



Synthesis, Spectral Characterization and Antibacterial Studies of Mixed Ligand La(III) and Ce(III) Complexes Derived From 1-nitroso-2-naphthol and Some Amino acids

GANESH ANANT THAKUR^{1*}, UTTAM NARAYAN DHAIGUDE¹
and PRAMOD BHAGAWAN THAKUR¹

¹Department of Chemistry, Rayat Shikshan Sanstha's, Mahatma Phule Arts, Science and Commerce College, Panvel, Raigad, Maharashtra, 410206, India.

*Corresponding author E-mail: ganeshthakur68@yahoo.co.in

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ABSTRACT

The synthesis of mixed ligand La(III) and Ce(III) complexes with formula $[M(1N2N)_2 \cdot (L) \cdot 2H_2O]$ is reported by using 1-nitroso-2-naphthol (1N2N) as a primary ligand and L-phenylalanine, L-threonine (HL) as secondary ligands. The structures of reported complexes were established by UV-Visible, infrared spectroscopic techniques, magnetic susceptibility measurements, molar conductance study, elemental analysis and thermal studies. The conductance study showed non-electrolytic nature of complexes. The thermal study was found in accordance with structure of complexes. The metal-ligand coordination was confirmed by FT-IR spectral study. Furthermore the lanthanum and cerium complexes were found diamagnetic and paramagnetic in nature respectively. The intraligand and LMCT transitions were confirmed by UV-Visible spectral study. The study to investigate antibacterial nature of compounds was done in vitro against *E. coli*, *S. typhi*, *Gram-negative* and *S. aureus*, *C. diphtheria*, *Gram-positive* bacteria.

Keywords: Antibacterial activity, Lanthanum, Cerium, Amino acid, 1-nitroso-2-naphthol.

INTRODUCTION

The coordination compounds comprising of transition metals containing amino acid ligands known for their biological importance such as cytotoxic and antimicrobial activities¹. The spectrophotometric determination of metal ions by using 1-nitroso-2-naphthol have been investigated². The complexes of some transition metals with 1-nitroso-2-naphthol and L-phenylalanine exhibit potent antibacterial

activity³. The lanthanide complexes with 1-nitroso-2-naphthol and trioctylphosphine oxide have been reported⁴. The metal complexes of lanthanum (III) possess significant antibacterial activity⁵. The synthesis, spectral characterization and antibacterial study of cerium complexes have been reported⁶⁻⁸.

Therefore, it was decided to study the complexation and antibacterial activity of synthesized complexes of lanthanum and cerium. In



present work, we explore the synthesis, structural elucidation and antibacterial behaviour of lanthanum (III) and cerium (III) mixed ligand complexes by adopting primary ligand 1-nitroso-2-naphthol and L-phenylalanine, L-threonine secondary ligands.

MATERIALS AND METHODS

Materials

The analytical grade lanthanum(III) chloride heptahydrate, cerium(III) chloride heptahydrate metal salts and 1-nitroso-2-naphthol, L-phenylalanine, L-threonine ligands were used without further purification. The used solvents for characterization of complexes were distilled and purified according to standard procedure^{9,10}.

Instrumentation

The C, H, N composition in lanthanum and cerium complexes were determined on Thermo Finnigan elemental Analyzer. The lanthanum content from their complexes was estimated by complexometric method¹¹⁻¹³. Similarly, the amount of cerium in their complexes were determined by the gravimetric method¹⁴. The electronic spectra of 10⁻³ molar solution of La(III) and Ce(III) complexes in DMF were recorded on UV/Vis-1800 spectrophotometer. The electrical conductivities of metal complexes were measured with digital conductivity meter (EQ-667 model). FT-IR spectra of synthesized complexes, primary and secondary ligands were recorded on Shimadzu FT-IR spectrophotometer using KBr disc in 400-4000-cm⁻¹ range. TG-DTA Perkin-Elmer Diamond instrument was used for thermal study of complexes. The magnetic susceptibility of reported complexes in present work were measured using Gouy's balance adopting Hg[Co(SCN)₄] as a calibrant.

