Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2011, **12** (2), pp. 127 – 140

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

Cu(II) AND Zn(II) COMPLEX COMPOUNDS WITH BIGUANIDES AROMATIC DERIVATIVES. SYNTHESIS, CHARACTERIZATION, BIOLOGICAL ACTIVITY

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Received: June 30, 2010
Accepted: March 02, 2011

Abstract: In this paper we report the synthesis, physical-chemical characterization and antimicrobial activity of some new complex compounds of hetero-aromatic biguanides ligands, chlorhexidine base *(CHX)* and chlorhexidine diacetate *(CHXac$_2$)* with metallic ions Cu(II) and Zn(II), in different molar ratio. The synthesized complexes were characterized by elemental chemical analysis and differential thermal analysis. The stereochemistry of the metallic ions was determined by infrared spectra, UV-Vis, EPR spectroscopy and magnetic susceptibility in the aim to establish the complexes structures. The biological activity of the new complex compounds was identified in solid technique by measuring minimum inhibition diameter of bacterial and fungal culture, against three standard pathogen strains, *Escherichia coli* ATCC 25922*, Staphilococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. The results show an increased specific antimicrobial activity for the complexes chlorhexidine:Cu(II) 1:1 and 1:2 compared with the one of the Zn(II) complexes.

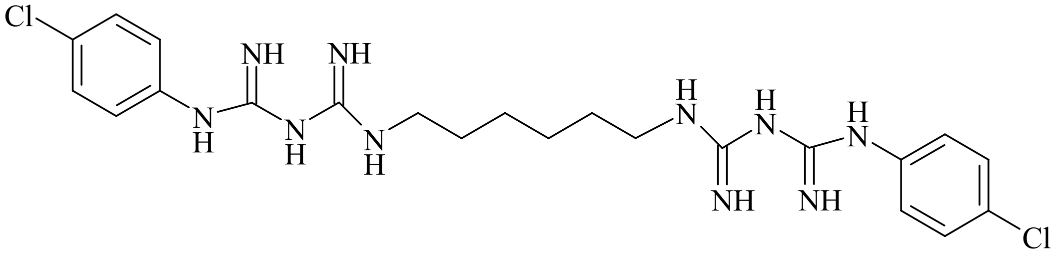
Keywords: *biguanides derivatives, biological activity, complex compounds, copper(II), zinc(II)*

INTRODUCTION

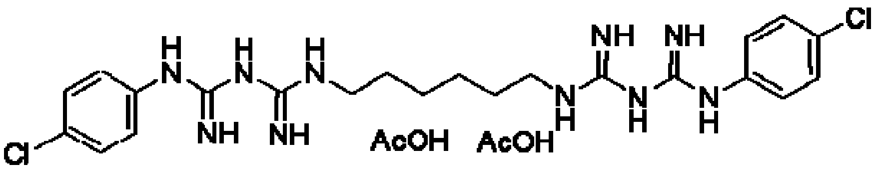
Chlorhexidine and its salts (diacetate, dihydrochloride, digluconate) are well known for their antimicrobial properties and have been used as oral solution to prevent plaque and cavities formation in the oral cavity. They also present an action on several Gram positive and Gram negative germs. Literature data show that many researches have been geared towards the introduction of metal ions, in various concentrations, in chlorhexidine-based preparations, both in order to potentiate its activity, and to diminish its side effects. Most used for this purpose are Cu^{2+}, Zn^{2+}, and Ag^{+} and less often Sn^{2+}, Fe^{2+} [1]. Choosing ions of transitional metals (Zn^{2+}, Cu^{2+}, Ag^{+}) has at the base the therapeutic role of biological systems which include such cations and the use of Cu^{2+}, Zn^{2+} and Ag^{+} ions in combination with chlorhexidine is due mainly to the antibacterial and antifungal action that these show even as simple salts form. Studies have shown that plaque reducing effect of chlorhexidine increases slightly by the introduction of zinc salts. Using a chlorhexidine and zinc complex (0.2% and 0.3%) had a better effect on plaque inhibition compared to chlorhexidine by itself [1 – 4].

In this paper were carried out five variants of the synthesis of chlorhexidine complex compounds with Cu(II) and Zn(II) in various conditions of *p*H, temperature, metallic salt, molar ratio Metal : Ligand. Syntheses were carried out using as ligands, chlorhexidine base (*CHX*) and monohydrated chlorhexidine diacetate (*CHXac*$_2$), Figure 1. Analysis of obtained complexes revealed that the chlorhexidine form and metal salt anion used have the greatest influence on the composition and stereochemistry of the complex obtained. The synthesis optimum *p*H belongs to 5.5 – 8. Also, because the compound of two different metal ions (copper and zinc) has been shown to contribute to intensifying the antimicrobial activity, there were synthesized also complex compounds of two different metal ions.

It is estimated that ligands (*CHX*), (*CHXac*$_2$) can function as bi- or monodentate ligands through *N*-imine atoms, with high values of electronic density.



