Synthesis And Structural Investigation Of Some Trivalent Lanthanide Complexes Of Cloxacillin

Rajesh Kumar Mishra¹ & B.G.Thakur²
¹²Department Of Chemistry, C.M.Sc. College
L.N.M.U Darbhanga, Bihar-846004
North to Badri Narayan Mandal, New Colony Shubhankarpur, Darbhanga, Bihar-846006
INDIA

Abstract: Complexes of some trivalent lanthanides with Cloxacillin have been synthesized. The complexes have been formulated as [Ln(Clox)(H₂O)₂]Cl Where Ln = La(III), Pr(III), Nd(III), Sm(III), Dy(III), Ho(III) and Er(III). The ligand and its metal complexes were characterized by their elemental analysis, molar conductance, magnetic susceptibility, IR and electronic spectral studies. Elemental analysis indicate 1:2 stoichiometry of synthesized complexes. In all the complexes, cloxacillin acts as a tridentate ligand with coordination involving the carboxylate-O, endocyclic-N of the β-Lactam ring and N-of amide. Complexes are eight coordinated. Finally the complexes have been screened for their antibacterial activity against E.Coli, K.Pneumoniae, S.Aureus and P.Aeruginosa...etc and found to be more potent against uncomplexed Cloxacillin.

(Keywords : Ln(III)-Cloxacillin complexes, IR, Electronic, Antimicrobial, Disc-Diffusion Method)

I. INTRODUCTION

Cloxacillin (Clox) ,[2S,5R,6R]-6-[[3-(2-chlorophenyl)-5-methylloxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid) [Figure-1] is a commonly used biologically important drug which has been shown to exert pronounced biological effects on various bacterial strains. Cloxacillin is used against staphylococci that produce beta-lactamase, due to its large R chain, which does not allow beta-lactamases to bind. This drug has a weaker antibacterial activity than benzylpenicillin, and is devoid of serious toxicity except for allergic reactions. Cloxacillin is white crystalline powder, freely soluble in water, methanol and soluble in alcohol.

Most living systems contain metal ions for their proper functioning¹-⁴. Many studies concerning the biochemical and pharmaceutical effects of antibiotics when complexed with metal ions have been a subject of great interest for many scientists⁵-²⁰. Based on these observations, we report here the synthesis, characterization and antimicrobial activity of a few Lanthanide(III)-Cloxacillin complexes.

II. EXPERIMENTAL

Chemicals used for synthesis were of AR grade and used without further purification. Metal salts i.e, LaCl₃, PrCl₃, NdCl₃, SmCl₃, DyCl₃, HoCl₃ & ErCl₃ (of 99.97% Purity) were purchased from Indian Rare Earth Udyog Mandal, Kerala, India and the ligand i.e, Cloxacillin was purchased from CDH. Molar conductance of the newly synthesized metal complexes was measured by Systronics Conductivity Meter Model-304 in 1x10⁻³ M DMF Solution. Melting points of the complexes were obtained in sealed glass capillary and are still uncorrected. Magnetic Moment of the complexes were measured Gouys method in Bohr-Magneton unit using Hg[CO(NCS)]₄ as the calibrant in INORGANIC RESEARCH LABORATORY, L.N.M.U.
Darbhanga. Molecular weight of the complexes were determined by Camphor Rast Method. C, H & N were determined at CDRI Lucknow. Chloride in the complexes was estimated using Volhard’s Method. IR Spectra of the complexes were recorded by the courtesy of CDRI using KBr Pellets on a Perkin Elmer IR Spectrometer in the range of 4000-400 cm⁻¹. Electronic spectra of the complexes were recorded by the courtesy of Dept. of Chemistry, IIT Delhi in the ranges of (10-35)kk.

III. BIOLOGICAL ACTIVITY

For determining antibacterial activity, the synthesized metal complexes have been screened against E.Coli, K.Pneumoniae, S.Aureus, P.aeruginosa using Agar-Plate diffusion technique. Two to eight hours old bacterial inoculums containing approximately 10⁵-10⁶ colony forming units (CFU)/ml were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24mm. Recommended concentration (100μl) of the test sample (1mg/ml in DMSO) was introduced into the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenem served as negative and positive controls respectively. The plates were incubated immediately at 37°C for 20h. Activity was determined by measuring the diameter of zones (mm) showing complete inhibition. Growth inhibition was compared with the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions of DMSO alone which showed no activity against any bacterial strains.

