



Preparation Of Mixed Ligand Complexes Of Cerium (III) Metal Using Multidentate Ligands And Investigation Of Their Physicochemical, Spectral, Thermal, And Antibacterial Properties.

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Abstract

This study involves the synthesis and characterization of Cerium (III) Mixed-ligand complexes employing 2-hydroxybenzaldehyde oxime (BO) as the primary ligand and organic compounds 2-amino-3-(1H-indol-3-yl)propanoic acid (L₁), 2-amino-4-(methylthio)butanoic acid (L₂), 2-amino-3-(4-hydroxyphenyl)propanoic acid (L₃), 2-Amino-3-sulfhydrylpropanoic acid (L₄) as secondary ligands. The resulting complexes exhibit a brown color and display stability, as evidenced by conductometric measurements indicating non-electrolyte behavior. FTIR analysis reveals coordination through O and N donor atoms, while thermogravimetric studies highlight stability with coordinated water molecules, supported by high decomposition temperatures. UV studies demonstrate intra-ligand and ligand-to-metal charge transfer transitions. Elemental analysis confirms a 1:2:1 ratio for the complexes. Antibacterial assays using agar cup and tube dilution methods affirm the complexes' antibacterial activity against specific bacterial strains.

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Keywords: ligand, complexes, metal, antibacterial.

Introduction

The intricate field of metal complexation, particularly involving transition and inner transition elements, has been a focal point of research for numerous years. Throughout this period, a growing body of evidence has established that mixed ligand complexes exhibit greater stability compared to their simple counterparts. The significance of these complexes extends into biological systems, where they play pivotal roles [1,2]. Researchers have extensively delved into the synthesis of mixed ligand complexes, revealing their profound impact on crucial biological activities, including antimicrobial, antibacterial, antifungal, and antidiabetic properties [3-7]. Organic compounds forming bonds with metal atoms, featuring diverse donor atoms such as oxygen (O) and nitrogen (N), have been identified as crucial components in the creation of stable metal complexes. These complexes, in turn, have demonstrated the ability to display notable activities, such as antituberculosis and antidiabetic effects [8-9]. Lanthanides, with a particular emphasis on cerium metal, have been subjects of interest in the synthesis and characterization of metal complexes. Studies focusing on their biological activities, especially antibacterial and antimicrobial effects, have contributed significantly to the

understanding of these systems [10-13].

In recent years, organic compounds with donor atoms like nitrogen (N), oxygen (O), and sulfur (S), coupled with active functional groups including -COOH, -NH₂, -SH, and -OH, have attracted considerable attention. The combination of these elements has demonstrated the potential to form stable mixed ligand complexes, marking a significant development in the field [14-17]. Notably, these mixed ligand complexes have been found to exhibit catalytic properties in a variety of reactions, encompassing reduction, oxidation, oxidative cleavage, hydroformylation, and hydrogen peroxide decomposition.

The synthesis, characterization, and antibacterial investigation of these complexes have been extensively reported by researchers globally, underscoring their importance in various scientific disciplines [18-20]. In light of the increasing recognition of the multifaceted nature of complexes in literature, we have embarked on a research endeavor to prepare mixed ligand complexes involving cerium metal. This investigation employs 2-Hydroxybenzaldehyde oxime as the primary ligand, complemented by secondary ligands such as 2-amino-3-(1H-indol-3-yl)propanoic acid, 2-amino-4-(methylthio)butanoic acid, 2-amino-3-(4-hydroxyphenyl)propanoic acid, and 2-Amino-3-sulphydrylpropanoic acid. Following synthesis, a comprehensive characterization is undertaken utilizing spectral, thermal, and physicochemical methods, including molar conductance, elemental analysis, pH, UV-Visible, FTIR spectral, and thermal analysis. The antibacterial investigation of the synthesized mixed ligand complexes further contributes to the exploration of their potential applications in biological systems.

Methodology

2.1 Materials :

The cerium metal salt, 2-Hydroxybenzaldehyde oxime (BO), and the organic compounds 2-amino-3-(1H-indol-3-yl)propanoic acid, 2-amino-4-(methylthio)butanoic acid, 2-amino-3-(4-hydroxyphenyl)propanoic acid, and 2-Amino-3-sulphydrylpropanoic acid were procured from S.D. Fine Chemicals, Mumbai. To ensure the purity of the solvents employed in the synthesis, including DMSO, DMF, and Ethanol, standard purification procedures were followed [21-22].

