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Synthesis, characterization and biological activity of new Ni(II), Pd(II) and Cr(III) complex compounds with chlorhexidine

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Abstract: Six new coordination compounds of Ni(II), Pd(II) and Cr(III) with chlorhexidine, 1,1′-hexamethylenebis[5-(4-chlorophenyl)biguanide], were prepared, characterized and examined for their potential as antimicrobial agents, as well as for their antioxidant activity. The metal complexes correspond to the formulas: $[Ni(CHX)]Cl_2·2H_2O$, $[Ni(CHX)]Br_2·2H_2O$, $[Ni(CHX)](CH_3COO)_2·C_2H_5OH$, $[Pd(CHX)][PdCl_4]·2H_2O$, $[Pd(CHX)](CH_3COO)_2$ and $[Cr(CHX)Cl_2](CH_3COO)$, where CHX = chlorhexidine. Investigations on the *in vitro* antimicrobial activity of the complexes indicated that all have high activity against the tested bacteria, but are less active against fungi. Among the six complexes, those of Pd(II) showed the highest antibacterial activity, $[Pd(CHX)][PdCl_4]·2H_2O$ being more active against Gram-positive and Gram-negative bacteria than chlorhexidine diacetate. The antioxidant activity of the metal complexes was investigated by photochemiluminescence and the results showed that the palladium(II) complexes have high antioxidant activities.

Keywords: biguanide metal complexes; antimicrobial agents; transition metal complexes.

INTRODUCTION

Biguanides and related compounds have been extensively studied in recent years, mainly due to their biological activity as antihyperglycemic, antimalarial, antibacterial and antifungal agents.[1–4] Moreover, their excellent ability to coordinate different metal ions has resulted in a large number of coordination compounds, some of them showing a better biological activity than the ligands.[5–9]

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The most used antimicrobial agent of the biguanide class is chlorhexidine, 1,1′-hexamethylenebis[5-(4-chlorophenyl)biguanide] (Fig. 1), a broad spectrum biocide effective against Gram-positive bacteria, Gram-negative bacteria and fungi.[10–12]

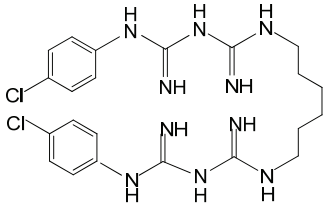

Fig. 1. Chlorhexidine.

Since chlorhexidine is practically insoluble in water, the commonly used forms are its salts, chlorhexidine diacetate or digluconate, which are soluble in water and ethanol.

Chlorhexidine is a positively charged molecule and is therefore attracted to the negatively charged Gram-positive cell wall, and can bind to the carboxyl, phosphate or hydroxyl groups of the membrane. Thus, at low concentrations, chlorhexidine has bacteriostatic action (inhibits bacterial growth) by disrupting the cell membrane.[13] At high concentrations, chlorhexidine causes the coagulation of cytoplasmic proteins, probably through denaturation; its action is bactericidal.[13]

As the Gram-positive bacteria *Streptococcus mutans* are sensitive to chlorhexidine, chlorhexidine is the most widely used agent against dental plaque.[14–17] Thus, chlorhexidine salts, especially the digluconate, are reported as not only being used as the active ingredient in mouthwashes, but also in shampoos, body lotions and face cleansers. Other non-dental applications of chlorhexidine are general skin cleaning, as a surgical scrub, catheter and pre-operative skin disinfection.[11]

For dental products, chlorhexidine is usually used in combination with other agents with antimicrobial activity, such as cetrimide or some metal ions, especially Cu(II), Zn(II), Sn(II) or Ag(I), for a possible synergistic effect against oral bacteria.[18,19]

Attempts towards obtaining and using coordination compounds of chlorhexidine as antimicrobial agents have hitherto been limited. They refer to the synthesis and characterization of some complexes of Cu(II), Zn(II) and Ag(I) with chlorhexidine.[20–22]

The purpose of this study was to establish the products of the interaction of chlorhexidine diacetate with Ni(II), Pd(II) and Cr(III) and the action of the formed metal complexes against some bacteria and fungi. The antioxidant activity of the metal complexes was also evaluated by the photochemiluminescent method.

