Synthesis, Characterization and in vitro Screening of Amoxicillin and its Complexes with Ag (I), Cu (II), Co (II), Zn (II) and Ni (II)

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Abstract: New complexes of Amoxicillin with some transition metal ions such as Ag(I), Cu(II), Co(II), Zn(II) and Ni(II) has been synthesized and characterized on the basis of physical, spectral and analytical data. These complexes have also been screened for their antibacterial activity against several bacterial strains such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa. The metal complexes showed enhanced antibacterial activity as compared to simple antibiotic. The present study was carried out in the search of target antibiotic moiety instead of broad-spectrum antibiotic.

Key words: Amoxicillin, metal ions, antibacterial studies, coordination compounds

INTRODUCTION

Many studies (Shields et al., 1960; Kirchner et al., 1966; Clear, 1974) concerning the biochemical and pharmacological effects of Antibiotics when complexed with metal ions have been a subject of great interest in recent years. A number of known biological reactions (Wacker et al., 1959; Berling, 1970) are catalyzed by enzymes containing metal ions such as Zn, Co and Mo etc. The bioavailability of drugs at their site of action can be enhanced or reduced by interaction with other groups or molecule, is well established (Albert and Rees, 1953). Most of the organic drugs used against bacterial and viral infection contain donor atoms (N, O and S) which easily coordinate with metal ions. Different studies (Gupta et al., 1979, Shotton and Ridgway, 1974; Chohan and Sheikh, 1987) show that upon coordination bioavailability of drug is effectively altered. The wide use of antibiotics in man and animals and their extensive use in areas other than the treatment and prophylaxis of disease have resulted in a serious problem of drug resistance. More and more bacterial strains have become resistant to the available drugs. Various strategies have been worked out and tried upon to cope with the resistance problems and enhance the activity, or broaden the spectrum of drugs (Brown and Roberts, 1984). Synthesis of various synthetic derivatives of antibiotics based on structure-activity relationship has been one of the best approaches. Relationship between structure of the complexes and their antibacterial activity can observed (Anaconda, 2001).

Amoxicillin is semi-synthetic penicillinase-susceptible broad-spectrum penicillin. Spectrum of Action is similar to penicillin. Less effective in Shigellosis and 50% excreted in active form in urine. In the present study, complexes of Amoxicillin with Ag(I), Cu(II), Co(II), Zn(II) and Ni(II) has been synthesized, characterized and screened for their antibacterial activity. This study has been carried out in search of target antibiotic instead of broad-spectrum antibiotic.

MATERIALS AND METHODS

All chemicals and solvents used were of Analytical Reagent grade. Metal ion was used as their chloride salt. Amoxicillin was obtained in pure form from local pharmaceutical company. IR spectra were recorded on Philips Analytical PU 5800 FTIR. UV/Vis. spectra was obtained in DMF by SPECORD-200 using software ACUTA710. Molar conductance of the complex was determined at room temperature using CMD 750 WPA conductivity meter. Melting point was determined on Gallenk Kemp apparatus. Synthetic study was carried out in Institute of Chemistry, while Antibacterial study was carried out at the Department of Molecular Biology, University of The Punjab Lahore.

Preparation of Amoxicillin-Ag complex: Equimolar solutions of Amoxicillin and AgNO₃ (in methanol) were mixed in equal quantities and mixture was refluxed for 24 h under reduced pressure. Dark crimson colored solution was obtained. Solution was concentrated at low pressure. Evaporation-resulting product was filtered and dried.
Preparation of Amoxicillin-Cu (II), Zn (II), Co (II) and Ni (II) complexes: Cu (II), Zn (II) and Ni (II) metal complexes were prepared by refluxing methanolic (10 mL) solution of ligand (0.02 mol) with metal (II) chloride (0.01 mol) in methanol (10 mL) for 2 h. The resulting colored solution was concentrated and cooled at room temperature. The colored precipitates were formed which were filtered, washed with distilled water, methanol and dried.

Antibacterial studies: Antibacterial activity of the complexes/Ampicillin was investigated in the laboratories of Department of Microbiology, University of the Punjab, Lahore. Antibacterial activity against different bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* was determined using the paper disc diffusion method (Chohan et al., 2003). The zone inhibition of complexes were measured and are compared with parent drug.

RESULTS AND DISCUSSION

The present study was carried out in the search of target Antibiotic moiety instead of broad-spectrum antibiotics.

The metal complexes of amoxicillin has been made using 1:1, 1:2 ratio of metal and medicine. Amoxicillin contains number of potential donor atoms such as N, O and S. The model studies show that it acts as a ligand (Kirschner et al., 1966). The complexes formed are less soluble in inorganic solvents and more soluble in organic solvents. Micro analytical data confirmed the ML composition of the Amoxicillin-Ag complex and ML₂ for Cu (II), Zn (II) and Ni (II) complexes. The observed molar conductance value of the complexes (Table 1) is consistent with their non-electrolytic nature.

