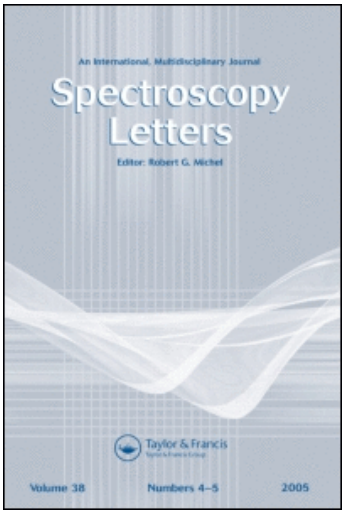

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Synthesis and Characterization of a Biologically Active Lanthanum(III)–Catechin Complex and DNA Binding Spectroscopic Studies

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ABSTRACT A lanthanum(III) complex of catechin has been synthesized and characterized by elemental analysis, molar conductance, UV-Vis spectra, infrared spectra, thermal analysis, and ^{1}H NMR. The complex behaves as a nonelectrolyte in methanol solvent. The spectral and thermal properties of the complex are examined. A thermogravimetric (TGA) study showed the hydrated nature of the complex. ^{1}H NMR spectra of the lanthanum and the catechin (CT) ligands measured in CD_3OD-d_4 also show metal ligand coordination. The lanthanum–catechin complex shows bright luminescence in methanol solution. The interaction of the complex with calf thymus DNA has been investigated by absorption and emission spectroscopic measurements. Experimental spectral results suggest CT–DNA binding with catechin complex via an intercalative mode.

KEYWORDS calf thymus DNA (CT–DNA), FTIR, ^{1}H NMR, lanthanum catechin complex, luminescence, UV-Vis

INTRODUCTION

Design and synthesis of lanthanide coordination complexes with polyphenolic ligands has attracted great attention from many researchers because of their potential applications in various fields of medicinal, biological, and material sciences, such as contrast agents in magnetic resonance imaging, and as mild reagents and catalysts in organic synthesis.[1–13] These applications require a precise knowledge of the coordination behavior of lanthanide(III) ions in complex formation with organic ligands.[14] Nevertheless, the number of La(III) complexes with oxygen donor ligands that are being isolated and studied is rapidly increasing.[14–19] This surge of interest in lanthanide complexes with polydentate aromatic O-donor ligands is derived from their photophysical properties and their application as supramolecular light conversion devices.[20–25] Also, flavonoids (polyphenolic) oxygen donor ligands are of interest in view of their free radical scavenging ability, and their antioxidant properties have been shown.[25–28] Catechin is

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one of the most biologically active and common dietary flavonols.[1–4,29] It is present in grapes, grapefruits, onion, berries, green veggies, and legumes as well as blue-green algae, and the average dietary intake of catechin has been documented.[29–31] Many of the beneficial effects of catechin are related to its antioxidant properties, which may result from its ability in scavenging free radicals (i.e., peroxyl radicals) and in chelating metal ions [Fe(II) and Fe(III), Cu(II), etc.].[2,5,32–35] Therefore, the free radical scavenging ability of flavonoids was enhanced after coordination with the metal ions.[5,33–35] Many transition metal complexes with flavonoids have demonstrated antitumor activity in tumor-bearing animals.[31–35] Flavonoids exert their antitumor effects through binding to DNA thereby changing the replication of DNA and inhibiting the growth of the tumor cell, which is the basis of designing new and more efficient antitumor drugs, and their effectiveness depends on the mode and affinity of the binding. Therefore, interaction of ligand to the metal ion is very important to understand the mechanism of their antioxidant activities. Many investigations[5,32–35] have proved that binding of a drug to a metalloelement enhances its activity, and in some cases the complex possesses even more healing properties than the parent drug. This has prompted us to investigate the metal-binding properties of several flavonoid complexes.

In the current paper, we have synthesized the trihydrated La-tris(catechin) complex and have characterized it by spectroscopic methods, and we have investigated the interaction of the complex with calf thymus DNA (CT–DNA) by absorption and emission spectroscopic methods.

MATERIALS AND METHODS

Materials

Lanthanum oxide (99.9%; Lieco Chemicals, NY, USA) was converted into the chloride by adding conc. HCl in metal oxide. Catechin (99.9%; Sigma–Aldrich, USA), methanol, xylenol orange (SD Fine Chemicals, Mumbai, India), and EDTA (BDH, England) were used as such in this study.

