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Study of the ternary complexes of lanthanum with 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one and some Amino Acids

B. D. Aghav* and R. S. Lokhande

Department of Chemistry, Changu Kana Thakur Arts, Commerce and Science College, New Panvel, Dist. Raigad, Maharashtra, 410206, India

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ABSTRACT

The ternary complexes of lanthanum (III) with 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one (HL) and L-tryptophan, L-phenylalanine, L-isoleucine, L-proline and L-serine have been studied. The mixed ligand complexes have been prepared and characterized on the basis of elemental analysis, conductivity data, magnetic susceptibility measurements, UV-visible, infrared spectra, ^{1}H NMR spectra and thermal analysis data. In these complexes, the Schiff base acts as a primary ligand and amino acids acts as secondary ligand which coordinates through the carboxylate oxygen and the amino nitrogen. The thermal stability of the complexes was studied and the weight losses were correlated with structure of the complexes. La (III) complexes were screened for their antimicrobial activities and show the potent biological activities.

Keywords: Ternary complexes, Lanthanum, Schiff base, Amino acids, Thermal analysis

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INTRODUCTION

The study of rare earth complexes with organic ligands is gaining considerable importance in recent years [1-3]. Rare earth ions possess the properties of antibacterial [4], antitumor [5] and antivirus [6] agents when co-ordinated with organic ligands and participate effectively in many important life processes. Many researchers have studied preparation, characterization, antimicrobial, and toxicological activity of mixed ligand complexes of transition metal, lanthanide metal and actinide metal ions [7-10]. Lanthanide complexes have been studied for their interesting and important properties like their reversibly ability to bind oxygen, catalytic activity in hydrogenation of olefins, structural probes in biological systems [11]. Lanthanides (III) with ionic radii of 1.06-0.85 Ǻ and +3 charge fulfill the optimum conditions for higher coordination [12]. Lanthanide (III) salts have been reported to exert moderate antiproliferative effects in vitro and in vivo. However, there is a continuing interest in mixed ligand metal complexes of Schiff bases and some nitrogen and / or oxygen donor ligands due to their unusual magnetic properties, novel structural features and relevance to the biological system. Hence it is required to develop new series of mixed ligand complexes with various metals and understand their role in biological processes.

Antipyrine derivatives are reported to exhibit analgesic, anti-inflammatory, antiviral, antibacterial effect and also have been used as hair colour additives [13-15]. These compounds have been widely used in spectrophotometric determination of metal ions. Antipyrine Schiff base derivative can serve as antiparasitic agents and their complexes with platinum (II) and cobalt (II) ions have been shown to act antitumor substances [16]. This encouraged us to synthesis the Schiff base ligand from 4-aminoantipyrine.

In the present paper we report the synthesis, characterization and biological studies of the ternary complexes of lanthanum (III) with 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one (HL) and some amino acids. The amino acids used were L-tryptophan, L-phenylalanine, L-isoleucine, L-proline and L-serine. The complexes were synthesized and characterized based on elemental analysis, molar conductivity, spectroscopic methods and thermal studies. The complexes were also screened for their biological activities.

EXPERIMENTAL SECTION

2.1 Materials

All the chemicals used were of analytical grade and used without further purification. Lanthanum nitrate hexahydrate, L-tryptophan, L-phenylalanine, L-isoleucine, L-proline, L-serine, 4-aminoantipyrine and salicylaldehyde were obtained from S.D. Fine Chemicals, Mumbai. Solvents like methanol, ethanol, dimethylformamide and dimethylsulphoxide whenever used were distilled and purified according to standard procedures [17].