2.2Instrumentation:

For elemental analysis, the quantification of carbon (C), hydrogen (H), and nitrogen (N) was conducted using the Thermo Finnigan Elemental Analyzer. The conductance of the complexes was measured employing a Conductivity Meter Model No. EQ-667. Infrared (IR) spectra of the complexes were recorded utilizing a Shimadzu FT-IR spectrophotometer, specifically the IR Affinity-1 model, with KBr discs, spanning the range of 4000-400 cm⁻¹. UV-Visible spectra were obtained using a Shimadzu UV/VIS-1800 spectrophotometer. Both UV-Visible and IR spectra analyses of the complexes were carried out at the Central Instrumentation Laboratory, Changu Kana Thakur ACS College, New Panvel (Autonomous). To investigate the thermal behavior of the cerium complexes, recordings were made using a Perkin-Elmer Diamond TG-DTA Instrument at the Institute of Science, Mumbai.

2.3Synthesis of Complexes:

The synthesis procedure involved the combination of Cerium (III) chloride heptahydrate and 2-Hydroxybenzaldehyde oxime in an alcoholic solution, followed by the gradual addition of an aqueous solution containing a secondary ligand. The reaction mixture was heated and stirred, undergoing a series of steps to ensure proper complex formation. By adjusting the pH of the resulting solution, the complex precipitated, and the solid product was isolated through filtration and washed with ethanol. The obtained complex was then air-dried over 48 hours. This meticulous process aimed to yield mixed ligand complexes of cerium metal, laying the foundation for subsequent characterization studies to explore their properties and potential applications.

2.4Antibacterial study:

2.4.1 Agar Cup Method

The antibacterial activity of all cerium complexes was investigated using the agar cup method against selected bacterial strains, namely *S. typhi*, *P. aeruginosa*, *S. aureus*, and *C. diphtheria* [23]. The procedure involved pouring agar solution containing the target bacteria into a sterile petriplate to a height of 5 mm, allowing it to solidify. A sterile cork was then used to cut a single cup with an 8 mm diameter from the middle portion of the agar plate. This cup was subsequently filled with a solution of the cerium complex at a concentration of 1000 µg/cm³. The petriplate containing the complex was placed in a refrigerator for 10 minutes to allow the complex solution to solidify within the cup. Following this, the plate was incubated at 37 °C for 24 hours to observe and

assess the antibacterial activity of the cerium complexes against the specified bacterial strains.

2.4.2 Tube Dilution Method

The determination of the Minimum Inhibitory Concentration (MIC) of the complexes was conducted through an in vitro screening method against bacterial strains, utilizing Muller Hinton broth as the culture medium. A test compound, comprising 10 mg, was dissolved in 10 cm³ of DMSO solvent to achieve a concentration of 1000 µg/cm³, serving as the stock solution. Bacterial inoculums were prepared in sterilized Muller Hinton Broth. Various aliquots ranging from 5 to 250 µg/cm³ were derived in the test broth from the stock solution. Borosilicate tubes with a diameter of 150 x 20 mm were used, and the aliquots were distributed into them. Subsequently, 0.1 cm³ of the desired bacterial strain was inoculated into each test tube. All tubes were then placed on a rotary shaker at a temperature of 37°C for a 24-hour incubation period. After the incubation period, the growth of the test organisms was observed to determine the MIC of the complexes. Tetracycline served as the standard compound in this method, providing a reference point for the antibacterial activity of the tested complexes. The MIC, representing the lowest concentration of the complexes that inhibits visible bacterial growth, was determined through this systematic approach.

3. Results and discussion

3.1 Synthesis of metal complexes

Synthesis of Cerium(III) metal complexes may be represented as follows



Where,

HBO is 2-Hydroxybenzaldehyde oxime

HL is secondary ligand.

