Research Paper

Synthesis and Characterization of Bioactive Transition Metal Complexes of Zr(IV) and Th(IV) using natural sources

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Abstract: Cardanol, a naturally occurring aliphatic side chain substituted phenol derived from cashew nut shell liquid (CNSL) was used for the preparation of Schiff base ligand using Glutamine. The ligand formed Schiff base complexes with Zr(IV) and Th(IV) ions. The ligand and complexes were characterized by UV-visible, IR, and the elemental analysis, melting point, metal ion intake, SEM, XRD antibacterial, antifungal, anti-inflammatory and DNA cleavage were studied. The result indicate that the complexes of Zr(IV) nd Th(IV)) are hexacoordinated and have moderate antibacterial and antifungal activity. The metal ion intake indicated the ligand can be used for the removal of these metals from water. The SEM and XRD studies revealed the nano crystalline nature of the complexes.

Keywords: Cardanol, Formaldehyde, Epichlorohydrin, Glutamine, Schiff base.

Introduction
Schiff base complexes of transition metals are highly useful due to their structure and physico-chemical properties. Cardanol is the main component obtained by vacuum distillation of roasted cashew nut shell liquid (CNSL) and was used for the preparation of bioactive transition Schiff base metal complexes. The preparation involves (i) conversion of cardanol into bis(3-pentadecenyl phenol)methane (BPPM) (ii) conversion of BPPM to DFMPM (iii) condensation of DFMPM with glutamine to give Schiff base ligand and finally (iv) Schiff base complexes with transition metal salts. The ligand and complexes were characterised by UV-visible, FTIR and the elemental analysis, melting point, conductivity, metal ion intake, anti bacterial, anti fungal, anti inflammatory and DNA cleavage activity were studied[1-3]. The result indicate that the complexes of Zr(IV) and Th(IV) were bioactive and also used for the removal of such ions from water the nano crystalline nature of complexes were confirmed by SEM and XRD studies. Antibacterial, antifungal, anti inflammatory and DNA cleavage study indicates the complexes are potential agents.

Material and Methods
Cardanol was obtained from M/S Sathya Cashew Chennai India, formaldehyde (37% solution), hydrochloric acid, epichlorohydrin, L-glutamine, sodium hydroxide and other chemicals were used of GR/AR grade quality obtained from Merck chemicals. All the solvents used were purified by standard methods[4]. The micro analytical data (C, H, N) were collected using Perkin Elmer 2400 instrument. The metal ion intake were estimated by standard methods[5] IR spectra were obtained by using PEIR spectrum instrument Model : 2000. SEM, XRD, anti bacteria, anti fungal, anti inflammatory and DNA cleavage were studied by standard methods.

Synthesis of Schiff base ligand with DFMPM and L-glutamine
The Schiff base ligand was prepared by the reported methods[6-7]. Equimolar ethanolic solution of DFMPM
and L-glutamine were mixed and refluxed for about an hour. Pour the reaction product in ice, (1+2) Schiff base ligand was obtained. The precipitated yellow compound was filtered washed with water and dried over anhydrous calcium chloride. The crude sample was recrystallised from 50% absolute alcohol yield=62%. Melting point =223°C.

Synthesis of Zr(IV) and Th(IV) Schiff base metal complexes
All the metal complexes were prepared by mixing ethanolic solution of Schiff base ligand with the corresponding aqueous metal salt solution of Zr(IV) nitrate, and Th(IV) nitrate in 2:1 molar ratio. The resulting mixture was refluxed for about twelve hours at 70-80°C. The complexes were filtered, washed with ethanol, diethyl ether, acetone and hot water and finally dried under vaccum at 90°C. yield=60%

Estimation of metal ion intake
The filtrates obtained in the above method were collected. The collections were used for the estimation of Zr(IV) and Th(IV) intake for complexation using standard methods.

Results and Discussion
The metal complexes of Zr(IV) and Th(IV) are coloured solids, stable towards air and have high melting points above (250°C). The complexes are insoluble in water and common organic solvents but are soluble in DMF, CDCl₃, and DMSO. Analytical data (Table 1) suggest that the metal to ligand ratio in all the complexes to be 1:2. Conductivities of solutions of the complexes are non electrolytes because their conductivity value were in the range 12-15 ohm⁻¹ cm⁻¹ mol⁻¹. However the conductivity value is higher than expected for non electrolytes probably due to partial solvolysis of the complexes in DMF medium.