EXPERIMENTAL

Materials and physicochemical analyses

All the chemicals were of AR grade purchased from Sigma–Aldrich or Merck and used without further purification.

Carbon, hydrogen and nitrogen analyses were performed with a Euro EA elemental analyser. The nickel and palladium contents were determined gravimetrically, by precipitation with dimethylglyoxime. The chromium content was also estimated gravimetrically, as Cr_2O_3. Thermogravimetric analysis was performed under a static air atmosphere, at a heating rate of 10 °C min^{-1}, using an STA 6000 Perkin Elmer derivatograph. The infrared spectra (in KBr pellets) were recorded on a BIORAD FTIR 135 spectrophotometer, in the range 4000–400 cm^{-1}. The UV–Vis diffuse reflectance spectra in the range 200–900 nm were measured on a UV–Vis Jasco 650 spectrophotometer. Magnetic susceptibility measurements were performed by the Faraday method, on a Gouy balance, at room temperature, using $Hg[Co(SCN)_4]$ as the calibrant. The ^{1}H-NMR (300 MHz) and ^{13}C- NMR (75 MHz) spectra of chlorhexidine and its Ni(II) and Pd(II) complexes in DMSO-d_6 were recorded on a Gemini 300BB spectrometer using TMS as an internal standard. The total antioxidant capacity of the metal complexes was registered using a photochemiluminometer, Photochem, Analytik Jena AG, Germany.

Synthesis of the coordination compounds

The nickel(II) complexes, chlorhexidinenickel(II) chloride dihydrate (complex **1**), chlorhexidinenickel(II) bromide dihydrate (complex **2**) and chlorhexidinenickel(II) acetate ethanolate (complex **3**), were prepared by the following general method. Chlorhexidine diacetate monohydrate, 0.6435 g (1 mmol), was dissolved in 30 mL ethanol, under slight heating. The required solid metal salt, *i.e.*, $NiCl_2{\cdot}6H_2O$ (0.2377 g, 1.0 mmol for complex **1**), $NiBr_2{\cdot}$(0.2185 g, 1.0 mmol for complex **2**) and $Ni(CH_3COO)_2{\cdot}4H_2O$ (0.2486 g, 1.0 mmol for complex **3**), was added slowly under stirring, keeping the temperature below 40 °C. The color of the solutions became orange after a few minutes. The precipitate began to form after 2 h of stirring with heating. The mixture was left overnight and then the orange precipitate was filtered off, washed with ethanol and dried under air.

The palladium(II) complexes, chlorhexidinepalladium(II) tetrachloridopalladate(II) dihydrate (complex **4**) and chlorhexidinepalladium(II) acetate (complex **5**), were obtained by mixing a solution of $PdCl_2$ dissolved in acetonitrile with a solution of chlorhexidine dissolved in ethanol, using a 2:1 metal:ligand mole ratio for complex **4** and a 1:1 mole ratio for complex **5**. Complex **4** was prepared by adding an ethanolic solution of chlorhexidine diacetate monohydrate (0.6435 g, 1.0 mmol, in 30 mL ethanol) to a refluxing solution containing 0.3546 g (2.0 mmol) of $PdCl_2$ dissolved in acetonitrile. The resulting suspension that was immediately formed was stirred at room temperature for 2 h and then filtered under vacuum. The orange precipitate was washed with ethanol and dried under air. Complex **5**, orange, was obtained in the same way as complex **4**, but using a 1:1 metal:ligand mole ratio, by mixing 0.6435 g (1.0 mmol) of ligand dissolved in 30 mL ethanol and 0.1773 g (1.0 mmol) of $PdCl_2$ in acetonitrile.

The chromium(III) complex, dichloridochlorhexidinechromium(III) acetate (complex **6**) was prepared by adding a solution of $CrCl_3{\cdot}6H_2O$ (0.266 g, 1.0 mmol) in ethanol to a solution of chlorhexidine ligand in ethanol (0.6435 g, 1.0 mmol) and the reaction mixture was heated at 40–50 °C, under constant stirring, for 1 h. The light blue precipitate obtained was separated by filtration under vacuum, washed with ethanol and dried under air.