3.2 Physico-Chemical Properties

The metal complexes are dark brown in colour and non-hygroscopic in nature. (Table No.1)

Table No. 1 :

Complex	Empirical Formula	Molecular Weight	Colour
[Ce(BO) ₂ ((L ₁)).2H ₂ O]	CeC ₂₅ H ₂₇ O ₈ N ₄	651.62	Brown
[Ce(BO) ₂ ((L ₂)).2H ₂ O]	CeC ₁₉ H ₂₆ O ₈ N ₃ S	596.61	Brown
[Ce(BO) ₂ ((L ₃)).2H ₂ O]	CeC ₂₃ H ₂₆ O ₉ N ₃	628.58	Brown
[Ce(BO) ₂ ((L ₄)).2H ₂ O]	CeC ₁₇ H ₂₂ O ₈ N ₃ S	568.55	Brown

The decomposition temperature of all the cerium metal complexes was observed within the range of 148-160°C and pH of the metal complexes between 6.90 to 7.05. This temperature range confirms the presence of robust metal-ligand bonding within the complexes. Furthermore, the complexes exhibited insolubility in various organic solvents such as methanol, ethanol, and acetone. However, they displayed partial solubility in polar aprotic solvents like dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). This observation suggests the formation of stable complexes with distinctive solubility characteristics, providing valuable insights into their potential applications and behavior in different solvents.

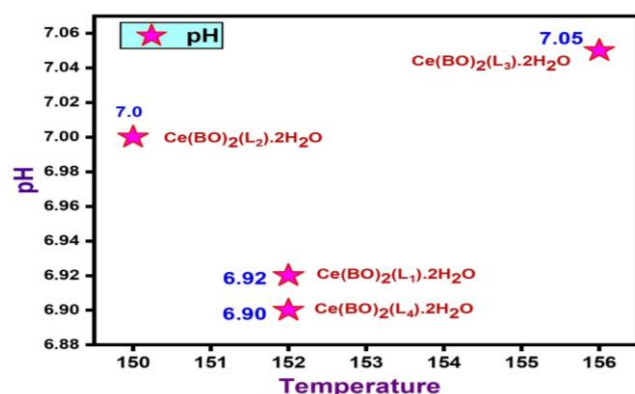


Figure: 1

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Table No-2 :

Complex	Elemental Analysis Found (Calculated)				Molar Conductance (Mhos cm ² mol ⁻¹)
	% C	% H	% N	% M	
[Ce(BO) ₂ ((L ₁)).2H ₂ O]	46.08 (46.07)	04.16 (04.18)	08.62 (08.60)	21.52 (21.50)	0.000192
[Ce(BO) ₂ ((L ₂)).2H ₂ O]	38.24 (38.22)	04.40 (04.36)	07.06 (07.04)	22.46 (23.48)	0.000224
[Ce(BO) ₂ ((L ₃)).2H ₂ O]	43.92 (43.94)	04.18 (04.17)	06.62 (06.68)	22.27 (22.29)	0.000230
[Ce(BO) ₂ ((L ₄)).2H ₂ O]	35.85 (35.88)	03.90 (03.87)	07.37 (07.39)	24.66 (24.65)	0.000195

The molar conductance of complexes measured by using Conductivity Meter Model No. EQ-667 by preparing 0.001 M solution of all complexes in N,N-dimethylformamide and obtained conductance values are recoded in Table No. 2 which reveals that all the cerium metal complexes exhibit very low molar conductance values, indicating their non-electrolyte nature [25]. The elemental composition analysis of the complexes was carried to find the carbon, hydrogen and nitrogen content which indicates the general formula of 1:2:1 for all complexes [24]. This suggests that the complexes are formed through the coordination of metal ions with ligands in a specific stoichiometric ratio, resulting in a stable non-electrolytic structure. The observed molar conductance values provide valuable information about the electrical conductivity of the complexes, further contributing to the understanding of their chemical nature and potential applications.

Electronic absorption spectra:

Table No. 3 : Electronic Absorption Spectral data of complexes

Complex	λ (nm)	ν (cm ⁻¹)	Proposed Assignments
[Ce(BO) ₂ ((L ₁)).2H ₂ O]	262	38168	$\pi \rightarrow \pi^*$
	330	30303	$n \rightarrow \pi^*$
	402	24875	Charge-transfer
[Ce(BO) ₂ ((L ₂)).2H ₂ O]	266	37594	$\pi \rightarrow \pi^*$
	322	31056	$n \rightarrow \pi^*$
	404	24753	Charge-transfer
[Ce(BO) ₂ ((L ₃)).2H ₂ O]	256	39063	$\pi \rightarrow \pi^*$
	327	30581	$n \rightarrow \pi^*$
	395	25316	Charge-transfer
[Ce(BO) ₂ ((L ₄)).2H ₂ O]	267	37453	$\pi \rightarrow \pi^*$
	326	30675	$n \rightarrow \pi^*$
	398	25126	Charge-transfer

