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Synthesis, Characterization and Antimicrobial Study of Lanthanide (III) Complexes of Cefepime

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ABSTRACT

The new lanthanide (III) complexes of Cefepime, [Ln(Cefepime)(H_2O)$_4$](NO_3)$_3$, where (Ln=La, Pr, Nd, Sm, Gd and Ho (III)), have been synthesized. The metal complexes were characterized on the basis of elemental analysis, UV-Visible Spectra and FTIR. The spectral data revealed that the Cefepime behaves in tridentate fashion coordinating through carbonyl oxygen of amide and lactum, and carboxylate oxygen. Cefepime and all the complexes were screened for their antimicrobial study against Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes using the disc diffusion method. An enhancement of antimicrobial activity of the Cefepime was observed on complexation.

Keywords: Lanthanide; Cefepime; FTIR; Antimicrobial activity

INTRODUCTION

Cefepime has been described as a semisynthetic, broad spectrum, fourth-generation cephalosporin [1]. It is active against some bacteria that are resistant to other antibiotics and is used for the treatment of susceptible infections caused by gram-negative and gram-positive organisms [2]. It is indicated for respiratory tract infections, skin and soft tissue infections, urinary tract infections and febrile neutropenia [3]. Some transition metal (II) coordination compounds of a Cephalexin Schiff base (HL) derived from the condensation of cephalexin antibiotic with sulphathiazole were synthesized by .J.R. Anacona et.al. [4]. Apart of membrane permeability, antibacterial activity of cephalexin Schiff base and its metal complexes depends mainly on the metal ion and the type of microorganism. J. R. Anacona and Heidy Rodriguez [5] have been synthesized Cefepime transition metal (II) ions complexes with (M=Mn(II), Co(II), Ni(II), Cu(II), and Zn(II)), which were characterized by physicochemical and spectroscopic methods. The complexes have been screened for antibacterial activity against several bacteria and showed activity less than that of free Cefepime.

The design and synthesis of complexes of lanthanide metal ions with organic ligands is fascinating area of research not only because owing to their structures, but also of potential applications of their luminescent properties [6]. These complexes have good physical and chemical properties as well as anti-inflammation, antitumor, and antithrombogenic properties because of their electron configuration [7,8]. For their special, photo physical and biological properties, lanthanide complexes are used as biological probes in the areas of clinical chemistry and molecular biology [9]. Some lanthanide complexes have a potential role in the treatment of tumor cell lines [10]. The complex of rare earth chloride with nicotinic acid or 8-hydroxyquinoline was synthesized by Xu li. The bioactivity of the product is significantly stronger than those of rare earth ions, nicotinic acid, or 8-hydroxyquinoline alone [11]. Due to their special electronic configuration, lanthanide complexes have inspired many efforts on the design and synthesis as potential anticancer and antibacterial agents. The importance of the applications of lanthanides, as an excellent diagnostic and prognostic probe in clinical diagnostics, and an anticancer material, is remarkably increasing. Lanthanide complexes based X-ray contrast imaging and lanthanide chelates based contrast enhancing agents for magnetic resonance imaging (MRI) are being excessively used in radiological analysis in our body systems [12]. Some of the lanthanide complexes are used in biomedical analysis as magnetic resonance imaging (MRI) contrast agents and also as effective catalysts for the hydrolytic cleavage of phosphate

ester bonds hydrolysis of nucleic acids [13]. These study remarkable mainly because it is essential for further development in biotechnology, molecular biology, therapy and related fields [14].
Literature presents several reports on the use of metal complexes for antimicrobial studies. Rajesh Kumar Mishra et al. have synthesized some trivalent lanthanides metal complexes of La(III), Pr(III), Nd(III), Sm(III), Dy(III), Ho(III) and Er(III) with Cloxacillin [15]. The synthesized ligands and their metal complexes were screened for their antimicrobial activity. The activity data showed that the metal complexes to be more potent against uncomplexed Cloxacillin. Kanti Pachori et al. [16] prepared Cefadroxil complexes with different metal ions of 1:1 metal to antibiotic stoichiometry and were screened for their antimicrobial activity. The results obtained showed that most of the compounds were biologically active and showed enhanced antimicrobial activities compared to the free ligand.
The aim of this study was to evaluate the antimicrobial activity of the synthetic lanthanide complexes with a Cefepime against some medically important bacteria and to investigate factor relevant to the biological activity of these compounds.