The prepared complexes are insoluble in acetone, ethanol and acetonitrile but readily soluble in DMF and DMSO.

RESULTS AND DISCUSSION

The results of the elemental analysis and the proposed formulas based on analytical and spectral data are presented in Table I.

TABLE I. Analytical data of the metal complexes

Compound	Found content (calculated content), %			
	C	H	N	Metal
[Ni(CHX)]Cl_2·2H_2O (**1**)	39.12 (39.31)	4.95 (5.06)	20.68 (20.85)	8.45 (8.78)
[Ni(CHX)]Br_2·2H_2O (**2**)	34.40 (34.71)	4.65 (4.47)	18.16 (18.41)	7.55 (7.75)
[Ni(CHX)]$(CH_3COO)_2$·C_2H_5OH (**3**)	45.98 (46.12)	5.57 (5.76)	19.29 (19.21)	8.21 (8.09)
[Pd(CHX)][$PdCl_4$]·2H_2O (**4**)	29.31 (29.45)	3.72 (3.79)	15.70 (15.61)	23.31 (23.74)
[Pd(CHX)]$(CH_3COO)_2$ (**5**)	42.59 (42.74)	4.58 (4.93)	19.29 (19.18)	14.26 (14.57)
[Cr(CHX)Cl_2](CH_3COO) (**6**)	41.73 (41.89)	4.69 (4.80)	20.41 (20.36)	7.52 (7.56)

The interaction of chlorhexidine with Ni(II) salts yields monomeric complexes corresponding to the formulas: [Ni(CHX)]Cl_2·2H_2O (**1**), [Ni(CHX)]Br_2·2H_2O (**2**) and [Ni(CHX)]$(CH_3COO)_2$·C_2H_5OH (**3**). It is important to note that only Ni(II) complexes at a 1:1 metal:ligand mole ratio were obtained irrespective of the metal:ligand mole ratio in the reaction mixture.

The palladium(II) complexes were obtained in the reaction of chlorhexidine diacetate with palladium chloride dissolved in acetonitrile. When a 2:1 metal:ligand ratio was used, a compound corresponding to the formula [Pd(CHX)][$PdCl_4$]·2H_2O (**4**) was obtained. When a 1:1 metal:ligand ratio was used, the resulting compound had the formula [Pd(CHX)]$(CH_3COO)_2$ (**5**). All complexes of Ni(II) and Pd(II) are orange, suggesting a square–planar symmetry around the metal ion.

The chromium(III) complex obtained through the reaction of chlorhexidine diacetate with chromium(III) chloride at a 1:1 mole ratio corresponds to the formula [Cr(CHX)Cl_2](CH_3COO) (**6**).

Infrared spectra

The important infrared bands of chlorhexidine diacetate monohydrate and its complexes are listed in Tables II and III.

In the region of 2900–3400 cm^{-1}, chlorhexidine diacetate monohydrate has a large number of absorption bands due to the presence of >NH (secondary amine), =NH (imine), $=NH_2^+$ (resulting by protonation) and OH (of crystalline water) groups. Thus, the strong broad absorption band at 3338 cm^{-1} could be assigned to the stretching vibrations of the hydroxyl group, overlapping the stretching vibration of the =NH and $=NH_2^+$ groups.[23–25] The in-plane deformation band, $\delta(NH_2^+)$, appears at 1613 cm^{-1}.[23,26] The presence of these two bands indicates protonation of chlorhexidine in the diacetate salt, in the solid state.

TABLE II. Characteristic bands in the IR spectra ($\bar{\nu}_{max}$ / cm^{-1}) of the ligand and its Ni(II) complexes; (vs – very strong, s – strong, m – medium, w – weak)

