Spectral and Biological Characterization of Some Copper(II) Complex Compounds with Chlorhexidine

MIRELA CALINESCU*, TICUTA NEGREANU-PIRJOL†, OCTAVIAN CALINESCU*, RODICA GEORGESCU

1University of Bucharest, Faculty of Chemistry, 23 Dumbrava Rosie Str., 020464, Bucharest, Romania
2Ovidius University, Faculty of Pharmacy, I Universitati Str., 900470, Constanța, Romania
3Research Development National Institute for Physics and Nuclear Engineering Horia Hulubei, Bucharest-Măgurele 077125, Romania

Three copper(II) complex compounds with chlorhexidine diacetate as ligand have been characterized by magnetic susceptibility measurements, infrared, electronic and EPR spectra. The complexes correspond to the formulae: [Cu(CHX)Cl2].2H2O, [Cu(CHX)Br2].1.5H2O and [Cu(CHX)](CH3COO)2 . C2H5OH, where CHX = chlorhexidine. Chlorhexidine acts as neutral tetradentate NNNN donor, coordinating through the four imine nitrogen atoms. The bonding parameters of the Cu(II) complexes determined from the EPR spectra indicate strong in-plane π-bonding, showing that the chlorhexidine is not only a good σ-donor, but also a good π-donor. Disc diffusion method was used to evaluate the antimicrobial activity against four bacterial and one fungal cultures and the complexes show to be moderate antibacterial and weak antifungal agents.

Keywords: Biguanides, chlorhexidine, copper(II) complexes

The interest in the chemistry of biguanides arises from their importance as antimalarial, antidiabetic, antimicrobial and antifungal agents [1-4]. Most of the studies were aimed at preparation and characterization of transition metal and antifungal agents [1-4]. Most of the studies were aimed at preparation and characterization of transition metal and antifungal agents [1-4]. A few studies have reported the synthesis and characterization of some copper(II) complex compounds using chlorhexidine diacetate and copper(II) chloride, bromide and acetate, respectively, in 2:1 metal:ligand ratio [17]. The screening data for the inhibition diameter of microbial culture show an increase of activity for the complex containing chloride against Gram negative bacteria comparatively with chlorhexidine diacetate.

In order to study the effect of the metal:ligand ratio on the biological activity of the complexes, we have prepared and characterized other three new complex compounds of copper(II) with chlorhexidine diacetate in 1:1 metal:ligand ratio. The results concerning the synthesis, the elemental analysis and the thermal decomposition of these complexes are described in a recent paper [18]. The aims of this study was to investigate the spectral, magnetic and antimicrobial properties of these new complex compounds of copper(II) with chlorhexidine diacetate in 1:1 metal:ligand ratio.

Experimental part
Preparation of the complexes

The three metal complexes were prepared according to the procedure described in the previous paper [18], by mixing together an ethanolic solution containing 1 mmol of chlorhexidine diacetate with an ethanolic solution containing 1 mmol of the metal salt: CuCl2 . 2H2O for the complex (1), CuBr2 for the complex (2) and Cu(CH3COO)2 . H2O for the complex (3). The resulting solutions were stirred at 40-50°C for 1 h, when solid colored products were separated out. They were filtered off, washed with ethanol and dried at air. The colours of the metal complexes are very different from those of the corresponding 2:1 metal:ligand complexes: dark-violet for the complex (1), dark-brown for the complex (2) and pink-violet for the complex (3). All these complexes are quite stable at air, at room temperature and can be stored for several months.

* email: mirela_calinescu@hotmail.com; Tel.: 0726317237

Fig. 1. Chlorhexidine (CHX)
are partly soluble in acetone, easily soluble in DMF, DMSO and acetonitrile.

**Physico-chemical analyses**

Infrared spectra (in KBr pellets) were recorded on a BIORAD FTIR 135 spectrophotometer, in the range 4000-400 cm\(^{-1}\). Molar conductance measurements were made on a Consort C-533 conductometer, in DMF solutions 10\(^{-4}\) M. UV-Vis diffuse reflectance spectra were registered on a UV-Vis Jasco 650 spectrophotometer, in the range 200-900 nm. K-band electron paramagnetic resonance spectra were recorded on a MiniScope MS 200 spectrophotometer, on powdered samples. Magnetic susceptibility measurements were performed by the Faraday method, on a Gouy balance, at room temperature, using Hg[Co(SCN)\(_4\)] as calibrant.