Methods of Physical Measurements

Microanalysis (carbon and hydrogen) was carried out with a FISONS EA-1108 (USA) elemental analyzer. The metal content of the complex was estimated by complexometric titration. Molar conductance of 10^{-3} M methanol solution of the complex was measured by an Orion conductivity meter. The thermogram was recorded on a DuPont TA 2000 TGA machine under nitrogen atmosphere at a heating rate of 10°C min^{-1}. Melting point was measured with a Gallenkamp MBF-595 apparatus. A Shimadzu UV-2501PC spectrophotometer (Japan) was used to obtain the electronic spectra in the region 200–900 nm in methanol solvent. Fourier Transform Infrared (FTIR) spectra in the 4000–400 cm^{-1} region were recorded from KBr pellets on a Perkin-Elmer spectrophotometer. ^1H NMR chemical shift was recorded on a Bruker DRX-300 NMR spectrometer (USA) with CD_3OD-d_4 as solvent and $SiMe_4$ as an internal standard.

All the experiments involving interaction of the complex with DNA were conducted in deionized water buffer of phosphate. Solution of CT-DNA gave ratios of UV absorbance at 260 and 280 nm of about 1.8:1 to 1.9:1, indicating that the DNA was sufficiently free of protein.[36] The DNA concentration per nucleotide was determined spectroscopically by assuming $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$.[37]

Synthesis of La(Catechin)$_3 \cdot 3H_2O$ Complex

Hydrated lanthanum chloride 0.300 g (1 mol) was dissolved in 50 mL methanol, and the solution was stirred on hot plate. This hot metal ion solution was added dropwise to the hot and stirred catechin ligand 0.226 g (3 mol) solution (50 mL). These two solutions were in 1:3 molar ratios, mixed thoroughly, and constantly stirred for about 6 h on a hot plate at about 100°C. The volume of the solution was reduced approximately 20 mL and the resulting solution kept at room temperature for slow evaporation. After 2 days, a pale yellow crystalline product was obtained. This crystalline product was washed with chloroform and recrystallized in methanol, dried *in vacuo* over P_4O_{10}. Anal. Calc. for $La_1C_{45}\ H_{39}\ O_{18}.3H_2O$: La, 13.79; C, 53.68; H, 3.90. Found: La, 13.98; C, 54.48; H, 4.01%.

RESULTS AND DISCUSSION

Synthesis and Characterization

Reaction of La^{3+} with catechin afforded a greenish-yellow complex, which was found stable in solid and

solution medium. The complex is soluble in polar organic solvents but scarcely soluble in chloroform, water, acetonitrile, acetone, and ether. The molar conductance of complex in methanol is low (5–8 Ω^{-1} cm^2 mol^{-1}) revealing that the complex is a nonelectrolyte.[38] The observed analytical data of the complex support the stoichiometry La(catechin)$_3 \cdot 3H_2O$. We assumed that catechin acted as bidentate ligand and formed a mononuclear complex where one La(III) ion is bound to three catechin molecules. This assumption is in accord with elemental analysis, molar conductance, thermogravimetric analysis (TGA), UV-Vis, FTIR, and ^{1}H NMR spectroscopic studies of the complex. The complex was crystalline solid having no melting point up to 360°C but decomposed over the temperature ranges 275–280°C. Synthesis of the complex was carried out in air, and no precaution was taken to exclude moisture. The complex is air stable and can be handled without effect of air or moisture. Thermal analysis data of the lanthanum complex exhibit a hydrated nature and revealed three lattice water molecules (Fig. 1).

UV-Vis Spectral Analysis

Electronic absorption spectra of La(catechin)$_3 \cdot 3H_2O$ complex with the parent ligand were measured in methanol solvent within the spectral range 200–500 nm. As reported, two characteristic absorption bands at 280 and 216 nm were observed in the free catechin.[1,39,40] Band I at 280 nm is due to the ring A (quinolic ring) and band II at 216 nm represents ring B (catechol) of catechin. Most significant changes in the lanthanum–catechin complex occurred in band II, which is shifted toward higher wavelength at 239 nm (~23 nm). This bathochromic shift in band II suggests that 3′-hydroxyl group of ring B associated to the metal ion that can most easily dehydrate and take part in a complexation process. Moreover, band I is slightly moved to higher wavelength (lower energy) at 288 nm (~8 nm), and display ring A is associated with metal ion through the ring (C–O–C) in coordination mode. The coordination of transition metal ion to the catechol moiety has been previously described and can be explained on the basis of the chelating effects of the two vicinal hydroxyl groups on ring B.[5,39–42]