The organic compounds utilized in the formation of metal complexes are amino acids, and many of these

amino acids exhibit a single peak below 280 nm attributed to $n \rightarrow \pi^*$ transitions [26]. In the UV spectrum of the free ligand, prominent peaks are observed at 220 and 250 nm, corresponding to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. The UV spectra of cerium complexes was obtained by using 0.001 M solution in DMF solvent. It was found that in the cerium complexes, these peaks shift to higher wavelengths, indicating the occurrence of metal-ligand bonding. This shift in peaks is indicative of three types of transitions in the complexes [27]. The alterations in the UV spectra provide valuable insights into the nature of the metal-ligand interactions within the complexes, contributing to the characterization of their electronic structure and bonding behavior.

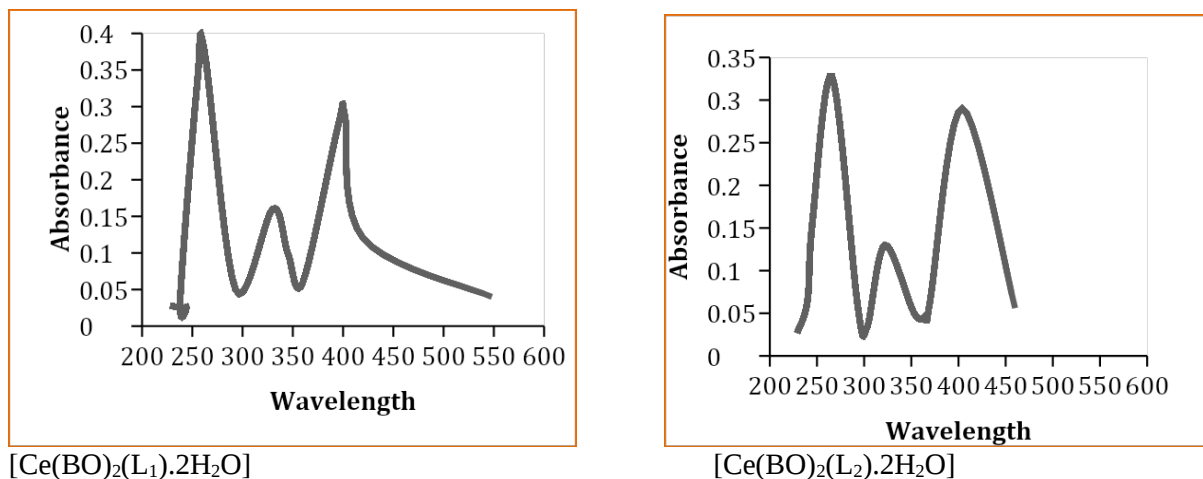


Figure: 2

Infra-Red Spectral details of the complexes:

In the infrared spectra of the compound 2-Hydroxybenzaldehyde oxime (BO), the broad band observed at 3550 cm^{-1} is primarily attributed to the presence of the phenolic $-\text{OH}$ group. However, this band is absent in the cerium complexes, indicating the deprotonation of the $-\text{OH}$ group and the bonding of the phenolic oxygen with the cerium metal. Peaks observed in the range of 3220 to 3230 cm^{-1} confirm the presence of the free oxime $-\text{OH}$ group, which is inherent in the primary ligand, 2-Hydroxybenzaldehyde oxime (BO), and remains unaffected by the metal complexation reaction.

The band at 1625 cm^{-1} in the free primary ligand, corresponding to $\text{C}=\text{N}$ vibrations, shifts to a lesser wavenumber in the range of 1590-1587 cm^{-1} in the cerium metal complexes, providing evidence for the bonding of the oxime group of the ligand to cerium metal through the nitrogen atom. In the complexes, weak bands in the range of 1540-1531 cm^{-1} and medium peaks in the range of 3355-3315 cm^{-1} indicate the O-H symmetric stretching vibrations and O-H asymmetric stretching vibrations, respectively. This suggests the presence of coordinated water molecules in the complexes, a finding further supported by the thermal behavior of the complexes.

