Novel Complexes of Schiff Base of Cefadroxil: Synthesis, Spectral Characterization and Biological Evaluation

Thirumagal. B*, Suman Malik1 and Bharati Jain2

1Department of Chemistry, Sadhuvaswami College, Bairagarh, Bhopal - 462 030, India.
2Department of Chemistry, SNGG PG College, Shivaji Nagar, Bhopal - 462 016, India.

ABSTRACT: Metal complexes of â –lactum antibiotic Cefadroxil, having the general composition ML2 (where M= Ca(II)/ Co(II)/ Cu (II) and L=Deprotonated Schiff base of Cefadroxil) have been prepared. The ratio of metals to Schiff base ligand was 1:2, as confirmed through Job’s method of continuous variation. Further the complexes were characterized by elemental and chemical analysis, molar conductance, thermal and spectral (UV, IR, and mass) studies. The IR spectral data suggests the involvement of phenolic oxygen and azomethine nitrogen in coordination to the central metal ion. On the basis of spectral studies, octahedral geometry has been assigned for the complexes. The free ligand and its metal complexes have been tested in vitro against a number of microorganisms in order to assess their antimicrobial properties.

Key words: Complexes, Schiff base, spectral analysis, thermal analysis, and antibacterial activity.

INTRODUCTION

Cefadroxil is a first generation semi-synthetic cephalosporin recommended for oral use. Cefadroxil has been shown to have antibacterial activity against 602 clinical isolates of different kinds of bacteria. It has primary amino group which can condense with salicylaldehyde to form Schiff base similar to Cephlexin. Schiff bases are very versatile ligands. They can coordinate to metals as neutral molecules or, after deprotonation, as anionic ligands, and can adopt a variety of different coordination modes. The efficacies of Schiff bases of some therapeutic agents are known to increase upon co-ordination with metals. Survey of literature shows that cefadroxil forms complexes with different metal ions. This work involves the synthesis, characterization and biological evaluation of complexes of Cefadroxil (CFDX) through Schiff base (SACFDX) formation.

EXPERIMENTAL

Materials and Methods

Pure sample of cefadroxil monohydrate (molecular formula C16H17N3O5S.H2O) with mol.wt.381 was obtained from Lupin Ltd., Bhopal. The assay of antibiotics was done by disc plate method. They were used as such without further purification. Calcium(II) chloride, cobalt(II) nitrate, copper (II) acetate, salicylaldehyde (SAL), potassium hydroxide and all other solvents used were of Analar grade or Merck products, which are available commercially.

Synthesis of Schiff base of cefadroxil- SACFDX

Twenty five milliliters of 0.01M methanolic solution of cefadroxil monohydrate was added to methanolic solution of salicylaldehyde (0.01M, 25 ml) in a beaker. The pH of the reaction mixture was adjusted between 7.4 – 7.6, using 1% methanolic potassium hydroxide solution. The contents were transferred into a round bottom flask, refluxed for three hours and cooled to room temperature. On cooling, Schiff base (SACFDX) separates out. It was washed with methanol and dried under vacuum.

Synthesis of SACFDX– Calcium(II), cobalt(II)/ copper(II) Complex

Methanolic solution of calcium(II), cobalt(II)/ copper(II) salts (0.01M, 25ml) was added to methanolic solution of SACFDX (0.02M, 25 ml) in a beaker, with constant stirring. The pH was adjusted between 7.4 – 7.8 using 1% methanolic potassium hydroxide solution. The contents were
transferred into a round bottom flask. The mixture was refluxed for 2 to 3 hrs and cooled to room temperature. On cooling, brown precipitate was formed, which was filtered, washed with methanol and dried by vacuum suction. Dark brown crystals of SACFDX-copper (II) complex were obtained.

**Determination of Physical Properties of the Schiff Base and its Complexes**

Microanalyses of carbon, hydrogen, nitrogen and sulphur were carried out using Elemental analyzer at Central Drug Research Institute (CDRI), Lucknow. Metal content was determined by Atomic Absorption Spectrometer. IR spectra were recorded on a Spectrum –BX series spectrometer, using KBr discs. Molecular mass was determined by Rast's method. Molecular mass was determined by Rast’s method. Mass spectra were recorded using Varian Winuv UV-Vis spectrophotometer. Conductivities of $10^{-3}$ M solutions were carried out using WTWD-812 Weilheum conductivity meter, fitted with cell model LTA 100. DTA-DTG-TG was carried out using Perkin –Elmer (Pyris Diamond) analyzer, in the temperature range 20 - 1000°C. The antimicrobial activities of both the ligand as well as its complexes were screened against gram positive bacteria such as Staphylococcus epidermis, Bacillus cereus, Bacillus subtilis and gram negative bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella and E.coli using agar cup plate method at Meru Research Institute, Bhopal.