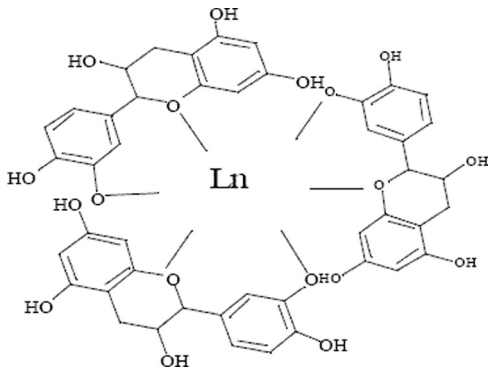

FIGURE 1 The structure of La(catechin)$_3 \cdot 3H_2O$ complex.

In general, ligand so far studied contains two possible sites at which the metal could attack. Bodini et al.[39] studied the redox properties of catechin through UV-Vis and cyclic voltammetry, suggesting higher proton acidity at 3′-hydroxyl group correlates well with its easy deprotonation and enhanced metal-binding ability. In opposition to these studies, the chelating behavior of catechin with metal is different with respect to its analogous family members such as quercetin and chrysin.[43,44]

Vibrational Spectra

The infrared absorption spectrum of lanthanum (III)–catechin complex with the parent free ligand is observed in the 4000–400 cm^{-1} spectral ranges; prominent vibrations with their tentative assignments are presented in Table 1. The spectra of ligand and complex are virtually identical except for the appearance of additional bands owing to the metal–ligand coordination. The IR spectra of ligand revealed a

TABLE 1 IR Absorption Frequencies of Lanthanide-Catechin Complex

Functional groups	Catechin	La
ν(O–H)	3341	3392
ν(C–H)	2926	2968
ν(C=C)	1629	1632
	1521	1522
δ(O–H) C–O–H	1460	1464
ν(C–O) C–O–C C–C–O	1373	1384
νC–C)	1285,	1287
C – C – C	1146	1147
‖ O	1112	1116
ρ(O–H) in-plane deformation	1079	1081
(C–H) in-plane deformation	1030	1033
(C–C) in-plane deformation	975	983
		877
(O–H) out-of-plane deformation	822	818
(C–H) out-of-plane deformation	765	768
(C–C) out-of-plane deformation	669	670
M–O	—	525

broad band with high intensity at 3341 cm^{-1} that is the main characteristic ν(O–H) stretching vibration of phenolic groups.[45,46] This O–H stretching vibration of the ligand is displaced ~51 cm^{-1} at 3392 cm^{-1} in the complex spectrum. Displacement of O–H stretching mode in the complex spectrum provides good evidence of association to the metal ion through oxygen atom. On comparing the IR absorption frequencies of ligand to the metal complex, essential changes in the complex spectrum clearly occur (Fig. 2). Conclusively, all the changes in the infrared spectrum lay in the region of (C–O–C) and ν(O–H) absorptions. They must be due to the removal of hydrogen from the 3′-OH group and formation of the M–O bonding.[43] However, any change in the peak shapes, their appearance, and disappearance of new peak shapes are informative. It should also be mentioned that interpretation of the 1600–400 cm^{-1} regions is difficult, because it is the so-called fingerprint region where a large number of different vibrations takes place.