CHX = chlorhexidine base



CHXac$_2$ *= chlorhexidine diacetate*

Figure 1. *Structures of ligands*

EXPERIMENTAL

For the synthesis of new complex compounds were used salts: $ZnCl_2$, $ZnSO_4\cdot 7H_2O$, $Zn(CH_3COO)_2\cdot H_2O$, $Cu(NO_3)_2\cdot 3H_2O$, $CuSO_4\cdot 5H_2O$, $Cu(CH_3COO)_2\cdot H_2O$ and *CHX*, *CHXac₂* (Merck) ligands, used without further purification, dissolved in ethanol. Content in metal ions was determined by atomic absorption, using a Perkin Elmer Aanalyst 400.

Spectra of compounds in solid state were recorded using a Jasco V560 UV-Vis spectrophotometer, with integrating sphere for diffuse reflectance in 200-850 nm domain.

Infrared spectra were recorded using a BIORAD FTIR 135 spectrophotometer (in KBr pellet) and a Bruker Equinox55 tool, for 4000 – 400 cm^{-1} domain.

Magnetic susceptibility measurements were performed on a magnetic balance using Faraday method, at room temperature, with $Hg[Co(SCN)_4]$ as standard.

EPR spectra were recorded on a high sensitivity spectrophotometer (1 x 1010 spins/G) MiniScope MS 200 (Magnettech Ltd., Germany) in X band (9.6 GHz). One has worked in 10 mW microwave field, Hm = 4 G magnetic field domain, at room temperature and at liquid nitrogen temperature (77 K).

In Table 1 are presented the synthesized complex compounds, their systematization being done by chemical form of the ligand: chlorhexidine base (*CHX*) and chlorhexidine diacetate (*CHXac₂*).

***Table 1**. Synthesized complex compounds with chlorhexidine base (CHX) and chlorhexidine diacetate (CHXac₂)*

Ligand	Metallic ion	Counter-ion	Complex notation	Molar ratio
Chlor-hexidine base (*CHX*)	Zn^{2+} Cu^{2+}	Cl^- NO_3^-	**(1)**	*CHX* : $ZnCl_2$: $Cu(NO_3)_2\cdot 3H_2O$ 1:1:1
	Cu^{2+}	SO_4^{2-}	**(2)**	*CHX* : $CuSO_4\cdot 5H_2O$ 1:1
	Cu^{2+}	NO_3^-	**(3)**	*CHX* : $Cu(NO_3)_2\cdot 3H_2O$ 1:2
Chlor-hexidine diacetate (*CHXac₂*)	Zn^{2+} Cu^{2+}	CH_3COO^- Cl^-	**(4)**	*CHXac₂*:$Zn(CH_3COO)_2\cdot 2H_2O$:$CuCl_2\cdot 2H_2O$ 1:1:1
	Zn^{2+} Cu^{2+}	Cl^- Cl^-	**(5)**	*CHXac₂* : $ZnCl_2$:$CuCl_2\cdot 2H_2O$ 1:1:1

Complexes synthesis

Complex (**1**): Chlorhexidine : $ZnCl_2$:$Cu(NO_3)_2\cdot 3H_2O$ in molar ratio 1:1:1 (*p*H = 5,5). 0.5054 g (1 mmol) ligand (chlorhexidine base) was dissolved in 50 mL methanol, at heat. To this solution were added consequently corresponding metallic salts 0.136 g (1 mmol) anhydrous $ZnCl_2$ and 0.242 g (1 mmol) $Cu(NO_3)_2\cdot 3H_2O$, each dissolved into a minimum quantity of methanol. Adjustment of *p*H by adding in drops of aq. 0.1 N KOH solution until *p*H = 8, then the mixture is brought to boiling and refluxed for 4 hours, under continuous agitation. A crystalline precipitate separates, of pale violet color. After cooling the solution, the product was filtered (G4), washed with methanol and ethyl ether and dried for 2-3 days in the vacuum desiccator, on $(P_2O_5)_2$. The final product resulting as fine, crystalline powder, fine, of pale violet color, stable in air and light, is soluble in dimethyl sulfoxide (DMSO), dimethylformamide (DMF), acetonitrile (AN), acetone, partially soluble in ethyl alcohol and methyl alcohol, insoluble in water, ethyl ether, chloroform, benzene, *n*-hexane, CCl_4. Reaction yield is of about 90%.

Complex (**2**): Chlorhexidine : $CuSO_4 \cdot 5H_2O$ in molar ratio 1:1 (pH = 5.5). For a suspension containing 0.5054 g (1 mmol) chlorhexidine (*CHX*) in 25 mL ethanol, at heating were added 0.2500 g (1 mmol) of $CuSO_4 \cdot 5H_2O$. Reaction mixture was maintained at slight heating for ½ h. A dark violet precipitate was formed. After another 1 h of shaking without heating, the solid compound that was formed was separated through vacuum filtration, washed with ethyl alcohol and dried in air. Complex obtained is insoluble in all the usual solvents.

Complex (**3**): Chlorhexidine : $Cu(NO_3)_2 \cdot 3H_2O$ in molar ratio 1:2 (pH = 5,5). For a suspension containing 0.5054 g (1 mmol) chlorhexidine (*CHX*) in 25 mL ethanol were added, at room temperature and under continuous shaking, 0.4830 g (2 mmol) of $Cu(NO_3)_2 \cdot 3H_2O$ dissolved in minimum quantity of ethanol. Immediately a blue precipitate was formed, which in approximately 30 minutes changed into a violet precipitate. This was separated by vacuum filtration, washed with alcohol and dried in air. The compound is partially soluble in ethanol, methanol and acetone at heat and soluble in DMF and DMSO at cold.