Preparation of mixed ligand complexes of lanthanum and cerium

The 20 mL solution of 1-nitroso-2-naphthol in ethyl alcohol (346 mg, 2mmol) was slowly added in 10 mL aqueous solution of metal salts (371 mg LaCl₃·7H₂O, 372 mg CeCl₃·7H₂O, 1mmol). The resulting mixture in 1:2 molar proportion was refluxed in water bath for 10 minutes. To this refluxed solution,

10 mL solution of L-phenylalanine/L-threonine (1mmol) in distilled water was added. The resulting solution with 1:2:1 molar proportion was kept in water bath, stirred well and warmed for 10 minutes. The complex formation was accomplished by adjusting the 7.0 pH of the reaction mixture with slow addition of aqueous ammonia. The obtained precipitate was filtered, successively washed by water and then small amount of ethyl alcohol. In oven, the final coloured product was dried and used for spectral characterization and antibacterial study.

Antibacterial Study

The synthesized complexes of lanthanum and cerium was assayed for their antibacterial activity against *Escherichia coli*, *Corynebacterium diphtheria*, *Salmonella typhi* and *Staphylococcus aureus* bacteria by agar cup method^{15,16} and serial tube dilution method¹⁷.

Agar cup method

The petriplate was filled with sterile nutrient agar containing test organism at 5mm height and was kept to solidify. Further single hole was punched in middle portion of petriplate employing sterile cork borer with 8mm internal diameter. The 1ppm DMSO solution of metal complexes was filled to this hole and petriplate was incubated for 24 h at 37°C. Thereafter, zone of inhibition surrounding to hole was measured in mm and noted as activity of complexes. Finally, activity of metal complexes were compared with the activity of standard tetracycline compound.

Tube dilution method

The 10 mg of synthesized lanthanum and cerium complexes were dissolved in 10 mL DMSO. In sterile Muller Hinton broth (MHB), bacterial inoculums were prepared and incubated at 37°C for 24 hours. The 5 mL bacterial inoculums were dispersed in each test tubes of borosilicate. To these tubes, solution of test compound in DMSO (10 mg/10 mL) was mixed to obtain final concentration in the range of 1 to 300 µg/mL. Further, inoculums (0.1mL) of respective bacteria was inoculated in the tubes. Again tubes were incubated at 37°C for 24 hours. All tubes were examined on next day for absence or presence of bacterial growth.

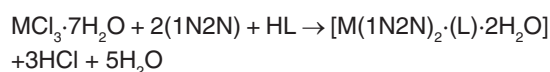
The concentration at which visible growth was not observed was considered as MIC. In this study, tetracycline compound was used as a control. The result of complexes were compared with tetracycline control which was screened simultaneously.

RESULTS AND DISCUSSION

The lanthanum complexes were green in color whereas cerium complexes were brown in color. All complexes were partly soluble in DMF, DMSO and were unsolvable in solvents like water, benzene, toluene, methanol, ethyl alcohol etc. Synthesized complexes of lanthanum and cerium were thermally stable (Table 1), amorphous nonhygroscopic solids.

The elemental analysis of lanthanum and cerium complexes were studied. The estimated data is presented in Table 2. The elemental analysis data was in accordance as of 1:2:1 type formula $[La(1N2N)_2 \cdot (L) \cdot 2H_2O]$ and $[Ce(1N2N)_2 \cdot (L) \cdot 2H_2O]$.

The general reaction of formation of mixed ligand complexes of lanthanum and cerium is shown below,



Where, M=lanthanum chloride heptahydrate/cerium chloride heptahydrate, 1N2N = 1-nitroso-2-naphthol, HL = L-phenylalanine/L-threonine.