Table 1: Physical characteristics and analytical data of complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>yield</th>
<th>Colour</th>
<th>molecular formula</th>
<th>Molecular Weight</th>
<th>Melting point</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand(L)</td>
<td>60</td>
<td>Brown</td>
<td>C₅₇H₁₀₀N₂O₈</td>
<td>968</td>
<td>229</td>
<td>C:6.51 H:1.26 N:10.05</td>
</tr>
<tr>
<td>[ZrL(NO₃)₂]·2H₂O</td>
<td>57</td>
<td>Light green</td>
<td>C₁₁₄H₃₄N₆O₂₄Zr</td>
<td>2131</td>
<td>&gt;250</td>
<td>Zr:10.10</td>
</tr>
<tr>
<td>[ThL(NO₃)₂]·2H₂O</td>
<td>56</td>
<td>Grey</td>
<td>C₁₁₄H₃₂N₆O₂₃Th</td>
<td>2272</td>
<td>&gt;250</td>
<td>Th:10.10</td>
</tr>
</tbody>
</table>

IR Spectra
IR spectra of the complexes were compared with the free ligand in order to determine the involvement of co-ordination sites in chelation. Characteristic peaks in the spectra of the ligand and complexes were considered and compared. The selected IR spectral data are given in (Table 2). The IR spectrum of the ligand (Fig.1) showed characteristic bands at 2856 cm⁻¹, 2923 cm⁻¹, 1606 cm⁻¹, 1489 cm⁻¹, 1455.02 cm⁻¹, 3013 cm⁻¹, 780 cm⁻¹, 702 cm⁻¹, due to the νC=O, νC=N, νC=O, νCOOH, νN=H₂, νM=N, νM=O, respectively. The IR spectra of the complexes (Figure 2-4) exhibited ligand bands with the appropriate shifts due to complex formation. The IR broad bands of metal complexes in the range of 3505-3486 cm⁻¹ indicate the presence of co-ordinated or lattice water molecule. The νC=O phenolic stretching frequency is observed around 2854-2852 cm⁻¹ which get shifted to lower or higher frequency region indicating co-ordination of phenolic oxygen. Bands at 2923-2922 cm⁻¹ and 1610-1590 cm⁻¹ were assigned to C-H and C=N respectively. The imine peaks in the metal complexes showed changes in the ligand indicating co-ordination of the imine nitrogen atom to the metal ion due to co-ordination.

Table 2: Selected FT-IR frequencies (cm⁻¹) and UV of the ligand and complexes

<table>
<thead>
<tr>
<th>Ligand/ Complexes</th>
<th>ν₀-Η</th>
<th>ν₀-C</th>
<th>ν₀-H</th>
<th>ν₀-N</th>
<th>ν₀-O</th>
<th>ν₀-CO</th>
<th>ν₀-CO₂</th>
<th>ν₀-CO₂</th>
<th>ν₀-CO₂</th>
<th>ν₀-CO₂</th>
<th>ν₀-CO₂</th>
<th>ν₀-CO₂</th>
<th>λ max(nm)</th>
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<tr>
<td>Ligand(L)</td>
<td>-</td>
<td>2856</td>
<td>2923</td>
<td>1606</td>
<td>1489</td>
<td>1455.02</td>
<td>3013</td>
<td>780</td>
<td>702</td>
<td>-</td>
<td>2856</td>
<td>2923</td>
<td>1606</td>
</tr>
<tr>
<td>[ZrL(NO₃)₂]·2H₂O</td>
<td>3486</td>
<td>2854</td>
<td>2923</td>
<td>1610</td>
<td>1590</td>
<td>1447</td>
<td>3853</td>
<td>772</td>
<td>687</td>
<td>368</td>
<td>231</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ThL(NO₃)₂]·2H₂O</td>
<td>3505</td>
<td>2852</td>
<td>2922</td>
<td>1590</td>
<td>1588</td>
<td>1445</td>
<td>3786</td>
<td>770</td>
<td>695</td>
<td>372</td>
<td>228.8</td>
<td>324.8</td>
<td></td>
</tr>
</tbody>
</table>
Another absorption bands at 772-770 cm\(^{-1}\) is assigned to M-N bond and 695-687 cm\(^{-1}\) is assigned to M-O bond\(^{[16-17]}\). The absorption bands at 3853-3786 cm\(^{-1}\) is assigned to free NH\(_2\) group and 1447-1445 cm\(^{-1}\) is assigned to free COOH group and 1590-1588 cm\(^{-1}\) is assigned to C=O group. It shows that the terminal NH\(_2\) group is not involved in bonding.