Complex (**4**): Chlorhexidine diacetate : $Zn(CH_3COO)_2 \cdot 2H_2O$: $CuCl_2 \cdot 2H_2O$ in molar ratio of 1:1:1 (pH = 5.5). 0.6435 g (1 mmol) ligand (chlorhexidine diacetate $CHX \cdot 2CH_3COOH$) were dissolved in 30 mL ethanol, at heating. Were added 0.2194 g (1 mmol) of $Zn(CH_3COO)_2 \cdot 2H_2O$, dissolved in the minimum quantity of ethanol. The solution remains clear even after 1 h of reflux. By adding $CuCl_2 \cdot 2H_2O$, 0.1704 g (1 mmol) an intense violet color precipitate was formed. The complex was separated by vacuum filtration, washed for several times with ethyl alcohol and dried in air. It is partially soluble, at heating, in methanol, ethanol and acetone, easy soluble in DMF at cold and insoluble in benzene, chloroform, CCl_4.

Complex (**5**): Chlorhexidine diacetate : $ZnCl_2$: $CuCl_2 \cdot 2H_2O$ in molar ratio of 1:1:1 (pH = 5.5). 0.6435 g (1 mmol) ligand (chlorhexidine diacetate $CHX \cdot 2CH_3COOH$) were dissolved in 30 mL ethanol, at heating. Metallic salts: 0.1362 g (1 mmol) of $ZnCl_2$ and of 0.1704 g (1 mmol) of $CuCl_2 \cdot 2H_2O$ were dissolved separately in the minimum quantity of ethanol. By adding the metallic salts solution immediately was formed a light violet, abundant precipitate. The compound obtained is soluble in methanol, ethanol and acetone, benzene, chloroform; it is soluble in DMF and DMSO.

Antimicrobial activity

Were tested complexes (**1 - 5**) compared with the ligands (*CHX*), (*CHXac₂*) on their action on a two standard bacterial pathogen or conditioned pathogen strains, belonging to Gram positive (*Staphylococcus aureus* ATCC 25923*)* and Gram negative (*Escherichia coli* ATCC 25922*)* species and one fungal strain, *Candida albicans* ATCC 10231. By measuring the minimum inhibition diameter of the bacterial growth, the "bactericide power" of the tested substances was established. A filter disc (F = 6 mm) was impregnated with 10 mL solution 10^{-3} M of the ligand or complex in acetone and then placed on an agar surface inoculated with bacterial and fungal cultures. At this concentration all the six complexes and ligands are soluble in acetone. Acetone was used to dissolve the compounds 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 the zone of inhibition was then measured and compared with the standard antibiotics (ampycillin and ciprofloxacin for bacteria and miyconazol for fungi) [5].

RESULTS AND DISCUSSION

The new complex compounds isolated as colored powders were characterized by elemental chemical analysis (Table 2), thermal analysis, FTIR UV-Vis, EPR spectroscopy and magnetic susceptibility.

Table 2. *Results of chemical analysis for synthesized complexes*

Notation	Complex	% Metal	
		Exp.	Calc.
(1)	$[CuZn(\mathit{CHX})(NO_3)_2Cl_2]\cdot 2C_2H_5OH$	7.03 7.00	6.94 (Cu) 6.95 (Zn)
(2)	$[Cu(\mathit{CHX})SO_4]\cdot C_2H_5OH$	9.18	8.99
(3)	$[Cu_2(\mathit{CHX})(NO_3)_4]$	14.30	14.52
(4)	$[CuZn(\mathit{CHX})(CH_3COO)_2]Cl_2$	7.93 8.02	7.77 (Cu) 7.78 (Zn)
(5)	$[CuZn(\mathit{CHXac_2})Cl_4]$	8.15 8.10	8.24 (Cu) 8.25 (Zn)

IR spectra

To obtain information on the nature of the bond between the central metal ion and anions and also about the coordination manner of ligands were recorded IR vibration spectra of these complexes in the 400 – 4000 cm^{-1} domain.

IR spectra of chlorhexidine base complexes

Because of the protonation absence, infrared spectrum of chlorhexidine base shows some important differences to that of the chlorhexidine diacetate. At large wave lengths were observed only two major bands: at 3471 and 3377 cm^{-1}, due to valence vibrations of N-H bonds in NH-Alkyl-Aryl groups; $(Alkyl)_2NH$, respectively (=NH). It was not observed any band due to protonated amino group.

Valence band characteristic to azomethine group appears at 1667 cm^{-1}, while coupled vibrations $\delta(NH) + \nu(C\text{-}N)$ give characteristic bands at 1598 and 1373 cm^{-1} [6, 8, 17 – 19].

In the spectra of complex compounds with chlorhexidine base the movement of valence vibration of the C = N group occurs for lower wave numbers than for the ligand, in agreement with the decrease of electronic density on these bonds, by complexation.

Coordination affects also positions of bands due to coupled vibrations δ(NH) + ν(C-N), these suffering slight movements, generally to higher wave numbers.

