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Studies of Mixed Ligand Lanthanum Complexes: Synthesis, Spectral Interpretations and Antibacterial Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The mixed ligand lanthanum complexes have been synthesized using polydentate ligands. The (2Z)-2-(N-hydroxyimino)-1,2-diphenylethan-1-ol (cupron) was used as a primary ligand and L-isolucine, L-threonine, L-lysine and L-glycine were used as secondary ligands for the synthesis. Conductometry, elemental analysis, magnetic susceptibility measurements, complexometric estimation, UV-Visible spectroscopy, FTIR spectroscopy, thermal analysis and XRD methods were used for structural interpretation of all synthesized complexes. All complexes were solid and white and slight yellow in colour. They are non-electrolytic and diamagnetic in nature, as confirmed by conductometric and magnetic susceptibility methods respectively. All the complexes were synthesized by combination of lanthanum metal ion, primary ligand and secondary ligands in 1:2:1 ratio which was confirmed by elemental analysis. During reaction between lanthanum ion, primary ligand and secondary ligands there was transition of electrons which was confirmed by UV-Visible spectroscopy. Identification of functional group in the complexes was carried out and confirmed by

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FTIR spectroscopy. The decomposition temperature and thermal behaviour of the complexes have been concluded by thermal and XRD techniques. Tube dilution and agar cup methods have been employed to study antibacterial activities of all synthesized lanthanum complexes. The inhibition potential was seen to some extent.

Keywords: Lanthanum complexes; synthesis; structural interpretation; antibacterial study.

1. INTRODUCTION

Synthesis of mixed ligand complexes is the important aspect for researchers to study their spectral interpretation and antibacterial study [1-2]. Number of mixed ligand complexes showed biological activities such as antifungal, anticancer, etc. [3-5]. Several complexes have been derived from transition metal ion to study their significance in biological activities [6-8]. Many polydentate ligands were used for formation of mixed ligand complexes and the synthesized complexes were studied for antituberculosis and antidiabetic properties [9-10].

Most of the amino acids are the prime choice for the researchers as ligand to synthesized mixed ligand complexes and study their biological activities [11-13]. Lanthanide complexes were also studied for their synthesis, spectral investigation and antimicrobial studies [14]. Thus, the present study corresponds to synthesis of mixed ligand complexes using lanthanum metal ion, (2Z)-2-(N-hydroxyimino)-1,2-diphenylethan-1-ol (primary ligand) and different amino acids, L-isolucine, L-threonine, L-lysine and L-glycine (secondary ligand). The synthesized complexes were interpreted using various physico-chemical and spectral methods. Conductometry, elemental analysis, magnetic susceptibility measurements, complexometric estimation, UV-Visible spectroscopy, FTIR spectroscopy, thermal analysis and XRD techniques were applied for the interpretation.

2. METHODOLOGY

2.1 Materials

The analytical grade chemicals and solvents were used during entire work of the study. The metal salt, cupron and all amino acids used in the reaction were of analytical grade. The purified and distilled solvents were used whenever necessary in the laboratory work as per the standard protocol [15-17].

2.2 Synthesis of Complexes

The 10 cm^3 of 1 mmol of solution lanthanum salt was prepared in distilled water and 10 cm^3 2 mmol solution of cupron was prepared in ethanol. Both solutions were mixed thoroughly by stirring. The resultant mixture was heated on boiling water bath for 15 minutes. The 10 cm^3 of 1 mmol solution of amino acid was prepared in distilled water and the same solution was added to the mixture of lanthanum salt and cupron. The mixture was again heated with stirring for about 10 minutes. The complex was obtained from the mixture by increasing the pH of reaction mixture. The increase in pH was achieved by adding diluted ammonia solution to the resultant mixture. The synthesized complex was cooled and filtered on Buckner funnel. It was washed with water and then alcohol. After washing the complex was dried under vaccum and then it was used for the further study.

2.3 Instrumentation

The complexes synthesized by using above method were tested using physico-chemical methods. The physico-chemical methods were conductometry, elemental analysis, magnetic susceptibility measurements, electronic spectroscopy, FTIR spectroscopy, thermal analysis and XRD analysis. The molar conductance of all complexes was measured to conclude non-electrolytic nature of the complexes. The magnetic behaviour of all complexes were studied using magnetic susceptibility measurements. The electronic spectroscopy studies revealed the transition of electrons during synthesis of complex. Various functional groups present in the complexes were concluded from FTIR studies. The thermal behaviour of the complexes was studied by thermal and XRD analysis. The coordinated water molecules present in the complexes were also confirmed by thermal studies. Thus the physico-chemical methods were used to elucidate the structure of synthesized lanthanum complexes.

