INTRODUCTION

The literature survey revealed that mixed ligand complexes of some transition metals with amino acids have been studied for their synthesis, spectral and biological importance [1], like antibacterial [2-4], antimicrobial [5,6], antituberculosis assay [7] and as a potential therapeutic agents [8]. The mixed ligand complexes of 1-nitroso-2-naphthol with transition and inner transition metals have been reported [9-11]. Similarly, metal complexes of 1-nitroso-2-naphthol and amino acids have been studied for their antibacterial activity [12,13]. The lanthanide inner transition cerium(IV) compounds plays the great important role in water gas-shift reaction [14-16] as well as a catalyst [17-19]. Recently, it has been found that the metal complexes of cerium(III) also possess antibacterial activity [20-22].

Thus, it was considered to study the complexation and to determine the biological activity of new cerium(III) complexes. The present paper includes synthesis and characterization of new mixed ligand ternary complexes of cerium(III), employing 1-nitroso-2-naphthol as a primary ligand and different amino acids such as L-valine, L-serine and L-isoleucine as a secondary ligand and were screened for their antibacterial properties.

EXPERIMENTAL

The AR grade cerium(III) chloride heptahydrate and ligands like 1-nitroso-2-naphthol and different amino acids such as L-valine, L-serine and L-isoleucine was used as such as obtained from S.D.Fine Chemicals, Mumbai. The solvents used ethyl alcohol, N,N-dimethyl formamide and dimethyl sulphoxide were purified by standard procedure [23].

The metal content of the synthesized mixed ligand ternary complexes of cerium(III) was estimated gravimetrically by standard procedure [24]. The electronic absorption spectra of all complexes were recorded in DMF solution (0.001M) on Shimadzu UV/VIS-1800 spectrophotometer. The Thermo Finnigan Elemental Analyzer was used for C, H and N elemental analysis. The conductance measurement study was carried out in DMF (0.001 M) on an Equiptronics Digital Conductivity Meter. Infrared spectra of all metal complexes as well as ligands were recorded on KBr disc on using Shimadzu FT-IR spectrophotometer in the region 4000-400 cm\(^{-1}\). In the controlled nitrogen atmosphere the thermal analysis of the complexes was carried on a Perkin-Elmer Diamond TG-DTA instrument at SAIF, IIT Bombay. Magnetic susceptibility measurements...
for all the complexes were reported at room temperature by the Gouy’s method using calibrant as a mercury tetrathio-
cyanatocobaltalate (II).

Preparation of mixed ligand complexes: The mixed ligand ternary complexes of cerium(III) were prepared from
CeCl₃·7H₂O, 1-nitroso-2-naphthol (1N2N) primary ligand and various amino acids (L-valine, L-serine and L-isoleucine) as
a secondary ligands. 10 mL of 0.001 M aqueous solution of
CeCl₃·7H₂O (0.372 g, 1 mmol) was prepared and warmed. To
a secondary ligands. 10 mL of 0.001 M aqueous solution of
dilute ammonia solution. The solution was cooled at room
temperature and the isolated solid product was filtered, washed
with water two to three times and finally with ethanol and
dried under vacuum.

Antibacterial screening

Agar cup method: The antibacterial activity of the synthesized mixed ligand ternary complexes of cerium(III) was tested
by using an agar cup method [25] against two Gram-positive
and two Gram-negative bacteria namely S. aureus, C. diphtheria,
E. coli and S. Typhi, respectively.

In this method, the sterile nutrient agar along with the
desired bacterial strain was allowed to solidify in a sterile plate
up to 5 mm height. Thereafter, by using sterilized cork borer,
single cup with diameter 8 mm was made by scooping out
agar medium from the middle portion of the plate and was
filled with test solution of 0.001 g/mL concentration. The plate
was kept in refrigerator for 10 min to diffuse the test solution
in surrounding agar and plate was incubated for 24 h at 37 °C.

The zone of inhibition was measured from the edge of the
cup and reported as antibacterial activity of complexes.
The results were compared with tetracycline standard, which
was screened simultaneously.