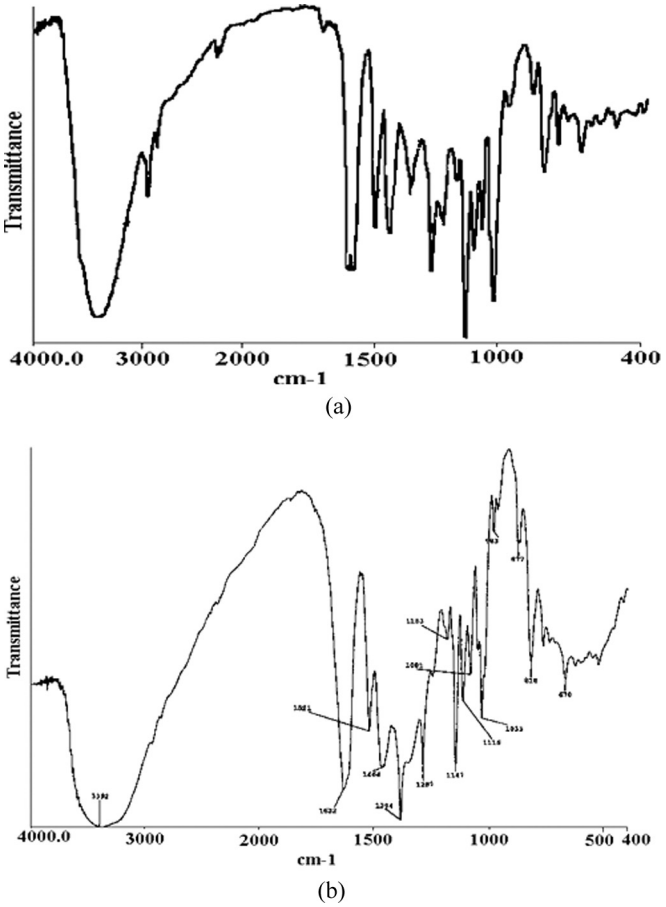

FIGURE 2 **FTIR absorption spectra: (a) free catechin molecule and (b) La(catechin)$_3$·3H$_2$O complex.**

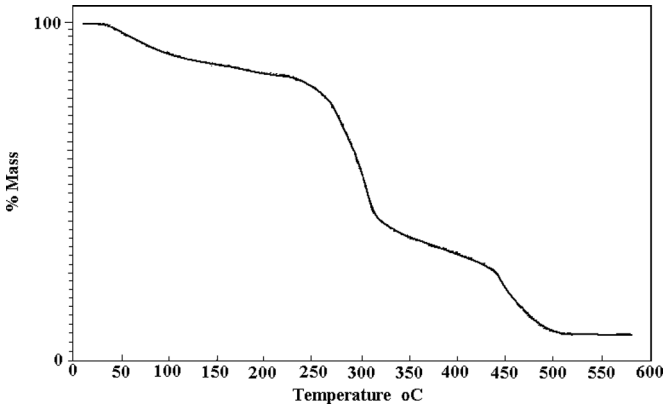

FIGURE 3 **TGA spectra of La(catechin)$_3$·3H$_2$O complex.**

Thermal Analysis

The thermal stability of the complex is determined by thermogravimetric analysis between the temperature ranges 30°C and 700°C under nitrogen atmosphere with a heating rate of 10°C per min. The main objectives of the thermal analysis are to assign the number and nature of water molecule(s) present in the complex. The thermogravimetric curve of the complex is shown in Fig. 3. According to the observed spectral results, the complex is not volatile, and decomposition commences after 100°C. The water content of the complex has been verified by TGA, which indicates that three water molecules in metal ion are lost in a one-step process at relatively low temperature (95°C). Elimination of water molecule below 100°C displays presence of lattice water, which is associated with the metal ion in outer coordination sphere as lattice form. An endothermic peak was observed at 95°C with a mass loss of 5.86% (calc. mass loss for three water molecules is 5.09%). A second inflexion point was observed between the temperature ranges 200°C and 308°C with a mass loss of 55.37%, which is equivalent to two catechin molecules (calc. mass loss for two molecules of catechin is 54.35%). Another mass loss of 25.83% was observed in the temperature range 350–520°C, corresponding to one catechin molecule (calc. mass loss for one molecule is 27.17%). (Table 2).

^{1}H NMR Spectra

^{1}H NMR spectra of the studied lanthanum complex, as well as of the free ligand in CD_3OD-d_4 solution, are presented in Fig. 4. Proton chemical shift data of the complex and the respective free

TABLE 2 Thermal Analysis Data of Lanthanum–Catechin Complex

Complex	Temperature (°C)	% Weight loss		Constituents eliminated
		Calc.	Obs.	
Lanthanum	95	5.09	5.86	3 molecules of H_2O
	200–308	54.35	55.37	2 molecules of catechin
	350–520	27.17	25.83	1 molecule of catechin