2.4 Antibacterial Study

2.4.1 Tube dilution method

The minimum inhibitory concentration was estimated using tube dilution method. A stock solution of concentration 1 mg/cm^3 was prepared by dissolving the complexes in DMSO. From this stock solution, the aliquots of concentrations 20, 40, 60, 80 and 300 µg/cm^3 were prepared using Mueller Hinton broth as a diluent. The complexes were tested against four bacterial strains viz. Staphylococcus aureus, Corynebacterium diphtheriae, Salmonella typhi and Pseudomonas aeruginosa, for the inhibition potential of the complexes. The cultures used were 18-24 hours old and their density was adjusted to 10^6 using a sterile Mueller Hinton broth. The 5 cm^3 of above prepared aliquots were taken in sterile borosilicate tubes and inoculated with 0.1 cm^3 of the inoculums of selected bacteria. The test control of DMSO was taken for every aliquot considering the corresponding dilution. Tetracycline (10 µg/cm^3) was used as standard control. A positive control of corresponding bacterium in the Mueler Hinton broth was also maintained. The tubes were incubated at 37^{o}C for 24 hours. The minimum concentration at which complete growth is inhibited was observed by measuring the turbidity.

2.4.2 Agar cup method

To estimate the zone of inhibition, agar cup method was used. Sterile petri plates were poured with sterile nutrient agar mixed with 1 cm^3 of bacterial inoculum of the bacterial density 10^6 cm^3. After solidification of the agar, in each plate 3 wells of 5 mm were punched using cork borer. Tetracycline (10 µg/cm^3) was used as control. 200 µl sample of the strength 300 µg/cm^3 of each complex was introduced in the wells. Each sample was tested in triplicate. The plates were kept in refrigerator for an hour and then incubated at 37^o C for 24 hours. The zone of inhibition was measured.

3. RESULTS AND DISCUSSION

3.1 Synthesis of mixed ligand complexes

The metal complexes were synthesized as per following reaction.

$LaCl_3 \cdot 7H_2O + 2HCup + HL \longrightarrow [La(Cup)_2(L) \cdot 2H_2O] + 3HCl + 5H_2O$

Where,
HCup:(2Z)-2-(N-hydroxyimino)-1,2-iphenylethan-1-ol [i.e. Cupron]
HL: Amino acid

It is concluded from the reaction that the metal salt, primary ligand (cupron) and secondary ligand (amino acid) were reacted in 1:2:1 proportion for formation of complexes.

3.2 Physico-Chemical Properties

The synthesized lanthanum complexes are white and slight yellowish in colour (Table No. 1). The complex $[La(Cup)_2(Iso) \cdot 2H_2O]$, $[La(Cup)_2(Thr) \cdot 2H_2O]$ and $[La(Cup)_2(Gly) \cdot 2H_2O]$ are in white colour and the complex $[La(Cup)_2(Lys) \cdot 2H_2O]$ is in slight yellowish colour. They are non-hygroscopic in nature. The Table No. 2 shows decomposition temperature of all synthesized complexes. The range of decomposition temperature is 268-279 ^{o}C. The high value of decomposition temperature in the table shows formation of strong bond between metal ion and primary and secondary ligand. The synthesized complexes are found partially soluble in DMF and DMSO and insoluble in common organic solvents like alcohol, acetone, etc. The value of molar conductance of all synthesized lanthanum complexes are summarized in Table No. 3. The 10^{-3} M solution of lanthanum complexes were prepared to measure their molar conductance. The values of molar conductance are in the range of 0.0012–0.0018 mhos cm^2 mol^{-1}. These very small values of molar conductance indicate the nature of complexes is non-electrolytic [18].

3.3 Elemental Analysis

During synthesis of mixed ligand complexes, the metal salt, primary ligand and secondary ligand were reacted in 1:2:1 proportion. The general formula 1:2:1 of the synthesized complexes is confirmed by study of elemental analysis. The elemental analysis data is included in Table No. 3. Elemental study revealed the general formula of all complexes i.e. 1:2:1 [19].

3.4 Magnetic Properties

The magnetic properties of synthesized complexes have been studied by using Gouy's method. The magnetic susceptibility of all complexes were obtained by applying diamagnetic corrections. The negative values of

effective magnetic moment indicate diamagnetic nature of all synthesized lanthanum complexes [20] (Table No. 4).

3.5 Electronic Absorption Spectra

The 10^{-4} M solutions of mixed ligand complexes in DMF were used to obtain their electronic absorption spectra. The transition of electrons from ligands to metal during formation of mixed ligand complexes is confirmed by interpretation of electronic absorption spectra. The electronic absorption spectra shows three peaks at the range of wavelengths, 273-278 nm (35971-36630 cm^{-1}), 331-338 nm (29585-30211 cm^{-1}) and 388-391 nm (25575-25773 cm^{-1}). These three peaks correspond to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and charge-transfer transitions of electrons respectively during the formation of metal complexes [21-22] (Table No.5).