Tube dilution method: In the present investigation the synthesized mixed ligand ternary complexes of cerium(III)
has been subjected to antibacterial screening skills conducted
in vitro study so as to determine their activity against different
microorganisms such as Staphylococcus aureus, Corynebac-
terium diphtheria, Escherichia coli and Salmonella typhi by
the serial tube dilution system. The stock solution of cerium
complexes in 1 ppm was prepared by using dimethyl sulphoxide. The bacterial inoculums were prepared in Sterilized
Muller Hinton broth and incubated for 4 h at 37 °C. From
these bacterial inoculums 5 mL were spread into a borosilicate
test tube. The test sample solution was added in order to attain
a final concentration as 5 to 1000 µg/mL. Again, 0.1 mL of
inoculums of desired bacteria was added in the tubes. The
tubes were kept on a rotary shaker for 24 h for incubation.
On next day results were observed for the presence or absence
of growth to estimate minimum inhibition concentration (MIC).
The result of synthesized cerium complexes was compared
with the antibacterial activity of standard tetracycline which
was used as a control.

RESULTS AND DISCUSSION

The synthesis reaction of mixed ligand ternary complexes of
cerium(III) can be represented as follows:

\[
\text{CeCl}_3\cdot7\text{H}_2\text{O} + 2(\text{1N2N}) + \text{HL} \rightarrow [\text{Ce}(\text{1N2N})_2\cdot(\text{L})\cdot2\text{H}_2\text{O}] + 3\text{HCl} + 5\text{H}_2\text{O}
\]

where, (1N2N) denotes 1-nitroso-2-naphthol and HL represent
amino acid.

All the complexes are brown in colour, partly soluble in common organic polar aprotic solvents like dimethyl
sulphoxide and N,N-dimethyl formamide and are insoluble in
solvents like acetone, ethyl alcohol, benzene, toluene, methanol etc. These complexes were non-volatile, non-hygrosco-
and thermally stable amorphous solids (Table-1). The complexes
exhibit strong metal-ligand bond.

The cerium complexes were analyzed for carbon, hydrogen
and nitrogen content and the estimated data (Table-2) of
metal complexes are in accordance with their 1:2:1 stoichio-
metric ratios of the type [Ce(1N2N)₂·(L)·2H₂O].

The 0.001 M solution of cerium complexes was prepared in N,N-dimethylformamide and molar conductance values
were measured by using Equiptronics Autoranging Conduc-
tivity Meter. The recorded electrical conductance data (Table-2)
are in the range of 0.000151-0.000162 Mhos cm² mol⁻¹
which indicates nonelecrolyt nature of complexes [21].

<table>
<thead>
<tr>
<th>Complex</th>
<th>Molar conductance (Mhos cm⁻¹ mol⁻¹)</th>
<th>Elemental analysis (%): Found (calcd.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ce(1N2N)₂·(Val)·2H₂O]</td>
<td>0.000162</td>
<td>C (47.17) H (4.12) N (6.60) M (22.01)</td>
</tr>
<tr>
<td>[Ce(1N2N)₂·(Ser)·2H₂O]</td>
<td>0.000151</td>
<td>C (44.23) H (3.55) N (6.73) M (22.40)</td>
</tr>
<tr>
<td>[Ce(1N2N)₂·(Iso)·2H₂O]</td>
<td>0.000162</td>
<td>C (48.00) H (4.34) N (6.46) M (21.51)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complex</th>
<th>EMPIRICAL FORMULA, MOLECULAR WEIGHT, COLOUR, DECOMPOSITION TEMPERATURE AND pH OF CERIUM(III) COMPLEXES</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ce(1N2N)₂·(Val)·2H₂O]</td>
<td>C₉H₆N₃O₈Ce</td>
</tr>
<tr>
<td>[Ce(1N2N)₂·(Ser)·2H₂O]</td>
<td>C₂₃H₂₁N₃O₉Ce</td>
</tr>
<tr>
<td>[Ce(1N2N)₂·(Iso)·2H₂O]</td>
<td>C₂₅H₂₆N₃O₈Ce</td>
</tr>
</tbody>
</table>

1N2N represents deprotonated 1-nitroso-2-naphthol primary ligand, where as Val, Ser, Iso represents secondary ligands valine, serine and isoleucine, respectively.
**Magnetic study:** The calculated magnetic moment values (Table-3) of synthesized mixed ligand ternary complexes of cerium(III) by using magnetic susceptibilities measurements employing diamagnetic corrections were observed in the range of 1.712-1.771 B.M. which indicates their paramagnetic nature [22].

