A large number of uranium and thorium complexes and their mixed chelates have been reported (1-7). Dioxouranium(VI), UO$_2^{2+}$, is one of the most studied oxocations for which a large number of complexes with varying geometries are possible (8). The high charge on thorium(IV) along with its effective ionic size enables Th(IV) to form complexes with higher coordination numbers (9). The coordination numbers ranging from 7 to 12 for metal chelates of Th(IV) and UO$_2$(VI) have been reported (10, 11). It has been found that a majority of metal complexes of 8-hydroxyquinoline possess biological activity (12). Ternary complexes containing an amino acid as a secondary ligand have a significance as they are potential models for enzyme metal ion substrate complexes (13). Antimicrobial (14-18) and cytotoxic (19-21) studies on some mixed ligand complexes have been reported. Recently, it was stated that the Th(IV) and UO$_2$(VI) complexes show antimicrobial (22-24) and cytotoxic (25) activity.

In view of the above, the present paper reports synthesis and characterization of mixed ligand dioxouranium(VI) and thorium(IV) complexes of 8-hydroxyquinoline as a primary ligand and amino acids such as L-proline (Pro) and 4-hydroxy-L-proline (Hyp) as secondary ligands. These complexes have been screened for their antibacterial and cytotoxic (IC$_{50}$) characteristic properties.

**EXPERIMENTAL**

**Materials**

Analytical grade thorium nitrate pentahydrate and uranyl nitrate hexahydrate were used as received. 8-Hydroxyquinoline, and the solvents, N, N-dimethyl formamide and dimethyl sulfoxide were purchased from E. Merck, whereas L-proline and 4-hydroxy-L-proline were purchased from S.D. Fine Chemicals, Mumbai, India. Laboratory grade chemicals whenever used were purified by standard procedures (26).
Preparation of mixed ligand complexes

Mixed ligand dioxouranium(VI) and thorium (IV) complexes were prepared as follows. To an aqueous solution (10 mL) of uranyl nitrate hexahydrate (502 mg, 1 mmol) ethanolic solution (10 mL) of 8-hydroxyquinoline (145 mg, 1 mmol) was added. The mixture was stirred and kept in a boiling water bath for 10 min. To this hot solution an aqueous solution (10 mL) of amino acids (1 mmol) was added with constant stirring. The mixture was again heated in a water bath. The complexes were obtained by raising pH of the reaction mixture by adding diluted ammonia 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.

The thorium complexes were prepared in a similar way by taking 1:2:1 proportion of a metal salt, primary ligand and a secondary ligand, respectively.

Instrumentation

The complexes were analyzed for C, H, and N contents on Thermo Finnigan elemental analyzer, Model No. FLASH EA 1112 Series at SAIF, I.I.T. Mumbai. Metal content was estimated gravimetrically by standard procedure (27). The molar conductance values were measured in DMF (10⁻¹ M) on an Equiptronics digital conductivity meter Model No.EQ-DCM-P. Room temperature magnetic susceptibilities were measured by a Guoy balance using Hg[Co(SCN)₄] as a calibrant. Electronic absorption spectra in DMF (10⁻³ M) in the UV-VIS range were measured on a Shimadzu UV-160A and Spectronic-20 spectrophotometers. FT-IR spectra were recorded in KBr discs on a Perkin-Elmer FT-IR spectrophotometer model 160. Thermal studies of the complexes were made on a Perkin-Elmer Diamond TG-DTA instrument at SAIF, I.I.T. Mumbai, by recording the change in weight of the complexes on increasing temperature up to 900°C in the nitrogen atmosphere at the heating rate of 10°C per min.

Antibacterial screening

The antibacterial activity of the complexes was assayed against the bacteria, *Staphylococcus aureus* and *Escherichia coli* by tube dilution method (28). The solvent used was dimethyl sulfoxide (DMSO) and sample concentrations from 1 to 200 μg/mL were used.