3.6 Infra-red Spectra

The 4000-400 cm^{-1} range was used in the study of FTIR of mixed ligand complexes. The samples were taken in KBR disc to obtain the FTIR spectra of the complexes. The common feature of all spectra is absence of band at ~3440 cm^{-1} which revealed deprotonation of hydroxyl group of cupron during formation of complexes. The strong band observed in the range of 1015-1010 cm^{-1} ascribed for C-O stretching vibration in the complexes which indicates shifting of band of free cupron from 1100 cm^{-1}. The weak band observed in the range of 1510-1485 cm^{-1} ascribed for C=N vibrations in the complexes. The same band is observed at 1650 cm^{-1} for free cupron. The difference is observed due to shifting of band ascribed for C=N vibrations from 1650 cm^{-1} to lower wavenumber. The shifting indicates metal coordinated with oxime nitrogen of cupron moiety. The medium band is observed in the range of 3290-3280 cm^{-1}. The observed band indicates free oxime –OH group is present in the cupron moiety of the complexes. The weak band observed in the range of 822-814 cm^{-1} ascribed for M-N vibrations in the complexes. The same band is observed at ~780 cm^{-1} for free cupron. The difference is observed due to shifting of band ascribed for M-N vibrations from ~780 cm^{-1} higher wavenumber. The shifting indicates nitrogen atom of cupron coordinated with metal ion. The weak bands observed in the range of 3142-3132 cm^{-1} and 2992-2983 cm^{-1} ascribed for N-H asymmetric and N-H symmetric vibrations in the complexes respectively. The same bands are observed at ~3050 cm^{-1} and ~2960 cm^{-1} for free amino acid. The difference is observed due to shifting of bands ascribed for N-H asymmetric and N-H symmetric vibrations to higher wavenumbers. The shifting indicates nitrogen atom of amino acid coordinated with metal ion. The strong band observed in the range of 1623-1618 cm^{-1} and a weak band observed in the range of 1385-1377 cm^{-1} ascribed for COO- asymmetric vibrations and symmetric vibrations respectively in the complexes. The same bands are observed at ~1595 and ~1410 cm^{-1} for free amino acid. The difference is observed due to shifting of bands to higher and lower wavenumbers respectively. The shifting indicates carboxylic acid group coordinated with metal ion through oxygen. Medium band observed in the range of 3360-3278 cm^{-1} and weak band observed in the range of 1595-1580 cm^{-1} ascribed for O-H asymmetric and O-H symmetric stretching vibrations in the complexes. These bands revealed that coordinated water molecules are present in the complexes. The medium band observed in the range of 410-400 cm^{-1} and 620-615 cm^{-1} ascribed for M-N and M-O vibrations in the complexes. These bands conclude the bonding between metal ion with oxygen as well as nitrogen of primary and secondary ligands [23-26]. (Table No. 6).

3.7 Thermal Studies

Controlled nitrogen atmosphere were used for the study of thermal behaviour of all synthesized complexes. They were heated by 10^oC per minute. All complexes were heated upto 900^oC and they have shown same thermal behaviour. A gradual weight loss is obtained with increasing temperature and upto 840^oC. After 840^oC, no weight loss has been observed and the TG-DTA curve shows straight line which indicates completion of decomposition of mixed ligand complex. The first weight loss was observed in the range 139-184^oC corresponds to loss of two water molecules from the complexes. The second weight loss was observed in the range of 262-416 ^oC corresponds to loss one molecule of amino acid. The third weight loss was observed in the range of 638-828 ^oC corresponds to simultaneous loss of two cupron molecules from the complex. Finally metal powder was observed as a decomposition product of the complexes which was converted into its oxide spontaneously. The oxygen was present in trace quantity with nitrogen used to study thermal behaviour of the complexes which reacted with metal powder and formed metal oxide. The same nature is confirmed by XRD study [27]. (Table No. 7)

Table 1. Empirical formula, molecular weight and colour of the complexes

Complex	Empirical Formula	Molecular Weight	Colour
$[La(Cup)_2(Iso)\cdot 2H_2O]$	$C_{34}H_{40}LaN_3O_8$	757.60	White
$[La(Cup)_2(Thr)\cdot 2H_2O]$	$C_{32}H_{36}LaN_3O_9$	745.54	White
$[La(Cup)_2(Lys)\cdot 2H_2O]$	$C_{34}H_{41}LaN_4O_8$	772.61	Slight Yellow
$[La(Cup)_2(Gly)\cdot 2H_2O]$	$C_{30}H_{32}LaN_3O_8$	701.49	White

Table 2. Decomposition Temperature and pH of the complexes

Complex	Decomposition Temperature (^oC)	pH
$[La(Cup)_2(Iso)\cdot 2H_2O]$	270	7.01
$[La(Cup)_2(Thr)\cdot 2H_2O]$	268	7.00
$[La(Cup)_2(Lys)\cdot 2H_2O]$	273	7.00
$[La(Cup)_2(Gly)\cdot 2H_2O]$	279	7.00

Table 3. Elemental analysis data and molar conductance of the complexes

Complex	Elemental Analysis Found (Calculated)				Molar Conductance
	% M	% C	% H	% N	
$[La(Cup)_2(Iso)\cdot 2H_2O]$	18.31 (18.33)	53.88 (53.90)	5.30 (5.32)	5.52 (5.55)	0.0014
$[La(Cup)_2(Thr)\cdot 2H_2O]$	18.60 (18.63)	51.50 (51.55)	4.82 (4.87)	5.63 (5.64)	0.0012
$[La(Cup)_2(Lys)\cdot 2H_2O]$	19.42 (19.49)	57.29 (57.30)	5.76 (5.80)	7.83 (7.86)	0.0016
$[La(Cup)_2(Gly)\cdot 2H_2O]$	19.77 (19.80)	51.33 (51.36)	4.56 (4.60)	5.95 (5.99)	0.0018

Table 4. Magnetic Susceptibility data of the Complexes (- 10-6 c.g.s. units)