### Table 3: Magnetic Susceptibility Data of Cerium(III) Complexes (10⁻⁶ c.g.s. Units)

<table>
<thead>
<tr>
<th>Complex</th>
<th>χ&lt;sub&gt;b&lt;/sub&gt;</th>
<th>χ&lt;sub&gt;f&lt;/sub&gt;</th>
<th>μ&lt;sub&gt;eff&lt;/sub&gt; (B.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ce(1N2N)₂(Val)-2H₂O]</td>
<td>1.6148</td>
<td>1027.9820</td>
<td>1.712</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Ser)-2H₂O]</td>
<td>1.7660</td>
<td>1102.9553</td>
<td>1.749</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Iso)-2H₂O]</td>
<td>1.6960</td>
<td>1103.4700</td>
<td>1.771</td>
</tr>
</tbody>
</table>

**Electronic absorption spectra:** The electronic spectra of 0.001M solution of synthesized mixed ligand ternary complexes of cerium(III) in DMF were carried out in UV-visible region. The UV-visible spectrum of all cerium complexes show three transitions (Table-4). The high intensity band in the range 263-266 nm (38023-37594 cm⁻¹) assigned to π→π* transitions due to conjugated π system. The low intensity second band was observed at wavelength range 328-334 nm (30488-29940 cm⁻¹) attributed to n→π* transition and the third intensity band at wavelength range 393-405 nm (25445-24691 cm⁻¹) ascribed to L-M charge transfer transitions.

### Table 4: Electronic Spectral Data of Cerium(III) Complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>λ (nm)</th>
<th>ν (cm⁻¹)</th>
<th>Proposed assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ce(1N2N)₂(Val)-2H₂O]</td>
<td>263</td>
<td>38023</td>
<td>π→π*</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Ser)-2H₂O]</td>
<td>329</td>
<td>30395</td>
<td>n→π*</td>
</tr>
<tr>
<td></td>
<td>396</td>
<td>25253</td>
<td>Charge-transfer</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Iso)-2H₂O]</td>
<td>264</td>
<td>37879</td>
<td>π→π*</td>
</tr>
<tr>
<td></td>
<td>334</td>
<td>29940</td>
<td>n→π*</td>
</tr>
<tr>
<td></td>
<td>405</td>
<td>24691</td>
<td>Charge-transfer</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Iso)-2H₂O]</td>
<td>266</td>
<td>37594</td>
<td>π→π*</td>
</tr>
<tr>
<td></td>
<td>328</td>
<td>30488</td>
<td>n→π*</td>
</tr>
<tr>
<td></td>
<td>393</td>
<td>25445</td>
<td>Charge-transfer</td>
</tr>
</tbody>
</table>

**Infrared spectra:** The FT-IR spectrum analysis of the cerium complexes were recorded in potassium bromide disc in range 4000-400 cm⁻¹ and collected data have been summarized in (Table-5).

The broad band around 3435-3431 cm⁻¹ is assigned to asymmetric and symmetric O-H stretching vibrations which imply the presence of coordinated water molecule [26] in the synthesized cerium complexes. This was further authenticated by thermal study.

### Table 5: FT-IR Spectral Data of Cerium(III) Complexes (cm⁻¹)

<table>
<thead>
<tr>
<th>Complex</th>
<th>v(O-H) H₂O</th>
<th>v(N-H) Asym. (A.a.)</th>
<th>v(N-H) Sym. (A.a.)</th>
<th>v(C=O) (A.a.)</th>
<th>v(C-O) (A.a.)</th>
<th>v(C-N) 1N2N</th>
<th>v(N=O) 1N2N</th>
<th>v(C-O) 1N2N</th>
<th>v(C-N) 1N2N</th>
<th>v(C-O)</th>
<th>v(C-N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ce(1N2N)₂(Val)-2H₂O]</td>
<td>3341 b</td>
<td>3122 w</td>
<td>3061 w</td>
<td>1616 s</td>
<td>1400 w</td>
<td>839 m</td>
<td>1554 s</td>
<td>1213 m</td>
<td>1155 w</td>
<td>636 w</td>
<td>524 w</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Ser)-2H₂O]</td>
<td>3429 b</td>
<td>3132 w</td>
<td>3061 w</td>
<td>1616 s</td>
<td>1400 w</td>
<td>839 m</td>
<td>1554 s</td>
<td>1211 m</td>
<td>1155 w</td>
<td>636 w</td>
<td>522 w</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Iso)-2H₂O]</td>
<td>3435 b</td>
<td>3115 w</td>
<td>3061 w</td>
<td>1614 s</td>
<td>1398 w</td>
<td>840 m</td>
<td>1554 s</td>
<td>1211 m</td>
<td>1155 w</td>
<td>638 w</td>
<td>526 w</td>
</tr>
</tbody>
</table>

A.a = Amino acids, 1N2N = 1-nitroso-2-naphthol

The spectrum of cerium complexes shows two characteristic bands around 1616-1614 and 1400-1398 cm⁻¹ which ascribed to asymmetric and symmetric vibrations of the carboxylate groups (COO⁻) of amino acids. However, these asymmetric and symmetric vibration modes of (COO⁻) group of free amino acids were observed in the range 1600-1560 and 1415 cm⁻¹. This indicates coordination between carboxylic acid group of amino acid and cerium metal ion via oxygen atom.