The strong band at 987 cm^{-1} shifts to a lower wavelength (913-910 cm^{-1}) due to C-O stretching vibrations. The N-H asymmetric and N-H symmetric bonding vibrations, observed in the range of 3095-3065 cm^{-1} and 2990-2985 cm^{-1} in the cerium complexes, are seen at lower wavelengths, around ~3025 cm^{-1} and ~2943 cm^{-1} , respectively, in the secondary ligand, i.e., free amino acids. This shift indicates the bonding of the nitrogen atom of secondary ligands to the cerium metal in the complexes.

In the infrared spectra of cerium complexes, medium bands observed in the range of 410-419 cm^{-1} and 610-594 cm^{-1} are assigned to M-N and M-O vibrations in the complexes, confirming the bonding of cerium metal with oxygen and nitrogen of 2-Hydroxybenzaldehyde oxime and secondary ligand [29-30]. These spectral features provide valuable information about the coordination and bonding patterns within the synthesized cerium metal complexes.

Table No.4 : Infrared spectral data of complexes in cm⁻¹

Sr. No.	Complex	v	v	v	v	v	v	v	v	v	v	v
		(-OH) (Oxime)	(C=N)	(C-O)	(HOH) (Asym)	(HOH) (Sym)	(N-H) (Asym)	(N-H) (Sym)	(COO-) (Asym)	(COO-) (Sym)	(Ce-N)	(Ce-O)
		(BO)	(BO)	(BO)	(H ₂ O)	(H ₂ O)	(L)	(L)	(L)	(L)	(Complex)	(Complex)
1.	[Ce(BO) ₂ (L ₁).2H ₂ O]	3224 (m)	1590 (m)	910 (s)	3348 (m)	1535 (w)	3090 (w)	2988 (w)	1628 (s)	1400 (w)	410 (m)	600 (m)
2.	[Ce(BO) ₂ (L ₂).2H ₂ O]	3230 (m)	1587 (m)	913 (s)	3315 (m)	1540 (w)	3086 (w)	2990 (w)	1628 (s)	1398 (w)	417 (m)	594 (m)
3	[Ce(BO) ₂ (L ₃).2H ₂ O]	3220 (m)	1589 (m)	910 (s)	3355 (m)	1538 (w)	3065 (w)	2985 (w)	1630 (s)	1404 (w)	412 (m)	598 (m)
4	[Ce(BO) ₂ (L ₄).2H ₂ O]	3226 (m)	1590 (m)	912 (s)	3345 (m)	1531 (w)	3078 (w)	2986 (w)	1626 (s)	1402 (w)	419 (m)	610 (m)

L= secondary ligands (Amino acids), m = medium, s = strong, w = weak, BO = 2-Hydroxybenzaldehyde oxime, Ce = cerium

Antibacterial studies:

The antibacterial activity of the metal complexes was assessed based on the zone of inhibition (mm), revealing a clear manifestation of biological activity in cerium complexes. The study showed that cerium complexes exhibit significant sensitivity against bacterial strains, particularly *S. aureus* and *S. typhi*, when compared to *C. diphtheria* and *P. aeruginosa*. Interestingly, the metal complexes demonstrated greater antibacterial activity than the ligands used in their formation [31]. The antibacterial effect of metal chelates can be attributed to their ability to impede bacterial growth. Chelation reduces the polarity of metal ions in the complexes, enhancing the hydrophobic character of the complex compounds. This modification aids in the permeation of the complexes through the lipid layer of microorganisms, including bacteria. The altered characteristics of metal chelates, therefore, contribute to their efficacy in inhibiting bacterial growth [32].