Complex	X_g	X_m	□□□
$[La(Cup)_2(Iso)\cdot 2H_2O]$	-1.03×10^{-6}	-7.83×10^{-4}	Diamagnetic
$[La(Cup)_2(Thr)\cdot 2H_2O]$	-1.05×10^{-6}	-7.83×10^{-4}	Diamagnetic
$[La(Cup)_2(Lys)\cdot 2H_2O]$	-1.01×10^{-6}	-7.86×10^{-4}	Diamagnetic
$[La(Cup)_2(Gly)\cdot 2H_2O]$	-1.05×10^{-6}	-7.42×10^{-4}	Diamagnetic

Table 5. Electronic absorption spectra of the complexes

Sr. No.	Complex	λ (nm)	ν (cm^{-1})	Proposed Assignments
1	$[La(Cup)_2(Iso)\cdot 2H_2O]$	278	35971	$\pi \rightarrow \pi^*$
		338	29585	$n \rightarrow \pi^*$
		391	25575	Charge-transfer
2	$[La(Cup)_2(Thr)\cdot 2H_2O]$	277	36101	$\pi \rightarrow \pi^*$
		336	29761	$n \rightarrow \pi^*$
		391	25575	Charge-transfer
3	$[La(Cup)_2(Lys)\cdot 2H_2O]$	273	36630	$\pi \rightarrow \pi^*$
		333	30030	$n \rightarrow \pi^*$
		389	25706	Charge-transfer
4	$[La(Cup)_2(Gly)\cdot 2H_2O]$	274	36496	$\pi \rightarrow \pi^*$
		331	30211	$n \rightarrow \pi^*$
		388	25773	Charge-transfer

3.8 Biological Studies

3.8.1 Tube dilution method

Complexes showed inhibition potential against the *Staphylococcus aureus, Corynebacterium diphtheriae, Salmonella typhi* and *Pseudomonas aeruginosa*. The complexes were more potent in inhibiting the growth of microorganisms than the metal salt and ligand.

Gram positive bacteria, *viz., S. aureus* and *C. diptheriae* were more sensitive to the complexes than gram negative *S.typhi* and *P. aeruginosa*. Complexes with Isoleucine and threonine are more potent than the complexes with lysine and glycine. The test control of DMSO showed very negligible inhibition (Tables. 8 and 9).

3.8.2 Agar cup method

The zone of inhibition shows the potential of complexes to inhibit the bacterial growth. The zone ranged between 11-14 mm in *S. aureus* and *C. diptheriae* for all the compexes, whereas for *S. typhi* and *P. aeruginosa* the zone of inhibition ranged between 8-12 mm.

The inhibition potential is again seen more against positive strains than negative strains. (Table No. 10) [28].

The coordination number 8 may be proposed for all synthesized complexes. The Fig. 1-4 shows the structure of synthesized mixed ligand lanthanum complexes.