EXPERIMENTAL SECTION

Materials and physical measurements

All the reagents, starting materials as well as solvents were purchased commercially and used without any further purification. The ligand (Cefepime) was purchased from Lupin Ltd. The metal was determined as lanthanum oxide after destroying the organic matter by using fuming HNO_3 several times [17]. Elemental analysis for C, H and S were performed on ELTRA CHS 500. Double beam spectrophotometer 2203 was used to determine the UV- Vis spectra of complexes. The infrared spectra of the complexes, in the range 4000-400 cm^{-1}, were recorded on a FTIR (Perkin Elmer - Spectrum RX-IFTIR).

Preparation of complexes

The ligand (1mmol) was dissolved in 20mL of water and then pH was adjusted to 7–8 by drop wise addition of aqueous solution of NaOH (0.01mol/L). After dissolution of all the ligand, a solution of $Ln(NO_3)_3 \cdot 6H_2O$ (0.5mmol) in water (10mL) was added drop wise to the system. Immediately there was a precipitate in the solution. The solution was stirred for 4 h at room temperature, the precipitate was separated. The precipitate was washed several times with water and one time with ether and finally dried. All the complexes were synthesized by the same way.

Antimicrobial activity

For antimicrobial study of ligand and complexes, the disc diffusion method [18] was adopted. For this 200mL of Nutrient Agar Media was prepared and autoclaved for 30min at 120^oC and 15 Ibs pressure. 15mL of Nutrient Agar Media was poured in sterile Petri dishes. The solution was allowed to solidify. The test organisms were grown on agar medium in Petri dishes. The test compounds were added drop wise to a 5mm diameter filter paper disk placed at the centre of the each Petri dish. The plates were incubated for 24 hrs at 36ºC. The inhibition zone was evaluated after 24 hrs.

RESULT AND DISCUSSION

All of complexes were soluble in Dichloromethane and DMSO.

Elemental analysis

The analytical data of the complexes are summarized in Table 1. The values obtained are in good agreement with the theoretical values calculated for the suggested general formula of the complexes such as $[Ln(Cefepime)(H_2O)_4](NO_3)_3$.

Electronic Spectra

UV-Visible spectra are usually helpful in the estimation of results equipped by other methods of structural investigation. The electronic spectra of metal (Pr, Nd, Sm and Ho) complexes are compared with the corresponding pure metal ions. The data are summarized in Table 2. La and Gd complexes showed no f-f bands in the measured region [19].
The tabulated data indicated that the f-f transitions energy of complexes is slightly reduced compared to the corresponding aquo ions. The reduction in the energy of f-f transitions in complexes can be attributed to covalent interaction of the 4f orbitals of metals with vacant ligand orbitals, by increased nuclear shielding of the orbitals, or leading to some delocalization with ensuing reduction in interelectronic repulsion, due to a slight covalent ligand-metal electron drift [20].

The nephelauxetic ratio β is calculated by using the following expression [21]:

$$(1- \beta) = (\nu_{aquo} - \nu_{complex})/ \nu_{aquo} \ldots. (1)$$

Table 1.Analytical data for the complexes (calculated values in parentheses)

S.N.	Compound	Elemental analysis (%)			
		C	H	S	Ln
1.	Cefepime	46	5.02	12.95	-
		-47.5	-5	-13.3	
2.	$[La(Cefepime)(H_2O)_4](NO_3)_3$	33.82	6.68	8.66	20
		-33	-6.7	-9.26	-20.1
3.	$[Pr(Cefepime)(H_2O)_4](NO_3)_3$	31.49	4	8.95	19.98
		-32.9	-4.6	-9.23	-20.3
4.	$[Nd(Cefepime)(H_2O)_4](NO_3)_3$	32.36	4.65	9.67	20.65
		-32.8	-4.6	-9.19	-20.7
5.	$[Sm(Cefepime)(H_2O)_4](NO_3)_3$	32.35	4.66	9.45	21.01
		-32.5	-4.6	-9.11	-21.4
6.	$[Gd(Cefepime)(H_2O)_4](NO_3)_3$	32.02	5.01	9.53	22.53
		-32.2	-4.6	-9.02	-22.1
7.	$[Ho(Cefepime)(H_2O)_4](NO_3)_3$	32.28	5.43	9.48	23.29
		-32.2	-4.5	-9.05	-23.3

