Spectroscopic Analysis and Antimicrobial Studies for New Metal Oxytetracycline Drug Complexes

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ABSTRACT

The 1:1 and 2:1 M ratio metal complexes of new Cu(II), Co(II), Fe(III), Zn(II) and Zr(II) complexes of Oxytetracycline hydrochloride have been synthesized. Attempts have been made to ascertain their probable structures on the basis of elemental analysis, spectra (IR, Electronic) and NMR studies. The ligand forms a complexes in a molar ratio 1:1 having an empirical formula (MLSO₄.nH₂O) and 2:1 molar ratio having an empirical formula (M₂LSO₄.nH₂O) where M = Cu(II), Co(II), Fe(III), Zn(II) and Zr(II), L = Oxytetracycline and n = 2 – 6. The infrared spectral data and NMR studies were support the postulate that Oxytetracycline reacts with metal ions as an ionic bidentate ligand through its carboxylate oxygen and the amide carbonyl oxygen. The antibacterial evaluation of the Oxytetracycline drug and their complexes were also performed against some grampositive and negative bacteria as well as fungi.

Keywords: Metal complexes, oxytetracycline, bacterial Studies

INTRODUCTION

Metals and metal complexes have played a key role in the development of modern chemotherapy[1, 2]. For example, complexation of non-steroidal anti-inflammatory drugs to copper overcomes some of the gastric side effects of these drugs[3-7]. A number of drugs and potential pharmaceutical agents also contain metal-binding or metal-recognition sites, which can bind or interact with metal ions and potentially influence their bioactivities and might also cause damages on their target biomolecules. Numerous examples of these metallo drugs and metallo pharmaceuticals and their actions can be found in the literature, for instance: (a) several anti-inflammatory drugs, such as aspirin and its metabolite salicylglycine [8-11], suprofen[12], and paracetamol [13], are known to bind metal ions and affect their antioxidant and anti-inflammatory activities; (b) the potent histamine-H2-receptor antagonist cimetidine [14] can form complexes with Cu²⁺ and Fe³⁺, and the histidine blocker antiulcer drug famotidine can also form stable complex with Cu²⁺ [15, 16]. (c) the anthelmintic and fungi static agent thiabendazole, which is used for the treatment of several parasitic diseases, forms a Co⁵⁺ complex of 1:2 metal to drug ratio[17] and (d) the Ru²⁺ complex of the anti-malaria agent chloroquine exhibits an activity two to five times higher than the parent drug against drug resistant strains of Plasmodium falciparum[18]. However, it is known that some drugs act via chelation or by inhibiting metalloenzymes but most of the drugs act as potential ligands, a lot of studies are being carried out to ascertain how metal binding influences the activities of the drugs[19]. Metal complexes are gaining increasing importance in the design of drugs on coordination with a metal. The therapeutic importance of Oxytetracycline drug was behind the development of numerous methods for its determination.

The different method techniques adapted to the analysis of Oxytetracycline drug have been reported [20-30]. Literature survey fell to reveal any previous work or literature regarding the complexation of Oxytetracycline drug with some transition metals [31, 32]. An attempt of synthesizing, characterizing and biological screening of Oxytetracycline drug ligand and their metal complexes of Calcium and Magnesium have been successfully achieved. The characteristic structure of the molecule is...
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apparent in the existence of the carboxylic and carbonyl electron-rich ligand. However, literature survey has revealed that no attempt has been made to study the complexes of some alkali earth metal ions with the above mentioned drug ligand compounds. It is a thought of interest to study the synthesis and characterization and biological screening of metal complexes of new Cu(II), Co(II), Fe(III), Zn(II) and Zr(II) complexes of Oxytetracycline drug. Drug compounds are biologically active, these compounds have become of interest to be studied biologically, and compared their activities against four species of bacteria (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa), and two fungal species (Aspergillusflavus and Candida albicans).