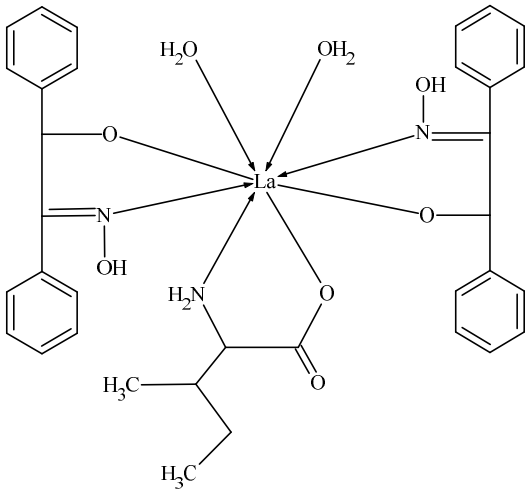

Fig. 1. Proposed Structure of $[La(Cup)_2(Iso).2H_2O]$

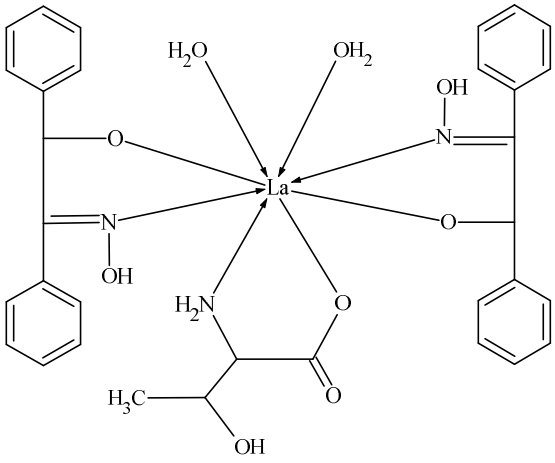

Fig. 2. Proposed Structure of $[La(Cup)_2(Thr).2H_2O]$

Table 6. FTIR Data of the Complexes

Complex	ν (C-O) (Cup)	ν (C=N) (Cup)	ν (-OH) (Oxime) (Cup)	ν (M-N) (Cup)	ν (N-H) (Asym) (A.a.)	ν (N-H) (Sym) (A.a.)	ν (COO-) (Asym) (A.a.)	ν (COO-) (Sym) (A.a.)	ν (HOH) (Asym) (HOH)	ν (HOH) (Sym) (HOH)	ν (M-N) (Complex)	ν (M-O) (Complex)
$[La(Cup)_2(Iso)\cdot 2H_2O]$	1010 (s)	1485 (w)	3285 (m)	817 (w)	3142 (w)	2988 (w)	1615 (s)	1377 (w)	3278 (m)	1595 (w)	407 (m)	620 (w)
$[La(Cup)_2(Thr)\cdot 2H_2O]$	1012 (s)	1585 (w)	3295 (m)	819 (w)	3132 (w)	2985 (w)	1615 (s)	1385 (w)	3360 (m)	1590 (w)	405 (m)	615 (w)
$[La(Cup)_2(Lys)\cdot 2H_2O]$	1015 (s)	1510 (w)	3290 (m)	814 (w)	3138 (w)	2992 (w)	1618 (s)	1382 (w)	3320 (m)	1580 (w)	400 (m)	615 (w)
$[La(Cup)_2(Gly)\cdot 2H_2O]$	1013 (s)	1505 (w)	3280 (m)	822 (w)	3135 (w)	2983 (w)	1623 (s)	1381 (w)	3315 (m)	1585 (w)	410 (m)	615 (w)

Table 7. Thermal Data of the Complexes

Sr. No.	Complex	Temperature Range (^oC)	Weight Loss Due to Loss of	% Weight Loss Found	% Weight Loss Calculated
1.	$[La(Cup)_2(Iso)\cdot 2H_2O]$	142-178	Two water molecules	4.73	4.76
		263-412	Amino acid	17.16	17.18
		638-824	Two cupron molecules	59.70	59.71
2.	$[La(Cup)_2(Thr)\cdot 2H_2O]$	144-182	Two water molecules	4.80	4.83
		268-416	Amino acid	15.83	15.84
		642-828	Two cupron molecules	60.61	60.68
3.	$[La(Cup)_2(Lys)\cdot 2H_2O]$	139-177	Two water molecules	4.64	4.66
		265-413	Amino acid	18.72	18.78
		645-825	Two cupron molecules	58.53	58.56
4.	$[La(Cup)_2(Gly)\cdot 2H_2O]$	146-184	Two water molecules	5.12	5.13
		262-415	Amino acid	10.51	10.55
		641-827	Two cupron molecules	64.18	64.20

Table 8. MIC Data of the Complexes by Tube Dilution Method

Sr. No.	Complex	MIC (µg/cm^3)			
		S. aureus	C. diphtheriae	S. typhi	P. aeruginosa
1.	$[La(Cup)_2(Iso)\cdot 2H_2O]$	80	80	120	100
2.	$[La(Cup)_2(Thr)\cdot 2H_2O]$	80	80	120	120
3.	$[La(Cup)_2(Lys)\cdot 2H_2O]$	100	140	140	140
4.	$[La(Cup)_2(Gly)\cdot 2H_2O]$	100	150	140	160

Table 9. MIC Data of Metal Salts, Ligand and Tetracycline by Tube Dilution Method

Salt / Ligand / Tetracycline	MIC (µg/cm^3)			
	S. aureus	C. diphtheriae	S. typhi	P. aeruginosa
$LaCl_3.7H_2O$	100	150	150	200
Cupron	110	200	160	140
Tetracycline	1.5	2.0	1.5	8.0

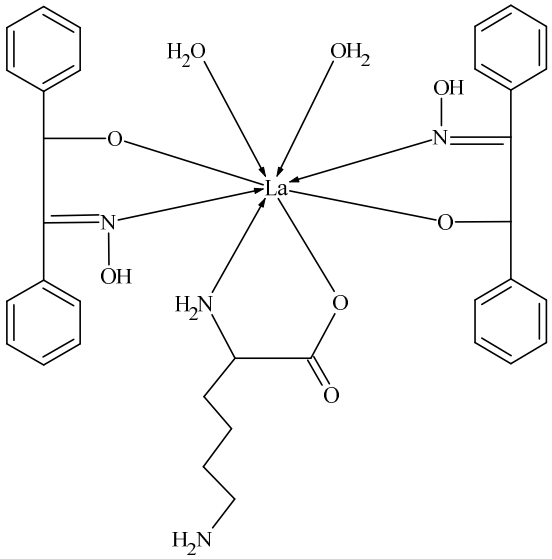

Fig. 3. Proposed Structure of $[La(Cup)_2(Lys).2H_2O]$

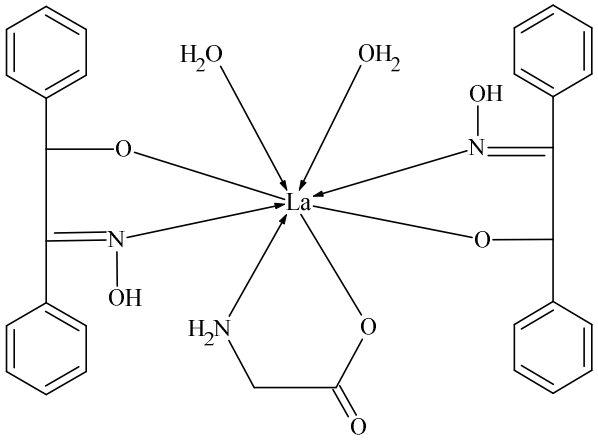

Fig. 4. Proposed Structure of $[La(Cup)_2(Gly).2H_2O]$

Table 10. Antibacterial Activity (mm) of the Complexes by Agar Cup Method

Sr. No.	Complex	Antibacterial Activity (mm) with			
		S. aureus	C. diphtheriae	S. typhi	P. aeruginosa
1.	$[La(Cup)_2(Iso)\cdot 2H_2O]$	14	14	11	12
2.	$[La(Cup)_2(Thr)\cdot 2H_2O]$	14	14	11	11
3.	$[La(Cup)_2(Lys)\cdot 2H_2O]$	13	12	10	10
4.	$[La(Cup)_2(Gly)\cdot 2H_2O]$	13	11	10	08
5.	Tetracycline	30	25	26	18

