molecules. The first stage of decomposition in the range 212-303° C due to loss of three nitrate ions with a weight loss of 23.21% is consist with theoretical value(23.16%).The second stage of decomposition in the range 304- 800 corresponds to the elimination and/or decomposition of ligand [11].

f. Cyclic voltammetry

Redox behavior of the lanthanide(III) complexes has been investigated by cyclic voltammetry using 0.1M tetrabutylammonium hexafluorophosphate (TBAHEP) as supporting electrolyte. The cyclic voltammetric profiles of [Ce(APBH)₂(NO₃)₃] complex are given in Fig 6.5. A plot of i_p vs $v^{1/2}$ (scan rate) is linear (Fig. 6.6) pointing towards diffusion controlled nature of reduction wave. In the reverse scan there is no anodic peak confirming the irreversible nature of electrode process. All these facts suggest the diffusion-controlled nature of the electrode process. The cyclic voltammograms of La(III), Ce(III), Pr(III), Nd(III) and Sm(III) gave irreversible waves. Their cathodic peak potentials are -1.15 [La(III)], -0.92 [Ce(III)], -0.83[Pr(III)], -0.94[Nd(III)] and -0.81[(Sm(III)]. The cathodic peak is due to the reduction of Ln(III) to Ln(II) [12].

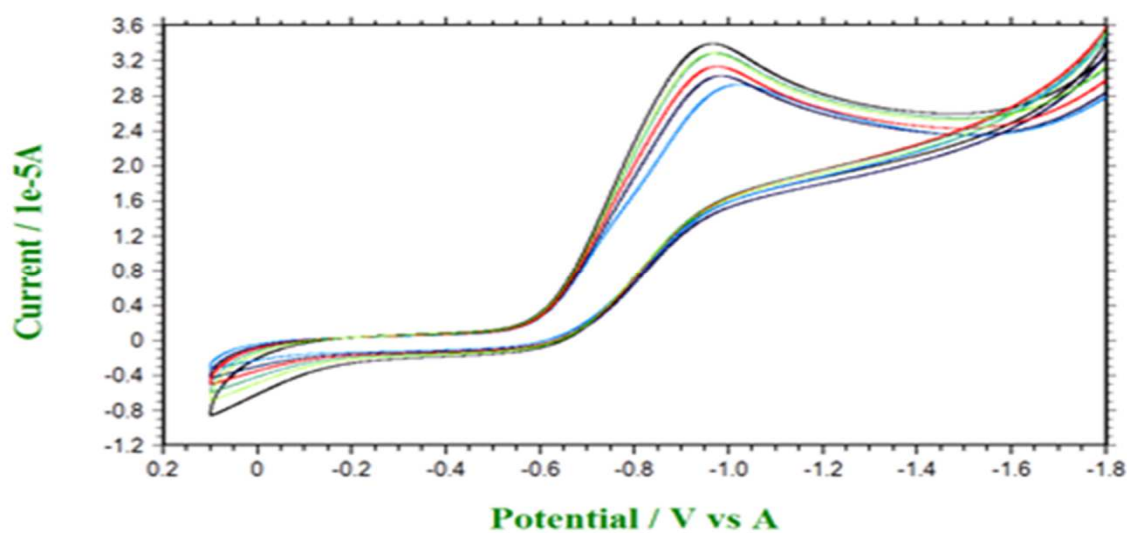


Fig 6.5: Cyclicvoltammetric profiles of the cerium complex at different scan rates 25-125 mV/s.