The electronic spectra of Cu (II), Co (II), Zn (II), Ni (II) and Ag (I) complexes were recorded in DMF and the data of these complexes is reported in (Table 2). The electronic spectrum shows a λ_max at 291 nm for Amoxicillin whereas 371 nm for Amoxicillin-Ag complex, the difference is due to variation in the perturbing influence of central metal ion on the ligand group. The shift of band together with color change indicates Amoxicillin-Ag complex formation. While the complexes of Cu (II), Co (II), Zn (II) and Ni (II) show less intense shoulder at 567-670 nm which are assigned as d-d transitions of these metal ions. The former band is probably due to the 3A_1g−²T_2g(P) for Co (L), 3A_1g−²T_2g(F) for Ni(L₂) and 2T_1g−²E(G) for (CuL₂) transition of tetrahedral geometry.

Making comparative study of complexed and uncomplexed compounds using IR Spectroscopy also authenticates formation of complexes. The important

<table>
<thead>
<tr>
<th>Compound</th>
<th>Color</th>
<th>Decomposition/MP (°C)</th>
<th>Solubility</th>
<th>Yield (%)</th>
<th>Molar conductance (µmhos mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>White</td>
<td>180</td>
<td>Decompose</td>
<td>Soluble in, Methanol</td>
<td>--</td>
</tr>
<tr>
<td>Ag(L)</td>
<td>Crimson</td>
<td>152</td>
<td></td>
<td>Soluble*DMF, **DMSO</td>
<td></td>
</tr>
<tr>
<td>Cu(L)</td>
<td>Yellow</td>
<td>200</td>
<td></td>
<td>Soluble*DMF, **DMSO, Methanol (on heating)</td>
<td>56</td>
</tr>
<tr>
<td>Ni(L)</td>
<td>Brown</td>
<td>235</td>
<td></td>
<td>Soluble*DMF, **DMSO, ethanol</td>
<td>62</td>
</tr>
<tr>
<td>Co(L)</td>
<td>Orange</td>
<td>278</td>
<td></td>
<td>Soluble*DMF, **DMSO, ethanol</td>
<td>53</td>
</tr>
<tr>
<td>Zn(L)</td>
<td>White</td>
<td>245</td>
<td></td>
<td>Soluble*DMF, **DMSO, Methanol (on heating)</td>
<td>68</td>
</tr>
</tbody>
</table>

L = Amoxicillin, *DMF = Dimethylformamide, **DMSO = Dimethylsulphoxide

<table>
<thead>
<tr>
<th>Compound</th>
<th>IR (cm⁻¹)</th>
<th>UV (nm)</th>
<th>Elemental analysis</th>
<th>Observed % (calculated %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>1775, 1583, 1684, 1713, 1248, 1613, 1313</td>
<td>291</td>
<td>C</td>
<td>39.70 (40.85)</td>
</tr>
<tr>
<td>Ag(L)</td>
<td>1730, 1694, 1505, 1455, 1610, 1250</td>
<td>371</td>
<td>2.80 (3.19)</td>
<td>8.20 (8.90)</td>
</tr>
<tr>
<td>Cu(L)</td>
<td>1780, 1693, 1495, 1456, 1610, 1258</td>
<td>567</td>
<td>49.93 (49.23)</td>
<td>2.54 (2.23)</td>
</tr>
<tr>
<td>Ni(L)</td>
<td>1784, 1694, 1505, 1455, 1613, 1256</td>
<td>659</td>
<td>50.38 (50.13)</td>
<td>2.57 (2.22)</td>
</tr>
<tr>
<td>Co(L)</td>
<td>1787, 1689, 1508, 1455, 1612, 1251</td>
<td>670</td>
<td>50.45 (50.08)</td>
<td>2.57 (2.20)</td>
</tr>
<tr>
<td>Zn(L)</td>
<td>1782, 1691, 1507, 1455, 1609, 1257</td>
<td>667</td>
<td>50.93 (49.53)</td>
<td>2.59 (2.20)</td>
</tr>
</tbody>
</table>

L = Amoxicillin, C = Carbon, H = Hydrogen, N = Nitrogen, M = Metal
bands of IR. Spectra were interpreted and data is shown in Table 2. The band responsible for carboxylic group of Amoxicillin appeared at 1713 and 1248 cm⁻¹ but it disappeared in the spectra of Amoxicillin-metal complexes. This indicates that the carboxylic group is taking part in complex formation. The disappearance of band 1713 cm⁻¹ due to carboxylic acid and 1775 cm⁻¹ due to carboxylic oxygen after deprotonation. Similarly the shifting of band of Amoxicillin to 1780-1790 cm⁻¹ in complexes indicates that keto group in the ring is also taking part in coordination but the IR data for Amoxicillin-Ag indicate that a salt of silver-CooAg⁺ has been formed instead of complex. IR data (Table 2) suggests that the Amoxicillin act as monodentate ligand coordinating the metal ion through carboxylate as well as through the lactamic carbonyl group. The above discussion supports the proposed structure Fig. 1.

The Amoxicillin-metal complexes were tested for their antibacterial activity against bacterial strains (a), (b) and (c) in comparison with pure Amoxicillin and the results of this study are reported in Table 3. The data shows that the metal ion significantly increases the antibacterial activity of Amoxicillin on evaluation against all bacterial species. Such increased activity of metal chelate can be explained on the basis of overtone’s concept (Anjaneyulu and Rao, 1986) and Tweedy’s chelation theory (Mishra and Singh, 1997). According to Overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials due to which liposolubility is an important factor which controls the antimicrobial activity. On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of II-electrons over the whole chelate ring and enhances the lipophilicity of complexes. This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restrict further growth of organism.

**REFERENCES**