Tube dilution method

Bacterial inoculum was prepared in sterilized Mueller Hinton broth and incubated for 4 h at 37°C. Opacity was adjusted to 10⁷ organisms/mL by Macfarland standard containing 0.1 mL of 1% aqueous BaCl₂ and 9.9 mL of 1% dil. H₂SO₄.

The complexes were dissolved in DMSO (1 mg/mL) and added into 5 mL of broth to give final concentration ranging from 1 to 200 μg/mL. To this solution 0.1 mL of inoculum of respective bacteria was added. The tubes were then kept on a rotary shaker and incubated at 37°C for 24 h. On the next day the results were observed for the presence or absence of bacterial growth to determine Minimum Inhibition Concentration (MIC). The lowest concentration at which there was no visible growth was taken as MIC.

Cytotoxicity studies

*In vitro* cytotoxicity (IC₅₀) study on the representative complexes, [Th(Q)₂(Hyp)NO₃H₂O] and [UO₂(Q)(Hyp)₂H₂O] was carried out at Amala Cancer Research Centre, Thrissure, Kerala, on established tumor cell lines Ehrlich ascites cells and Dalton’s lymphoma ascites cells, respectively.

The Ehrlich ascites cells were originally procured from Cancer Institute, Mumbai and Dalton’s lymphoma ascites cells from Cancer Institute, Adayar, Chennai. For the experiment, these cells were aspirated, washed with phosphate buffered saline and made up to a concentration of 1 million/mL.

Different concentrations of the complexes (5 to 100 μg) in DMSO (20 μL) were incubated with 1 million cells in a total volume of 1 mL with phosphate buffered saline (PBS) used as a control in the experiment. Cells were incubated at 37°C for 3 h.

After incubation 0.1 mL of 1% trypan blue was added and cytotoxicity was determined by counting live and dead cells using Haemocytometer.

RESULTS AND DISCUSSION

Characterization of metal complexes

The synthesis of mixed ligand uranyl and thorium complexes may be represented as follows:

\[
\text{UO}_2(\text{NO}_3)_2\cdot 6\text{H}_2\text{O} + \text{HQ} + \text{HL} \rightarrow [\text{UO}_2(\text{Q})(\text{L})\cdot 2\text{H}_2\text{O}] + 2 \text{HNO}_3 + 4 \text{H}_2\text{O}
\]

\[
\text{Th(NO}_3)_4\cdot 5\text{H}_2\text{O} + 2 \text{HQ} + \text{HL} \rightarrow [\text{Th(Q)}_2(\text{L})\text{NO}_3\cdot \text{H}_2\text{O}] + 3 \text{HNO}_3 + 4 \text{H}_2\text{O}
\]

(where HQ is 8-hydroxyquinoline and HL is an amino acid).

All the complexes are colored, non-hygroscopic and thermally stable solids (Table 1), indicating a strong metal-ligand bond. The complexes are insoluble in common organic solvents such as ethyl alco-
hol, acetone, chloroform etc. but are partially soluble in DMF and DMSO. The elemental analysis data (Table 1) of uranyl and thorium complexes are consistent with their general formulation as 1:1:1 and 1:2:1 mixed ligand complexes of the type \[\text{[Th(Q)2(L)NO3]}\sum\text{H2O}\] and \[\text{[UO2(Q)(L)}\sum\text{2H2O]}\]. The molar conductance values of the complexes in DMF at 10^{-3} M concentration are found to be (0.007-0.065) Mhos cm^{-2} mol^{-1}, indicating their non-electrolytic nature (29).

Magnetic studies

The magnetic moments of the complexes were calculated from the measured magnetic susceptibilities after employing diamagnetic corrections and revealed diamagnetic nature of the complexes (30).

Electronic absorption spectra

The electronic spectra of the metal complexes in DMF were recorded in the UV-visible region. The spectra show three transitions in the range 37037-36363 cm^{-1}, 30303-29411 cm^{-1} and 27027-26315 cm^{-1} ascribed to \(\pi \rightarrow \pi^*\), \(n \rightarrow \pi^*\) and the charge transfer transitions from the ligands to the metal, respectively.