![Figure 1: FTIR Spectrum of ligand (L)](image1)

![Figure 2: FTIR Spectrum of Zr(IV) complex](image2)

![Figure 3: FTIR Spectrum of Th(IV)) complex](image3)

\(^{1}\)H NMR Spectra

On examining the \(^{1}\)H NMR spectrum of ligand (Figure 4), it exhibited a multiplet signed at \(\delta = 7.004 \text{ ppm} - 7.032 \text{ ppm}\) is due to substituted aromatic ring protons\(^{[16]}\). The presence of H – C = N group is indicated by the singlet at \(\delta = 7.024 \text{ ppm}\). The multiplet at \(\delta = 6.539 \text{ ppm} - \delta = 6.640 \text{ ppm}\) and \(\delta = 5.158 \text{ ppm} - \delta = 5.676 \text{ ppm}\) were due to the olfinic protons of the
side chain and – O-CH₂ -group of the ligand respectively.

Figure 4: The ¹H NMR Spectrum of Ligand

Figure 5: The ¹H NMR Spectrum of Zr(IV) Complex

The ¹H NMR spectrum of the Zr(IV) complex (Figure 5) it exhibited a multiplet signed at δ=7.100 ppm – 7.139 ppm is due to substituted aromatic ring protons. The presence of H – C = N- group is indicated by the

Figure 6: The ¹H NMR Spectrum of Th(IV) Complex
singlet at $\delta = 7.119$ ppm, $\delta = 5.277$ ppm $– \delta = 5.313$ ppm were due to the olifinic protons of the side chain and $–\text{O-CH}_2$-group. A multiplet at $\delta = 4.063 - 4.089$ ppm is due to substituted NH-C=O- proton. Thus $^1H$ NMR study also confirms the binding of metal with Schiff base ligand through pyridine nitrogen and azomethine nitrogen.

The $^1H$ NMR spectrum of the Th(IV) complex (Figure 6) gave a multiplet signed at $\delta = 7.098$ppm $–7.140$ ppm is due to substituted aromatic ring protons. The presence of H $–$ C = N- group is indicated by the singlet at $\delta = 7.117$ppm, $\delta = 5.274$ ppm due to the olifinic protons of the side chain and $–\text{O-CH}_2$-group. A multiplet at $\delta =4.061 - 4.086$ ppm is due to substituted NH-C=O- proton. Thus $^1H$ NMR study also confirms the binding of metal with Schiff base ligand through pyridine nitrogen and azomethine nitrogen.

**UV-visible spectra**

The UV visible spectra (Figure 5-7) are often very helpful in the evaluation of results furnished by other methods of structural investigation. The ligand showed a broad band at 360nm which is assigned to $\pi-\pi^*$ transition of the C=N chromophore$^{18}$. On complexation this bond was shifted to lower wavelength suggesting the co-ordination of imine nitrogen with central metal ion. The UV spectrum of Zr(IV) complex showed two absorption bands at 368 and 231.2 nm giving an octahedral geometry with field transition $^2B_{1g}$$-$$^2A_{1g}$,$^2B_{2g}$$-$$^2B_{2g}$ and $^2B_{1g}$$-$$^2E_{2g}$ respectively$^{19-20}$. The UV spectrum of Th(IV) complexes showed absorption bands at 372.8nm and 228.8nm and 324.8 nm respectively suggesting octahedral geometry.