Infrared spectra of the complexes confirmed the presence of coordinated nitrate in complexes (**1**) and (**3**), by strong enhancing of the band from ~1090 cm^{-1} [14].

Other additional bands appearing in complexes spectra are due to vibrations of monodentate ligand (complex **2**) [14], of bidentate coordinated acetate (**3**), to vibrations of O-H group from water or ethanol in complexes (**1**) and (**2**) [7, 8].

IR spectra of chlorhexidine diacetat complexes

Monohydrated chlorhexidine diacetate presents, in the region 2900 – 3400 cm^{-1} characteristic absorption bands: two bands of medium intensity, in 3326 and 3140 cm^{-1}, due to the valence vibrations of NH bonds in Alkyl-NH-Aryl groups, respectively $(Alkyl)_2NH$ and an absorption band of moderate intensity, at 3180 cm^{-1}, attributed to the valence vibration of the group = NH [7, 8]. In the same region appears also valence vibration band characteristic of O-H group from water molecule, which overlaps the N-H vibration, causing a band stretching from 3326 cm^{-1}.

Vibrations of valence (symmetrical and asymmetrical) of NH bond in NH_2^+ give an absorption band at 3140 cm^{-1}, while the deformation vibration in plane corresponding to this relationship, $\delta(NH_2^+)$, gives an absorption band at 1613 cm^{-1} [9]. The presence of these two bands proves that chlorhexidine is found in protonated form.

Intense band, with maximum at 1644 cm^{-1} is due to the valence vibrations of C=N bond [10 – 13]. Intensity and position of this band in the IR spectra of complex compounds are the most importance to provide ligand coordination to metallic ions.

Bands at 1574 and 1337 cm^{-1} are due to coupled vibrations δ(NH) + ν(C-N) and are also affected by coordination. Other bands characteristic to ligand are due to valence vibrations of C=C bonds of aromatic nuclei (1550, 1537, 1487 cm^{-1}), generally less affected by coordination.

Medium intensity band, with maximum at 1249 cm^{-1} is due to valence vibration of $C_{aliphate}$-N [9]. Coordination of ligand to metal ions leads to light movements, usually to lower wave numbers, of the maximum of this band.

Medium intensity bands from 1514 and 1412 cm^{-1} are due to asymmetrical valence vibrations of C = O bonds, namely C-O from acetate group [14].

In the spectra of all synthesized complex compounds with chlorhexidine diacetate is observed the movement towards higher wave numbers of the band due to valence vibration of imine group, ν(C=N), according to the coordination of ligand to metal ions through non-participant electrons pair of imine nitrogen [10 – 13]. Coordination through imine nitrogen atoms is confirmed by the slight displacement towards higher wave numbers of the band from 1574 cm^{-1} δ(NH) + ν(C-N) and movement to lower wave numbers of the band from 1249 cm^{-1} due to the valence vibration $C_{aliphate}$-N.

Changes appearing in the region of large wave numbers, which is observed in the case of all complexes, a single band at ~3200 cm^{-1}, as well as band disappearing from 1613 cm^{-1}, indicates that at the formation of complex compounds occurs ligand deprotonation. Moreover, the shift to larger wave numbers of the azomethine group valence vibration, by complexing, is an indication of ligand participation in the deprotonated form (by deprotonation increases the electron density on C=N bonds).

Additional bands appearing in spectra of complex compounds are due to vibration of metal-nitrogen bonds or, by case, to metal-oxygen bonds (650 – 520 cm^{-1}) [15, 16].

Acetate group bands appear in complexes spectra (**4**), being characteristic to bidentate acetate [14].

Comparative study of IR spectra for ligands and complexes allowed establishing the coordination manner of ligands to metal ions. In all cases, coordination takes place through imine nitrogen atoms and the ligand was found in deprotonated form in all complexes obtained from the chlorhexidine base and chlorhexidine diacetate [17 – 19].

Thermal analysis

In conformity with TG and DTA curves, complex $[CuZn(\mathit{CHX})(NO_3)_2Cl_2]\cdot 2C_2H_5OH$ (**1**) is thermally decomposed in four clearly defined stages (Table 3). After removal of solvent molecules in a first step, follows the loss of two molecules of chlorobenzene resulted from the thermal decomposition of the chlorhexidine ligand. It follows a stage corresponding to nitrate anions decompose, to removal of chloride ions simultaneously with copper oxide formation. In the last stage takes place the thermal decomposition of the organic rest result from chlorhexidine after the loss of two chlorobenzene molecules ($C_{10}H_{20}N_{10}$).

Thermal decomposition of complex $[CuZn(\mathit{CHX})(NO_3)_2Cl_2]\cdot 2C_2H_5OH$ (**2**) ensue similarly, being observed four clearly defined stages (Table 4). The first two correspond to the elimination of two molecules of chlorobenzene. Thermal decomposition of the organic rest followed and finally the sulfate decomposition with formation of corresponding oxide, CuO, respectively ZnO [20, 21].