IV. PREPARATION OF METAL COMPLEXES

For the preparation of [Ln(Clox)₃(H₂O)₂]Cl Complexes, Cloxacillin(5mmol, 2.1794g) was mixed with 2.5mmol of Ln(III) chlorides in a mixture of water-ethanol(25ml, 1:1v/v). The pH of the solution was adjusted to 7-8 with sodium acetate using digital pH meter. The mixture was refluxed for 1h on a water bath and concentrated to half volume. Then on cooling to room temperature, the colored complexes got precipitated slowly, which was filtered, washed repeatedly with distilled water and ethanol. Now, the complexes were dried over anhydrous calcium chloride in dessicator.

V. RESULTS AND DISCUSSION

All the Ln(III) complexes were obtained in powder form with characteristic color. All these complexes are non-hygrosopic. Analytical data, Magnetic moment, %yield, Molar conductance, Decomposition temperature, Melting points and color of all the seven complexes are reported in Table-I. At room temperature magnetic moment of the complexes are in good agreement with the theoretical values calculated by Van-Vleck. Complexes are insoluble in common organic solvents, only soluble in DMF and DMSO. All the metal complexes decomposed above than 300°C.

VI. IR SPECTRAL STUDIES

IR Spectra of cloxacillin and their Ln(III)-complexes comparing mainly the IR frequencies of free and complexed cloxacillin are reported in Table-2. The IR Spectra of cloxacillin and their Ln(III) complexes were recorded in the range of 4000-400 cm⁻¹. The IR Spectra of all the complexes shows band at 3450-3400 cm⁻¹ indicate the involvement of water molecule in the coordination sphere. Ligand exhibits strong absorption bands at 1185 cm⁻¹, 2988 cm⁻¹ due to ν(C=N) of (β-Lactam) and ν(C=O) of Amide stretching vibrations which was shifted in the range of (1350-1040) cm⁻¹ and (2950-2900) cm⁻¹ respectively. The band at 1748 cm⁻¹ assigned due to ν(C=O) of carboxylic acid of thiazolidine nucleus of cloxacillin which was shifted to lower frequencies in the range of (1630-1590) cm⁻¹ in the spectra of all the Ln(III) complexes. A comparison of the IR Spectra of free ligand and complexed ligand provide evidence in support of mode of bonding i.e., Shifting of these bands in all the Ln(III) complexes indicate that there is a coordinate covalent bonding through endocyclic N of β-Lactam, N of Amide, carboxylate-O of cloxacillin and ‘O’ of water molecule with Ln(III) central metal ion. All of the IR spectral data confirms coordination number eight of the synthesized metal complexes.

VII. ELECTRONIC SPECTRAL STUDIES

Electronic spectral data for the solution of Ln(III)-Cloxacillin complexes investigated in CH₃CN are reported in Table-3. For comparison, the spectral data for the corresponding aqueous salt solution are also given in the same table. Lanthanum(III) has no significant absorption in the UV-Visible region. The absorption bands of the Pr³⁺, Nd³⁺, Sm³⁺, Dy³⁺, Ho³⁺, & Er³⁺ in the visible and near infrared region appears due to the transitions from ground
state i.e. $^3\text{H}_2$, $^4\text{i}_{9/2}$, $^4\text{i}_{11/2}$, $^4\text{i}_{15/2}$, $^1\text{h}$ and $^1\text{i}_{15/2}$ respectively to the excited states i.e. J-levels of 4f$^6$-configuration. The Nephelauxetic ratio ($\beta$) has been determined by the method of Jørgensen using the relation:

$$ (1-\beta) = \frac{V_{\text{aquo}} - V_{\text{complex}}}{V_{\text{aquo}}} $$

The covalence factor ($b^{1/2}$), metal – ligand covalency % i.e. sinh Parameter ($\delta$%) and covalency angular overlap parameter ($\eta$) have been calculated by using the following relations:

$$ b^{1/2} = \frac{1}{2} \left[ (1-\beta)^{1/2} \right] $$

$$ \delta\% = \left[ \frac{(1-\beta)}{\beta} \right] \times 100 $$

$$ \eta = \left[ \frac{1-\beta^{1/2}}{\beta^{1/2}} \right] $$

The +ve values of (1-\beta) and $\delta$% supports the evidence of covalent bonding in all the synthesized Ln(III) complexes. The spectral profile of hypersensitive bands of Nd(III), Ho(III) and Er(III) complexes closely resembles that of eight coordinated complexes reported by Karrakar and is in good agreement with the other physico-chemical investigations.