**Antibacterial Screening**

In vitro antimicrobial screening was performed by the cup plate method\textsuperscript{15, 16}. The antimicrobial activities of both the ligands as well as the complexes were screened against gram positive (Staphylococcus epidermis, Bacillus cereus, Bacillus subtilis) and gram negative (Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella and E.coli) bacteria. The selected strains of bacteria were inoculated into 10 ml of sterile nutrient broth and incubated at 37°C for 16 – 18 hours. Using sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. Agar wells were prepared with the help of sterilized cork borer with 10mm diameter\textsuperscript{17}. Using a micropipette, 100 microlitres of 250 µg concentration of the synthesized complexes were added to different wells in the plate. The petriplates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. The inhibition zones with diameter less than 12mm were considered as having no antibacterial activity.

**RESULTS AND DISCUSSION**

The condensation of cefadroxil with salicylaldehyde results in the formation of salicylidene cefadroxil (Schiff base). The complexes were synthesized by reacting Schiff base ligand with metal ions in the ratio 2:1 in the methanolic medium. The physico-chemical data of Schiff base and its complexes were studied and are listed in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Compound / Mol. formula / Mol. weight</th>
<th>Colour and decomp. temp (°C)</th>
<th>Yield (%)</th>
<th>% Composition</th>
<th>Found (Calculated)</th>
<th>Conductance cm² mole⁻¹ cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylidene Cefadroxil (Schiff base)</td>
<td>Reddish yellow, 195-198</td>
<td>75</td>
<td>58.90 (54.80)</td>
<td>4.43 (4.49)</td>
<td>8.90 (8.99)</td>
</tr>
<tr>
<td>Salicylidene Cephalexin-Ca(II)Complex</td>
<td>Orange red, 205-207</td>
<td>69</td>
<td>52.34 (52.47)</td>
<td>3.70 (3.80)</td>
<td>7.88 (7.98)</td>
</tr>
<tr>
<td>Salicylidene Cephalexin-Co(II)Complex</td>
<td>Chocolate Brown, 286-289</td>
<td>73</td>
<td>52.75 (53.75)</td>
<td>3.75 (3.89)</td>
<td>8.16 (8.18)</td>
</tr>
<tr>
<td>Salicylidene Cephalexin-Cu(II)Complex</td>
<td>Dark brown, 290-292</td>
<td>72</td>
<td>55.44 (55.46)</td>
<td>3.99 (4.01)</td>
<td>8.30 (8.43)</td>
</tr>
</tbody>
</table>
The complexes were found to be sparingly soluble in distilled water and acetone. However, it is soluble in hot methanol and ethanol. The molar conductance values of the calcium(II), cobalt(II), and copper(II) complexes of SACFDX in 50% methanol were 16.2, 13.7 and 14.2 ohm^{-1} cm^{2} mole^{-1} respectively, showing their non-electrolytic behaviour. The magnetic moment data indicate that Ca(II) complex is diamagnetic, Co(II) and Cu(II) complex is paramagnetic with $\mu_{\text{eff}}$ value of 4.90 and 1.72 B.M. respectively.

**IR Spectra**

The IR spectra of the ligand and its metal complexes were recorded in KBr pellets in the 4000 - 400 cm^{-1} range with a Spectrum BX Series spectrophotometer and compared. Some important characteristic IR frequencies of Schiff base ligand and its complexes are recorded in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Compound / Assignments</th>
<th>Salicylidene Cefadroxil</th>
<th>Salicylidene Cefadroxil</th>
<th>Salicylidene Cefadroxil</th>
<th>Salicylidene Cefadroxil</th>
</tr>
</thead>
<tbody>
<tr>
<td>for</td>
<td>(cm^{-1})</td>
<td>(cm^{-1})</td>
<td>(cm^{-1})</td>
<td>(cm^{-1})</td>
</tr>
<tr>
<td>v (OH)</td>
<td>3237</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>v (-CH=N-)</td>
<td>1672</td>
<td>1645</td>
<td>1645</td>
<td>1645</td>
</tr>
<tr>
<td>v (C-O)/phenolic</td>
<td>1251</td>
<td>1335</td>
<td>1327</td>
<td>1335</td>
</tr>
<tr>
<td>v (M-N)</td>
<td>—</td>
<td>701</td>
<td>699</td>
<td>698</td>
</tr>
<tr>
<td>v (M-O)</td>
<td>—</td>
<td>494</td>
<td>496</td>
<td>494</td>
</tr>
<tr>
<td>v (C=H)</td>
<td>1383</td>
<td>1366</td>
<td>1383</td>
<td>1392</td>
</tr>
<tr>
<td>def(methyl)</td>
<td>—</td>
<td>3379</td>
<td>3328</td>
<td>3392</td>
</tr>
<tr>
<td>v(Coordinated water)</td>
<td>—</td>
<td>998</td>
<td>1002</td>
<td>1005</td>
</tr>
<tr>
<td>v (OH)rocking of Coord. water</td>
<td></td>
<td>759</td>
<td>741</td>
<td>760</td>
</tr>
</tbody>
</table>

