

Preparation and Spectral Studies of Biologically Active Mixed Ligand Cerium (III) Complexes

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Abstract

*Cerium (III) complexes of type $[M(Q)(L).H_2O]$ have been prepared by using 8-hydroxy quinoline (HQ) as a primary Ligand and amino acids (HL) such as secondary ligands. The mixed ligand metal complexes have been studied spectrochemically and characterized by elemental analysis. Room temperature magnetic susceptibility measurement specifies that cerium (III) complexes are paramagnetic in nature. Electrical conductance studies of the complexes indicate their non-electrolytic nature. Electronic Absorption spectra of the complexes shows intra ligand, charge transfer and d-d transitions respectively. The thermal analysis data of the complexes indicates the presence of coordinated water molecules. FTIR spectra show bonding of the metal ion through N- and O- donor atoms of the ligand molecules. Tube dilution and agar cup methods were implemented for the study of antibacterial activity of the complexes against *S.aureus*, *C.diphtheriae*, *S.typhi* and *P.auruginosa*. The antibacterial activity of the prepared complexes have been determined by using tetracyclin as standard antibacterial compound and it was found that, the complexes show mild activity against selected stains of micro-organisms as compared to standard tetracycline.*

Keywords: *Mixed ligand lanthanum complexes, characterization, and antibacterial activity.*

1. Introduction

Bibliological studies exhibited that many researchers have studied characterization and biological study of mixed ligand complexes of transition metals^[1-6]. Mixed ligand metal complexes play a very important role in biological process^{[1],[8]}. This metal complexes with 8-Hydroxyquinoline exhibited biological activity^[9-11]. Mixed ligand complexes are formed with amino acids which are well known for their biological significance^[12]. Mixed ligand complexes of Ce(III) showed that antifungal activity^{[13]-[14]}. These complexes are also characterized for their cytotoxic activity^[15].

The author therefore thought to undertake the study of mixed ligand complexes of cerium, with 8-hydroxyquinoline (HQ) as a primary ligand and N- and/or O -donor amino acids (HL) such as, as L-isoleucine and L-hydroxyproline as secondary ligands. The metal complexes have been characterized by element analysis and various physic-chemical techniques such as molar conductance, room temperature magnetic susceptibility, electronic spectra, IR spectra and thermal studies.

2. Experimental

2.1 Materials

Cerium (III) chloride heptahydrate of analytical grade was used as such without purification. Amino acids, L-isoleucine and L-hydroxyproline were obtained from E. Merck.

Solvents like DMF and DMSO whenever used were distilled and purified according to standard procedure [16-18].

2.2 Preparation of mixed ligand complex

The mixed ligand cerium (III) complexes were prepared from cerium chloride heptahydrate with primary ligand (HQ) and as L-isoleucine and L-hydroxyproline as secondary ligand (HL) in 1:2:1 proportion. To an aqueous solution (10ml) of cerium (III) chloride heptahydrate (372mg, 1mmol), ethanolic solution (20 ml of 8-hydroxyquinoline (290mg, 2mmol) was added. The mixture was stirred and kept in boiling water bath for 10 minutes. To this hot solution, an aqueous solution (10ml) of amino acids (1mmol) was added with constant stirring. The mixture was again heated in a water bath till the temperature reached to 50° c. The complexes were precipitated by raising pH of the reaction mixture by adding diluted amino solution. The mixture was cooled and solid complex obtained was filtered, washed, with water followed by ethanol. The complexes thus prepared were dried under vacuum and were used for further studies.