Table 1: The decomposition temperature, empirical formula, molecular weight and color of synthesized lanthanum and cerium metal complexes

Complex	Decomposition Temperature (°C)	Empirical Formula	Molecular Weight (gms)	Color
$[La(1N2N)_2 \cdot (Phe) \cdot 2H_2O]$	220	$LaC_{29}H_{26}N_3O_8$	683.44	Green
$[La(1N2N)_2 \cdot (Thr) \cdot 2H_2O]$	205	$LaC_{24}H_{24}N_3O_9$	637.37	Green
$[Ce(1N2N)_2 \cdot (Phe) \cdot 2H_2O]$	215	$CeC_{29}H_{26}N_3O_8$	684.65	Brown
$[Ce(1N2N)_2 \cdot (Thr) \cdot 2H_2O]$	220	$CeC_{24}H_{24}N_3O_9$	638.58	Brown

1N2N = 1-nitroso-2-naphthol, Phe = phenylalanine, Thr = threonine

Table 2: Elemental analysis data of lanthanum and cerium complexes

Complex	Elemental Analysis Found (Calculated)			
	% M	% C	% N	% H
$[La(1N2N)_2 \cdot (Phe) \cdot 2H_2O]$	20.3	50.94	6.14	3.81
	-20.32	-50.96	-6.15	-3.84
$[La(1N2N)_2 \cdot (Thr) \cdot 2H_2O]$	21.78	45.22	6.58	3.8
	-21.79	-45.23	-6.59	-3.79
$[Ce(1N2N)_2 \cdot (Phe) \cdot 2H_2O]$	20.47	50.85	6.12	3.82
	-20.46	-50.87	-6.14	-3.83
$[Ce(1N2N)_2 \cdot (Thr) \cdot 2H_2O]$	21.93	45.13	6.55	3.77
	-21.94	-45.14	-6.58	-3.79

Molar Conductance and Magnetic study

The molar conductance values of lanthanum and cerium metal complexes was measured in DMF having 0.001M concentration. The obtained data summarized in Table 3 indicates that all synthesized complexes of lanthanum and cerium were non-electrolytes¹⁸.

The values of magnetic moment of lanthanum and cerium complexes (Table 3) was determined from magnetic susceptibility measurements after utilizing diamagnetic corrections which indicates that lanthanum complexes were diamagnetic^{19,20} in nature and cerium complexes were paramagnetic²¹⁻²³ in nature.

Table 3: Molar conductance and magnetic moment values of synthesized complexes of lanthanum and cerium metal

Complex	Molar conductance (Mhos $cm^2 mol^{-1}$)	Xm	Xg	μ_{eff} (B.M.)
$[La(1N2N)_2 \cdot (Phe) \cdot 2H_2O]$	0.157×10^{-3}	-2.10×10^{-4}	-3.08×10^{-7}	Diamagnetic
$[La(1N2N)_2 \cdot (Thr) \cdot 2H_2O]$	0.156×10^{-3}	-1.94×10^{-4}	-3.05×10^{-7}	Diamagnetic
$[Ce(1N2N)_2 \cdot (Phe) \cdot 2H_2O]$	0.154×10^{-3}	1.04×10^{-3}	1.53×10^{-6}	1.729
$[Ce(1N2N)_2 \cdot (Thr) \cdot 2H_2O]$	0.151×10^{-3}	1.24×10^{-3}	1.94×10^{-6}	1.85

UV-Visible Spectroscopy

The UV-Visible spectrum of 10^{-3} M solution of lanthanum and cerium complexes were recorded in DMF. The found data presented in Table 4 showed three absorption bands in the range 263-266 nm, 331-370 nm and 384-423 nm are assigned to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and LMCT transitions²⁴⁻²⁷.