The peaks observed in the range 3650-3200 cm⁻¹ in the spectrum of free amino acid due to O-H stretching vibrations were found absent in the spectra of cerium complexes. This confirms that -OH group of carboxylic acid bonded to metal ion through oxygen atom.

Similarly the band observed in the spectra of cerium complexes in the range of 3132-3115 and 3061 cm⁻¹ for N-H asymmetric and N-H symmetric vibrations. However in the free amino acid N-H asymmetric and N-H symmetric vibration mode was observed at 3040 and 2960 cm⁻¹, respectively.

Also the (C-N) symmetric stretching band observed at 900 cm⁻¹ in the spectra of free amino acids was shifted to lower wave number in the range of 840-839 cm⁻¹ in the spectra of the complexes. This N-H vibration shift to higher wave numbers and C-N stretching frequency mode to lower wave number indicates the coordination between amino group of amino acid with cerium metal ion through nitrogen atom.

The absorption band observed at 3448 cm⁻¹ can be assigned to free O-H group of 1-nitroso-2-naphthol which indicates that OH group of 1-nitroso-2-naphthol moiety undergoes deprotonation to form complexes. The strong v(C=O) band has been reported in the range 1170-1109 cm⁻¹ in silver metal complexes with 1-nitroso-2-naphthol [27]. The strong v(C=O) band in synthesized cerium(III) complexes at 1155 cm⁻¹ indicates that the complex contains 1-nitroso-2-naphthol and confirms the bonding between cerium metal and 1-nitroso-2-naphthol ligand via hydroxyl oxygen atom. The metal complexes with 1-nitroso-2-naphthol predict the variation in position of v(C=O) band.

The observed v(N=O) band at 1527 cm⁻¹ of 1-nitroso-2-naphthol free ligand was shifted to higher wave number 1554 cm⁻¹ in the spectra of synthesized cerium complexes, which indicates that 1-nitroso-2-naphthol coordinated to metal ion via nitrogen atom.

Further, some new bands were observed over the range of 526-522 and 638-636 cm⁻¹ with weak intensity which corresponds to M-N and M-O stretching vibrations, respectively, which were absent in both free ligand, 1-nitroso-2-naphthol and amino acids.

**Thermal studies:** The thermal study of mixed ligand ternary complexes of cerium(III) was recorded in an inert nitrogen atmosphere and gradual weight loss due to decomposition.
of the complex by fragmentation at 10 °C/min heating rate was noted (Table-6). The thermogram curves of synthesized cerium complexes show three different decomposition stages. In the first stage, the weight loss observed in the temperature range of 110-171 °C may be due to the loss of two water molecules. In the second stage, loss in weight in the temperature range 200-281 °C which may be due to decomposition of an amino acid moiety. In the third stage, weight loss observed in the temperature range 305-650 °C confirms decomposition of 1-nitroso-2-naphthol from complexes. The heating was carried out until there was no further loss in weight. Above 650 °C a constant plateau indicates that complexes undergo complete decomposition and forms the metal oxide as final product which was confirmed by X-ray diffraction study [28].

The DTA (differential thermal analysis) curve of all cerium(III) complexes shows a small endothermic peak in the temperature range 102-170 °C which indicates the presence of coordinated water molecules. The DTA curve shows small and broad exothermic peak in the range 200-281 and 305-650 °C due to decomposition of amino acids and 1-nitroso-2-naphthol moieties from cerium complexes.

From the physico-chemical studies the proposed structure and bonding of cerium(III) complexes may be represented as shown in Fig. 1.

**Antibacterial studies:** In the biological evaluation, the antibacterial activity of mixed ligand ternary complexes of cerium(III) was performed using the diffusion agar cup and a tube dilution technique. The cerium complexes indicates a promising biological activity against two Gram-positive and two Gram-negative bacteria namely *S. aureus*, *C. diphtheria*, *E. coli* and *S. typhi*.