The value of β is used to determine the covalency factor ($b^{1/2}$), Sinha parameter i.e. degree of metal–ligand covalency (δ%) and the covalency angular overlap parameter (η) by the following relations [22]

$$b^{1/2} = [(1- \beta)/2]^{½} \text{ …. (2)}$$

$$\delta\% = (1- \beta)*100/ \beta \text{ …. (3)}$$

$$\eta = (1- \beta^{1/2})/\beta^{1/2} \text{…. (4)}$$

Table 2: Electronic spectral data (cm^{-1}) and related bonding parameter of lanthanide (III) complexes

Ln^{+3}	$Ln(NO_3)_3$ Electronic spectral bands	$[Ln(Cefepime)(H_2O)_4](NO_3)_3$ Electronic spectral bands	J-levels	
Pr^{+3}	22545	22381	$^3H_4 \rightarrow {}^3P_2$	β = 0.9927
	22472	21240	$\rightarrow {}^3P_1$	$b^{1/2}$ = 0.0602
	20833	20678	$\rightarrow {}^3P_0$	δ% = 0.7310
	16949	16812	$\rightarrow {}^1D_2$	η = 0.0036
Nd^{+3}	23200	23116	$^4I_{9/2} \rightarrow {}^2P_{1/2}$	
	21913	21308	$\rightarrow {}^4G_{11/2}$	
	21530	20986	$\rightarrow {}^2G_{9/2}$	
	20188	19523	$\rightarrow {}^4G_{9/2}$	β = 0.9787
	19338	19138	$\rightarrow {}^4G_{7/2}$	$b^{1/2}$ = 0.1030
	17510	17406	$\rightarrow {}^4G_{5/2}$	δ% = 2.1681
	14644	14628	$\rightarrow {}^4F_{9/2}$	η = 0.0107
	13812	13498	$\rightarrow {}^4F_{7/2}$	
	12887	12613	$\rightarrow {}^4F_{5/2}$	
	11921	11611	$\rightarrow {}^4F_{3/2}$	
Sm^{+3}	27933	27624	$^6H_{5/2} \rightarrow {}^4D_{3/2}$	
	26624	26619	$\rightarrow {}^6P_{7/2}$	β = 0.9675
	25825	24987	$\rightarrow {}^6P_{3/2}$	$b^{1/2}$ = 0.1273
	24850	24539	$\rightarrow {}^6P_{5/2}$	δ% = 3.3516
	22870	22857	$\rightarrow {}^4G_{9/2}$	η = 0.0166
	21600	21505	$\rightarrow {}^4I_{13/2}$	
	21450	21110	$\rightarrow {}^4M_{15/2}$	
	20460	20040	$\rightarrow {}^4G_{7/2}$	
	19960	18018	$\rightarrow {}^4G_{5/2}$	
Ho^{+3}	27594	26042	$^5I_8 \rightarrow {}^5G_4$	
	24120	24015	$\rightarrow {}^5G_3, {}^3G_5$	β = 0.9965
	22240	22163	$\rightarrow {}^5F_2$	$b^{1/2}$ = 0.0416
	20800	20576	$\rightarrow {}^5F_3$	δ% = 0.3469
	18920	18601	$\rightarrow {}^5F_4$	η = 0.0017
	15860	15576	$\rightarrow {}^5F_5$	

he values of β are found to be less than unity and the values of the bonding parameter $b^{1/2}$ and Sinha's parameter δ% are found to be positive in these complexes which is indicating convalent characters in the metal–ligand bonding [23].
The interaction between the central ion and the surrounding can be expressed by the using the term of the Judd Ofelt Intensity Parameters (T_λ , λ=2, 4 and 6) [24,25]. These are derived from the observed oscillator strength. The measured intensity of an absorption band is related to the probability of radiant energy by the expression –

$$P_{obs} = 4.6 \times 10^{-9} \times \varepsilon_{max} \times \Delta\nu_{1/2} \ldots. (5)$$