***Table 3.** Thermal behavior data (in static air atmosphere) for complex (1)*

Complex compound	**Stage**	**Thermal effect**	**Temperature interval 20/900 °C**	**Δm_{exp} [%]**	**Δm_{calc} [%]**
(**1**) $[CuZn(\mathit{CHX})(NO_3)_2Cl_2]\cdot 2C_2H_5OH$	1	Endothermic	50 - 82	10.19	10.09
	2	Exothermic	82 - 275	24.61	24.67
	3	Exothermic	275 - 355	17.92	17.85
	4	Exothermic	355 - 730	30.65	30.72

***Table 4.** Thermal behavior data (in static air atmosphere) for complex (2)*

Complex compound	**Stage**	**Thermal effect**	**Temperature interval 20/900 °C**	**Δm_{exp} [%]**	**Δm_{calc} [%]**
(**2**) $[Cu(\mathit{CHX})SO_4]\cdot C_2H_5OH$	1	Endothermic	55 - 179	16.84	16.92
	2	Exothermic	179 - 273	16.99	16.92
	3	Exothermic	273-580	42.28	42.15
	4	Exothermic	580-630	11.94	12.04

Thermal decomposition of complex $[Cu(\mathit{CHX})SO_4]\cdot C_2H_5OH$ (**3**) is dominated by the presence of large amounts of nitrate, ion with marked tendency of thermal decomposition at a relatively low temperature, sometimes with explosion (Table 5).

Consequently, after eliminating a molecule of chlorobenzene ion nitrate decomposition occurs [22, 23], highly exothermic process, with formation of a mixture of copper oxide and organic rest from chlorhexidine. It follows the thermal decomposition of organic residue, a process that occurs in two endothermic steps, not very well defined, corresponding to elimination of another molecule of chlorobenzene and combustion of organic rest.

Table 5. *Thermal behavior data (in static air atmosphere) for complex (**3**)*

Complex compound	**Stage**	**Thermal effect**	**Temperature interval 20/900 °C**	**Δm_{exp} [%]**	**Δm_{calc} [%]**
(**3**) $[Cu_2(CHX)(NO_3)_4]$	1	Endothermic	55 - 196	12.70	12.78
	2	Exothermic	196 - 290	24.61	24.53
	3	Exothermic	290-405	12.65	12.78
	4	Exothermic	405-835	32.02	31.83

Differential thermal analysis in kinetic regime contributed to the elucidation of compound ratio of metal ion to ligand chlorhexidine, nature and role of solvent molecules in compounds composition, as well as the stability of complex combinations. Compounds studied behave alike at thermal decomposition. In all the cases it was highlighted a first stage of chlorhexidine decomposition in chlorobenzene and $C_{10}H_{20}N_{10}$. Subsequently this fragment either burns or decomposes further leading to $(CN)_{2n}$. In all cases the final decomposition residue was copper oxide, CuO, respectively zinc oxide or a mixture of the two in the case of heterobinuclear compounds.

UV-Vis and EPR spectra

UV-Vis spectra determinations, correlated with the values of experimental parameters of the paramagnetic resonance spectra revealed that the symmetry around Cu^{2+} ion and the estimation of copper-donor atoms bond nature.

Based on the values of bond parameters one can say that the metal-ligand bond in copper complexes with chlorhexidine is predominantly covalent character, an important ratio in the bond having π interaction in plan. This observation leads to the conclusion that imine nitrogen atoms in chlorhexidine molecule participate in coordination not only through non-participant electron pair, but also through π electron of the double bond. For all the Cu(II) complex compounds synthesized, magnetic moment values at room temperature, are between 1,30 and 1,80 MB, values lower than 1,73 MB (corresponding to an odd/unpaired electron) being characteristic to binuclear complexes with strong spin-spin interactions [24 – 27].

EPR spectra of complex (**4**) $[CuZn(CHXac_2)(CH_3COO)_2]Cl_2$ (Figure 3) indicates an axial symmetry, so that the maximums in the UV-Vis spectrum (Figure 2) could be attributed to transitions below, in symmetry D_{4h}: 45270 cm^{-1} (σ - σ^*) ; 31810 cm^{-1} (π - π^*) ; 26590 cm^{-1} (n - π^*) ; 19280 cm^{-1} $^2B_{1g} \rightarrow ^2A_{1g}$ + charge transfer; 15630 cm^{-1} $^2B_{1g} \rightarrow ^2E_g$; 12880 cm^{-1} $^2B_{1g} \rightarrow ^2B_{2g}$.