**Results and discussion**

**Infrared spectra**

A comparison between the important absorption bands in the IR spectra of chlorhexidine diacetate and those of its complexes allows to establish the groups involved in coordination to the metal ion.

In the IR spectrum of chlorhexidine diacetate monohydrate two medium absorption bands appearing at high wave numbers, at 3326 cm\(^{-1}\) and 3180 cm\(^{-1}\) may be assigned to the stretching vibrations N-H of the groups Alkyl-NH-aryl and (Alkyl)NH and to the stretching vibration of the group =NH, respectively [6,19,20]. ν(OH) absorption band may be observed in the same region, with the maximum at 3338 cm\(^{-1}\).

The presence of the bands due to the symmetric and asymmetric N-H stretching modes of NH\(_2\) group, at 3140 cm\(^{-1}\) and to the deformation vibration of the same bonding, at 1613 cm\(^{-1}\) are in accordance with the protonation of the chlorhexidine in its diacetate salt, in solid state [19,21].

The most important band which is expected to be influenced by coordination is the strong absorption, at 1644 cm\(^{-1}\), due to the stretching vibration of the imine function, ν(C=N) [19,22].

Other characteristic bands of the ligand occur at 1574 and 1337 cm\(^{-1}\) and may be attributed to δ(NH) + ν(C-N) and 1249 cm\(^{-1}\), attributed to ν(C=O) [6,20].

The IR spectra of the chlorhexidine diacetate ligand also shows bands due to the stretching vibrations of the acetate group: 1536 cm\(^{-1}\) - ν\(_{as}\) (COO) and 1417 cm\(^{-1}\) - ν\(_{s}\) (COO) [23]. The IR spectra of all the three complexes show important changes comparatively with the spectrum of the chlorhexidine ligand.

Thus, the strong band assigned to ν(C=N) shows a positive shift, indicating the coordination of the imine nitrogen atoms to the metal ion [6,19,21]. The upward shift of the bands due to δ(NH) + ν(C-N) and the downward shift of the band assigned to C\(_{aryl}\)-N are also in favour of the coordination of the ligand through the four imine nitrogen atoms. Further confirmation of the implication of the imine nitrogens in coordination is the upward shift of the stretching vibration ν(=NH). These observations are in accordance with the results of other studies regarding the metal complexes with biguanides [24,25]. The possibility of coordination through amino nitrogen atoms also exists, but the metal-N (imino) formation takes priority, probably due to the increase of stability by \(π\)-conjugation on the C-N-C system involving the imino nitrogen [24,25].

The band due to stretching vibrations of NH\(_3\)^+ group and that attributed to the deformation vibration, δ(NH\(_2\)^+) disappear in the IR spectra of the complexes, in accordance with the deprotonation of the chlorhexidine and involvement in complexation as neutral ligand [24].

The IR spectra of the complexes show supplementary bands at low wave numbers, which may be assigned to ν(Cu-N) [20]. The medium-strong bands, at 1530 and 1409 cm\(^{-1}\) in the spectrum of the complex (3) may be attributed to ν\(_{as}\) (COO) and ν\(_{s}\) (COO), respectively, of acetate group [23]. The value of \(Δ = ν\(_{as}\) (COO) - ν\(_{s}\) (COO) = 121 cm\(^{-1}\) is in the range of ionic acetate [23].

The free ν(OH) band is observed in the IR spectra of the complexes at 3350 - 3430 cm\(^{-1}\) and it is due to the presence of the crystalline water or ethanolic alcohol, as we can establish from the thermal analysis.

The presence of the crystalline water or ethanol are confirmed by the thermal analysis. The thermal decomposition steps as well as the mass lost for each of them were detailed analyzed in the previous paper [18].