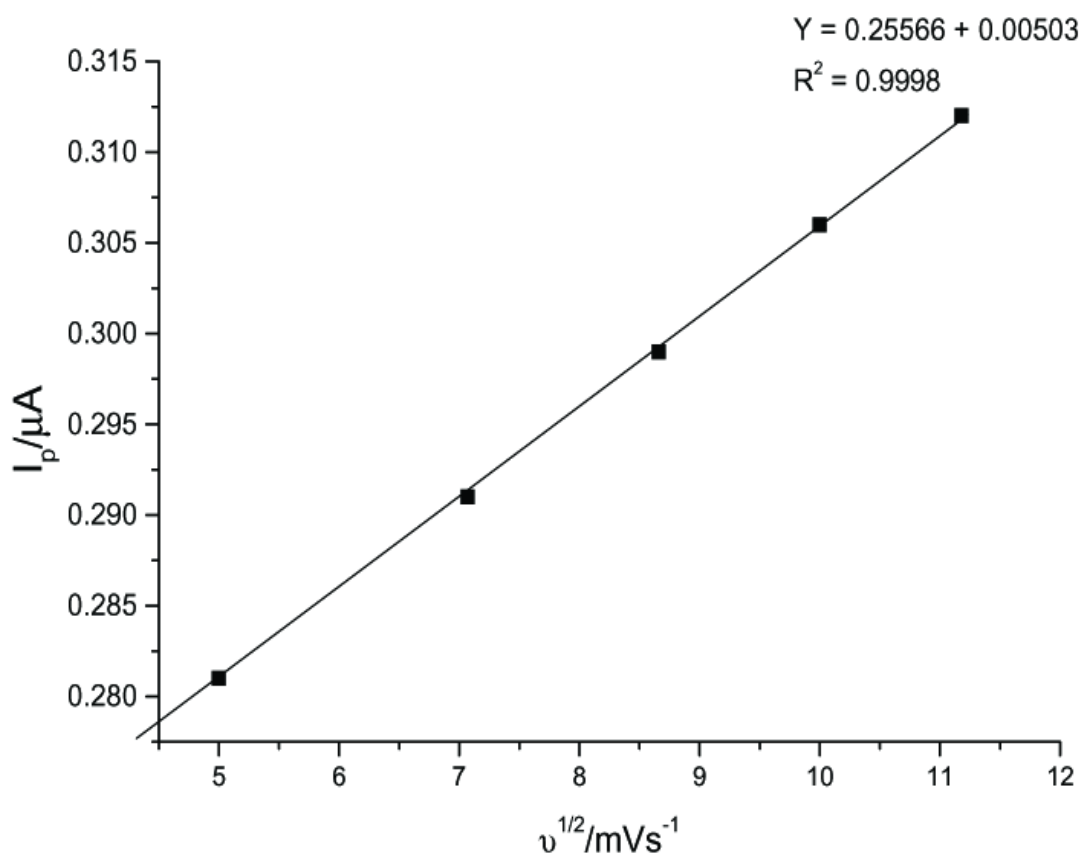


Fig 6.6: Plot of peak current vs scan rate for cerium complexes.

g. DNA binding studies

Electronic absorption spectroscopy is an effective method for examining the interaction of DNA with metal complexes. Hyperchromic and hypochromic effects are the spectral changes when a complex interacts with DNA and forms a new complex. In general, a complex binding with DNA through intercalation usually results in hypochromism and bathochromism of the absorption band due to the intercalative mode involving a strong π - stacking interaction between the aromatic chromophore and base pairs of DNA [13]. 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 in 298-300.5 nm region attributed to $\pi \rightarrow \pi^*$ transition. Absorption spectra of $[\text{La}(\text{APBH})_2(\text{NO}_3)_3]$ in the absence and in presence of CT-DNA are shown Fig 6.7. The intrinsic binding constants (K_b), was determined by using the equation,

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

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 6.4) for lanthanide complexes are almost similar and independent of metal ion. This observation suggests that the complexes do not bind DNA via coordination (No direct Metal- DNA bond formation). On addition of DNA, the absorbance of the complexes decreases (hypochromism). Negligible red shift (0.5 –

1.5 nm) in band position is observed for lanthanide complexes [14]. These observations suggest groove binding of complexes to DNA.