MATERIALS AND METHODS

The chelating agent oxytetracycline complexes was prepared by mixing ethanolic solution of oxytetracycline drug (1 gm) and Metal salts (0.5 gm) in the same solvent the reaction mixture was boiled under reflux in awater bath for 3 hours then left overnight until precipitated. Yields 70%. Identification of New complexes was carried out by elemental analysis and IR Spectral.

The complexes were prepared by adding EtOH solutions of equimolar amounts of Oxytetracycline and metal (II) salts. The mixture was heated on a water bath for 3 hrs. And allowed to evaporate slowly prior to filtration. The precipitates was washed with EtOH and left overnight until dried. Yield 70-80%.

RESULTS AND DISCUSSION

Transition elements were found to form stable complexes with many ligands containing heteroatoms. There is preference for amines, halogens, CN-, tertiary phosphrines and sulfides.

Nickel (II) is one of the transition elements was found to form complex of square shape with the general formula MLX2 where L is a neutral ligand and X anegativeIon [33]. Nickel(II) was found to form stable complexes with many drugs Acetylsalicylic acid [34], fluoxetine [35], vitamin C [36], and ephedrine [37]. Theoretically and from what was mentioned above Oxytetracycline could form chelate, through its nitrogen and oxygen atoms, with Nickel (II). Addition of NiSO4 to oxytetracycline produced black complex that is soluble in Dimethyl sulfoxide(DMSO).

Solution Studies

Effect of pH

The influence pH was studied over the range (1-10) on the absorbance of complex at 354.3 nm, the results were evaluated as shown in (Fig. 1). The shape of the absorption spectrum, the position of the absorption maximum and the apparent molar absorptivity of oxytetracycline-Nickel (II) complex do vary with pH, where the maximum absorbance obtained at pH (4), but a neutral and basic media the oxytetracycline-Nickel (II) complex was precipitate.

Effect of Reaction Time

The stability of the absorbance with the time was studied from 1-60 min. (Fig.2) shows the relation between absorbance and the time, where the maximum absorbance was reached at the 6 and 7 min. after the addition of NiSO4 to oxytetracycline, the absorbance after this optimal time decreased at 8 min. decreased and then the absorbance was almost stable till 60 min.
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Fig2. Effect of time on the absorbance of Oxytetracycline –Ni(II) complex

Effect of Temperature
The effect of temperature was studied in the range of (5-60°C) on the produce of complex of Oxytetracycline with Ni (II) as shown in (Fig.3), the absorbance was reduced as the temperature was increased.

Fig3. Effect of temperature on the absorbance of Oxytetracycline –Ni(II) complex

Effect of Volume of Ni (II) Salt
The volume of Ni(II) has a great effect on the formation of oxytetracycline-Ni(II) complex. (Fig.4) shows that as the volume of Ni(II) increase the absorbance slightly decrease.

Fig4. Effect of volume of 1x10⁻⁴M NiSO₄ on the absorbance

Solid Studies
Elemental Analysis
The Elemental analysis is applied for oxytetracycline complexes to suggest the Structure of Oxytetracycline Complexes, Carbon and hydrogen Percentage shown in Table (1). The proposal Structure for Oxytetracycline Complexes Shown in Scheme 1, 2, 3, 4 and 5.
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Scheme 1. Oxytetracycline Structure