2.3 Instrumentation

The synthesized cerium (III) complexes were analyzed for C, H, and N content on Thermo Finnegan Elemental Analyzer Model no. FLASH EA 1112 series at the department of chemistry, I.I.T. Mumbai. Metal content was estimated gravimetrically by standard procedure [19], [20]. The molar conductance values were measured in DMF (10^{-3} M) on an Equiptronics Auto ranging Conductivity Meter Model No. EQ-667. Room temperature magnetic susceptibility were measured by a Guoy method using $\text{Hg}[\text{Co}(\text{SCN})_4]$ as a calibrate at the Department of chemistry, I.I.T. , Mumbai. The electronic absorption spectra of all the complexes in DMF solution (10^{-4} M) in the ultraviolet and visible region were recorded on Shimadzu UV/VIS-160 spectrophotometer. FT-IR spectra were recorded in KBr disc on PerkinElmer FT-IR spectrophotometer model 1600 at Department of Chemistry, I.I.T. Mumbai. Thermal analysis (TG and DTA) were carried out in control Nitrogen atmosphere on a Perkin-Elmer Diamond TG-DTA Instrument Department of Chemistry, I.I.T., Mumbai by recording the change in weight of complexes on increasing temperature up to 900° C at the heating rate of 10° C per minute.

2.4 Antibacterial screening

2.4.1 Agar cup method

In this method, a single compound can be tested against number of organisms or given organism against different concentration of the same compound. It was found suitable for semisolid or liquid samples and was used in the present work. In agar cup method, a plate of sterile nutrient agar with the desired test strain was poured to a high of about of 5mm, allowed solidified and single cup of 8mm diameter was cut from the central of the plate with sterile cork borer. There after the cup was filled with the sample solution of $1000\mu\text{g}/\text{cm}^3$ concentration. The test solution was allowed to diffuse in surrounding agar by keeping in refrigerator for 10minutes and the plate was incubated at 37°C for 24 hours. The extent of inhibition of growth from the edge of the cup was a considered as a measure of the activities of give compound. By using several plates simultaneously, the activities of several samples could qualitative studied.

2.4.2 Tube dilution method

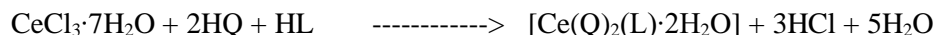
The test compounds were subjected to in *vitro* screening against *Staphylococcus aureus* *Corynebacterium diphtheriae* , *Salmonella typhi* and *P. aeruginosa* using Muller Hinton broth as the culture medium. The test compound (10mg) was dissolved in DMSO (10cm^3) so as to prepare a stock solution concentration $1000\mu\text{g}/\text{cm}^3$. From the stock solution, aliquots of 5,10,15,20 to

....., 250 µg/cm³ were obtained in test broth. Bacterial inoculums were prepared in a sterilized Muller Hinton broth and incubated for 24 hrs. at 37^oc. The aliquots were dispensed(5cm³) in each borosilicate test tube (150×20mm). The bacterial inoculums 0.1 cm³ of the desired bacterial strain (*S.aureus*, *C.diphtheriae*, *S.typhi* and *P.aeruginosa*) containing 10⁶ bacteria/cm³ was inoculated in the tube. The tube was incubated at 37^oc for 24 hrs. and then examined for the presence or absence of the growth of the test organisms. Tetracycline was used as standard drug again Gram positive and Gram negative bacteria by similar screening procedure. The solvent DMSO was also tested as control to see that it did not affect the growth of the culture .MIC of tetracycline was found to be 1.5µg/cm³ against *S.aureus* 2.0µg/cm³ against *C.diphtheriae* 1.5µg/cm³ against *S.typhi* and 8.0µg/cm³ against *P.aeruginosa*.

3. Result and Discussion

3.1 Characterization of Metal Complexes

The synthesis of mixed ligand Ce(III) complexes may be represented as follows:



(Where, HQ is 8-hydroxyquinoline and HL is an amino acid). All the complexes are colored, non-hygroscopic and thermally stable solid (Table 1 and 2), indicating a strong metal-ligand bond. The complexes are insoluble in common organic solvents. Such as ethyl alcohol, acetone, etc. but are partially soluble in DMF and DMSO. The elemental analysis data (Table 3) of metal complexes is consistent with their general formulation as 1:2:1, mixed ligand complexes of the type [Ce(Q)₂(L)·2H₂O]. The molar conductance values of the complexes in DMF at 10⁻³ M concentration are found to be 0.0019 – 0.0023 mhos cm² mol⁻¹ indicating their non- electrolytic nature [21].