VIII. MAGNETIC SUSCEPTIBILITY STUDIES

Except La(III) all the Ln(III) complexes are paramagnetic showing close agreement with the calculated values except for Sm(III), indicating an insignificant participation of the 4f-electrons in the bonding. Unlike the d-electrons of the transition metal ions, the f-electrons of the lanthanide ions are almost unaffected by the chemical environment and the energy levels are same as in the free ion due to very effective shielding by the overlying 5s$^2$ and 5p$^6$ shells. The relatively high value obtained in the case of samarium(III) complex, which may be due to small J-J separation, which leads to the thermal population of the higher energy levels and show susceptibilities due to first order Zeemann effect.

IX. BIOLOGICAL EVALUATION

A comparison of the diameter of inhibition zone of complex investigated showed that all the Ln(III) complexes exhibit higher antibacterial activity than the uncomplexed cloxacillin (Table-4).

**Table-1 : Analytical data of “Ln(III)-Cloxacillin” Complex.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Complex</th>
<th>M. Formulae</th>
<th>M. Wt (Obs./Cal.)</th>
<th>% Yield</th>
<th>Color</th>
<th>Decomposition Temp°C</th>
<th>M.Pt °C</th>
<th>$\Lambda_{\text{m}}$ (Ohm$^{-1}$ cm$^{-1}$mol$^{-1}$)</th>
<th>$\mu_{\text{el}}$(in B.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cloxacillin</td>
<td>C$_3$H$_7$ClIN$_2$O$_5$S</td>
<td>435.88/435</td>
<td></td>
<td>White</td>
<td>52.47 (52.41)</td>
<td>4.19 (4.13)</td>
<td>9.69 (9.65)</td>
<td>8.07 (8.04)</td>
</tr>
<tr>
<td>2.</td>
<td>[La(Clox)$_2$ (H$_2$O)]Cl</td>
<td>C$_5$H$_8$N$_2$O$_2$S$_2$LaCl</td>
<td>1076.25 (1076)</td>
<td></td>
<td>White</td>
<td>42.41 (42.37)</td>
<td>3.41 (3.34)</td>
<td>7.84 (7.80)</td>
<td>9.81 (9.75)</td>
</tr>
<tr>
<td>3.</td>
<td>[Pr(Clox)$_2$ (H$_2$O)]Cl</td>
<td>C$_5$H$_8$N$_2$O$_2$S$_2$PrCl</td>
<td>1078.15 (1078)</td>
<td></td>
<td>White</td>
<td>42.35 (42.50)</td>
<td>3.55 (3.33)</td>
<td>7.82 (7.79)</td>
<td>9.78 (9.74)</td>
</tr>
<tr>
<td>4.</td>
<td>[Nd(Clox)$_2$ (H$_2$O)]Cl</td>
<td>C$_5$H$_8$N$_2$O$_2$S$_2$NdCl</td>
<td>1081.23 (1081)</td>
<td></td>
<td>White</td>
<td>42.24 (42.18)</td>
<td>3.37 (3.33)</td>
<td>7.82 (7.77)</td>
<td>9.78 (9.71)</td>
</tr>
<tr>
<td>5.</td>
<td>[Sm(Clox)$_2$ (H$_2$O)]Cl</td>
<td>C$_5$H$_8$N$_2$O$_2$S$_2$SmCl</td>
<td>1087.11 (1087)</td>
<td></td>
<td>White</td>
<td>42.01 (41.95)</td>
<td>3.38 (3.31)</td>
<td>7.77 (7.72)</td>
<td>9.69 (9.65)</td>
</tr>
<tr>
<td>6.</td>
<td>[Dy(Clox)$_2$ (H$_2$O)]Cl</td>
<td>C$_5$H$_8$N$_2$O$_2$S$_2$DyCl</td>
<td>1099.09 (1099)</td>
<td></td>
<td>White</td>
<td>41.57 (41.49)</td>
<td>3.32 (3.27)</td>
<td>7.68 (7.64)</td>
<td>9.59 (9.55)</td>
</tr>
<tr>
<td>7.</td>
<td>[Ho(Clox)$_2$ (H$_2$O)]Cl</td>
<td>C$_5$H$_8$N$_2$O$_2$S$_2$HoCl</td>
<td>1102.21 (1102)</td>
<td></td>
<td>White</td>
<td>41.40 (41.37)</td>
<td>3.34 (3.26)</td>
<td>7.64 (7.62)</td>
<td>9.54 (9.52)</td>
</tr>
<tr>
<td>8.</td>
<td>[Er(Clox)$_2$ (H$_2$O)]Cl</td>
<td>C$_5$H$_8$N$_2$O$_2$S$_2$ErCl</td>
<td>1104.07 (1104)</td>
<td></td>
<td>White</td>
<td>41.33 (41.30)</td>
<td>3.29 (3.26)</td>
<td>7.65 (7.60)</td>
<td>9.53 (9.51)</td>
</tr>
</tbody>
</table>