Table 1. Elemental analysis of oxytetracycline and their metal complexes

<table>
<thead>
<tr>
<th>Oxy.Complexes formula</th>
<th>Color</th>
<th>Elemental analysis %</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Found</td>
<td>Calculated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C %</td>
<td>H %</td>
<td>C %</td>
</tr>
<tr>
<td>[Ni(L)SO(_4)]_H(_2)O (689.856)</td>
<td>Black crystalline powder</td>
<td>38.31</td>
<td>4.24</td>
<td>38.26</td>
</tr>
<tr>
<td>Ni(_2)(L)(SO(_4))_2_H(_2)O(_2) (808.580)</td>
<td>Black crystalline powder</td>
<td>32.68</td>
<td>3.12</td>
<td>32.92</td>
</tr>
<tr>
<td>Cu(L)SO(_4)_5H(_2)O(_2) (694.646)</td>
<td>Black</td>
<td>38.04</td>
<td>4.4</td>
<td>37.32</td>
</tr>
<tr>
<td>Zr(L)(Cl)_H(_2)O(_2) (642.322)</td>
<td>Brown near to be Orange</td>
<td>41.14</td>
<td>4.2</td>
<td>41.62</td>
</tr>
<tr>
<td>Fe(L)(H(_2)O)(_2) (570.954)</td>
<td>Black</td>
<td>46.28</td>
<td>4.77</td>
<td>46.02</td>
</tr>
<tr>
<td>[Fe(_2)(L)(Cl)(_3)_2H(_2)O(_2) (750.68)</td>
<td>Black</td>
<td>35.2</td>
<td>3.9</td>
<td>34.52</td>
</tr>
</tbody>
</table>

IR spectral studies

The infrared absorption bands were one of the important tools of the analyses used for determining the mode of chelation. Oxytetracycline HCl behaves as an ionic bidentate molecule and was coordinated to the metals one through oxygen atoms of hydroxyl and carbonyl groups and the other one through amide and another oxygen atom of another carbonyl group. Therefore, in these complexes one metal ion was coordinated to one molecule of the Oxytetracycline. The assignments of bonding sites of the ligand with metal ions are based on the following evidences:

- The band at 3000–3700 cm\(^{-1}\) in all complexes was broad. This was due to the presence of the hydroxyl group. The ligand band at 3080 cm\(^{-1}\) assigned to the secondary amide NH\(_2\)[38] was either absent or very weak in all complexes, indicating the interference of the OH and NH\(_2\) bands in this region (3000–3700 cm\(^{-1}\)).

- The resolved bands at 3020 and 3000 cm\(^{-1}\) in the free Ligand and copper complex due to the aromatic mCH.

- The next diagnostic band in the free ligand was that of the amide carbonyl group which appears at 1583.27 cm\(^{-1}\). This band mC=O is slightly positively shifted in the spectra of the complexes due to the coordination between amide and carbonyl group (tautomeric), indicating the involvement of the C=O of the amide in the chelation process [39].

- The appearance of a mMAO band at 676–640 cm\(^{-1}\) and 601–525 cm\(^{-1}\) can be attributed to the carbonyl, these bands support the chelation through the O atoms[40].

- A broad band at 3200 cm\(^{-1}\) which is assigned to the enolic OH group. The broadness of this band indicates the presence of hydrogen bond. This band disappear in the complexes indicates deprotonation of enolic group, which lead to a six-membered ring structure around metal ions.

- Infra spectra of the complexes also showed non-ligand band in the region 430-460 cm\(^{-1}\) and 510-520 cm\(^{-1}\), which could be assigned to v(M-O) and v(M-N) modes respectively.

The UV spectral bands in the metal complexes, the v(C=N) is displaced to lower wave number by about 20-30 cm\(^{-1}\) on bond stabilization of the azomethine moiety upon coordination. The bond corresponding to the ester v(C=O) has been shifted to lower frequency by about 35-35 cm\(^{-1}\) in the metal complexes indicating coordination by ester function.

Proton NMR spectral data of the ligand supported the conclusion drawn on the basis of UV and IR spectral data. The absence of NH\(_2\)
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proton signal in the NMR spectrum of ligand in DMSO-d 6 indicates successful Schiff base formation by replacement of the C=O group.

A signal at 13.2 δ indicates the enolic proton and therefore the weakest shielded proton in the molecule. The small shift and presence of the signal at 13.2 was confirmed to the fact that the drug reacts with metal ions in the enolic form during complexation with the metal ions. The signal at 1.50 δ (d) and 4.30 δ (m) can be assigned for methyl and methylene proton respectively. Twomultiples centered at 2.6-2.7 δ and doublet at 1.2 δ in the ligand and metal complexes are due to different hydrogen atom of the tetrahydrobenzothiophene ring. A signal at 5.8 δ and 2.0 δ is due to methine and two methyl group proton respectively.