However UV – visible spectra could not provide structural details of these complexes$^{15-18}$. Zr(IV) has the coordination number of 4, 6, 7 and 8. Earlier researchers are indicated the coordination number of Th(IV) is also 6, 8 or 10. The Zr(IV) and Th(IV) formed 1:1 complexes. Hence the complexes of Zr (IV) and Th(IV) are believed to have the coordination number of 8. The $–\text{NO}_3$ group is present in the coordination sphere because conductance data showed the complexes are non-electrolytes and the NO$_3$ groups were coordinated with the central Zr(IV) or Th(IV) ion. On the basis of foregoing observation the probable structure of Zr(IV) and Th (IV) complexes may be presented as in (Figure 4,5). Earlier researchers also reported the octacoordination of Zr(IV) and Th (IV) Schiff base complexes$^{20-22}$. 

![Figure 4: UV-Visible Spectrum Zr(IV) complex of ligand](image4.png)

![Figure 5: UV-Visible Spectrum Th(IV) complex of ligand](image5.png)
SEM analysis
The surface morphology of the complexes has been examined using scanning electron microscope. The SEM image of Zr(IV) complex is given below. The SEM images showed that the complex is microcrystalline in nature. Careful examination of single crystal, clearly indicate the nanoscale size of the single crystal of the complexes\(^{[23]}\). Lower magnification showed grain like appearance.

X-Ray Diffraction Analysis
The powder XRD for the Th(IV) was performed. The diffractogram is given in Figure 12. It is evident that the strong and broad peaks confirm the complex formation and the appearance of large feeble peaks indicate micro crystalline. The grain size of the complexes was calculated using Scherrer’s formula. The calculated grain size of the complexes is in the range of 1.6544nm. These values suggested that the complexes are in nano crystalline size\(^{[24]}\).

Figure 6: Structure of ligand

Figure 7: Structure of Schiff base complexes, M=Zr(IV), Th(IV)

Figure 8: SEM image of Schiff base of Zr(IV) in ethanol

Figure 9: SEM image of powder sample of Zr(IV)
study indicates that the metal ion intake decreased from Zr to Th (Table 4). This order can be explained by Pearson’s proposal [25], hard acid preferred to combine with hard base and soft acid preferred to combine with soft base. It was found that the interaction of Th(IV) is normally more intense than other divalent metal ion with Schiff base ligand [28]. Nature of the ligand and the chelate effect were the factors involved [29].

**Antibacterial activity**

Antibacterial activity of the ligand complexes and standard drugs were screened by the disc diffusion method in ethanol as solvent. The result of antibacterial study is given in Table 3 [30]. Under identical conditions the Schiff base complexes of Zr and Th have moderate antibacterial activities against these bacteria [31]. The results of antibacterial activity substantiate the findings of earlier research [32]. The biological inactive compound become active and less biologically active compounds become more active upon coordination [33].

**Metal ion intake**

The complexation behaviour of cardanol based Schiff base was affected by structural parameters [25]. This study indicates that the metal ion intake decreased from Zr to Th [26] (Table 4). This order can be explained by Pearson’s proposal [25], hard acid preferred to combine with hard base and soft acid preferred to combine with soft base. It was found that the interaction of Th(IV) is normally more intense than other divalent metal ion with Schiff base ligand [28]. Nature of the ligand and the chelate effect were the factors involved [29].

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**Table 3: Grain size of the Cu(II) complexes**

<table>
<thead>
<tr>
<th>Complex</th>
<th>Grain size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ThL(NO$_3$)$_2$] 2H$_2$O</td>
<td>1.6544</td>
</tr>
</tbody>
</table>

**Table 4: Antibacterial and Anti fungal activity data of complexes**

<table>
<thead>
<tr>
<th>Ligand / Complex</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>Klebsiella pneumoniae</th>
<th>Staphylococcus aureus</th>
<th>Candida albicans</th>
<th>PC</th>
<th>NC</th>
<th>Metal Ion Intake (meq/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ZrL(NO$_3$)$_2$] 2H$_2$O</td>
<td>6.0</td>
<td>15.0</td>
<td>6.0</td>
<td>19.0</td>
<td>16.0</td>
<td>23.00</td>
<td>6.0</td>
<td>0.4532</td>
</tr>
<tr>
<td>[ThL(NO$_3$)$_2$] 2H$_2$O</td>
<td>6.0</td>
<td>17.0</td>
<td>6.0</td>
<td>10.0</td>
<td>6.0</td>
<td>25.00</td>
<td>6.0</td>
<td>0.6201</td>
</tr>
</tbody>
</table>

*Media: Mueller Hinton Agar for bacteria * PC - Chloramphenicol

Such enhancement in biological activity of metal complexes can be explained on the basis of Overstone’s concept of cell permeability, the lipid membrane that surrounds the cell flavours the passage as only lipid soluble materials due to which lipho solubility is an important factor that controls antimicrobial activity [34]. Also other factors such as solubility, conductivity and dipole moment may also be among the possible reason causing enhancement of bactericidal activity of the metal complexes as compared to the uncomplexed Schiff base compound. Under identical conditions the Schiff base complex of Zr (IV) shows maximum antibacterial activity than Th(IV) against these bacteria [35].