2.2 Analytical methods and physical measurements

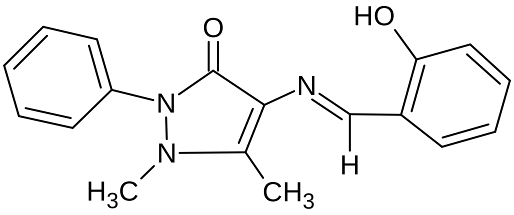
The content of elements (C, H, N) were obtained on Thermo Finnigan, Elemental Analyzer, Model No. FLASH EA 1112 at Sophisticated Analytical Instrumentation facility (SAIF), IIT, Bombay. The metal content was estimated gravimetrically [18, 19]. The conductance measurements were carried out on an Equiptronics Auto ranging Conductivity Meter. Magnetic susceptibility measurements for all the complexes reported in the present study were recorded at room temperature by the Gouy's method using $Hg[Co(SCN)_4]$ as a calibrant. The electronic spectra of the complexes were recorded in DMSO solution (10^{-3} M) on a Shimadzu UV/VIS-160 Spectrophotometer. Infrared spectra of the ligand and all metal complexes were recorded in KBr disc on a Perkin-Elmer FTIR Spectrophotometer in the region 4000-400 cm^{-1}. The ^1H NMR spectra of the Schiff base ligand and their lanthanum (III) complexes were recorded in DMSO-d_6 at room temperature on a VARIAN USA, Mercury plus 300 NMR Spectrometer using TMS as an internal standard at Sophisticated Analytical Instrumentation facility (SAIF), IIT, Bombay. The thermal analysis of the complexes were carried out in controlled nitrogen atmosphere on a Perkin-Elmer Diamond TG-DTA instrument at Sophisticated Analytical Instrumentation facility (SAIF), IIT, Bombay by recording the change in weight of the complexes on increasing temperature up to 900^oC at the heating rate of 10^oC per minute.

The antibacterial activity of the ligands and complexes was evaluated by agar cup and tube dilution methods using Muller-Hinton agar medium [20]. The antibacterial effect was studied after 24 h incubation at 37 ^oC. The MIC of the complexes was studied in liquid Muller-Hinton medium. Test compounds were dissolved to different concentrations in nutrient broth. The MIC was determined after 24 h incubation at 37 ^oC.

2.3 Preparation of 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one (HL)

The organic Schiff base ligand (HL) was prepared from condensation between salicylaldehyde and 4-aminoantipyrine.

Equimolecular amounts of salicylaldehyde and 4-aminoantipyrine were mixed in ethanol and refluxed for 3 h, then cooled. The Schiff base obtained was filtered, washed with ethanol and dried under vacuum. The Schiff base was purified by re-crystallization from ethanol and washed thoroughly with diethyl ether.



HL

2.4 Preparation of Ternary Complexes

Mixed ligand La (III) complexes were prepared from lanthanum (III) nitrate hexahydrate, 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one (HL) as a primary ligand and different amino acids (AA) such as L-tryptophan, L-phenylalanine, L-isoleucine, L-proline and L-serine as secondary ligands.

All the complexes were prepared by the following general procedure:

To a solution of 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one (HL) (1mmol) in hot methanol (25 cm^3), an aqueous solution (10 cm^3) of metal salt (1mmol) was added. To this solution, an aqueous/alcoholic solution (10 cm^3) of amino acids (1 mmol) was added with constant stirring. The mixture (1:1:1 molar proportion) was again heated till it reaches to boiling. The complexes were obtained by raising pH of the reaction mixture by adding dilute ammonia solution. The mixture was cooled and solid complexes obtained were filtered, washed with water, methanol and then with diethyl ether. The complexes thus prepared were dried under vacuum.

RESULTS AND DISCUSSION

The reaction of Schiff base ligand (HL) as a primary ligand and different amino acids as secondary ligands with lanthanum (III) nitrate hexahydrate salt yielded different ternary complexes. The following representative equation illustrates the formation of ternary complexes:

$$\mathrm{La\ (NO_3)_3{\cdot}6H_2O + HL + HAA \longrightarrow [La\ (L)\ (AA)\ NO_3]\ {\cdot}2H_2O + 2HNO_3 + 4\ H_2O}$$

Where, L is deprotonated 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one as ONO donor primary ligand, and AA is different amino acids as deprotonated N and / or O donor secondary ligands.

All the complexes are non-hygroscopic, stable solids, insoluble in water and in common organic solvents such as ethyl alcohol, acetone, chloroform, etc., but moderately soluble in DMF and DMSO.

Table 1 Molecular Weight, Colour and Decomposition Temperature of Lanthanum (III) Complexes

Complex	Empirical Formula	Molecular Weight	Colour	Decomposition Temperature (°C)
[La (L) (Trp) NO_3] .$2H_2O$	$C_{29}H_{31}LaN_6O_9$	746.49	Light Yellow	175
[La (L) (Phe) NO_3] ·$2H_2O$	$C_{27}H_{30}LaN_5O_9$	707.46	Light Yellow	170
[La (L) (Ile) NO_3] ·$2H_2O$	$C_{24}H_{32}LaN_5O_9$	673.44	Light Yellow	180
[La (L) (Pro) NO_3] ·$2H_2O$	$C_{23}H_{28}LaN_5O_9$	657.4	Dark Yellow	170
[La (L) (Ser) NO_3] ·$2H_2O$	$C_{21}H_{26}LaN_5O_{10}$	647.36	Light Yellow	175