Table 1: Empirical Formula, Molecular Weight and Colour

Complex	Empirical Formula	Molecular Weight	Colour
[Ce(Q) ₂ (Iso)·2H ₂ O]	CeC ₂₄ H ₂₈ O ₆ N ₃	594.61	Brown
[Ce(Q) ₂ (HPro)·2H ₂ O]	CeC ₂₃ H ₂₄ O ₇ N ₃	594.57	Brown

Table 2: Decomposition temperature and P^H

Complex	Decomposition Temperature (°C)	P ^H
[Ce(Q) ₂ (Iso)·2H ₂ O]	225	6.99
[Ce(Q) ₂ (HPro)·2H ₂ O]	230	7.01

Where Q represents the deprotonated primary ligand 8- Hydroxyquinoline whereas Iso and HPro represents deprotonated secondary ligands, L-Isoleucine and L-Hydroxylproline respectively.

Table 3: Elemental Analysis Data and Molar Conductance of Cerium Complexes

Complex	Elemental Analysis found(Calculated)	Molar Conductance
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	% M	%C	%H	%N	(mhos cm ² mol ⁻¹)
[Ce(Q) ₂ (Iso)·2H ₂ O]	23.58 (23.56)	48.50 (48.48)	04.77 (04.75)	07.08 (07.07)	0.0018
[Ce(Q) ₂ (HPro)·2H ₂ O]	23.58 (23.57)	46.49 (46.46)	04.09 (04.07)	07.08 (07.07)	0.0010

3.2 Magnetic Studies:

The magnetic moment of the mixed ligand cerium (III) complexes (Table 4) were calculated from the measured magnetic susceptibilities after employing diamagnetic corrections and revealed their paramagnetic nature.

Table 4: Magnetic susceptibility data of Cerium (III) complexes (10⁻⁶ c.g.s. unit)

Complex	X _g	X _m	μ _{eff} (B.M)
[Ce(Q) ₂ (Iso)·2H ₂ O]	- 2.13 x 10 ⁻⁶	1.27 x 10 ⁻³	1.85
[Ce(Q) ₂ (HPro)·2H ₂ O]	- 2.16 x 10 ⁻⁶	1.28 x 10 ⁻³	1.86

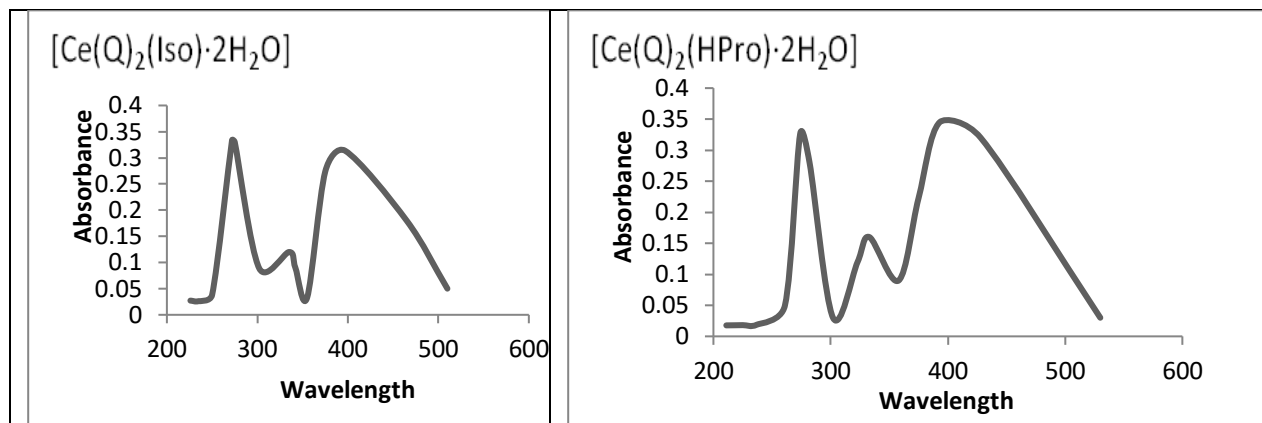
3.3 Electronic Absorption Spectra

The electronic spectra of the metal complexes in DMF were recorded in the UV-Visible region (Table 5). The spectra shows three transitions π → π*, n → π* and charge transfer transition from ligand to the metal respectively [24].