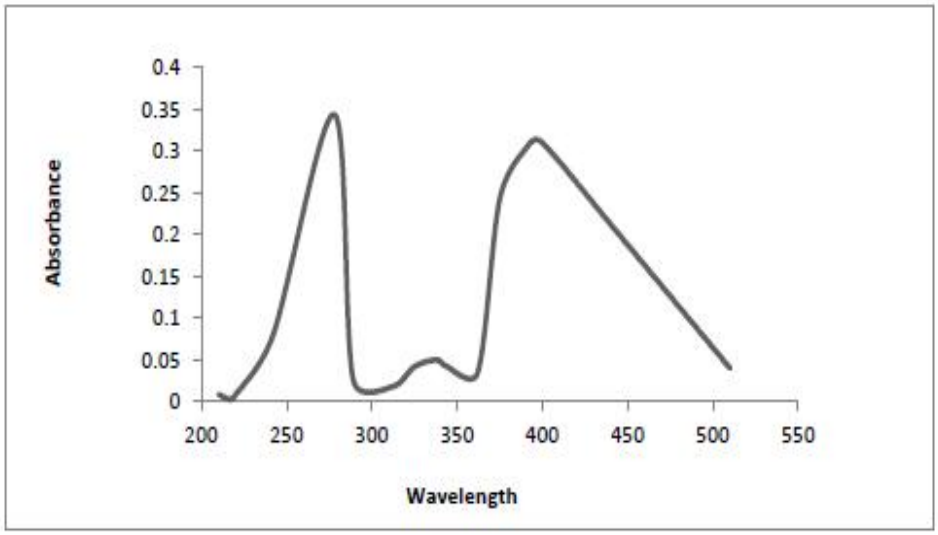

Fig. 5. Uv-Visible Spectra Of [La(Cup)2(Iso)·2h2o] Uv-Visible spectra of [La(Cup)2(Iso)·2h2o]

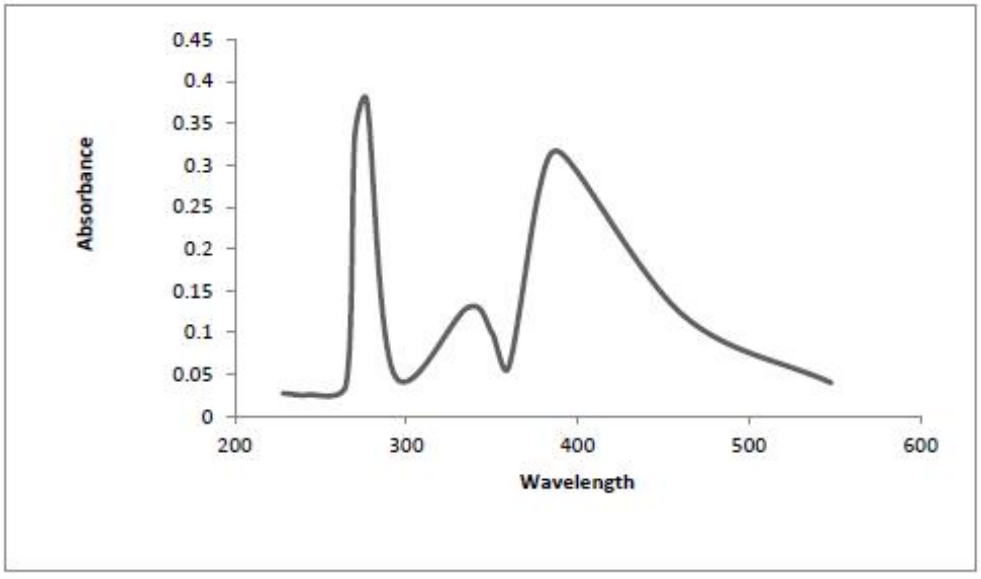

Fig. 6. UV-Visible Spectra of [La(Cup)2(Thr)·2H2O]

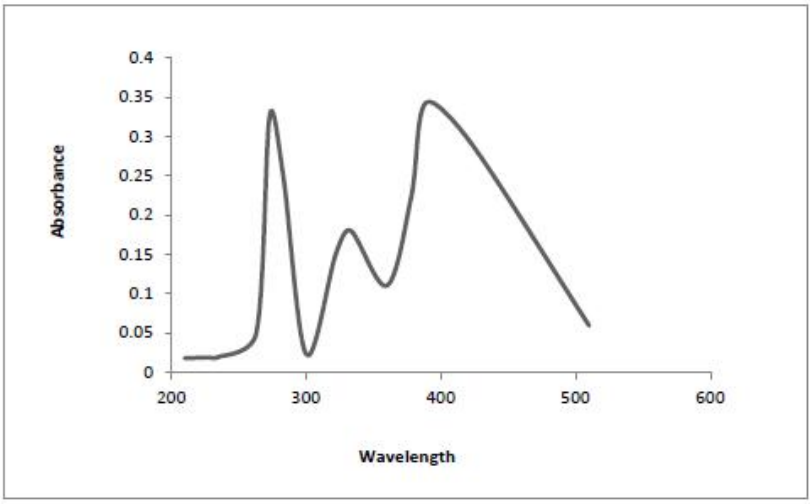

Fig. 7. UV-Visible Spectra of [La(Cup)2(Lys)·2H2O]