The electronic absorption spectrum of the ligand in alcohol showed three band at 285,340 and 360nm.the first one may be assigned to intraligand π→π* transition which is nearly unchanged on complexation, where as the second and third band may be assigned to the n→π* and charge transfer transition of the aromatic group proton respectively.

In alcohol showed three band at 285, 340 and 360 nm. The first one may be assigned to CH=O group in coordination with the metal ions. In complexation, indicating participation of this group in coordination with the metal ions. In addition, the spectra of the complexes showed new bands observed in the 420-440 nm range which may be attributed to the charge transfer transitions. The electronic spectrum of Co(II) show absorption band at 9100, 14950 and 18500 cm-1 attributed to the transition 4T1g(F)→4T2g(F) (υ1), 4T1g(F)→4A2g(F) (υ2) and 4T1g(F)→4T1g(P)(υ3) respectively. This confirms octahedral geometry for the Co(II) complex. The υ3/υ1 value in the Co(II) complex is 2.03 and it lies in the usual range (2.00-2.80) reported of octahedral complex. The electronic parameters were calculated using the standard method and the values are as follows:

Table 2. Significant IR spectral bands (cm⁻¹) of the ligand of Oxytetracycline HCl and their metal complexes

<table>
<thead>
<tr>
<th>Assignments</th>
<th>The compounds</th>
<th>Cu(1:1)</th>
<th>Cu(2:1)</th>
<th>Ni(1:1)</th>
<th>Ni(2:1)</th>
<th>Fe(1:1)</th>
<th>Fe(2:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>υ(OH)</td>
<td>3382.5 cm⁻¹</td>
<td>3418.2 cm⁻¹</td>
<td>3424.9 cm⁻¹</td>
<td>3381.5 cm⁻¹</td>
<td>3410.4 cm⁻¹</td>
<td>3411.4 cm⁻¹</td>
<td>3418.2 cm⁻¹</td>
</tr>
<tr>
<td>υ(NH2)</td>
<td>3080 cm⁻¹</td>
<td>3100 cm⁻¹</td>
<td>3100 cm⁻¹</td>
<td>3250 cm⁻¹</td>
<td>3250 cm⁻¹</td>
<td>3220 cm⁻¹</td>
<td>3220 cm⁻¹</td>
</tr>
<tr>
<td>CH-aliph</td>
<td>2926.4 cm⁻¹</td>
<td>2362.3 cm⁻¹</td>
<td>2337.3 cm⁻¹</td>
<td>2362.3 cm⁻¹</td>
<td>2928.3 cm⁻¹</td>
<td>1559.1 cm⁻¹</td>
<td>1580 cm⁻¹</td>
</tr>
<tr>
<td>CH=N</td>
<td>1622.8 cm⁻¹</td>
<td>1621.8 cm⁻¹</td>
<td>1621.8 cm⁻¹</td>
<td>1636.3 cm⁻¹</td>
<td>1639.2 cm⁻¹</td>
<td>1580 cm⁻¹</td>
<td>1624.7 cm⁻¹</td>
</tr>
</tbody>
</table>
| ¹H-NMR and IR for oxytetracycline, 1:1 and 2:1 Zn(II) complexes

1. Oxytetracycline: ¹H-NMR (Bruker 400 MHz pulsed FT NMR): δ 2.3 (2H), 2.5 (w), 2.6 (w), 2.8 (s), 2.9 (s), 3.0 (w), 3.37 (3H), 3.6 (w, 1H), 4.6 (m, 1H), 5.9 (w, 1H), 7.4 (t, 2H, Ar-H), 7.54 (m, 4H, Ar-H), 7.6 (m, 4H, Ar-H), 9.1 (d, 1H, Ar-H), 9.6 (d, 1H, NH), 11.64 (s, 1H, OH) ppm. (KBr): υ(OH), 3382; υ(C=O), 1622; υsym(COO), 1583; υasym(COO), 1398; bend(OH), 1138.