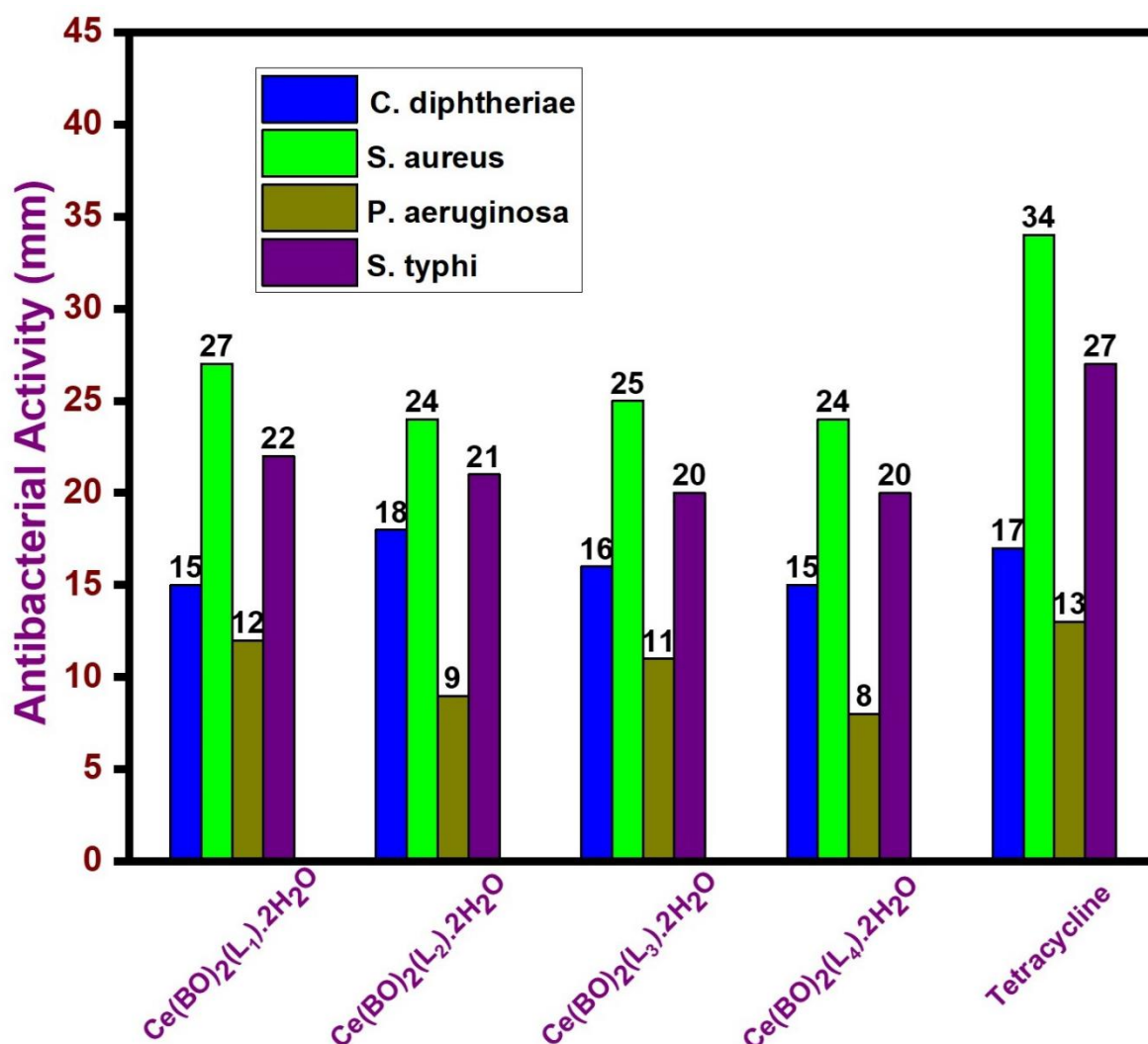
**Figure: 3**

Table 05: MIC ($\mu\text{g/mL}$) Data of Cerium(III) metal Complexes

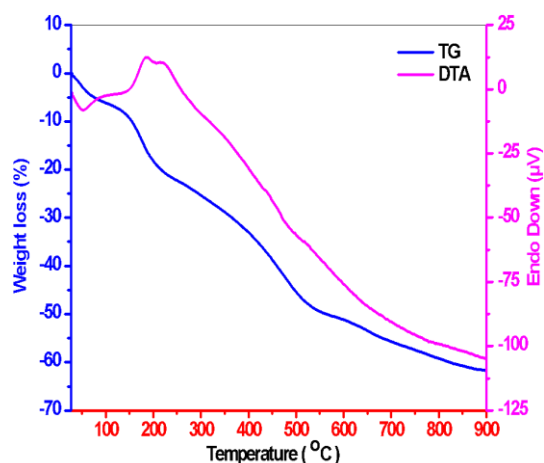
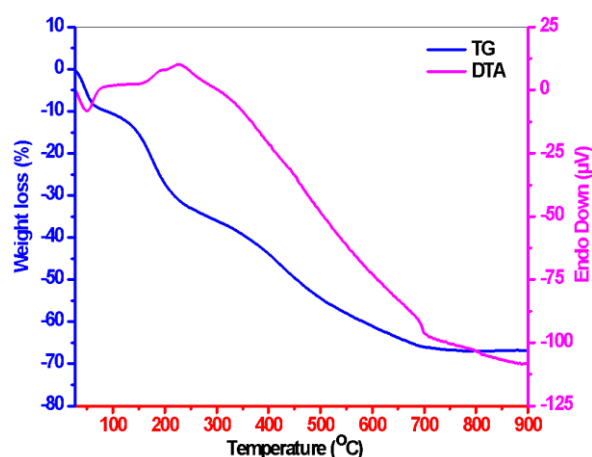
Complex	MIC ($\mu\text{g/mL}$)			
	<i>C. diphtheriae</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
$[\text{Ce}(\text{BO})_2((\text{L}_1)).2\text{H}_2\text{O}]$	30	15	35	15
$[\text{Ce}(\text{BO})_2((\text{L}_2)).2\text{H}_2\text{O}]$	35	10	25	20
$[\text{Ce}(\text{BO})_2((\text{L}_3)).2\text{H}_2\text{O}]$	30	15	30	10
$[\text{Ce}(\text{BO})_2((\text{L}_4)).2\text{H}_2\text{O}]$	35	15	25	15
$\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$	156	80	195	75
2-Hydroxybenzaldehyde oxime	194	100	186	130
Tetracycline	2.0	1.5	8.0	1.5

Thermal behavior of Ce-complexes

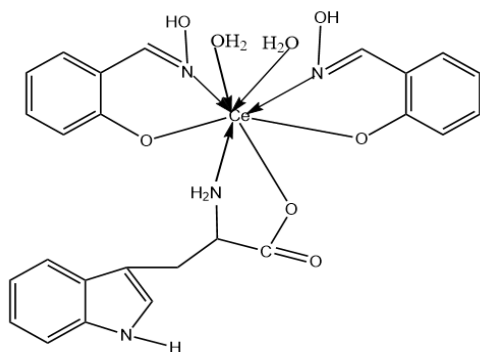
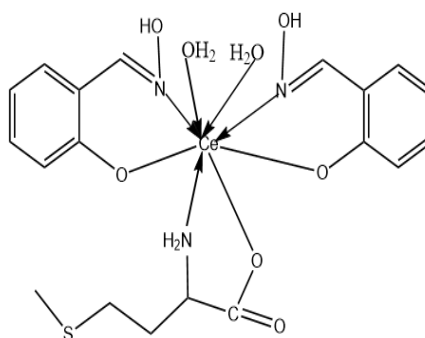
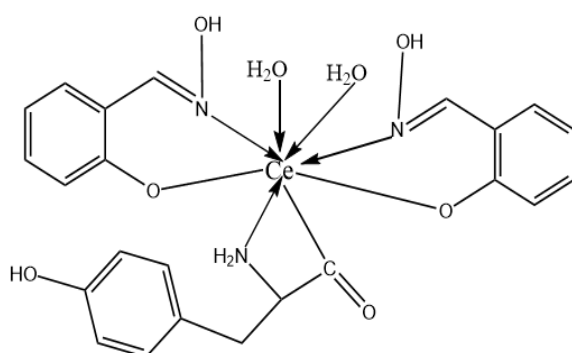
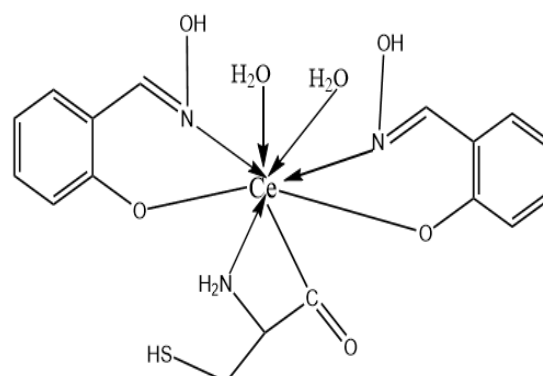
The thermal study of metal chelates was conducted using TG (Thermogravimetry) and DTA (Differential Thermal Analysis) techniques in a controlled atmosphere of N_2 gas with a heating rate of 100°C per minute. All metal complexes were subjected to heating up to 900°C . The thermograms displayed three distinct types of behavior, indicating gradual weight loss with an increase in temperature. Notably, all cerium metal complexes exhibited similar patterns in both TG and DTA analyses. The first weight loss occurred in the temperature range of $30\text{--}130^\circ\text{C}$, attributed to the loss of two coordinated molecules of water. The second weight loss, in the range of $150\text{--}270^\circ\text{C}$, was associated with the loss of secondary ligands (amino acids). The third weight loss, observed in the temperature range of $310\text{--}740^\circ\text{C}$, occurred due to the loss of two molecules of 2-Hydroxybenzaldehyde oxime (BO) from the complex compounds. After 750°C , the thermogram displayed a constant plateau, indicating no further weight loss. The thermogram exhibited a straight line, confirming the completion of the decomposition process of cerium metal complexes at this point. This thermal analysis provides valuable insights into the stability and decomposition behavior of the cerium metal complexes under controlled conditions.