Infra-red spectra

The FT-IR spectra of the metal complexes were recorded as KBr discs over the range 4000-400 cm^{-1}. On the basis of the reported infra-red spectra of amino acids, 8-hydroxyquinoline and their metal complexes (31-33), some of the important bands have been assigned.

1. The \(\nu_{\text{asym}}(\text{COO}^-)\) band of free amino acid i.e. 1610-1590 cm^{-1} is shifted to lower wave number in the range 1570-1566 cm^{-1} and \(\nu_{\text{sym}}(\text{COO}^-)\) mode observed at ~1400 cm^{-1} in the spectra of free amino

<table>
<thead>
<tr>
<th>Complex</th>
<th>Temp. range (°C)</th>
<th>% Weight loss</th>
<th>Decomposition product</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\text{[Th(Q)2(Pro)NO3]}]</td>
<td>130-200</td>
<td>2.50</td>
<td>2.52</td>
</tr>
<tr>
<td>[\text{[Th(Q)2(Pro)NO3H2O]}]</td>
<td>220-350</td>
<td>15.35</td>
<td>15.96</td>
</tr>
<tr>
<td>[\text{[Th(Q)2(Hyp)NO3]}]</td>
<td>140-200</td>
<td>2.50</td>
<td>2.46</td>
</tr>
<tr>
<td>[\text{[Th(Q)2(Hyp)NO3H2O]}]</td>
<td>230-350</td>
<td>17.14</td>
<td>17.80</td>
</tr>
<tr>
<td>[\text{[UO2(Q)(Pro)2H2O]}]</td>
<td>170-240</td>
<td>5.33</td>
<td>6.38</td>
</tr>
<tr>
<td>[\text{[UO2(Q)(Hyp)2H2O]}]</td>
<td>140-230</td>
<td>5.25</td>
<td>6.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complex</th>
<th>Antibacterial activity (MIC µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\text{Th(NO3)4H2O}]</td>
<td>S.aureus 100  E.coli 200</td>
</tr>
<tr>
<td>[\text{UO2(NO3)2H2O}]</td>
<td>S.aureus 50  E.coli 100</td>
</tr>
<tr>
<td>8-Hydroxyquinoline</td>
<td>S.aureus 50  E.coli 100</td>
</tr>
<tr>
<td>[\text{[Th(Q)2(Pro)NO3H2O]}]</td>
<td>S.aureus 20  E.coli 40</td>
</tr>
<tr>
<td>[\text{[Th(Q)2(Hyp)NO3H2O]}]</td>
<td>S.aureus 20  E.coli 40</td>
</tr>
<tr>
<td>[\text{[UO2(Q)(Pro)2H2O]}]</td>
<td>S.aureus 05  E.coli 10</td>
</tr>
<tr>
<td>[\text{[UO2(Q)(Hyp)2H2O]}]</td>
<td>S.aureus 05  E.coli 10</td>
</tr>
</tbody>
</table>
acids is also shifted to lower wave numbers i.e. 1380-1378 cm⁻¹ in the spectra of complexes indicating the co-ordination of carboxylic acid group via oxygen with metal ion (31). Broad bands in the region 3130-3030 cm⁻¹ due to NH₂⁺ band of free amino acid moiety is shifted to higher wave numbers as N-H stretching vibrations i.e. in the range 3240-3047 cm⁻¹, respectively, in the spectra of metal complexes, suggesting coordination of the imino group through nitrogen with the metal ion (32). A band observed at 3280 cm⁻¹ due to the O-H stretching vibration of hydroxyl group of 4-hydroxy-L-proline could not be interpreted due to overlapping with the bands of coordinated water molecule. Hence, both the ligands coordinate as bidentate ligands (33).