The IR spectra confirm the participation of nitrogen atom of azomethine group as its complexes may be attributed to the C=O frequency of the CONH group. The IR spectra of the complexes show new bands at 494-496 cm^{-1} and 698-701 cm^{-1} respectively, showing their non-electrolytic behaviour. Some bands due to the coordination of ligands with metal ions with N and O as donors. The spectra of the complexes derived from Schiff base of cefadroxil indicates the deprotonation of phenolic proton of salicylaldehydic moiety prior to coordination. The IR spectra of the complex show the elimination of two water molecules in the temperature of 149-200°C. This suggests that both the water molecules in the complexes participate in coordination with the metal ion.

**Mass Spectra**

The mass spectrum of the Schiff base ligand shows a weak molecular ion peak (M^+) peak at m/z = 466 amu, corresponding to [C_{23}H_{29}N_{5}O_{3}S]^+. This confirms the proposed molecular formula for Schiff base. The other peaks with m/z values of 424, 406, 345, 328, 317, 277, 231, 210, 192,154, 136, 124 and 113 amu corresponds to various fragments of Schiff base ligand. Also, the spectra shows that the fragments corresponding to m/z values of 192 is the stableness fragment, the ones at 154, 136 and
124 are less stable while the other fragments are not stable.

**Electronic Spectra**

The electronic spectral bands\textsuperscript{36, 37} of the free Schiff base ligand of cefadroxil and its complexes were studied in methanol. The absorption band observed at 255 nm for the Schiff base of cefadroxil and the ones at 250nm, 230nm and 229nm in Ca(II), Co(II) and Cu(II) complexes can be assigned to the $\pi \rightarrow \pi^*$ transition originating in the azomethine (CH=N) chromophore. The bands at 343nm and 249nm in the Co(II) and Cu(II) complexes may be attributed to the charge transfer from nitrogen atom of Schiff base ligand to the metal ion (N$\rightarrow$M$\textsuperscript{38}$). In Co(II) complex, the bands at 502nm and 560nm can be assigned to $^4T_{1g}(F) \rightarrow ^4T_{1g}(P)$ and $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ transitions respectively. In Cu(II) complex, the bands at 540nm and 660nm can be assigned to $^2B_{1g} \rightarrow ^2E_g$ and $^2B_{1g} \rightarrow ^2B_{2g}$ transitions respectively. These transitions along with $\mu_{eff}$ value suggest an octahedral environment around Co(II) and Cu(II).

The physical, analytical and spectral study of the Schiff base and its complex discussed above confirms the coordination of metal to the Schiff base via phenolic deprotonated oxygen and the imino nitrogen, thus agreeing well with the proposed structure of the complex as presented in Figure 1.

**Antibacterial Activity**

The antimicrobial screening data shows that the ligand exhibits antimicrobial properties, and it is important to note that the Hg(II) metal chelates exhibit more inhibitory effect than the parent ligand. From Table 3, it is clear that the zone of inhibition is much larger for metal chelates against gram-positive (Staphylococcus epidermis, Bacillus cereus, Bacillus subtilis) than gram negative (Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella and E.coli) pathogenic bacteria. The increased activity of metal chelates can be explained on the basis of chelation theory. It is known that the chelation tends to make the ligand act as a more powerful and potent bactericidal agent, thus killing more of the bacteria than the ligand. It is observed that, in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligand, and there may be $\delta$-electron delocalization over the whole chelating site. This increases the lipophilic character of the metal chelate and favors its permeation through the lipid layer of the bacterial membranes.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Organisms</th>
<th>Org./ Samples</th>
<th>SACFDX</th>
<th>SACFDX-Ca</th>
<th>SACFDX-Co</th>
<th>SACFDX-Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Grampositive</td>
<td>Staphylococcus epidermis</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus cereus</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Bacillus subtilis</td>
<td>17</td>
<td>18</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Gramnegative</td>
<td>Klebsiella pneumoniae</td>
<td>17</td>
<td>20</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>5.</td>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>22</td>
<td>14</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Shigella</td>
<td>-</td>
<td>12</td>
<td></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>E.coli</td>
<td>18</td>
<td>19</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>
References