Where Trp, Phe, Ile, Pro, and Ser represents L-tryptophan, L-phenylalanine, L-isoleucine, L-proline and L-serine respectively

3.1 Elemental analysis and conductance measurement

Elemental analysis data shows that metal salt, lanthanum (III) nitrate hexahydrate reacts with a primary ligand 2,3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one and secondary ligands L-tryptophan, L-phenylalanine, L-isoleucine, L-proline and L-serine in the proportion 1:1:1 to form complexes of the type [La (L) (AA) NO_3]·$2H_2O$. The molar conductance values of these complexes in DMSO fall in the range of 8 to 16 Mhos cm^2 mol^{-1}, indicating their non-electrolytic nature. As the configuration of lanthanum atom has no unpaired electrons thus their complexes are expected to be diamagnetic. The magnetic moment of the lanthanum (III) complexes were calculated from the measured magnetic susceptibilities and found diamagnetic in nature [21, 22].

Table 2 Elemental Analysis Data, Molar Conductance and Magnetic Moments of Lanthanum (III) Complexes

Complex	Elemental Analysis Found (Calculated)				Molar Conductance (Mhos cm^2 mol^{-1})	μ_{eff} (B.M.)
	% C	% H	% N	% M		
[La (L) (Trp) NO_3] ·$2H_2O$	46.1 (46.64)	4.23 (4.19)	10.93 (11.26)	18.55 (18.62)	8	Diamagnetic
[La (L) (Phe) NO_3] ·$2H_2O$	45.3 (45.82)	4.33 (4.28)	9.73 (9.9)	19.55 (19.64)	12	Diamagnetic
[La (L) (Ile) NO_3] ·$2H_2O$	42.7 (42.79)	4.93 (4.79)	10.73 (10.4)	20.55 (20.64)	14	Diamagnetic
[La (L) (Pro) NO_3] ·$2H_2O$	42.1 (42.00)	4.53 (4.29)	10.73 (10.66)	21.55 (21.14)	16	Diamagnetic
[La (L) (Ser) NO_3] ·$2H_2O$	38.3 (38.94)	4.13 (4.05)	10.73 (10.82)	21.55 (21.47)	9	Diamagnetic

3.2 Infrared spectra and mode of bonding

The IR spectra of the complexes are compared with that of the free ligand to determine the changes that might have taken place during the complexation. The band at 1655 cm^{-1} is characteristic of the carbonyl group present in the Schiff base ligand. This group was shifted to lower frequency (59-62 cm^{-1}) in all complexes indicating the involvement of the carbonyl oxygen in coordination [23]. The band assigned to azomethine group in the free Schiff base ligand was observed at 1503 cm^{-1} and shifted to lower frequency in all metal complexes (13-58 cm^{-1}). This indicates the participation of the nitrogen atom of the azomethine group in coordination [24]. A broad vibration band at 3284 cm^{-1} in the free ligand is assigned to the phenolic OH group. The disappearance of this peak in the spectra of all the complexes indicates the deprotonation of phenol proton prior to coordination. The stretching frequency due to N-N in free ligand was observed at 1034 cm^{-1} is slightly affected in all metal complexes. This indicates the non-involvement of this linkage in coordination to the central metal ion. An important feature of infrared spectra of the metal complexes is the absence of band due to O-H stretching vibrations of either the free –OH group of 2,3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5one (HL) or of the –COOH group of the amino acid. This observation leads to the conclusion that the complex formation takes place by deprotonation of hydroxyl group of HL and carboxylic group of the amino acid moiety [25]. Broad band observed in the region between 3365-3284 cm^{-1} due to asymmetric and symmetric O–H stretching modes and a weak band in the range 1579-1574 cm^{-1} due to H–O–H bending vibrations indicating presence of water molecules, further confirmed by thermal studies.

Broad band observed at 3040 cm^{-1} and 2960 cm^{-1} due to N-H (asymmetric) and N-H (symmetric) vibrations of free amino acid moiety are shifted to higher wave numbers in the range 3180-3110 cm^{-1} and 3060-2965 cm^{-1} respectively in the spectra of metal complexes, suggesting coordination of the amino group through nitrogen with the metal ion.