Table No.6 : Thermal analysis of metal complexes :

Complex	Temp. Range ($^\circ\text{C}$)	Loss in Weight due to	% Weight Loss	
			Found	Calculated
$[\text{Ce}(\text{BO})_2((\text{L}_1)).2\text{H}_2\text{O}]$	30-130	Two water molecules	5.50	5.52
	152-250	Secondary ligand	31.16	31.19
	320-710	Two BO molecules	41.72	41.78
$[\text{Ce}(\text{BO})_2((\text{L}_2)).2\text{H}_2\text{O}]$	30-126	Two water molecules	6.01	6.03
	150-260	Secondary ligand	24.79	24.84
	325-720	Two BO molecules	45.61	45.64
$[\text{Ce}(\text{BO})_2((\text{L}_3)).2\text{H}_2\text{O}]$	30-128	Two water molecules	5.71	5.72
	156-257	Secondary ligand	28.62	28.66
	310-740	Two BO molecules	43.30	43.32
$[\text{Ce}(\text{BO})_2((\text{L}_4)).2\text{H}_2\text{O}]$	30-124	Two water molecules	6.32	6.33
	152-270	Amino acid	21.11	21.13
	340-730	Two SO molecules	47.86	47.89

[Ce(BO)₂(L₁).2H₂O][Ce(BO)₂(L₂).2H₂O]**Figure: 3**

Structure of Ce(III) mixed ligand complexes are as following

[Ce(BO)₂(L₁).2H₂O][Ce(BO)₂(L₂).2H₂O][Ce(BO)₂(L₃).2H₂O][Ce(BO)₂(L₄).2H₂O]

Conclusion

In conclusion, the successful preparation and characterization of cerium metal mixed ligand complexes utilizing ligands with N,O donor atoms have been achieved. The dark brown color and stability of the complexes at room temperature underscore their potential for practical applications. The low molar conductance values indicate the non-electrolyte nature of the cerium complexes. UV studies further support the formation of the complexes, revealing intra-ligand and charge transfer transitions. The bonding mechanism involving N- and O-donor atoms from both primary and secondary ligands establishes a coordinated interaction with the cerium metal ion. The high decomposition temperatures observed in thermogravimetric analysis indicate strong bonding between ligand molecules and the cerium metal, emphasizing the stability of the

complexes. The thermogravimetric study also suggests the presence of ligand molecules and two coordinated water molecules, implying an octahedral coordination for the metal complexes. Notably, the antibacterial study employing Tube dilution and Agar cup methods demonstrates the antibacterial activity of the complexes against selected bacterial strains. The complexes exhibit greater efficacy against *S. typhi* and *S. aureus* compared to *C. diphtheria* and *P. aeruginosa*, indicating potential applications in targeting specific pathogenic microorganisms. Overall, this research contributes valuable insights into the synthesis, characterization, and antibacterial properties of cerium metal mixed ligand complexes, paving the way for further exploration of their applications in diverse fields.

Acknowledgement

The corresponding author, **Digambar K. Patil**, expresses gratitude to Changu Kana Thakur Arts, Commerce & Science College, New Panvel (Autonomous) Navi Mumbai, for their support and provision of instrumental and laboratory facilities.

Conflict of interest

The authors declare that they have no conflict of interest.

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