2. An important feature of infra-red spectra of metal complexes with 8-HQ is the absence of the band at ~3440 cm⁻¹ due to the O-H stretching vibration of the OH group of HQ (34). This observation leads to the conclusion that the complex formation takes place by deprotonation of the hydroxyl group of HQ moiety through nitrogen with the metal ion (32). A band observed at 3280 cm⁻¹ due to the O-H stretching vibration of hydroxyl group of 4-hydroxy-L-proline could not be interpreted due to overlapping with the bands of coordinated water molecule. Hence, both the ligands coordinate as bidentate ligands (33).

2. An important feature of infra-red spectra of metal complexes with 8-HQ is the absence of the band at ~3440 cm⁻¹ due to the O-H stretching vibration of hydroxyl group of HQ moiety (34). This observation leads to the conclusion that the complex formation takes place by deprotonation of the hydroxyl group of HQ moiety through nitrogen with the metal ion (32). A band observed at 3280 cm⁻¹ due to the O-H stretching vibration of hydroxyl group of 4-hydroxy-L-proline could not be interpreted due to overlapping with the bands of coordinated water molecule. Hence, both the ligands coordinate as bidentate ligands (33).

3. A broad band observed in the region between 3565-3400 cm⁻¹ due to asymmetric and symmetric O-H stretching modes and a band in the range 1641-1630 cm⁻¹ due to H-O-H bending vibrations indicate the presence of a coordinated water molecule (35), further confirmed by thermal studies.

4. Charles et al. (36) reported that for several metal complexes with HQ, the ν(C-O) band is observed at ~1120 cm⁻¹. The position of this band varies depending on metal complex under study. A strong ν(C-O) band observed at ~ 1104 cm⁻¹ indicates the presence of oxime moiety in the complexes coordinated through its nitrogen and oxygen atoms as uninegative bidentate ligand (37). The ν(C=N) mode observed at 1580 cm⁻¹ in the spectra of free HQ ligand is found to be shifted to lower wave number i.e. ~1500-1497 cm⁻¹ in the spectra of complexes. A negative shift in this vibrational mode on complexation indicates the coordination through the tertiary nitrogen donor of HQ.

5. The FT-IR spectra of the Th(IV) complexes show no absorption bands near 1352 cm⁻¹, where ionic nitrate is known to absorb (38), indicating absence of ionic nitrate. Other bands observed at ~1463, ~1274, ~1035, and ~734 cm⁻¹ corresponding to ν₁, ν₂, ν₃, and ν₅ vibrations agree with frequencies reported for bidentate nitrate group (39, 40). The separation of highest frequency bands ν₁ and ν₄ (186-140 cm⁻¹) in the complexes favors bidentate character of the nitrate group (41).

6. The FT-IR spectra of UO₂(VI) complexes show strong absorption bands near 1352 cm⁻¹, where ionic nitrate is known to absorb (38), indicating absence of ionic nitrate. Other bands observed at ~1463, ~1274, ~1035, and ~734 cm⁻¹ corresponding to ν₁, ν₂, ν₃, and ν₅ vibrations agree with frequencies reported for bidentate nitrate group (39, 40). The separation of highest frequency bands ν₁ and ν₄ (186-140 cm⁻¹) in the complexes favors bidentate character of the nitrate group (41).
tively (44, 45). It may be noted that, these vibrational bands are absent in the spectra of the ligands.

**Thermal studies**

The TG and DTA studies of the complexes have been recorded in the nitrogen atmosphere at the constant heating rate of 10°C/min.

The TG of both the complexes indicate that they are thermally quite stable to varying degree. The complexes show gradual loss in weight due to decomposition by fragmentation with increasing temperature as presented in Table 2. The thermogram of thorium complexes show the loss in weight corresponding to one water molecule in the temperature range ambient to ~200°C, followed by weight loss due to amino acid moiety in the range 220-350°C. The final step of the decomposition observed in the range 380-760°C corresponds to the weight loss of nitrate as well as HQ moieties present in the complexes.

The thermograms of uranyl complexes indicate that they are stable up to 130°C, above which they loose two water molecules in a single step. The dehydrated products are stable up to 275°C, above which second step involving loss of a ligand begins. The third step involving loss of a ligand begins around 400°C and is completed at around 700°C. However, the steps corresponding to loss in weight due to individual ligand moieties could not be unambiguously resolved due to complexity of the reaction.