Table 4: UV-Visible spectral Data of complexes of lanthanum and cerium

Complex	ν (cm^{-1})	λ (nm)	Proposed Assignments
[La(1N2N) ₂ ·(Phe)·2H ₂ O]	37594	266	$\pi \rightarrow \pi^*$
	27027	370	$n \rightarrow \pi^*$
	23866	419	LMCT
[La(1N2N) ₂ ·(Thr)·2H ₂ O]	38023	263	$\pi \rightarrow \pi^*$
	27100	369	$n \rightarrow \pi^*$
	23641	423	LMCT
[Ce(1N2N) ₂ ·(Phe)·2H ₂ O]	37879	264	$\pi \rightarrow \pi^*$
	30211	331	$n \rightarrow \pi^*$
	25000	400	LMCT
[Ce(1N2N) ₂ ·(Thr)·2H ₂ O]	37736	265	$\pi \rightarrow \pi^*$
	30030	333	$n \rightarrow \pi^*$
	36042	384	LMCT

Infra-red spectra

Table 5 contains FT-IR spectral data of complexes of lanthanum and cerium. The data shows peak at $3435\text{-}3433\text{ cm}^{-1}$ of hydroxy group of water. The absence of peak at 3448 cm^{-1} indicates deprotonation of hydroxy group of 1-nitroso-2-naphthol during complexation. Furthermore, the presence of 1-nitroso-2-naphthol moiety in the complexes was indicated by 1155 cm^{-1} peak²⁸. Also the peak at 1554 cm^{-1} and 1211 cm^{-1} for nitroso and nitrile group respectively indicates that 1-nitroso-2-naphthol is coordinated with La and Ce metal ions via nitrogen atom. In addition to this, the coordination of amino group was confirmed from the band in $3120\text{-}3113\text{ cm}^{-1}$ range and at 3061 cm^{-1} as well as nitrile peak at 839 cm^{-1} . The presence of -COOH group of amino acid was identified from peak obtained at 1600 cm^{-1} and 1400 cm^{-1} . Finally, the metal-nitrogen and metal-oxygen bonding was confirmed from weak bands in $524\text{-}522\text{ cm}^{-1}$ range and at 636 cm^{-1} .

Table 5: FT-IR spectroscopy data of synthesized lanthanum and cerium complexes in cm^{-1}

Complex	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν
	(C=O) (A.a.)	(C-N) (A.a.)	(C-O) (A.a.)	(N-H) Sym. (A.a.)	(N-H) Asym. (A.a.)	(O-H) H ₂ O	(C-N) 1N2N	(N=O) 1N2N	(C-O) 1N2N	(M-N)	(M-O)
[La(1N2N) ₂ ·(Phe)·2H ₂ O]	1616	839	1400	3061	3120	3435	1211	1554	1155	524	636
	(s)	(m)	(w)	(w)	(w)	(b)	(m)	(s)	(w)	(w)	(w)
[La(1N2N) ₂ ·(Thr)·2H ₂ O]	1616	839	1400	3061	3113	3433	1211	1554	1155	522	636
	(s)	(m)	(w)	(w)	(w)	(b)	(m)	(s)	(w)	(w)	(w)
[Ce(1N2N) ₂ ·(Phe)·2H ₂ O]	1616	839	1400	3061	3120	3435	1211	1554	1155	524	636
	(s)	(m)	(w)	(w)	(w)	(b)	(m)	(s)	(w)	(w)	(w)
[Ce(1N2N) ₂ ·(Thr)·2H ₂ O]	1616	839	1400	3061	3113	3433	1211	1554	1155	522	636
	(s)	(m)	(w)	(w)	(w)	(b)	(m)	(s)	(w)	(w)	(w)

b = broad band, m = medium band, w = weak band, s = strong band, A.a = Amino Acids, 1N2N = 1-nitroso-2-naphthol, Sym. = Symmetric, Asym. = Asymmetric

Thermal studies

The TG and DTA data of synthesized La³⁺ and Ce³⁺ complexes is listed in Table 6 which showed steady weightloss with increasing temperature. In TG study of lanthanum complexes the loss of two coordinated water molecules, amino acid and 1-nitroso-2-naphthol moiety was observed at $110\text{-}171^\circ\text{C}$, $205\text{-}285^\circ\text{C}$ and $310\text{-}648^\circ\text{C}$ respectively. Furthermore in DTA study, endothermic peak at $110\text{-}171^\circ\text{C}$, a small exotherm at $205\text{-}285^\circ\text{C}$ and broad exotherm in the range $310\text{-}648^\circ\text{C}$ attributed

to decomposition two coordinated water molecules, amino acid and 1-nitroso-2-naphthol moiety of lanthanum complexes respectively. The constant weight plateau in thermogram after 648°C indicates accomplishment of the reaction and forms metal oxide as final product²⁹⁻³¹. The XRD study^{32,33} was used to confirm the formation of La₂O₃ as a final product.