On the base of the IR spectra we can conclude that the ligand acts as neutral tetradentate NNNN donor ligand in all the three complexes, coordinating through the four imine nitrogen atoms. Taking into account of molar conductance values which indicate a non-electrolytic nature for the complexes (1) and (2) \(λ_2\) in DMF = 43 \(Ω^{-1} cm^2 mol^{-1}\) and 47 \(Ω^{-1} cm^2 mol^{-1}\), respectively and 1:2 electrolyte for the complex (3) (\(λ_2\) in DMF = 153 \(Ω^{-1} cm^2 mol^{-1}\)), the metal ion is four-coordinated in the complex (3) and six-coordinated in the complexes (1) and (2).

According to the determinations presented above, the following structures have been proposed for the complexes (fig. 2):

![Fig. 2. Proposed structures for the Cu(II) complexes](image)

**Magnetic and electronic spectral data**

The magnetic moment values for the complexes 1-3 are 1.74, 1.89 and 1.70 MB, respectively and correspond to one unpaired electron [26].

A correlation between the EPR spectral data and the observed bands in the electronic spectra gave important information on the stereochemistry of the complexes and on the character of the metal-ligand bonds.

For all the compounds, the electronic spectra were recorded in solid state.

The chlorhexidine diacetate exhibits strong absorption bands in the UV region, at 208 nm (48100 cm\(^{-1}\)), 255 nm
(39215 cm⁻¹), 298 nm (33500 cm⁻¹) and 344 nm (29000 cm⁻¹), which can be assigned to n-σ*, π-π* and n-π* transitions, respectively [27]. These bands are also present in the electronic spectra of the copper(II) complexes, with a slight shift due to the complexation.

UV-VIS spectra of the complexes (1) and (2) are very different from the spectrum of the complex (3) (figs. 3-5). The main absorption band in the visible region for the complexes (1) and (2) is stronger than the corresponded band in the spectrum of the complex (3). Moreover, a very strong absorption band around 400 nm, observed in the spectra of these complexes may be attributed to a ligand → metal charge transfer process (LMCT) [28]. This band is stronger even than of the ultraviolet bands characteristic of the ligand.

The colours of the complex compounds evidence these differences. Thus, while the complex (3) is pink-violet, this colour being characteristic to the chromophore CuN₄ in the dibiguanide and bis(biguanide) Cu(II) complexes [5], the compounds (1) and (2) are coloured in dark-violet and dark-brown, respectively.

The electronic spectral bands of the complex compounds, with their assignments, are given in the table 1 [28].

A square-planar D₄h symmetry is predictable for the complex (3), because of the ionic character of acetate group, established by means of the IR spectra. In the case of the complexes (1) and (2), their non-ionic nature and the presence of the strong charge transfer band in the visible-ultraviolet spectra lead to attributing them a distorted octahedral symmetry, with the two halogen atoms in the coordinated sphere of the metal.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Observed bands (λmax, nm/cm⁻¹)</th>
<th>Assignments</th>
<th>Symmetry</th>
</tr>
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<tbody>
<tr>
<td>[Cu(CHX)Cl₂]·2H₂O (1)</td>
<td>250/40000 330/30300 410/24390 540/18518 650/15384 700/14285</td>
<td>π-π* n-π* LMCT ²B₁g→²E ²B₁t→²B₂g ²B₄g→²A₁g</td>
<td>Tetragonal distorted O₄h</td>
</tr>
<tr>
<td>[Cu(CHX)Br₂]·1.5H₂O (2)</td>
<td>240/41666 310/32258 390/25640 565/17700 600/16666 700/14285</td>
<td>π-π* n-π* LMCT ²B₁g→²E ²B₁t→²B₂g ²B₄g→²A₁g</td>
<td>Tetragonal distorted O₄h</td>
</tr>
<tr>
<td>[Cu(CHX)(CH₃COO)₂]C₂H₅OH (3)</td>
<td>240/41666 290/34482 500/20000 530/18867 770/12987</td>
<td>π-π* n-π* ²B₁t→²A₁g ²B₄g→²E ²B₄g→²B₂g</td>
<td>Square-planar D₄h</td>
</tr>
</tbody>
</table>
The EPR spectra of the Cu(II) complexes, recorded on powdered samples, in K band, are presented in Figures 6 and 7 and the experimental parameters are listed in Table 2.