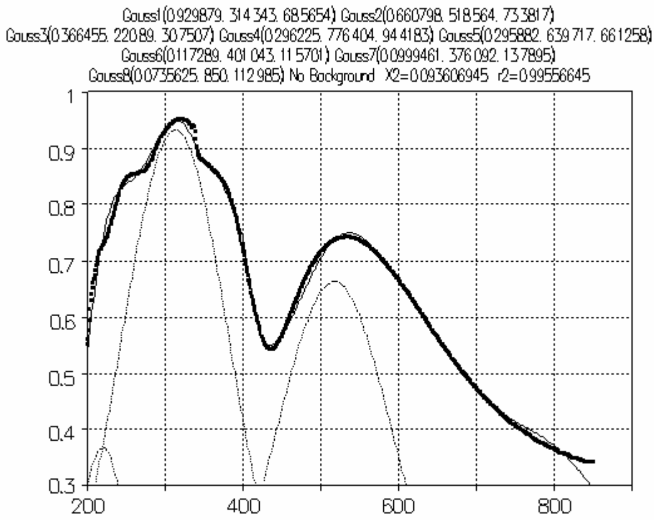


Figure 2. *UV-VIS spectrum of complex (4)*

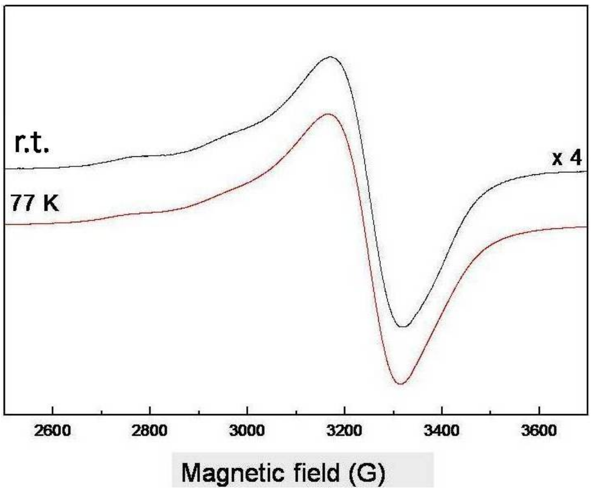


Figure 3. *EPR spectra of complex (4) recorded at room temperature and 77 K (P = 10 mW and modulation field intensity Hm = 4 G)*

Signals, for both temperatures, have broad lines and it is observed the shaping of hyperfine structure splitting, due to interaction of the odd electron with Cu^{2+} ion, but the hyperfine structure constant is difficult to determine accurately. If case of the spectrum recorded at liquid nitrogen temperature, hyperfine structure is seen even worse than in those recorded at room temperature (r.t.). Values of spectral parameters evaluated in these conditions are:

$A_{//} \approx 185$ G $g_{//} = 2.20$; $g_{\perp} = 2{,}064$ at r.t. and 2.065 at 77 K
Calculated parameters: $\lambda_{//} = -322\ cm^{-1}$; $\alpha^2 = 0.77$; $K_{//} = 0.38$
$\lambda_{\perp} = -507\ cm^{-1}$ $\alpha^2 = 0.77$; $K_{\perp} = 0.61$

The lower value of $K_{//}$ parameter compared to $K_{\perp}$ is an indication for the existence of strong π bonds in plane.

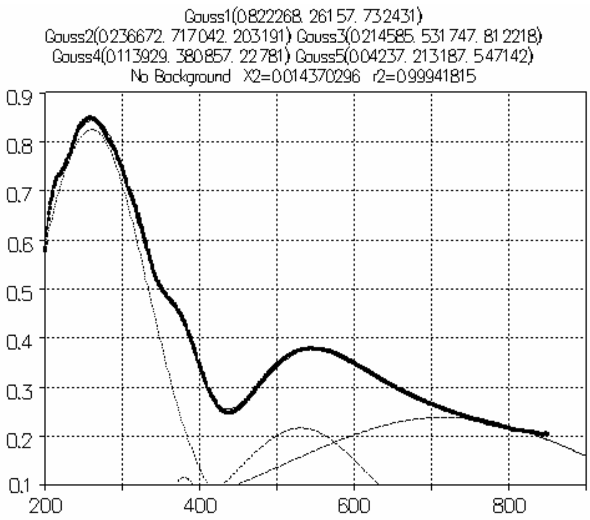


Figure 4. *UV-VIS spectrum of complex (**5**)*

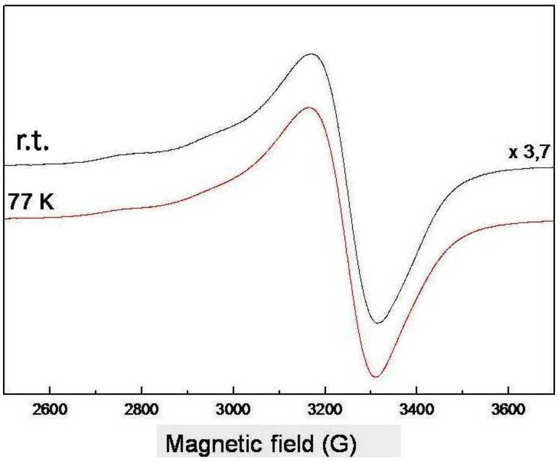


Figure 5. *EPR spectra of complex (**5**) recorded at room temperature and 77 K (P = 10 mW and modulation field intensity Hm = 4 G)*

Magnetic moment of complex (**5**) is 1.64 MB. EPR signals of complex (**5**) [CuZn(*CHXac₂*)Cl₄] (Figure 5) are practically identical with those obtained for complex (**4**) [CuZn(*CHXac₂*)(CH₃COO)₂]Cl₂. $g_\perp$ has a little bit higher value (2.065 at room temperature and 2.067 at 77 K), but the difference is insignificant. Because EPR spectrum is in accordance with an axial symmetry, and the analytical data show a tetra coordination around metallic ions, the maximums in the UV-Vis spectrum (Figure 4) can be attributed to transitions below, in symmetry D_{4h}: 46900 cm^{-1} (σ - σ^*); 38230 cm^{-1} (π - π^*) ; 26250 cm^{-1} (n - π^*) ; 18500 cm^{-1} $^2B_{1g}\rightarrow{}^2A_{1g}$, $^2B_{1g}\rightarrow{}^2E_g$; 13950 cm^{-1} $^2B_{1g}\rightarrow{}^2B_{2g}$. Bonding parameters calculated from spectral and magnetic data are:

$\lambda_{//} = -348$ cm^{-1}; $\alpha^2 = 0.77$; $K_{//} = 0.42$
$\lambda_\perp = -601$ cm^{-1} $\alpha^2 = 0.77$; $K_\perp = 0.72$

Figure 6 presents the UV-Vis spectrum for complex (**2**) [Cu(*CHX*)SO₄]·C₂H₅OH, attributions of the bands observed in visible being done under the hypothesis of a C_{4v}

symmetry for complex (**2**): 46730 cm^{-1} (σ - σ^*) ; 38910 cm^{-1} (π - π^*) ; 27000 cm^{-1} (n - π^*) ; 18200 cm^{-1} $^2B_1 \rightarrow ^2E$; 13150 cm^{-1} $^2B_1 \rightarrow ^2B_2$; 11760 cm^{-1} $^2B_1 \rightarrow ^2A_1$.

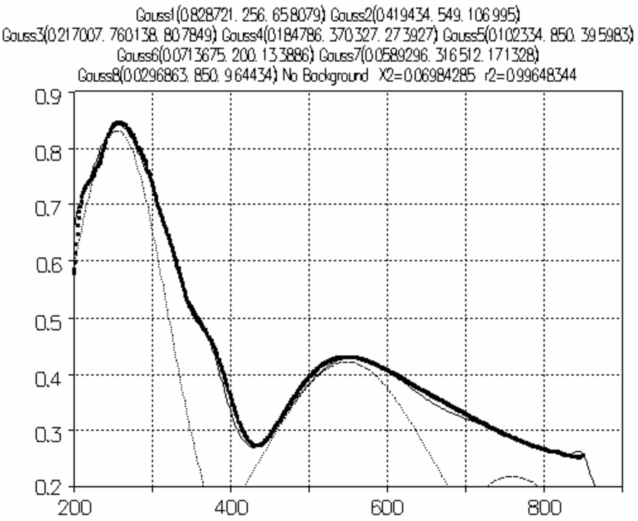


Figure 6. *UV-VIS spectrum of complex (**2**)*

Recorded magnetic moment is 1.76 MB for complex (**2**). EPR spectrum of complex (**2**) [Cu(*CHX*)SO_4]·C_2H_5OH presents hyperfine structure splits, due to odd electron interaction with Cu^{2+} ion.

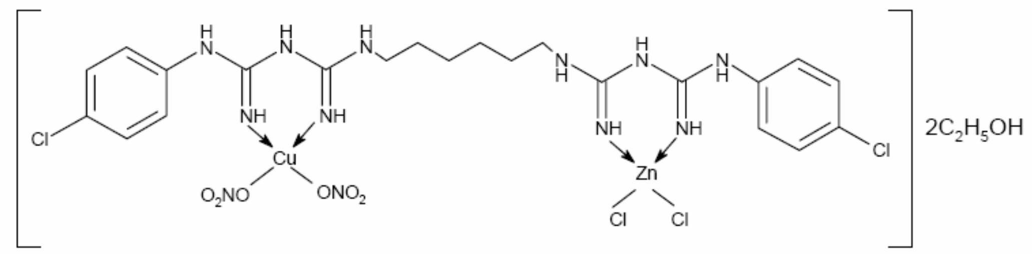
The proposed structures for the complexes (**1**) – (**5**) are presented in Figure 7.

Antimicrobial activity assay

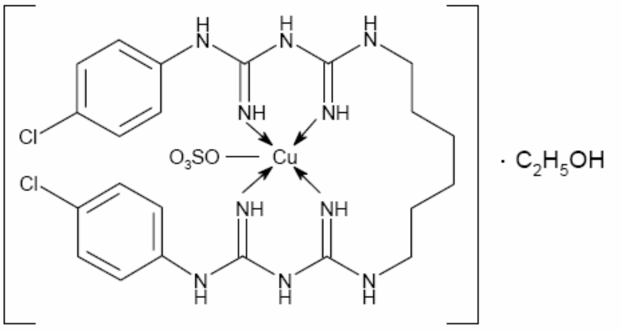
The results of measuring the minimum inhibition diameter (in mm) for the fifth complexes and two ligands are presented in Table 6. Since chlorhexidine is a cationic agent, it is more active against Gram positive bacteria than Gram negative bacteria.