Where ε_{max} is the molar extinction coefficient of the peak maximum and $\Delta\nu_{1/2}$ is half intensity band width.
The calculated oscillator strength is represented in terms of T_2, T_4 and T_6 (Judd Ofelt Intensity Parameters) parameters as

$$P_{cal} = [T_2 [U^2]^2 + T_4 [U^4]^2 + T_6 [U^6]^2] \bar{\upsilon} \ldots. (6)$$

The values of $[U^2]^2$, $[U^4]^2$ and $[U^6]^2$ have been taken as reported by Carnall et.al [26]. T_2 parameters show high sensitivity towards coordination changes while T_4 and T_6 have been found to exhibit more sensitivity towards symmetry changes.
Table 3 shows Judd Ofelt intensity parameters. The negative value of T_2 was found for Pr (III) systems. The behavior may be due to one or more of the following points. Strong f-d mixing: poor resolution method of the overlapped 3p-bands; small number of observed orfitted lines; or due to using incorrect value of reduced matrix elements of Pr (III) [27]. Table 3 is showing an appreciable variation in all the three T_λ parameters (T_2, T_4 and T_6). The variation of T_2, T_4, T_6 parameters clearly shows the high sensitivity towards coordination and symmetry changes. The ratio of T_4/T_6 showed smaller change, thereby suggesting that symmetry changes are less prominent. The low value of σ_{rms} Deviation indicates the applicability and suitability of Judd-Ofelt theory [28].

Table 3: Judd Ofelt parameters T_λ for Ln(III)-Complexes

Complex	$T_2 x10^9$(cm)	$T_4 x10^9$(cm)	$T_6 x10^9$(cm)	T_4/T_6	σ_{rms} $x10^6$
Pr	-10.251	0.974	3.9129	0.2489	0.2137
Nd	0.3202	0.7431	0.732	1.0151	1.9894
Sm	25.523	1.5941	0.9	1.771	2.1982
Ho	0.1571	0.2068	0.2742	0.7541	0.2613

FTIR spectra
IR spectroscopic studies are used to identify different groups of ligand involved in chelation sites to form complexes detected in the solution [29]. The IR absorption bands for complexes are given in Fig. 1. It can be found that the characteristic absorption peaks of all complexes are similar. A broad band in the region 3200-3600 cm^{-1} appeared, due to the overlapping of the symmetric and anti-symmetric OH stretching vibrations of lattice water [30]. A strong band in the spectrum of free Cefepime at 1676 cm^{-1} is assigned to υ(C=O) (amide) [31]. Whereas in IR spectra of the complexes, bands due to υ(C=O) was shifted to lower wave number, compared to the free ligand. This indicates coordination of the ligand through carbonyl oxygen. The lactam (C=O) band [32] appears at 1772 cm^{-1}in the spectrum of Cefepime, while this peak disappear in complexes. The asymmetric stretching ν(CO_2^-) carboxylate in ligand appeared at 1600 cm^{-1} shifted toward higher wavenumbers at 1630 cm^{-1}, and symmetric stretching ν(CO_2^-) carboxylate 1390 cm^{-1} shifted towards lower wavenumber at 1384 cm^{-1} in complexes indicating the participation of the carboxylate in the coordination. The Δν = [ν(COO)as - ν(COO)s] value is useful to determine the bonding properties of carboxylate anion to the metal atoms. Based on the infrared data, all the complexes showed that the Δν fall in the range of 350 cm^{-1} > Δν > 200 cm^{-1} which thus indicated that the carboxylate anions bonded to metal atom moiety in anisobidentate manner [33]. (Figure 1)

Antimicrobial activity
The ligand and some of their corresponding metal complexes were screened for their antimicrobial activity against one Gram-negative (Escherichia *coli*) and two Gram-positive (Staphylococcus *aureus* and Streptococcus *pyogenes*) bacterial strains. The results of antimicrobial activity were summarized in Table 4. The ligand was sensitive to all the organisms. It was found to be strong activity against the Gram positive bacterial species as compared to Gram negative specie.
All complexes are found to have enhanced activity compared to the ligand against the Gram-negative bacteria E. *coli* and Gram positive bacterial species Staphylococcus. All the complexes, except Pr(III) complex, show moderate activity against Streptococcus, while Pr(III) complex show good activity against the same species. A comparative study of the ligand and their metal complexes indicates that most of the metal complexes exhibit higher antimicrobial activity than that of the free ligand and the control. Hence complexation increases antimicrobial activity. The greater activity of the complexes compared to free ligand may be attributed to chelation which decreases polarity of the metal ion by partial sharing of the positive charge with donor atoms of the ligand. This increases the lipophilic character, favoring the permeation through lipid layers of the bacterial membrane [34,35].