The data of agar cup method listed in Table-7 show that the complexes were more sensitive against *S. aureus* and *S. typhi* in comparison with *C. diphtheria* and *E. coli*. The minimum inhibitory concentration (MIC) of the cerium complexes, ligands and cerium salts (Table-8) was found in the range of 0.5-35, 100-150 and 75-150 µg/mL, respectively. Thus complexes are found to be more active against *S. typhi* and *S. aureus* as compared to *C. diphtheria* and *E. coli*. Also the complexes show moderate activity as compared to tetracycline standard control.

### TABLE-6 THERMAL STUDY DATA OF CERIUM(III) COMPLEXES

<table>
<thead>
<tr>
<th>Complex</th>
<th>Temperature range (°C)</th>
<th>Weight loss due to</th>
<th>Loss in weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Found</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Val)·2H₂O]</td>
<td>110-152</td>
<td>Two water molecules</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>200-276</td>
<td>Amino acids</td>
<td>19.32</td>
</tr>
<tr>
<td></td>
<td>320-533</td>
<td>Two 1N2N molecules</td>
<td>54.01</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Ser)·2H₂O]</td>
<td>120-171</td>
<td>Two water molecules</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>205-281</td>
<td>Amino acids</td>
<td>17.32</td>
</tr>
<tr>
<td></td>
<td>343-650</td>
<td>Two 1N2N molecules</td>
<td>55.34</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Iso)·2H₂O]</td>
<td>115-148</td>
<td>Two water molecules</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>200-267</td>
<td>Amino acids</td>
<td>20.66</td>
</tr>
<tr>
<td></td>
<td>305-600</td>
<td>Two 1N2N molecules</td>
<td>52.0</td>
</tr>
</tbody>
</table>

### TABLE-7 ANTIBACTERIAL ACTIVITY (mm) OF CEIUM(III) COMPLEXES BY AGAR CUP METHOD

<table>
<thead>
<tr>
<th>Complex</th>
<th><em>S. aureus</em></th>
<th><em>C. diphtheriae</em></th>
<th><em>E. coli</em></th>
<th><em>S. typhi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ce(1N2N)₂(Val)·2H₂O]</td>
<td>26</td>
<td>15</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Ser)·2H₂O]</td>
<td>24</td>
<td>12</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Iso)·2H₂O]</td>
<td>26</td>
<td>15</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>32</td>
<td>15</td>
<td>15</td>
<td>26</td>
</tr>
</tbody>
</table>

### TABLE-8 MIC (µg/mL) DATA OF CERIUM(III) COMPLEXES

<table>
<thead>
<tr>
<th>Complex</th>
<th><em>S. aureus</em></th>
<th><em>C. diphtheriae</em></th>
<th><em>E. coli</em></th>
<th><em>S. typhi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ce(1N2N)₂(Val)·2H₂O]</td>
<td>15</td>
<td>30</td>
<td>25</td>
<td>05</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Ser)·2H₂O]</td>
<td>10</td>
<td>30</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>[Ce(1N2N)₂(Iso)·2H₂O]</td>
<td>10</td>
<td>35</td>
<td>30</td>
<td>05</td>
</tr>
<tr>
<td>1-Nitroso-2-naphthol</td>
<td>75</td>
<td>200</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>CeCl₃·7H₂O</td>
<td>75</td>
<td>150</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1.5</td>
<td>2.0</td>
<td>4.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Fig. 1. Proposed structures of (a) [Ce(1N2N)₂(Val)·2H₂O], (b) [Ce(1N2N)₂(Ser)·2H₂O], (c) [Ce(1N2N)₂(Iso)·2H₂O]
This indicates that metal complexes exhibit higher antibacterial activity than metal salts and free ligands. The antibacterial activity of metal complexes is enhanced due to chelate effect of ligands which increases the hydrophobic nature that permits the diffusion of the complex through the lipid layer of microorganisms [29].

Conclusion

The FT-IR spectral analysis of the complexes confirms the metal-ligand bonding through nitrogen and oxygen donor atoms of both ligands. High decomposition temperature in thermal study indicates strong metal-ligand bonding and the presence of a coordinated water molecules in the complexes. The complexes are non-electrolytic and paramagnetic in nature which was confirmed by electrical conductivity and magnetic susceptibility measurement study. All complexes show higher antibacterial activity than free ligands and metal salts. The coordination number of the proposed structure is eight.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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