Regarding the antimicrobial action of the complexes compared with the one of the ligands were observed some specific activities, as follows:

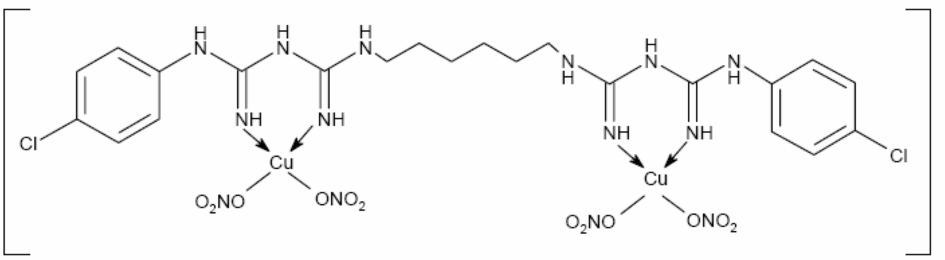
- complex (**2**) shows an increased biological activity against Gram negative bacteria and fungi compared with chlorhexidine base;
- complexes (**4**) and (**5**) present an increased activity against Gram negative bacteria compared to chlorhexidine diacetate;
- for the complex (**5**) were registered the highest values of microbial strains inhibition diameters between all the new complexes;
- complex (**1**) presents the most decreased activity against the tested microbial strains, compared to the other new complexes;
- chlorhexidine base, chlorhexidine diacetate and their complexes are less active against Gram negative and Gram positive bacteria than standard antibiotics.



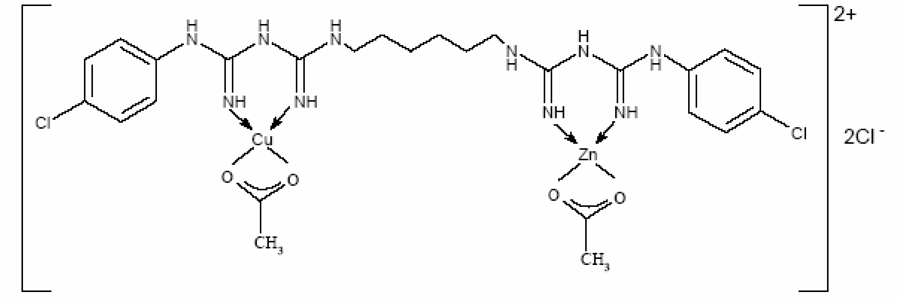
$[CuZn(CHX)(NO_3)_2Cl_2]\cdot 2C_2H_5OH$ **(1)**



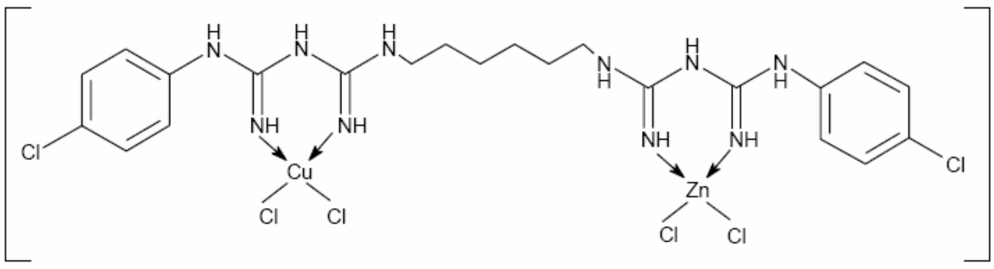
$[Cu(CHX)SO_4]\cdot C_2H_5OH$ **(2)**



$[Cu_2(CHX)(NO_3)_4]$ **(3)**



$[CuZn(CHX)(CH_3COO)_2]Cl_2$ **(4)**



$[CuZn(CHX)Cl_4]$ **(5)**

Figure 7. *Proposed structures for the complexes* ***(1)*** *–* ***(5)***

Table 6. *Microbiological activity of the ligands and new complex compounds*

Microbial strain		**Microbial culture inhibition diameter [mm]**						
		A	**C**	**M**	**Ligand *CHX***	**(1)**	**(2)**	**(3)**
Gram negative bacteria	*Escherichia coli*	18	28	-	7	8	12	11
Gram positive bacteria	*Staphyloccocus aureus*	28	25	-	22	11	16	13
Fungi	*Candida albicans*	-	-	21	10	2	11	3

Microbial strain		**Microbial culture inhibition diameter [mm]**					
		A	**C**	**M**	**Ligand *CHXac₂***	**(4)**	**(5)**
Gram negative bacteria	*Escherichia coli*	18	28	-	10	12	15
Gram positive bacteria	*Staphyloccocus aureus*	28	25	-	24	15	19
Fungi	*Candida albicans*	-	-	21	24	11	12

A - ampycillin (10 μg/disc); C - ciprofloxacin (5 μg/disc); M - myconazol (30 μg/disc)

CONCLUSIONS

Were prepared five new copper(II) and zinc(II) complex compounds with chlorhexidine base and chlorhexidine diacetate, in different molar ratio, using copper(II) nitrate, sulfate and chloride, respectively zinc(II) chloride and acetate.

The new complexes were characterized by elemental chemical analysis, thermal analysis, FTIR, UV-Vis, EPR spectra, magnetic susceptibility.

The screening data for the inhibition diameter of microbial strains show an increase of activity for all the complexes which contain copper(II) and zinc(II) chloride, nitrate sulfate or acetate in different molar ratio, against Gram negative bacteria comparatively with both ligands, chlorhexidine base and chlorhexidine diacetate.

Regarding their antibacterial activity, we recommend that copper(II) and zinc(II) complexes with chlorhexidine diacetate in molar ratio 1:1:1 could be used, as alcohol solution, for the disinfection of the external surfaces, pavements and medical instruments.

ACKNOWLEDGEMENTS

To CNMP for supporting the researches included in this paper by the PNCDI2-Project no. 61_035/2007.

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