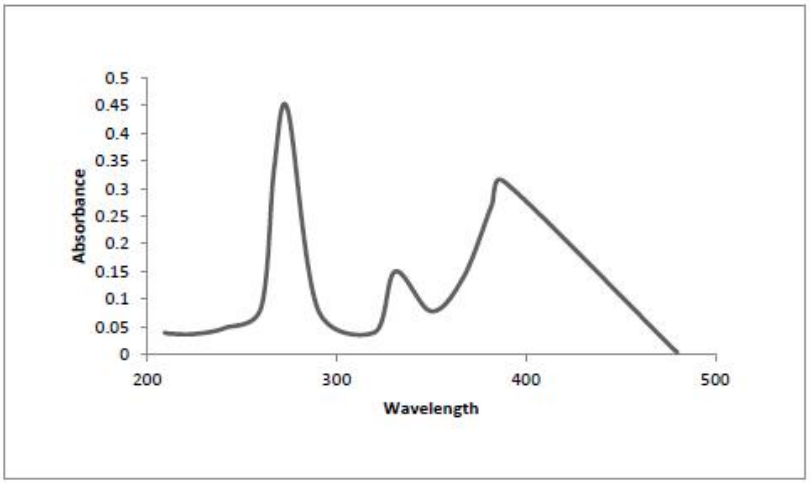

Fig 8. UV-Visible Spectra of [La(Cup)2(Gly)·2H2O]

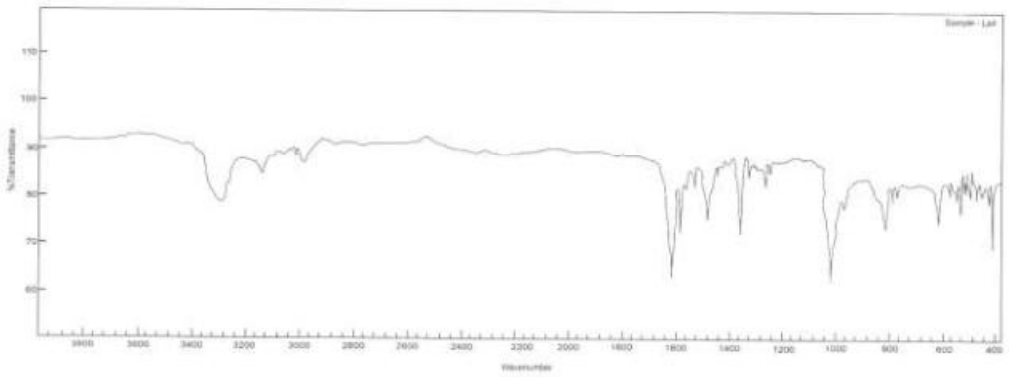

Fig. 9. FTIR Spectra of [La(Cup)2(Iso)·2H2O]

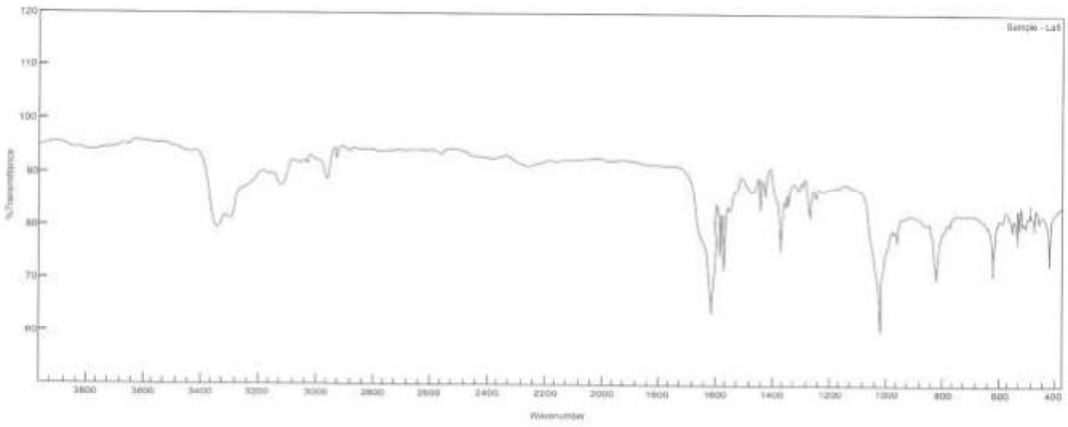

Fig 10. FTIR Spectra of [La(Cup)2(Thr)·2H2O]