The C-N symmetrical stretching frequency observed at ~950 cm^{-1} in the spectra of amino acids is found to be shifted to lower wave numbers in the range of 917-897 cm^{-1} in the spectra of the complexes, confirming coordination through the amino group of the amino acids. The presence of hydroxyl group in the molecule of carboxylic acid is readily established by the observation of intense band due to O-H stretching vibrations in the region 3650-3200 cm^{-1}. The absence of bands due to O-H stretching vibrations in the metal complexes can be used as evidence for replacement of proton of hydroxyl group and bonding via oxygen atom to the metal ion. Some new bands of weak intensity observed in the regions of 768-699 cm^{-1} and at 523-470 cm^{-1} may be ascribed to the M-O and M-N vibrations respectively. The M-O bond has much less covalent character than the M-N bond so the stretching bands of the former appear in high frequency region.

Table 3 Characteristic Infrared Spectral Bands (cm^{-1}) of Lanthanum (III) Complexes

Complex	**ν (O-H) H_2O**	**ν (N-H) Asym (A. a.)**	**ν (N-H) Sym. (A. a.)**	**ν (C=O) (HL) (A. a)**	**ν (C=N) (HL)**	**ν (C-O) (A. a.)**	**ν (C-O) (HL)**	**ν (N-N) (HL)**	**ν (C-N)**	**ν (M-O)**	**ν (M-N)**	**ν (NO_3)**
[La (L) (Trp) NO_3] ·$2H_2O$	3330 (b)	3150 (w)	3060 (w)	1594 (s)	1445 (m)	1327 (m)	1151 (m)	1036 (m)	916 (w)	748 (m)	523 (m)	1198(m) 890(m)
[La (L) (Phe) NO_3] ·$2H_2O$	3360 (b)	3180 (w)	3060 (w)	1594 (s)	1490 (s)	1308 (m)	1149 (m)	1054 (m)	905 (w)	699 (m)	480 (w)	1196(m) 755(m)
[La (L) (Ile) NO_3] ·$2H_2O$	3365 (b)	3110 (w)	2965 (w)	1593 (s)	1445 (s)	1308 (m)	1150 (s)	1032 (m)	897 (w)	755 (m)	470 (w)	1195(m) 854(m)
[La (L) (Pro) NO_3] ·$2H_2O$	3341 (b)	3130 (w)	3050 (w)	1596 (s)	1487 (s)	1390 (m)	1140 (m)	1023 (m)	905 (w)	768 (m)	478 (w)	1269(m) 935(w)
[La (L) (Ser) NO_3] ·$2H_2O$	3284 (b)	3135 (w)	3055 (w)	1596 (s)	1453 (m)	1385 (m)	1196 (m)	1061 (m)	917 (w)	767 (m)	470 (w)	1305(w) 893(m)

Where, s: strong, m: medium, b: broad, w: weak

3.3 Nuclear magnetic resonance spectral studies

The ^1H NMR spectra of the free Schiff base ligand in DMSO-d_6 exhibit a signal at δ 12.94 ppm (1H), which is due to the proton of the phenolic hydroxyl group. The large shift of this peak to downfield is due to the bonding of the hydrogen of this group with the nitrogen of the azomethine group. The disappearance of this signal in the spectrum of the complexes confirms the deprotonation of the hydroxyl group of the Schiff base ligand (HL) and participation of its oxygen in the formation of the metal complexes. The free ligand (HL) also shows signals at δ 9.7 ppm (1H), δ 6.9- 7.51 ppm (9H) and δ 2.40-3.33 ppm (6H) assigned to azomethine group proton, aromatic protons and methyl group protons respectively. These signals were slightly shifted downfield or upfield in the complexes. The ^1H NMR of La (III) metal complexes shows additional signals were assigned to amino acid protons.

The assignments of the main signals in the ^1H NMR spectra of the free Schiff base ligand (HL) and its diamagnetic lanthanum complexes are listed in the table 4.

3.4 Thermal analysis

The thermal behavior of the La (III) complexes was investigated by TG and DTA techniques. The thermogram of the complexes indicates that they are thermally pretty stable to varying temperature. All the complexes show the gradual loss in weight due to decomposition with increasing temperature. The decomposition products have been identified on the basis of percentage weight loss observed. The assignment of the different decomposition steps were given in Table 5. The complexes shows decomposition at initial stage in the temperature range of 25-180 ^0C corresponding to loss of two molecules of lattice water. TG shows the loss in weight due to decomposition of organic ligand and amino acid moiety in the range of 170-900 ^0C. The DTA of the La (III) complexes shows a small endothermic peak in the range of 170-200 ^0C indicates the presence of water molecules. A broad exothermic peak at increased temperature attributed to the decomposition of Schiff's base ligand (HL) moiety, nitrate group and amino acid moiety present in the complexes. The pattern of broad exothermic peak is probably due to simultaneous decomposition of the ligand moieties and their subsequent oxidation to gaseous products like CO, CO_2, H_2O, NO_3 etc. The weight loss of these complexes and assignments to the expelled groups exhibited a good agreement with proposed ratio of metal and ligands.