2. 1:1 Zn(II) metal complex: ¹H-NMR: δ 2.3 (w), 2.36 (m), 2.5 (s, 1H), 2.56 (s, 1H), 2.8 (s, 1H), 3.5 (m, 2H), 3.8 (w, 4H), 4.2 (m, 2H), 6.9 (w, 1H), 7.1 (w, 1H), 7.3 (s, 3H, Ar-H), 7.72 (m, 3H, Ar-H), 7.90 (m, 3H, Ar-
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H), 9.2(m,1H, Ar-H), 9.25(m,1H, Ar-H),11.6(w) ppm .IR(KBr): v(OH),3298; v(C=O), 1616; v asym(COO), 1507; v sym(COO), 1367; bend(OH), 1175; δ (Zn-N), 545 cm⁻³.

- 2:1 Zn(II) metal complex: ¹H-NMR: δ; 1.81 (s, 6H, CH3), 1.83(s,3H, CH2), 2.1(w), 2.17(w,1OH), 2.2(s,29H), 2.8(m,12H), 3.6(m,52H), 7.1(w,2H), 7.2(w,4H), 7.5(w,3H), 11.66(w,1H) ppm. IR(KBr): v(OH), 3068; δ (C=O),1659; v asym (COO),1585; v sym(COO), 1396; bend(OH), 1138; δ(Zn-N), 574; δ(Zn-O), 532 cm⁻³.

**BIOLOGICAL SCREENING**

The Oxytetracycline HCl ligand and their metal complexes tested for their antimicrobial activity against some species of bacteria (S. aureus, B. subtilis, E. coli, P. aeruginosa, N. gonorrhoea and S. faecalis), Table (3)

Using a modified Kirby-Bauer disc diffusion method [43] Briefly, 100 ml of the test bacteria/ Fungi were grown in 10 ml of fresh media until they reached account of approximately 108 cells/ml for bacteria or 105 cells/ ml for fungi [44]. 100 ml of microbial suspension spread onto agar plates corresponding to the broth in which they maintained.

Isolated colonies of each organism that might be playing a pathogenic role should selected from primary agar plates and tested for susceptibility by disc diffusion method[45].

Of the many media available, NCCLS recommends Mueller- Hinton agar due to it results in good batch – to – batch reproducibility.

Disc diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by the (NCCLS, 2002)[46]for evaluating the susceptibilities of filamentous fungi to antifungal agents.

Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the (NCCLS, 2003)[47].

Plates inoculated with filamentous fungi as Aspergillusflavus at 25degree c for 48 hours:

**Table3: Biological activity (sensitivity tests) by Kirby-Bauer Method**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bacillus subtilis (G+)</th>
<th>Escherhia coli (G+)</th>
<th>Neisseria gonorrhoeae (G+)</th>
<th>Pseudomonas aeruginosa (G-)</th>
<th>Staphylococcus aureus (G+)</th>
<th>Streptococcus faecalis (G+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: DMSO</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Antibiotics:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20</td>
<td>22</td>
<td>20</td>
<td>17</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Tetracycll</td>
<td>26</td>
<td>27</td>
<td>31</td>
<td>25</td>
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</table>
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<table>
<thead>
<tr>
<th>Cation</th>
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<td>36</td>
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<tr>
<td>Cu 1:1</td>
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<td>14</td>
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<td>13</td>
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<td>13</td>
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<tr>
<td>Cu 2:1</td>
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<tr>
<td>Fe 1:1</td>
<td></td>
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<td>12</td>
<td>13</td>
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<td>Fe 2:1</td>
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<td>Ni 1:1</td>
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<td>16</td>
<td>15</td>
<td>17R</td>
<td>14</td>
<td>14</td>
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<tr>
<td>Ni 2:1</td>
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<td>17</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

Scheme 2. The Proposal structure of Oxytetracycline – Nickel(II) (1:1) complex

Scheme 3. The proposal structure of oxytetracycline – Nickel(II) (2:1) complex

Scheme 4. The proposal structure of Oxytetracycline – Copper(II) (1:1) complex
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REFERENCES


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