The DTA of the thorium complexes display an endothermic peak in the range 140-170°C, which indicates the presence of coordinated water molecule. As the temperature is raised, the DTA curve shows a small exotherm in the temperature range 220-350°C, and a broad exotherm in the range 380-760°C attributed to decomposition of amino acid moiety, and nitrate along with 8-hydroxyquinoline moieties present in the complexes. Like most of the metal organic complexes, these complexes also decompose to a fine powder of metal oxide i.e. ThO₂ confirmed by the X-ray diffraction pattern of the decomposed product.

The DTA of the uranyl complexes display an endothermic peak in the range 140-240°C, which indicates the presence of coordinated water molecules. As the temperature is raised, the DTA curves show a small exotherm in the range 350-380°C and 390-550°C which could be attributed to the partial oxidation of UO₂ formed by the decomposition of the complexes, as well as the oxidation of the ligand moieties detached from the complexes in the presence of traces of oxygen present in the nitrogen gas. The X-ray diffraction pattern of the decomposed product confirmed the formation of a mixture of uranium oxides as UO₂ and U₂O₈, hence the steps corresponding to loss in weight due to individual ligand moieties could not be resolved.

On the basis of the physico-chemical studies, the bonding and structure for the metal complexes may be represented as shown in Figure 1.

**Antibacterial studies**

The minimum inhibition concentrations (MIC) of the metal salts, 8-hydroxyquinoline, metal complexes and standard antibacterial compound tetracycline against Staphylococcus aureus and Escherichia coli studied by tube dilution method are recorded in Table 3. It has been observed that the amino acids used, L-proline and 4-hydroxy-L-proline do not show antibacterial activity. The data show the enhancement in activity of the complexes due to chelation (46), as compared to the activity of metal salts and free ligand. Dioxouranium complexes show higher activity as compared to thorium complexes. The complexes are more active against Staphylococcus aureus. Compared to standard antibacterial compound, tetracycline, the present complexes show lower activity against selected strains of microorganisms.

**Cytotoxicity (IC₅₀) studies**

The results of the in vitro cytotoxicity studies on the representative complexes [Th(Q)₂(Hyp)NO₃ ∑H₂O] and [UO₂(Q)(Hyp)₂H₂O] are recorded in Table 4. The concentration which is sufficient to reduce the cell number by 50% (IC₅₀) were calculated from dose-response curves (47). The IC₅₀ values of dioxouranium and thorium complexes along with standard cytotoxic compound, curcumin, are recorded in Table 5. The results suggest that dioxouranium complexes show higher cytotoxic activity than thorium complexes at equivalent concentrations against Ehrlich ascites cells and Dalton’s lymphoma ascites cells. IC₅₀ values of the complexes when compared with the reported IC₅₀ values of standard cytotoxic compound curcumin (48), indicate very low cytotoxic activity of thorium complex but moderate cytotoxic activity of dioxouranium complex.

**CONCLUSIONS.**

The analytical data of the complexes confirm the stoichiometry of dioxouranium and thorium complexes to be 1:1:1 and 1:2:1, respectively.

High thermal stability of the complexes indicate a strong metal-ligand bond. Electrical conductance studies show non-electrolytic nature of the complexes. Magnetic studies indicate diamagnetic nature of the
complexes. IR spectra show bonding of the metal ion through N- and O- donor atoms of the two ligands. Thermal analysis confirms the presence of the coordinated water molecules. On the basis of above results a coordination number eight and nine are proposed for uranium and thorium complexes, respectively.

The antibacterial studies indicate that chelation enhances antibacterial activity of the complexes against selected strains of *S.aureus* and *E.coli*. Compared to tetracycline the present complexes show lower activity. The cytotoxicity (IC_{50}) studies on the representative complexes indicate that dioxouranium complexes show better cytotoxicity than thorium complexes.

**Acknowledgement**

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