Table 5: The electronic spectra of the metal complexes

Complex	λ nm	ν cm ⁻¹	Proposed Assignment
[Ce(Q) ₂ (Iso)·2H ₂ O]	272	36765	π → π*
	335	29851	n → π*
	397	25189	Charge-transfer
[Ce(Q) ₂ (HPro)·2H ₂ O]	274	36496	π → π*
	333	30030	n → π*
	393	25445	Charge-transfer

UV-Visible Spectra of Cerium (III) Complexes

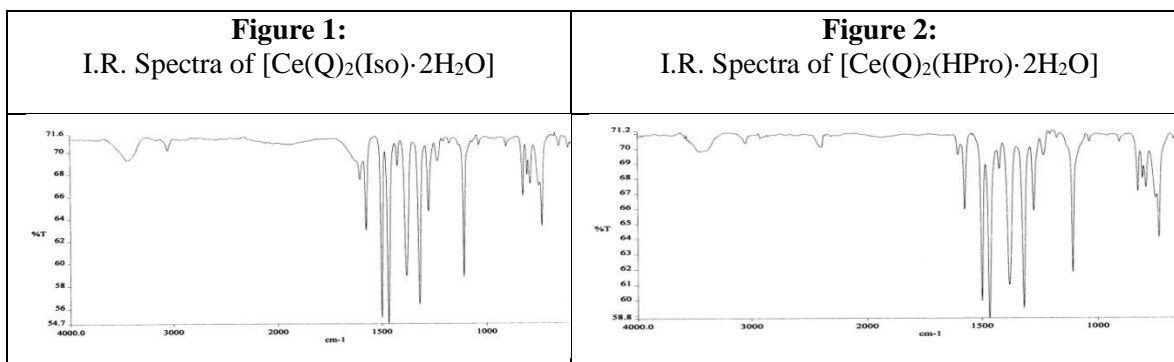


3.4 Infra-red Spectra

The FTIR spectra of the metal complexes were recorded in a KBr disc over the range $4000-400\text{cm}^{-1}$. These spectra's were complex due to presence of numerous bands with varying intensities, making the task quite difficult. However, an attempt has been made to assign some of the important bands on the basis of reported infrared spectra of several N and / or O donor ligands, 8-hydroxy quinoline and their metal complexes. An important feature of infrared spectra of the metal complexes is the absence of band at $\sim 3440\text{cm}^{-1}$ due to the O-H stretching vibration of the free O-H group of HQ. This observation leads to the conclusion that complex formation takes place by deprotonation of hydroxyl group of HQ moiety. A strong $\nu(\text{CO})$ band observed in the range of $1110-1105\text{cm}^{-1}$ in the spectra of the complexes, indicates the presence of the 8-hydroxyquinolate group in the complexes co-ordinating through its nitrogen and oxygen atoms in uninegative bidentate ligand. The $\nu(\text{C}=\text{N})$ mode observed at 1503cm^{-1} in the spectrum of free HQ ligand is found to be shifted to lower wave at 1503cm^{-1} in the spectrum of the complexes, suggesting co-ordination through the tertiary nitrogen donor of HQ. The in-plane and out-of-plane deformation modes observed at $\sim 500\text{cm}^{-1}$ and $\sim 780\text{cm}^{-1}$ respectively, in the spectrum of HQ are shifted to higher wave numbers 504cm^{-1} and 790cm^{-1} respectively, conforming co-ordination through the nitrogen atom of HQ with the metal ion.