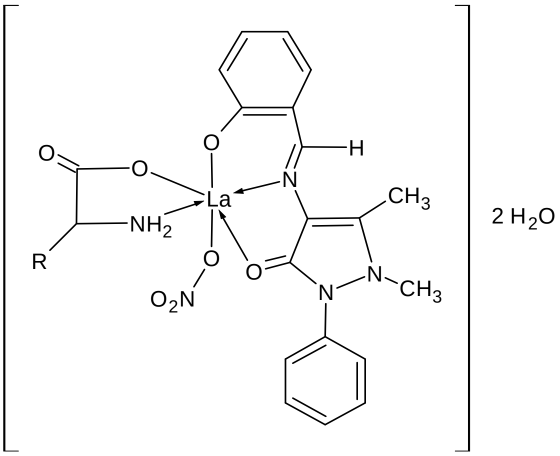
Table 4 ^{1}H NMR Spectral data of 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one (HL) and their La (III) Complexes

Compound	NMR Signals (δ, in ppm)				
	δ (OH)	**δ (HC=N)**	**δ (CH) aromatic**	**δ (CH) of CH_3**	**Additional Signals (Amino Acids)**
HL	12.94 (1H)	9.7 (1H)	6.90-7.51 (9H)	2.40-3.33 (6H)	-----------
[La (L) (Trp) NO_3] ·$2H_2O$	-----	9.7 (1H)	6.84-7.56 (13H)	2.37-3.31 (6H)	δ 4.46 (2H)- CH_2 of Trp δ 6.3(1H)- CH of Trp
[La (L) (Phe) NO_3] ·$2H_2O$	-----	9.68 (1H)	6.88-7.56 (14H)	2.39-3.20 (6H)	δ 3.40 (2H)- CH_2 of Phe δ 4.51(1H)- CH of Phe
[La (L) (Ile) NO_3] ·$2H_2O$	-----	9.7 (1H)	6.89-7.52 (9H)	2.45-3.34 (6H)	δ 0.92-1.43 (6H)- 2 CH_3 of Ile δ 1.82(1H)- CH of Ile
[La (L) (Pro) NO_3] ·$2H_2O$	-----	9.7 (1H)	6.89-7.56 (9H)	2.41-3.39 (6H)	δ 2.11 (4H)- CH_2 of Pro δ 4.48(1H)- CH of Pro
[La (L) (Ser) NO_3] ·$2H_2O$	-----	9.7 (1H)	6.89-7.56 (9H)	2.41-3.33 (6H)	δ 3.92 (2H)- CH_2 of Ser

Table 5 Thermal Data of Lanthanum (III) Complexes

Complex	Temperature Range (^{o}C)	% Weight Loss		Assignment of the expelled group
		Found	**Calculated**	
[La (L) (Trp) NO_3] ·$2H_2O$	25-175	5.88	4.82	Two molecules of lattice water
	175-900	49.72	49.3	$C_{18}H_{16}N_3O_2$ from ligand & One molecule of NO_3
[La (L) (Phe) NO_3] ·$2H_2O$	25-170	4.88	5.09	Two molecules of lattice water
	170-900	51.62	49.47	$C_{18}H_{16}N_3O_2$ from ligand & CO_2 from amino acid
[La (L) (Ile) NO_3] ·$2H_2O$	30-180	5.96	5.35	Two molecules of lattice water
	180-900	45.52	45.44	$C_{18}H_{16}N_3O_2$ from ligand
[La (L) (Pro) NO_3] ·$2H_2O$	35-170	3.27	2.74	One molecules of lattice water
	170-900	46.66	46.85	One molecule of lattice water & $C_{18}H_{16}N_3$ from ligand
[La (L) (Ser) NO_3] ·$2H_2O$	25-150	8.5	8.34	Two molecules of lattice water & H_2O from amino acid
	150-900	40.4	42.17	$C_{18}H_{15}N_3$ from ligand

On the basis of elemental analysis data and various physico-chemical studies, coordination number six is proposed for lanthanum (III) complexes. The bonding and structure for the lanthanum (III) complexes may be represented as shown in figure