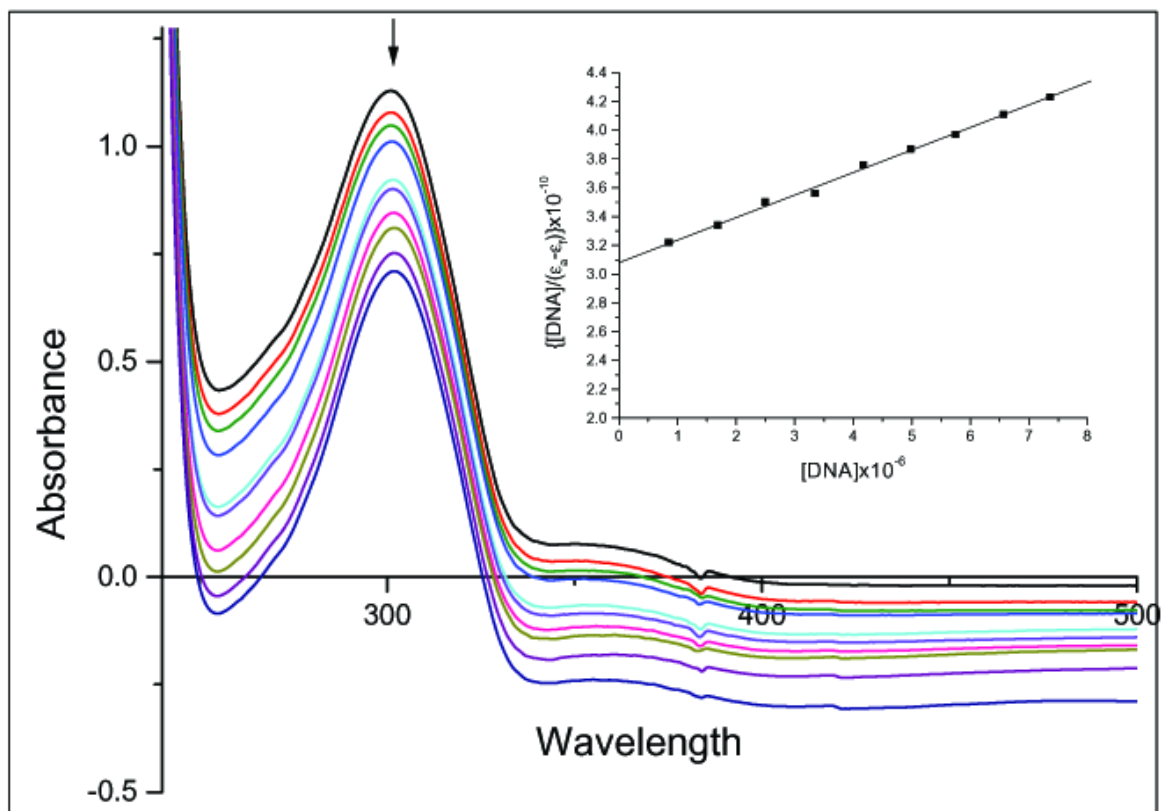


Fig 6.7: Absorption spectra of [La(APBH)₂(NO₃)₃] in the absence and in the presence of increasing concentration of CT-DNA; top most spectrum is recorded in the absence of DNA and below spectra on addition of 10 μl DNA each time.

Table 6.4. Electronic absorption data upon addition of CT-DNA to the complexes

Compound	λ_{\max} (nm)		$\Delta\lambda$	H(%)	K_b (M^{-1})
	Free	bound			
[La(APBH) ₂ (NO ₃) ₃]	298	299.5	1.5	29.71	1.32x10 ⁵
[Ce(APBH) ₂ (NO ₃) ₃]	298	299	1	32.50	1.40x10 ⁵
[Pr(APBH) ₂ (NO ₃) ₃]	299	300	1	34.46	1.43x10 ⁵
[Nd(APBH) ₂ (NO ₃) ₃]	299	299.5	0.5	31.42	1.39x10 ⁵
[Sm(APBH) ₂ (NO ₃) ₃]	299	300.5	1.5	30.42	1.38x10 ⁵

h. DNA Cleavage studies

Nuclease activity of lanthanide complexes derived from 2-acetylpyridine benzoylhydrazone (APBH) has been studied by agarose gel electrophoresis using pBR 322 plasmid DNA in Tris-HCl/NaCl (50mM/5mM) buffer(pH-7) in the presence and in absence of H₂O₂ as an oxidant at micro molar concentration for 30 min incubation period at 37° C. In the presence of H₂O₂ the super coiled DNA (form I) is changed into nicked form (form II). Fig. 6.8 and 6.9 show the cleavage activity of lanthanide complexes. In the presence of H₂O₂ the complexes cleave DNA more effectively [lanes 4, 6 and 8 in Fig 6.9; lanes 4, 6 in Fig 6.10], which may be due to the reaction of hydroxyl radical with DNA like Fenton mechanism [15]. These hydroxyl free radicals participate in the oxidation of the deoxyribose moiety [16]. The order of nuclease activity is as follow in the presence of hydrogen peroxide.