A broad band observed in the region between $3210-3207\text{cm}^{-1}$ due to asymmetric and symmetric O-H stretching modes and a weak band in the range of $1577-1575\text{cm}^{-1}$ due to H-O-H bending vibrations indicating presence of co-ordinate water molecule ^{[25] - [27]}, further confirmed by thermal studies. The N-H asymmetric and N-H symmetric vibrations observed at $\sim 3042\text{cm}^{-1}$ and $\sim 1372\text{cm}^{-1}$, respectively, in the free amino acids are shifted to higher wave numbers i.e. in the range $3178-3082\text{cm}^{-1}$ and $3055-3050\text{cm}^{-1}$, respectively, in the spectra of the complexes, suggesting co-ordination of amino group through nitrogen with the metal ion. The $\nu_{\text{symmetric}}(\text{COO}^-)$ band of the free amino acid i. e. $\sim 1590\text{cm}^{-1}$ is shifted to higher wave number, i.e. in the range $1639-1633\text{cm}^{-1}$ and $\nu_{\text{symmetric}}(\text{COO}^-)$ mode observed at $\sim 1400\text{cm}^{-1}$ in the spectra of free amino acids are shifted to lower wave numbers in the range of $1377-1372\text{cm}^{-1}$, in the spectra of complexes indicating the co-ordination of carboxylic acid group via oxygen with the metal ion.

Coordination through the amino group of the amino acids has been further confirmed by the C-N symmetrical stretching frequency. It is observed at $\sim 950\text{cm}^{-1}$ in the spectra of free amino acids and found to be shifted to lower wave numbers 912cm^{-1} in the spectra of the complexes. Some new bands of weak intensity observed in the regions around $605-600\text{cm}^{-1}$ and 409cm^{-1} may be ascribed to M-O and M-N vibrations respectively. It may be noted that these vibrational bands are absent in the infra-red spectra of HQ as well as amino acids.



3.5 Thermal Studies:

The TG and DTA studies of the complexes have been recorded in the nitrogen atmosphere at the constant heating rate of $10^{\circ}\text{C}/\text{minute}$. Thermal study on mixed ligand cerium complexes in controlled nitrogen atmosphere was carried out to understand stages of decomposition and temperature range of decomposition. The most probable decomposition pattern of the complexes is proposed on the basis of the careful examination of TG and DTA curves. The thermo analytical data is summarized in (Table 6, 7 and 8). The thermogram of these complexes shows the loss in weight corresponding to two water molecules in the temperature range $125\text{-}160^{\circ}\text{C}$, followed by weight loss due to amino acids moiety in the temperature range $230\text{-}410^{\circ}\text{C}$. The final step of decomposition observed in the temperature range $615\text{-}835^{\circ}\text{C}$ corresponds to two molecules of 8-hydroxyquinoline in the complex. The constant weight plateau in TG of cerium (III) after 835°C indicates completion of the reaction.

Table 6: Thermal data of Cerium Complexes showing % Loss due to Water Molecules

Sr. No.	Complex	% Weight Loss due to Water Molecules		
		Temperature Range($^{\circ}\text{C}$)	Found	Calculated
1	$[\text{Ce}(\text{Q})_2(\text{Iso})\cdot 2\text{H}_2\text{O}]$	125-150	6.09	6.05
2	$[\text{Ce}(\text{Q})_2(\text{HPro})\cdot 2\text{H}_2\text{O}]$	125-160	6.15	6.05

Table 7: Thermal data of Cerium Complexes showing % Loss due to Amino acid Molecules

Sr. No.	Complex	% Weight Loss due to Water Molecules		
		Temperature Range($^{\circ}\text{C}$)	Found	Calculated
1	$[\text{Ce}(\text{Q})_2(\text{Iso})\cdot 2\text{H}_2\text{O}]$	225-405	21.95	21.89
2	$[\text{Ce}(\text{Q})_2(\text{HPro})\cdot 2\text{H}_2\text{O}]$	230-390	21.95	21.89

Table 8: Thermal data of Cerium Complexes showing % Loss due to 8-HQ Molecules

Sr. No.	Complex	% Weight Loss due to Water Molecules		
		Temperature Range($^{\circ}\text{C}$)	Found	Calculated
1	$[\text{Ce}(\text{Q})_2(\text{Iso})\cdot 2\text{H}_2\text{O}]$	630-840	48.52	48.49

2	[Ce(Q) ₂ (HPro)·2H ₂ O]	610-835	48.67	48.49
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On the basis of physicochemical studies, the bonding and structure for the cerium complexes may be represented as.