R= $-CH_2-C_8H_6N$ *(Trp),* $-C_6H_5$ *(Phe), -* $CH-C_3H_8$ *(Ile) and* $-CH_2OH$ *(Ser)*

Figure 1 General proposed structure for the La-L-AA complexes

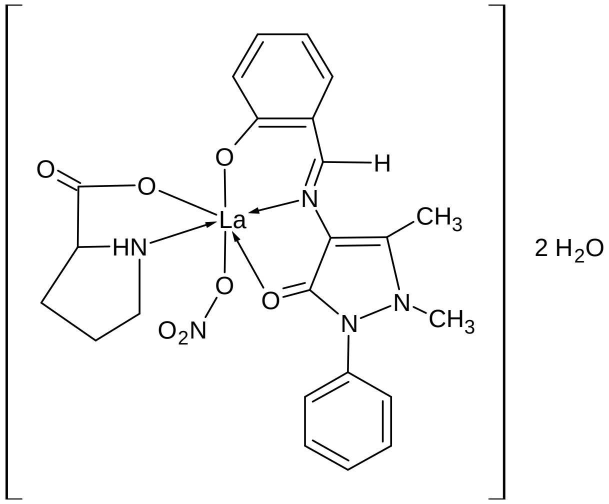


Figure 2 Proposed Structure of [La (L) (Pro) NO_3] ·$2H_2O$

3.5 Antibacterial study

The antibacterial activities of the complexes were evaluated by the agar cup method using tetracycline as a standard against the bacteria *Staphylococcus aureus, Corynebacterium diphtheriae, Pseudomonas aeruginosa* and *Escherichia coli*. The results, expressed as the diameter of growth inhibition area in millimeters, are given in Table 6. The minimum inhibitory concentration (MIC) of the test sample which is expressed in µg /cm^3 was determined by using Mueller-Hinton culture medium, are given in Table 7.

The antimicrobial activity results indicate that lanthanum (III) complexes exhibit good antimicrobial activity against *S. aureus, C. diphtheriae, P. aeruginosa* and *E. coli,* especially against *E. coli.* The complexes have better antibacterial activity than that of each ligand.

The enhancement in the activity is rationalized on the basis of the structures of the ligands by possessing an additional azomethine (C=N) linkage which is significant in determining the mechanism of transamination and resamination reaction in biological system [26, 27]. The ligand with nitrogen and oxygen donor system might inhibit enzyme production, since the enzymes which requires these groups for their activity appear to be more liable to deactivation by metal ions upon chelation.

Table 6 Antibacterial Activity of Lanthanum (III) Complexes by agar cup method

Complex	**Antibacterial Activity (mm)**			
	S. aureus	***C. diphtheriae***	***P. aeruginosa***	***E. coli***
[La (L) (Trp) NO_3] ·$2H_2O$	13	13	12	16
[La (L) (Phe) NO_3] ·$2H_2O$	14	12	13	15
[La (L) (Ile) NO_3] ·$2H_2O$	14	11	13	15
[La (L) (Pro) NO_3] ·$2H_2O$	13	12	11	15
[La (L) (Ser) NO_3] ·$2H_2O$	14	12	12	15
Tetracycline	30	25	26	18

Table 7 Antibacterial Activity of Lanthanum (III) Complexes by tube dilution method

	MIC (µg/cm^3)			
Complex	***S. aureus***	***C. diphtheriae***	***P. aeruginosa***	***E. coli***
[La (L) (Trp) NO_3] ·$2H_2O$	300	500	450	250
[La (L) (Phe) NO_3] ·$2H_2O$	350	400	400	300
[La (L) (Ile) NO_3] ·$2H_2O$	350	400	500	250
[La (L) (Pro) NO_3] ·$2H_2O$	300	500	450	250
[La (L) (Ser) NO_3] ·$2H_2O$	350	450	400	300
HL	450	500	450	400

CONCLUSION

In conclusion, we have reported the synthesis of ternary complexes of La (III) metal with 2, 3-dimethyl-1-phenyl-4-salicylidene-3-pyrazolin-5-one and some amino acids. The complexes were characterized and it is found that the Schiff base participated in the bonding to lanthanum as monobasic tridentate ONO ligand and the amino acids as monobasic bidentate ligand by deprotonation of the Schiff base phenolic OH and the amino acid COOH. The correlation of the elemental analysis data and various physico-chemical studies, coordination number six is proposed for lanthanum (III) complexes. These complexes exhibit excellent antibacterial activity. Thus the series of lanthanum complexes can hopefully become a novel kind of drugs.

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