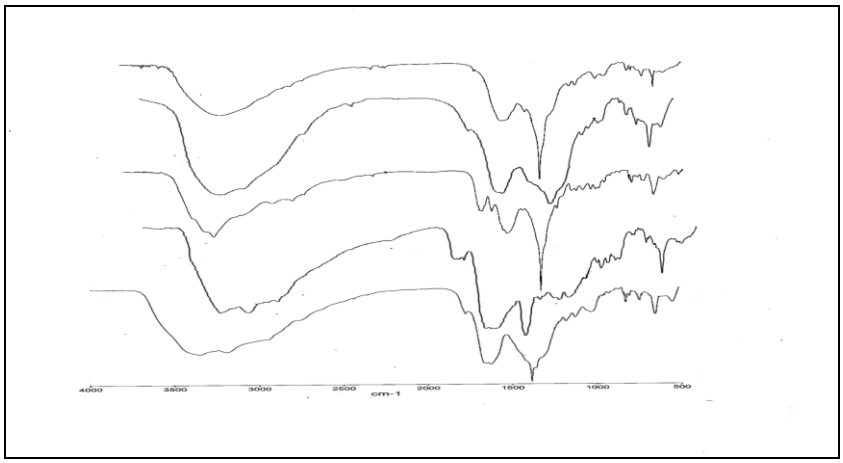

Figure 1: FTIR spectrum of [Ln(Cefepime)$(H_2O)_4$] complexes

Table 4: Antimicrobial activity of Cefepime and its complexes against different bacteria

Compounds	E.*c*.	S.*a*.	S.*p*.
HL	++	++++	++++
[La(Cefepime)$(H_2O)_4$] $(NO_3)_3$	++	+++	++
[Pr(Cefepime)$(H_2O)_4$] $(NO_3)_3$	+++	++++	++++
[Nd(Cefepime)$(H_2O)_4$]$(NO_3)_3$	+++	++++	+++
[Sm(Cefepime)$(H_2O)_4$]$(NO_3)_3$	+++	++++	+++
[Gd(Cefepime)$(H_2O)_4$]$(NO_3)_3$	++++	++++	+++
[Ho(Cefepime)$(H_2O)_4$]$(NO_3)_3$	++++	++++	+++

Key to interpretation: E.*c*. – Escherichia *coli*; S.*a*. – Staphylococcus *aureus*; S.*p*. - Streptococcus *pyogenes*. Inhibition Zone: 5mm–10mm = (+), 10mm–15mm = (++), 15mm–20mm = (+++) and >20mm = (++++)

CONCLUSION

In summary, a series of new lanthanide (III) complexes with Cefepime were synthesized and characterized by elemental and spectral analysis such as IR, UV-Vis. Based on the above data, ligand and metal are presented in 1:1 ratio in all the complexes. The proposed general formula of the complexes were [Ln(Cefepime)$(H_2O)_4$]$(NO_3)_3$, where (Ln= La, Pr, Nd, Sm, Gd and Ho (III)). The spectral data suggested that Cefepime coordinated as neutral tridentate ligand coordinating via two carbonyl oxygen and one carboxylate oxygen atom in addition to the coordination of water to the Ln(III) ion. Thus ligand and water coordinates to the Ln(III) ion with the coordination number seven. The deviation in the magnitude of energy interaction and intensity parameters we can recommend that minor coordination changes in the complexes are caused by the different coordinating sites of Cefepime, coordination number, denticity and nature of Ln(III)- Cefepime bond, which do induce significant variation in the intensity of f-f transitions. The antimicrobial study indicated that the complexes inhibited the growth of bacteria more than the free ligand.

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