Similarly, in TG study of cerium complexes the loss of two coordinated water molecules, amino acid and 1-nitroso-2-naphthol moiety was observed at $110\text{-}162^\circ\text{C}$, $214\text{-}300^\circ\text{C}$ and $400\text{-}548^\circ\text{C}$ respectively. Furthermore in DTA study, endothermic peak at $110\text{-}162^\circ\text{C}$, a small exotherm at $214\text{-}300^\circ\text{C}$ and broad exotherm in the range $400\text{-}548^\circ\text{C}$ attributed to decomposition two coordinated water molecules, amino acid and 1-nitroso-2-naphthol moiety of

cerium complexes respectively. The constant weight plateau in thermogram after 548°C indicates accomplishment of the reaction and forms metal oxide²⁹⁻³¹ as final product. The XRD study³²⁻³³ was

used to confirm the formation of Ce_2O_3 as a final product. The typical TG and DTA curves of reported lanthanum and cerium complexes are shown in Fig. 1-8 respectively.

Table 6: Thermal analysis data of lanthanum(III) and cerium (III) complexes

Compound	Temp. Range (°C)	Lost Species	% Mass Loss		Residue
			Found	Calculated	
[La(1N2N) ₂ ·(Phe)·2H ₂ O]	115-171	Two molecules of water	5.34	5.27	[La(1N2N) ₂ (Phe)]
	223-285	Amino acid	24.66	24.02	[La(1N2N) ₂]
	361-626	Two molecules of 1N2N	49.34	50.08	[La ₂ O ₃]
[La(1N2N) ₂ ·(Thr)·2H ₂ O]	110-160	Two molecules of water	5.34	5.65	[La(1N2N) ₂ (Thr)]
	205-271	Amino acid	19.33	18.53	[La(1N2N) ₂]
	310-648	Two molecules of 1N2N	54.67	53.71	[La ₂ O ₃]
[Ce(1N2N) ₂ ·(Phe)·2H ₂ O]	120 - 162	Two molecules of water	5.34	5.26	[Ce(1N2N) ₂ (Phe)]
	214 - 300	Amino acid	25.32	23.98	[Ce(1N2N) ₂]
	400 - 548	Two molecules of 1N2N	48.01	49.99	[Ce ₂ O ₃]
[Ce(1N2N) ₂ ·(Thr)·2H ₂ O]	110 - 145	Two molecules of water	5.34	5.65	[Ce(1N2N) ₂ (Thr)]
	220 - 285	Amino acid	17.99	18.49	[Ce(1N2N) ₂]
	400 - 510	Two molecules of 1N2N	55.34	53.6	[Ce ₂ O ₃]

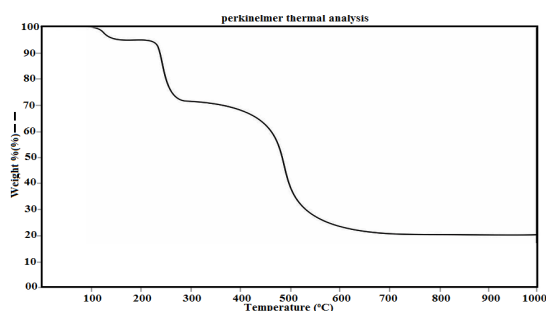


Fig. 1. TG Curve of [La(1N2N)₂·(Phe)·2H₂O]