Figure 3: Proposed Structure of [Ce(Q)₂(Iso)·2H₂O]

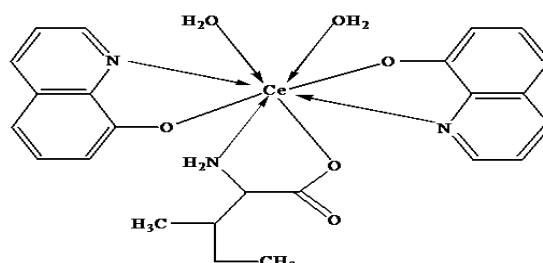
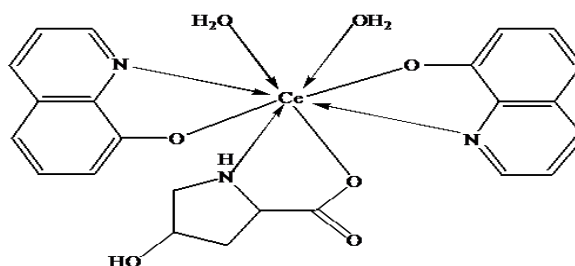


Figure 4: Proposed Structure of [Ce(Q)₂(HPro)·2H₂O]



3.6 Biological Studies:

The metal complexes were screened against *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Salmonella typhi* and *P. aeruginosa*. The studies based on agar cup method revealed that the complexes are more sensitive against *S. aureus* and *S. typhi* while less sensitive against *C. diphtheria* and *P. aeruginosa*. (Table 9) The minimum inhibitory concentration (MIC) of ligand and the metal salts ranges between 50 and 300 µg / mL while that of metal complexes ranges between 5 and 35 µg/mL (Table 10). The complexes are found to be more active against *S. aureus* and *S. typhi* as compare to *C. diphtheria* and *P. aeruginosa*. As compared to standard antibacterial compound tetracycline, the complexes show moderate activity against selected strains of microorganisms. The results show that, as compared to the activity of metal salt and free ligand, the metal complexes show higher activity. The activity of metal complexes is enhanced due to which in turn increases the hydrophobic character of the chelate and thus enables its permeation through the lipid layer of microorganism.

Table 9: Antibacterial Activity (mm) of Cerium Complexes by Agar Cup Method

Complex	Antibacterial Activity (mm) with			
	<i>S. aureus</i>	<i>C. diphtheria</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>
[Ce(Q) ₂ (Iso)·2H ₂ O]	24	16	22	12
[Ce(Q) ₂ (HPro)·2H ₂ O]	26	15	20	12

Table 10: MIC ($\mu\text{g/mL}$) Data of Cerium Complexes

Complex	MIC ($\mu\text{g/mL}$)			
	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>
[Ce(Q) ₂ (Iso)·2H ₂ O]	05	20	20	30
[Ce(Q) ₂ (HPro)·2H ₂ O]	05	20	25	25
8-hydroxyquinoline	50	200	150	250
Tetracycline	1.5	2.0	1.5	8.0

4. Conclusion

On the basis of the above observations, following conclusions are made. The higher decomposition temperatures of the complexes indicate a strong metal-ligand bond and electrical conduction studies show non-electrolytic nature of the complexes, respectively. Magnetic studies indicate paramagnetic nature of the complexes. Electronic absorption spectra of the complexes show intra ligand and charge transfer transitions, respectively. IR spectra show bonding of the metal ion through N- and O- donor atoms of the two ligands. Thermal analysis confirms the presence of two coordinated water molecules. On the basis of the above results coordination number eight is proposed for cerium complexes.

The antibacterial study shows that complexes are found to be more active against *S. aureus* and *S. typhi* as compare to *C. diphtheria* and *P. auruginosa*. Compared to standard antibacterial compound, tetracycline, the complexes show mild activity against the selected strains of microorganisms.

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