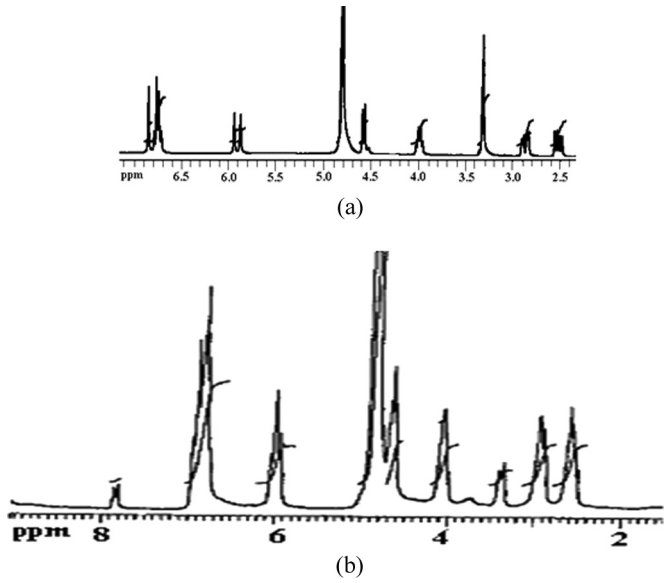

FIGURE 4 ^{1}H NMR spectra: (a) free catechin molecule, (b) La(catechin)$_3 \cdot 3H_2O$ complex in CD_3OD.

ligand is summarized in Table 3 with their tentative assignments. The signals of catechin, in diamagnetic lanthanum complex, have been found to shift to lower field compared with the free catechin ligand. These protons, which are shifted to lower fields, are subjected to no perturbing influence other than deshielding expected from the electron withdrawing inductive effect of coordination. Protons of the catechol aromatic ring were observed in the region 6.70–6.84 δ, corresponding with H-6′, H-5′ and H-2′ protons respectively.[47–50] The signals of aromatic ring, H-8 and H-6 were observed at 5.86 δ and 5.93 δ, respectively. Double resonance signal with doublet was observed in the region 2.47–2.89 δ corresponding with H-4 ring proton; another multiplet was observed at 3.97 for the H-3 ring proton. H-2 ring proton also observed at 4.55 δ with doublet.[47–50] The signals of catechin, in diamagnetic lanthanum complex, have been found to shift to lower field compared with free catechin. The lower field shifts of catechin resonance signals is strong evidence that the aromatic ring remains coordinated to the lanthanum metal in solution medium. The observed downfield shift of the signals is a good indication of complex formation (Fig. 4).

DNA Binding Studies

Absorption Spectroscopy

Electronic absorption spectroscopy is one of the most powerful experimental techniques for probing metal ion–DNA interaction.[5,33,35,44] The electronic spectrum of complex at room temperature in the presence of varying amounts of DNA and in the absence of DNA is shown in Fig. 5. Binding of the macromolecule leads to changes in the electronic spectrum of the metal complex. Base binding is expected to perturb the ligand field transitions of the metal complex. Intercalative mode of binding usually results in hypochromism and bathochromism due to the strong stacking interactions between an aromatic chromophore and the base pairs of DNA. With increasing amount of CT–DNA concentration (10–150 μM), the metal–ligand charge transfer (MLCT) transition of the complex at 400 nm exhibits bathochromism ~4–29 nm in the spectrum. These spectral characteristics suggest that the complex might bind to DNA by an intercalative mode. After intercalating the base pairs of DNA, the p* orbital of the intercalated ligand could couple with the p orbital of base pairs, thus decreasing the p–p* transition energy and further resulting in bathochromism. The

TABLE 3 ^{1}H NMR Chemical Shift Data of Lanthanum-Catechin Complex in CD_3OD-d_4 on 300 MHz

Ligand/Compound	7-OH, 5-OH, 3′-OH, 4′-OH, 3-OH,	H-2	H-3	H-4	H-6	H-8	H-2′	H-5′	H-6′
Catechin	not observed	4.55d	3.97 m	2.88dd 2.52dd	5.93s	5.86s	6.84s	6.75d	6.70d
La	not observed	4.63d	4.03 m	2.92dd 2.57dd	5.96s	5.89s	6.92s	6.86d	6.78d