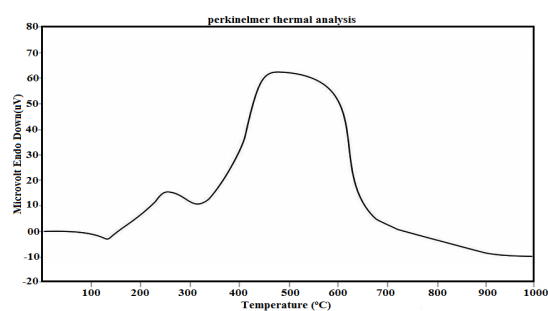


Fig. 4. DTA Curve of [La(1N2N)₂·(Thr)·2H₂O]

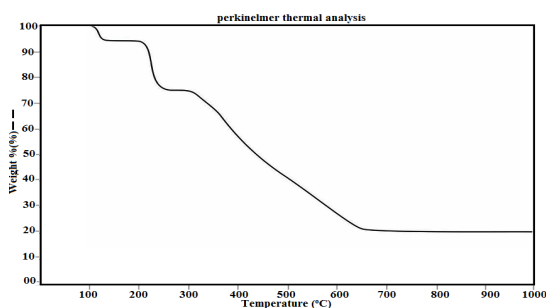


Fig. 2. TG Curve of [La(1N2N)₂·(Thr)·2H₂O]

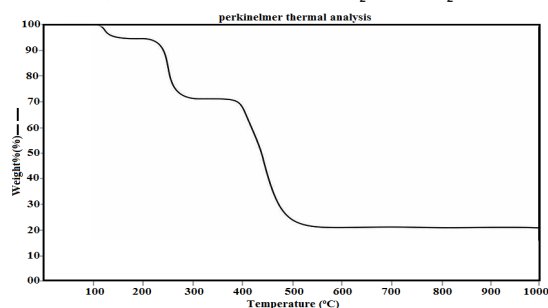


Fig. 5. TG Curve of [Ce(1N2N)₂·(Phe)·2H₂O]

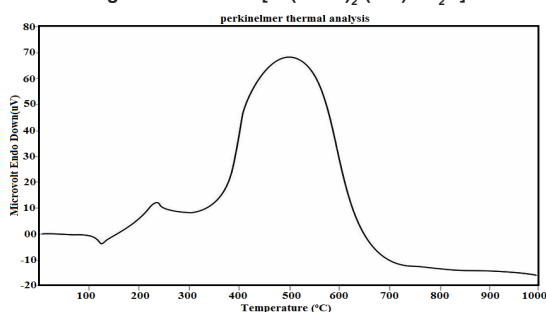


Fig. 3. DTA Curve of [La(1N2N)₂·(Phe)·2H₂O]