The EPR spectra of \([\text{Cu(CHX)}\text{Cl}_2] \cdot 2\text{H}_2\text{O}\) (1) and \([\text{Cu(CHX)}\text{Br}_2] \cdot 1.5\text{H}_2\text{O}\) (2) show broad lines, with weak hyperfine splitting in the low field region. These can be better observed from the second derivative of the signal, especially for the complex (2). For this compound we have determined the spectral parameters \(g_\parallel\), \(g_\perp\) and \(A_\parallel\). At 77 K, the shape of the signal is the same, but the hyperfine structure is more poorly resolved.

The EPR spectra of \([\text{Cu(CHX)}\text{(CH}_3\text{COO)}_2] \cdot \text{C}_2\text{H}_5\text{OH}\) (3) shows also an axial symmetry, with \(g_\parallel < g_\perp\), with hyperfine splitting in the parallel region.

On the basis of molecular orbital theory, using the experimental EPR parameters and the energies of \(d-d\) transitions we calculated the covalency parameters \(\alpha^2\), \(\beta^2\), and \(\beta_1^2\) listed in the Table 3 [29-31].

The coefficients \(\alpha^2\), \(\beta^2\), and \(\beta_1^2\) characterize the in-plane \(\sigma\)-bonding, in-plane \(\pi\) bonding and out-of-plane \(\pi\) bonding of \(\text{Cu}^{2+}\), respectively. \(\alpha^2\) can take the values between 0.5 and 1, corresponding to pure covalent and pure ionic metal-ligand bond, respectively.

The orbital parameters \(K_\parallel\) and \(K_\perp\), related to the covalency parameters discussed above by the relations:

\[K_\parallel = \alpha^2 \beta_1^2 \text{ and } K_\perp = \alpha^2 \beta^2\]

are a guide of the metal-ligand bonding nature: \(K_\parallel = K_\perp\) for the pure \(\sigma\) bonding, \(K_\parallel < K_\perp\) for strong in-plane \(\pi\) bonding and \(K_\parallel > K_\perp\) for strong out-of-plane \(\pi\) bonding [32].

The \(\alpha^2\) values show that the metal-ligand bond has an intermediate character, the covalent and the ionic interactions having the contributions almost equal.

The values of the parameter \(\beta_1^2\), as well as the \(K_\parallel\) values, which are smaller than \(K_\perp\), indicate strong in-plane \(\pi\) bonding, showing that the chlorhexidine is a good \(\pi\)-donor.

The parameter \(f = g_\parallel/A_\parallel\) is an index of tetragonal distortion and its values may vary from 105 to 135 for small to extreme distortion [32,33]. The \(f\) value calculated for the complex (2) is in accordance with a weak distortion from the octahedral symmetry, suggesting that the ligand field of the chlorhexidine is only a little stronger than the ligand field of the halogen. For the complex (3), the \(f\) value is very close from the inferior limit, according to the planarity of the molecule of chlorhexidine and thus to a square-planar geometry of the complex cation.

\[G = (g_\parallel - 2)/(g_\perp - 2)\] determined for the complexes (2) and (3) is smaller than 4, suggesting the strong spin-spin interactions between the copper centers.

### Biological activity

The *in vitro* antibacterial and antifungal activity of chlorhexidine diacetate, Cu(II) complexes and standard drugs (ampicillin, ciprofloxacin and miconazol) was estimated by agar disc diffusion method in acetone as solvent [34-36], following the same protocol as described in the previous work [17].