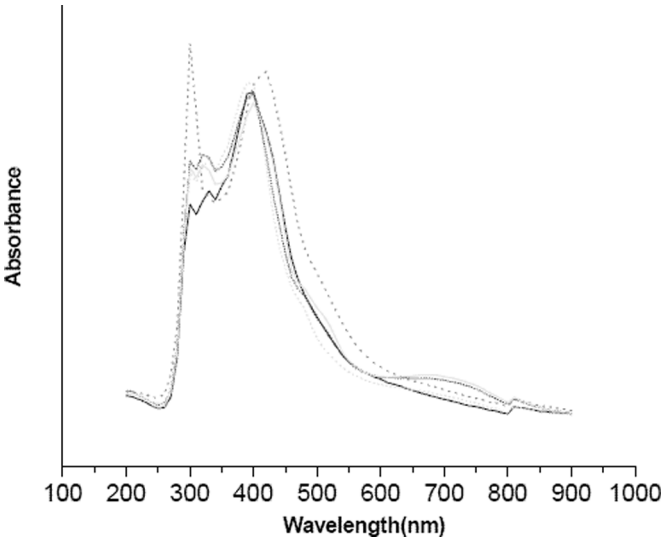

FIGURE 5 **Absorption spectra of La(catechin)$_3 \cdot 3H_2O$ complex in the absence and presence of increasing amounts of CT–DNA ([La] = 300 μM, [DNA] = 150–10 μM from top to bottom) concentrations.**

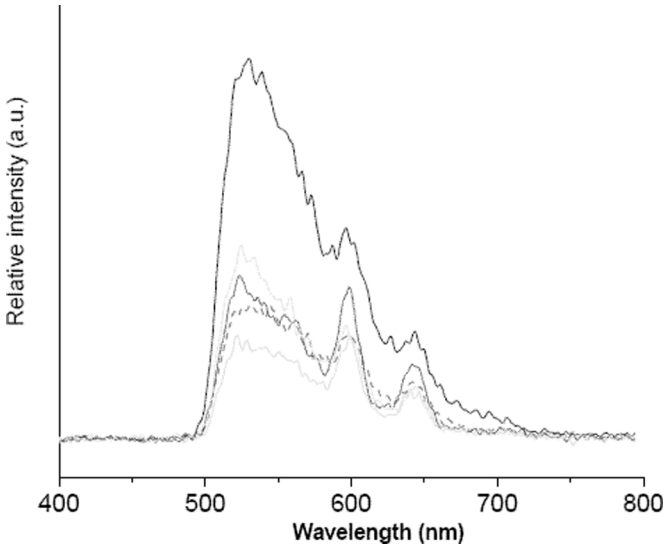

FIGURE 6 **Emission spectra of La(catechin)$_3 \cdot 3H_2O$ complex in the absence and presence of CT–DNA ([La] = 300 μM, [DNA] = 150–10 μM from top to bottom) concentrations.**

extent of hypochromism parallels the intercalative binding strength. The intensity of the intraligand band at 360 nm increases with increasing concentration of DNA. Addition of DNA also leads to changes in the position of absorption bands.

Luminescence

The emission spectral analysis shows that catechin complex exhibits a strong luminescence between 500 and 800 nm with two additional bands. The emission spectra of the complex in the presence of varying amounts of DNA concentration (10–150 μM) are presented in Fig. 6. In the absence of DNA, the complex can emit weak luminescence in phosphate buffer at ambient temperature, with a maximum appearing at 530 nm. As the concentration of CT–DNA is increased the emission intensity of the band increases appreciably with a small red shift. Thus, substantial enhancement in the emission band exhibits some interactions between the base pairs of CT–DNA and La(III) complex, which increases the emission intensity of the probe molecule. This implies that the complex can strongly interact with DNA and be protected by DNA efficiently, as the hydrophobic environment inside the DNA helix reduces the accessibility of solvent water molecules to the complex, and the complex mobility is restricted at the binding site, leading to decrease of the vibrational modes of relaxation. The emission intensities of the complex from its MLCT excited states upon a fixed excitation is found to depend on DNA concentration.

CONCLUSION

We have synthesized a new lanthanum (III)–catechin complex [La(catechin)$_3 \cdot 3H_2O$] and characterized it by spectroscopic methods. Preliminary studies show that the complex undergoes easy reaction involving displacement of hydrogen atom from the 3′-hydroxyl group of catechol leading to association with metal ion. The spectral and emission properties of the complex were investigated it and was found that three catechin units are still attached with metal ion through two binding sites in solid state as well as in solution medium. Absorption and emission data suggest an intercalative mode of DNA binding for this complex. The current study demonstrates that the ancillary ligands with hydrogen bonding potential support the intercalative interaction of ligands with extended aromatic rings and enhances the DNA binding affinity. Due to the presence of labile groups, it is expected that it may be used as a precursor for the synthesis of other interesting complexes and in the development of pharmaceutical agents or as MRI shift reagents.

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