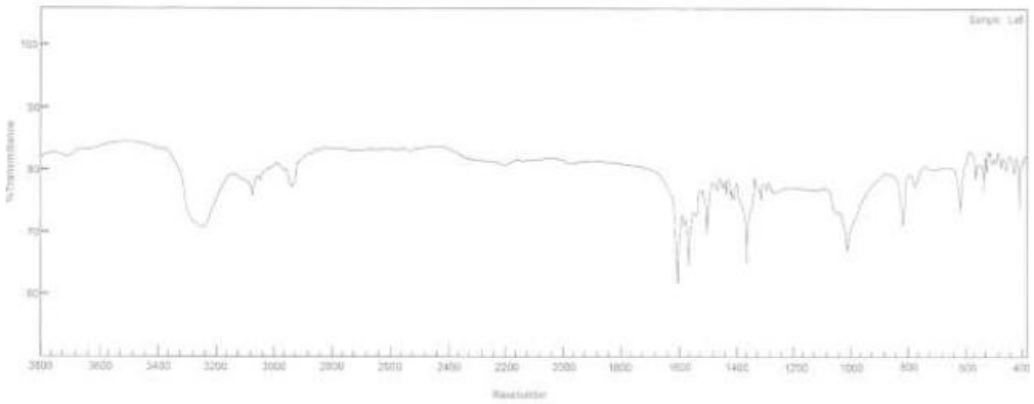

Fig 11. FTIR Spectra of [La(Cup)2(Lys)·2H2O]

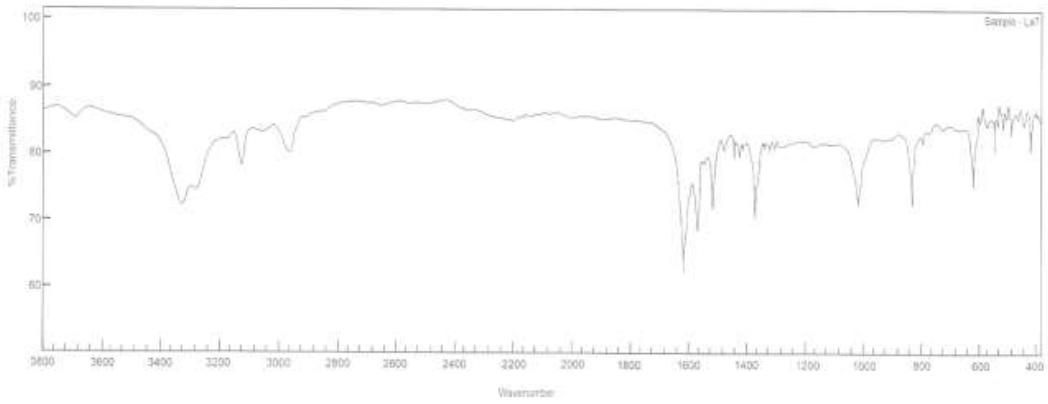

Fig 12. FTIR Spectra of [La(Cup)2(Gly)·2H2O]

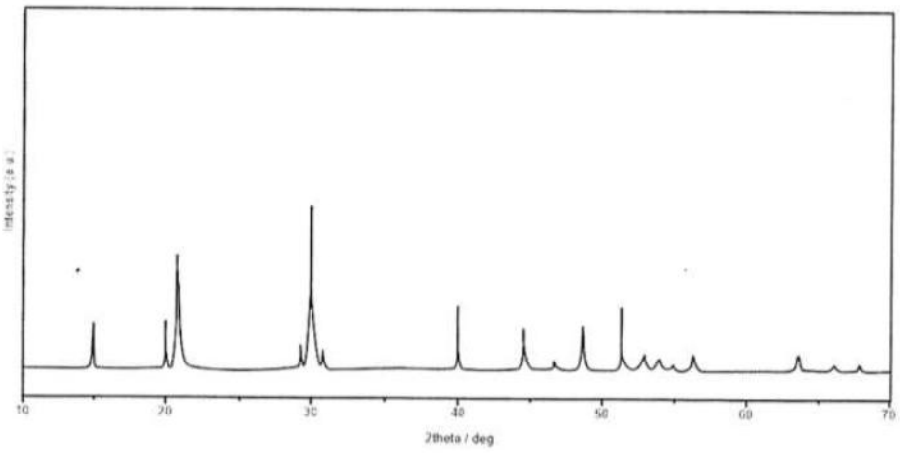

Fig 13. XRD of Decomposition Product of Lanthanum(III) Complexes

4. CONCLUSION

The high values of decomposition temperature reveal formation of strong bond between metal ion and primary and secondary ligand. Very low values of molar conductance reveal non-electrolytic nature of complexes. Negative values of effective magnetic moment indicate diamagnetic nature of complexes. The values of wavelength of electronic absorption spectra indicate the transition of electrons from ligands to metal ion during formation of metal complexes. The wavenumbers observed in FTIR spectra conclude the nature of bonding between metal ion and ligand. The gradual weight loss of the complexes in the TG-DTA study concludes that two coordinated water molecules and ligand moieties are present in the complexes. The antibacterial properties of the complexes were confirmed by Tube Dilution and Agar Cup methods. These methods indicate that the complexes are more active against *S. aureus*, *C. diptheriae* and less active against *S. typhi* and *P. aerruginosa*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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