The compounds were checked against three Gram negative bacteria (*E. coli*, *Pseudomonas aeruginosa* serotype IV and *Klebsiella pneumoniae*), one Gram positive bacteria (*Staphylococcus aureus*) and one fungal culture (*Candida albicans*). The organisms were collected in the Microbiology Laboratory of Faculty of Pharmacy, Ovidius University, Constanța: *E. coli*, from a urine sample, *Pseudomonas aeruginosa* serotype IV, from sewage water samples, *Klebsiella pneumoniae*, from a saliva sample,
The complexes reported in this work have a high symmetry, tetragonal distorted O₆ symmetry (complex 3), so they are poorly active against tested microorganisms.

**Conclusions**

The complex compounds obtained from chlorhexidine diacetate and various copper(II) salts in 1:1 metal:ligand ratio have tetragonal distorted octahedral symmetry: [Cu(CHX)Cl₂]. 2H₂O and [Cu(CHX)Br₂]. 1.5H₂O and square-planar symmetry: [Cu(CHX)](CH₃COO)₂.C₂H₅OH (3).

These complexes show moderate antibacterial and weak antifungal activities against microorganisms tested. The high symmetry of the complex cation leads to a diminution of the antimicrobial activity comparatively with that of the chlorhexidine and with a similar complexes, in 2:1 metal:ligand ratio.

**References**


**Table 4**

<table>
<thead>
<tr>
<th>Microbial strain</th>
<th>Microbial culture inhibition diameter [mm]</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
</tr>
<tr>
<td>-18</td>
<td>28</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>21</td>
</tr>
</tbody>
</table>

A – ampicillin (10 μg/disc); C – ciprofloxacin (5 μg/disc); M – miconazol (30 μg/disc)

**Staphylococcus aureus**, from a pharyngeal exudate and *Candida albicans*, collected from a pharyngeal exudate.

Filter paper discs (Φ = 6 mm) were impregnated with 10 μL solution 10⁻³ M of the ligand or complex in acetone and then placed on agar surface inoculated with bacterial and fungal cultures. The amounts of the compound/disc were between 10.1 and 11.2 mg/disc, comparable with those used for the 2:1 metal:ligand complexes [17]. For the ligand, the amount was 6.4 μg/disc. Acetone was used to dissolve the compounds due to its fast evaporation, so that the effect on microbial culture is only due to its fast evaporation, so that the effect on microbial culture is only due to the tested compound. The plates were incubated at 37°C, for 24 h for the bacteria and 72 h for the fungi. The diameter of zone of inhibition was then measured and compared with the standard antibiotics (ampicillin and ciprofloxacin for bacteria and miconazol for fungi). The results are presented in table 4.

The results indicate that these complexes show a diminution of antimicrobial activity comparatively of 2:1 metal:chlorhexidine complexes reported in our previous work [17].

Thus, while the diameter of inhibition zone against Gram negative bacteria is comparable or only slowly diminished comparatively to 2:1 metal:chlorhexidine complexes, the activity against *Staphylococcus aureus* and *Candida albicans* is considerably reduced. The great diminution is observed for [Cu(CHX)Cl₂]. 2H₂O comparatively to [Cu(CHX)Cl₂]. 2C₂H₅OH (for this last complex, the diameter of inhibition zone was 25 mm against *Staphylococcus aureus* and 20 mm against *Candida albicans* [17]).

Two assumptions can be considered to explain these results. First, we can suppose that a higher concentration of copper in the molecular weight for 2:1 metal:chlorhexidine complexes than for 1:1 metal:chlorhexidine complexes leads to an increase of antimicrobial activity. However, the activity of chlorhexidine diacetate against Gram positive bacteria and fungi is higher than that of copper(II) salts. Thus, while the diameter of zone of inhibition for chlorhexidine diacetate is 10 mm against Gram negative bacteria and 24 mm against Gram positive bacteria and fungi, for the copper(II) salts (chloride, bromide and acetate) the diameter of zone of inhibition varies between 14 and 19 mm for the same amount (6-7 μg/disc).

The second hypothesis is related to the symmetry around the metal ion. Several works have reported that the complexes with low symmetry display higher biological activity [33,37]. The complexes reported in this work have a high symmetry, tetragonal distorted O₆ symmetry (complexes 1 and 2) and square-planar symmetry (complex 3), so they are poorly active against tested microorganisms.

Manuscript received: 6.04.2012