**S.No** | Complex | % Yield | Color | Decomposition Temp°C | M.Pt °C | $\Lambda_{\text{m}}$ (Ohm$^{-1}$ cm$^{-1}$mol$^{-1}$) | $\mu_{\text{el}}$(in B.M.) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cloxacillin</td>
<td></td>
<td>White</td>
<td>330</td>
<td>260</td>
<td>10.7</td>
<td>Dia</td>
</tr>
<tr>
<td>2.</td>
<td>[La(Clox)$_2$ (H$_2$O)]Cl</td>
<td>64</td>
<td>Yellowish White</td>
<td>335</td>
<td>263</td>
<td>14.3</td>
<td>5.64</td>
</tr>
</tbody>
</table>
4. \[\text{[Nd(Clox)\(\text{H}_2\text{O})_3\text{Cl}]}\] 62 Yellow. 332 273 13.4 3.67

5. \[\text{[Sm(Clox)\(\text{H}_2\text{O})_3\text{Cl}]}\] 59 Deep Yellow. 347 267 10.3 1.63

6. \[\text{[Dy(Clox)\(\text{H}_2\text{O})_3\text{Cl}]}\] 67 Light Yellow 342 266 17.4 11.5

7. \[\text{[Ho(Clox)\(\text{H}_2\text{O})_3\text{Cl}]}\] 55 Pale Yellow 377 275 15.9 10.17

8. \[\text{[Er(Clox)\(\text{H}_2\text{O})_3\text{Cl}]}\] 52 Yellow 384 278 12.8 9.58

Table-2: IR Spectral data (in cm\(^{-1}\)) of ligand and complexes:

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Ligand</th>
<th>Cloxacillin</th>
<th>Complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ln(III)</td>
</tr>
<tr>
<td>N—(\beta)-Lactam</td>
<td>1185</td>
<td>1182 1178</td>
<td>1072 1317</td>
</tr>
<tr>
<td>C—OH</td>
<td>1748</td>
<td>1605 1592</td>
<td>1628 1614</td>
</tr>
<tr>
<td>-NH(_2)amine</td>
<td>2988</td>
<td>2918 2907</td>
<td>2944 2934</td>
</tr>
</tbody>
</table>

Table-3: Electronic spectral data along with band-assignment (in cm\(^{-1}\)) and related bonding parameters of “Ln (III)- Cloxacillin” Complex.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Band Assignments</th>
<th>Bands of Ln(^{3+})-aqutions (in kk)</th>
<th>Bands of Complex of (in kk)</th>
<th>Calculated Bonding Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>((1-\beta)) (\beta) (\beta^{2+}) (\delta(%)) (\eta)</td>
</tr>
<tr>
<td>[Pr(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>(\text{H}_2) → (\text{F}) (\text{F})</td>
<td>22.2 22.2 0.0134 0.9866 0.0578 1.3581 0.0068</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Nd(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>(\text{H}_2) → (\text{F}) (\text{F})</td>
<td>33.3 29.0 0.0175 0.9825 0.0641 1.7811 0.0088</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Sm(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>(\text{H}_2) → (\text{F}) (\text{F})</td>
<td>33.3 29.0 0.0175 0.9825 0.0641 1.7811 0.0088</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Nd(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>(\text{H}_2) → (\text{F}) (\text{F})</td>
<td>33.3 29.0 0.0175 0.9825 0.0641 1.7811 0.0088</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table-4. Antibacterial activities of the ligand & its Ln(III) complexes [Diameter(mm) of Zones Showing complete inhibition of growth]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pseudomonas Aeruginosa</th>
<th>S. Aureus</th>
<th>E. Coli</th>
<th>Klebs. Pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloxacillin</td>
<td>24</td>
<td>22</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>[La(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>24</td>
<td>25</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>[Pr(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>29</td>
<td>27</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>[Nd(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>32</td>
<td>28</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>[Sm(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>25</td>
<td>27</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>[Dy(Clox)(\text{H}_2\text{O})_3\text{Cl}]</td>
<td>32</td>
<td>36</td>
<td>24</td>
<td>23</td>
</tr>
</tbody>
</table>
X. CONCLUSION

On the basis of above discussion coordination number eight has been assigned for all the Ln(III)-Cloxacillin complexes. The tentative structure of the synthesized complexes may be as shown in the figure-2.

![Proposed structure of Ln(III)-Cloxacillin Complexes.](image-url)

Where Ln(II) = La(III), Pr(III), Nd(III), Sm(III), Dy(III), Ho(III) and Er(III)

REFERENCES

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