Ce complex > Pr complex > Nd complex > Sm complex > La complex

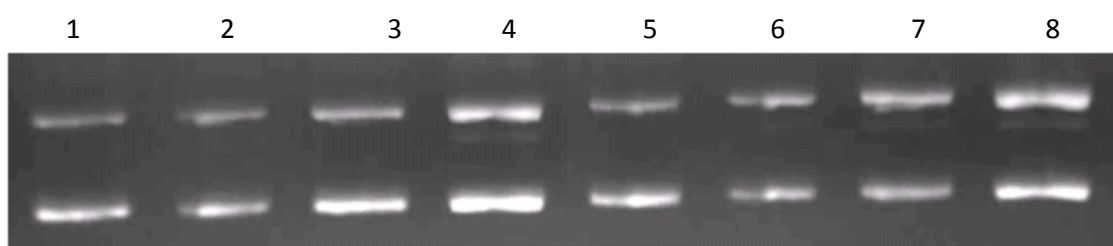


Fig 6.8: Agarose gel (0.8%) showing results of electrophoresis of 1 μ l of pBR322 Plasmid DNA; 4 μ l of Tris–HCl/NaCl (50 mM/5 mM) buffer (pH-7); 2 μ l of complex in DMF(1×10^{-3} M); 11 μ l of sterilized water; 2 μ l of H₂O₂ (total volume 20 μ l) were added, respectively, incubated at 37°C (30 min); Lane 1: DNA control; Lane 2: DNA control + H₂O₂; Lane 3: Lanthanum complex+ DNA ; Lane 4: Lanthanum complex + DNA + H₂O₂; Lane 5: Cerium complex+ DNA; Lane 6: Cerium complex+ DNA + H₂O₂; Lane 7: Samarium complex+ DNA; Lane 8: Samarium complex + DNA+H₂O₂.

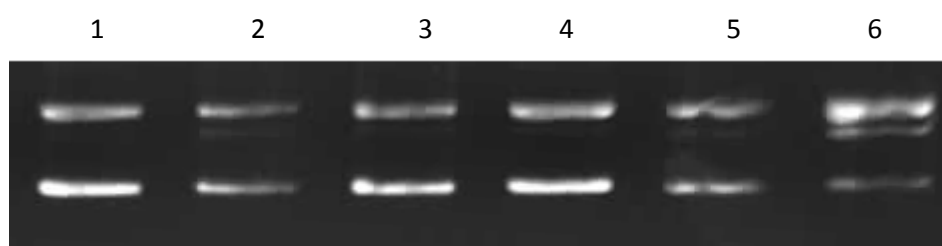


Fig 6.9: Agarose gel (0.8%) showing results of electrophoresis of 1 μ l of pBR322 Plasmid DNA; 4 μ l of Tris–HCl/NaCl (50 mM/5 mM) buffer (pH-7); 2 μ l of complex in DMF(1×10^{-3} M); 11 μ l of sterilized water; 2 μ l of H₂O₂ (total volume 20 μ l) were added, respectively, incubated at 37°C (30 min); Lane 1: DNA control; Lane 2: DNA control + H₂O₂; Lane 3: Praseodymium complex+ DNA; Lane 4: Praseodymium complex+ DNA + H₂O₂; Lane 5: Neodymium complex+ DNA; Lane 6: Neodymium complex+ DNA+H₂O₂;

Conclusions

La(III), Ce(III), Pr(III), Nd(III) and Sm(III) complexes of 2-acetylpyridine benzoylhydrazone(APBH) have been synthesized and characterized. Physico-chemical and spectral studies reveal that the complexes have general formula $[Ln(APBH)_2(NO_3)_3]$ (where Ln = La, Ce, Pr, Nd, and Sm). APBH acts as neutral tridentate ligand and NO_3^- acts as bidentate ligand. Two APBH ligands occupy six coordination sites and three NO_3^- ligands another six coordination sites to form 12-coordinate complexes mono nuclear complexes.

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