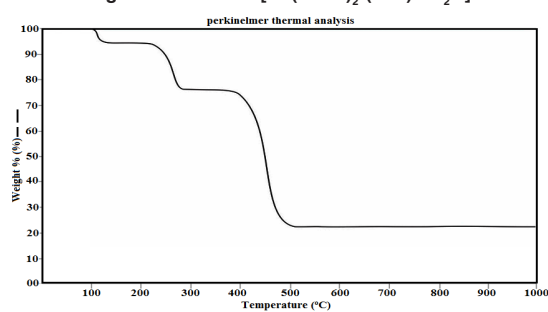
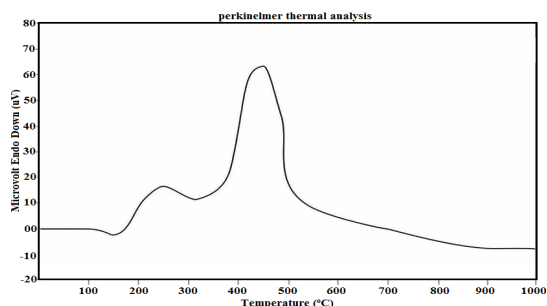
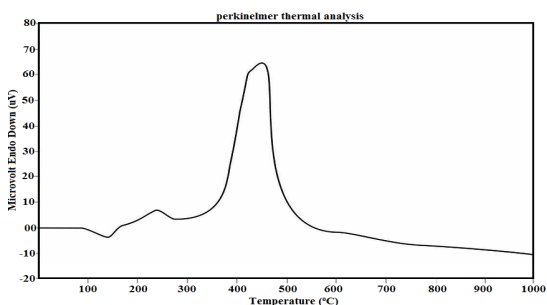
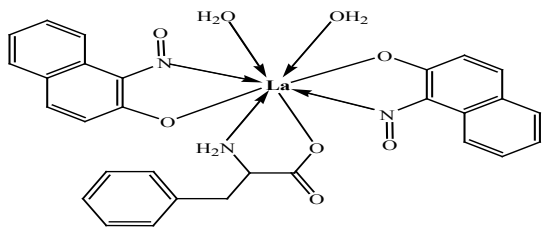
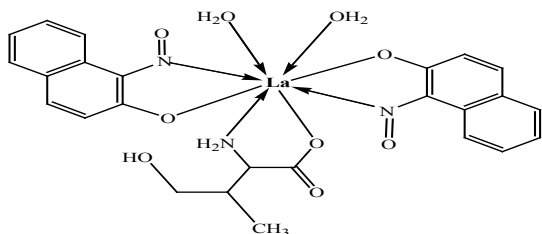
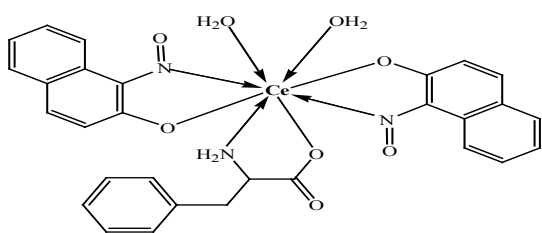
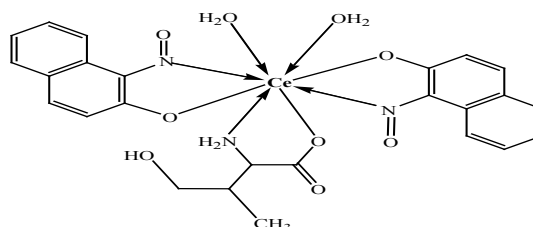


Fig. 6. TG Curve of [Ce(1N2N)₂·(Thr)·2H₂O]

Fig. 7. DTA Curve of [Ce(1N2N)₂•(Phe)•2H₂O]Fig. 8. DTA Curve of [Ce(1N2N)₂•(Thr)•2H₂O]

From the observations of physico-chemical and spectral study data, the structure of reported mixed ligand complexes of lanthanum and cerium can be represented as shown in Fig. 9,10,11,12 respectively.

Fig. 9. Anticipated representation of [La(1N2N)₂•(Phe)•2H₂O]Fig. 10. Anticipated representation of [La(1N2N)₂•(Thr)•2H₂O]Fig. 11. Anticipated representation of [Ce(1N2N)₂•(Phe)•2H₂O]Fig. 12. Anticipated representation of [Ce(1N2N)₂•(Thr)•2H₂O]

Antibacterial Study

The agar cup and serial tube dilution method was adopted to evaluate the antibacterial activity of synthesized complexes of lanthanum and cerium against *C. diphtheria*, *E.coli*, *S.aureus* and *S. typhi* respectively. In agar cup method, the zone of inhibition of test compounds against selected bacteria was measured in mm and recorded in Table 7 and Fig. 13. The result of agar cup method showed that all synthesized compounds of lanthanum and cerium were more sensitive against *S. typhi* and *S. aureus* as related to *E. coli* and *C. diphtheria*.

In tube dilution method, minimum inhibition concentration of metal complexes, metal salts, tetracycline standard compound and 1-nitroso-2-naphthol ligand were studied against selected bacterial strain and presented in Table 8. The MIC of synthesized lanthanum complexes and lanthanum metal salts was found in the range 10-35 μg/mL and 50-100 μg/mL respectively. Similarly, MIC of synthesized cerium complexes, cerium metal salts and 1-nitroso-2-naphthol ligand was observed in the range of 10-35 μg/mL, 75-150 μg/mL and 75-200 μg/mL respectively. The obtained result show that synthesized compounds were more active against *S. typhi* and *S. aureus* as related to *E. coli* and *C. diphtheria*. Further, synthesized compounds show moderate antibacterial activity as related to tetracycline standard compound.