**Proteinase Inhibitory Activity**

Proteinase inhibitory activity was performed according to the modified method. Concentration increases with increase in % inhibition, then the complex show more inflammation and it is more active. From Invivo analysis the percentage inhibition of [ThL(NO$_3$)$_2$] 2H$_2$O increases with increase in concentration and exhibit statistically significant Proteinase inhibitory activity [36]. From Invitro analysis the percentage inhibition of [ZrL(NO$_3$)$_2$] 2H$_2$O and [ThL(NO$_3$)$_2$] 2H$_2$O increases with increase in concentration and exhibit statistically significant Proteinase inhibitory activity. In Invitro more inflammation is shown by [ThL(NO$_3$)$_2$] 2H$_2$O [37].

**Table 5: Invivo Antiarthritic Activity by inhibition of protein denaturation method**

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Concentration</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ZrL(NO$_3$)$_2$] 2H$_2$O</td>
<td>25</td>
<td>91.27</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>86.13</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>86.665</td>
</tr>
<tr>
<td>[ThL(NO$_3$)$_2$] 2H$_2$O</td>
<td>25</td>
<td>56.42</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>61.319</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>77.08</td>
</tr>
</tbody>
</table>
Table 6: Invitro Antiarthritic Activity by inhibition of protein denaturation method

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Concentration (µg/ml)</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ZrL (NO₃)₂]2H₂O</td>
<td>25</td>
<td>10.77</td>
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<tr>
<td></td>
<td>50</td>
<td>23.46</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>26.51</td>
</tr>
<tr>
<td>[ThL (NO₃)₂]2H₂O</td>
<td>25</td>
<td>10.58</td>
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<td>25.61</td>
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<td>100</td>
<td>52.49</td>
</tr>
</tbody>
</table>

Nuclease Activity

The nuclease activity of Zr(IV) and Th(IV) complexes of ligand were studied using gel electrophoresis and the respective photograph is shown in Figure 11. The cleavage efficiency of the complexes is compared with the control DNA to study the binding ability. The presence of smear in the gel diagram indicates the radical cleavage\(^{[38]}\) by the abstraction of hydrogen from sugar units of DNA. The metal complexes were able to convert super coiled DNA into open circular DNA\(^{[39]}\). The reaction is modulated by the metallo complexes bound hydroxyl or peroxo radical generated from the oxidant H₂O₂. All the complexes of ligand L₃ showed enhanced nuclease activity.

![Figure 11: Gel diagram of Zr(IV) and Th(IV)](image)

Conclusion

The Schiff base of Zr(IV) and Th(IV) were synthesized from cardanol using L-glutamine. The ligands and complexes are insoluble in water, but are soluble in ethanol, acetone and DMSO. The ligands and their complexes were characterized using spectral and analytical data. From the spectral and stoichiometric analysis, a hexa coordinated nature was assigned for the metal complex. The nitrate group is present inside the coordination sphere. The XRD and SEM studies reveal that the complexes are nano crystalline. Antimicrobial activity and nuclease activity were studied. The antimicrobial study showed that Zr(IV) have more antibacterial activity than Th(IV) complexes. The antifungal study revealed that Th (IV) complexes have more antifungal activity than other complexes. In proteinase inhibitory activity of Th(IV) show more inflammatory activity. Nuclease activity studies of complexes showed greater cleavage.

References


23. Isac Sobuna Raj C., Christudhas M., and Allen Gana Raj G., Synthesis, Characterization, Metal ion Schiff base complexes of Zr(IV) and Th(IV) derived from Di- formyl methoxybis(3-pentadecenylphenyl) methane [DEMPM] and ethylenediamine, Asian J. Res. in chem., 4(11),1765-1770 (2011)