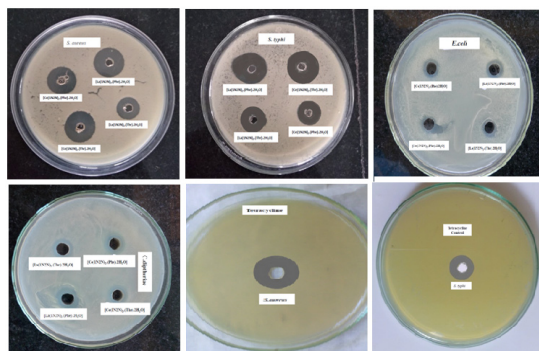
All compounds of lanthanum and cerium possesses higher antibacterial activity as compared only to ligand and metal salts. The antibacterial activity enhancement in metal compounds is because of chelate effect. The chelate effect decreases the polar nature of metal ions in the complexes and increases the hydrophobic character of metal chelates that allows the diffusion of compound through lipid layer of microorganisms³⁴⁻³⁵.

Table 7: Antibacterial activity (mm) of synthesized complexes of lanthanum and cerium by agar cup method

Compound	Zone of Inhibition (mm)			
	<i>E.coli</i>	<i>S.typhi</i>	<i>C.diphtheriae</i>	<i>S.aureus</i>
[La(1N2N) ₂ ·(Phe)·2H ₂ O]	12	24	14	25
[La(1N2N) ₂ ·(Thr)·2H ₂ O]	10	23	13	24
[Ce(1N2N) ₂ ·(Phe)·2H ₂ O]	11	22	13	25
[Ce(1N2N) ₂ ·(Thr)·2H ₂ O]	14	24	14	28
Tetracycline	18	25	20	30

Table 8: The values of MIC (mg/mL) of synthesized complexes of lanthanum and cerium

Compound	MIC (μg/mL)			
	<i>E.coli</i>	<i>S.typhi</i>	<i>C.diphtheriae</i>	<i>S.aureus</i>
[La(1N2N) ₂ ·(Phe)·2H ₂ O]	30	20	35	10
[La(1N2N) ₂ ·(Thr)·2H ₂ O]	25	15	30	15
[Ce(1N2N) ₂ ·(Phe)·2H ₂ O]	25	10	30	15
[Ce(1N2N) ₂ ·(Thr)·2H ₂ O]	25	15	35	15
1-nitroso-2-naphthol	150	100	200	75
LaCl ₃ ·7H ₂ O	100	75	100	50
CeCl ₃ ·7H ₂ O	100	75	150	75
Tetracycline	4.0	1.5	2.0	1.5

**Fig. 13. Zone of Inhibition of synthesized complexes of lanthanum and cerium**

CONCLUSION

In conclusions, we have successfully synthesized the mixed ligand La(III) and Ce(III)

complexes of the type $[M(1N2N)_2 \cdot (L) \cdot 2H_2O]$ by using 1-nitroso-2-naphthol (1N2N) as a primary ligand and L-phenylalanine, L-threonine (HL) as secondary ligands. The coordination of water molecules in complexes was corroborated by thermal study. The higher decomposition temperature indicate that there is a strong metal-ligand bond in the complexes. All complexes shows three stages of degradation corresponds to loss of two coordinated water molecules, amino acid and 1-nitroso-2-naphthol. Magnetic study indicates diamagnetic nature of lanthanum complexes while the cerium complexes were observed paramagnetic in nature. The non-electrolytic character of lanthanum and cerium complexes were confirmed by molar conductance study. The FT-IR spectra show bonding of primary and secondary ligands to metal ion through donor N- and O- atoms. UV-Visible spectra shows three transitions in the complexes namely $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and LMCT transitions, respectively. The antibacterial study show that metal complexes were more potent than corresponding ligands/metal salts. The above result confirms eight coordination number to the synthesized La and Ce complexes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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