Assignments	Chlorhexidine diacetate monohydrate	Complex **1**	Complex **2**	Complex **3**
ν(OH) H_2O / C_2H_5OH; ν(=NH)	3338 *s*	3304 *s*	≈3300 *s* (broad)	3340 *s*
ν(NH)Alkyl–NH–aryl; $(alkyl)_2NH$	3181 *s*	3202 *s*	3201 *s*	3246 *s*
$\nu_{as}(NH_2^+)$; $\nu_{sym}(NH_2^+)$	3338 *s* superp. ν(OH)	–	–	–
ν_{as}(C=O); ν_{as}(C–O)	1549 *vs*, 1417 *s*	–	–	1566 *s* 1406 *s*
ν(C=N)	1644 *s*	1660 *vs*	1660 *vs*	1667 *vs*
$\delta(NH_2^+)$	1613 *m*	–	–	–
δ(NH) + ν(C–N)	1574 *s*	1590 *m*	1588 *m*	1592 *m*
	1365 *m*	1378 *m*	~1360 *m*	1370 *m*
$\nu(C_{arom}–N)$	1249 *m*	1250 *s*	1247 *ms*	1252 *m*
$\nu(C_{aliph}–N)$	1165 *m*	1151 *w*	1150 *w*	1145 *m*

TABLE III. Characteristic bands in the IR spectra ($\bar{\nu}_{max}$ / cm^{-1}) of the ligand and its Pd(II) and Cr(III) complexes; (vs – very strong, s – strong, m – medium, w – weak)

Assignments	Complex **4**	Complex **5**	Complex **6**
ν(OH) H_2O / C_2H_5OH; ν(=NH)	3339 *ms*	3250 *m*	3307 *ms*
ν(NH) alkyl–NH–Aryl; $(alkyl)_2NH$	3226 *ms*	3202 *m*	3194 *ms*
$\nu_{as}(NH_2^+)$ $\nu_{sym}(NH_2^+)$	–	–	–
ν_{as}(C=O); ν_{as}(C–O)	–	1582 *m*; 1415 *s*	1564 *m*; 1414 *s*
ν(C=N)	1673 *vs*	1634 *s*	1630 *s*
$\delta(NH_2^+)$	–	–	–
δ(NH)+ν(C–N)	1591 *m*; 1342 *m*	1590 *m*	1581 *s*; 1362 *m*
$\nu(C_{arom}–N)$	1240 *m*	1241 *s*	1240 *ms*
$\nu(C_{aliph}–N)$	1151 *m*	1145 *w*	1147 *m*

The stretching vibrations ν(N–H) of the alkyl–NH–aryl and $(alkyl)_2NH$ groups give a strong absorption band at 3181 cm^{-1}.[24–26]

The most important band in the IR spectrum of chlorhexidine diacetate occurs at 1644 cm^{-1} and was assigned to the stretching vibration of the imine group, ν(C=N).[24,27]

The characteristic bands of the coupling vibrations δ(NH)+ν(C–N) were identified at 1574 and 1365 cm^{-1}.[27] Two other medium absorption bands, appearing at 1249 and 1165 cm^{-1} may be assigned to the stretching vibrations C_{arom}–N and C_{aliph}–N, respectively.[27,28]

The most important changes observed in the IR spectra of the metal complexes compared to that of the free ligand are the disappearance of the band due to $\delta(NH_2^+)$, in accordance with the deprotonation of chlorhexidine on complexation and the shift of the band due to the stretching vibration of the imine group, ν(C=N).[26,29]

The shift of the band assigned to ν(C=N) indicates the coordination of the imine nitrogen atoms to the metal ion. The infrared spectra of complexes **1**–**4** show a positive shift of this band at wavenumbers between 1660 and 1667 cm^{-1}. This positive shift could be explained by the increase in the electron density on C=N bonds, as a result of deprotonation.[30,31] In the IR spectra of complexes **5** and **6**, the band assigned to ν(C=N) appears at lower wavenumbers and a stronger shift of the electronic density from the donor atoms to the metal could be assumed in these cases. This leads to a decrease in the electronic density on the C=N bonds.

The coordination of chlorhexidine through the imine nitrogen atoms for all the metal complexes is also supported by the shift of the band due to δ(NH)+ +ν(C–N) to higher wavenumbers and the shift of the band assigned to C_{aliph}–N to lower wavenumbers.

Absorption bands characteristic for the acetate group, present in the IR spectrum of the ligand at 1549 cm^{-1}, ν_{as}(COO), and 1417 cm^{-1}, ν_{sym}(COO), could also be found in the spectra of complexes **3**, **5** and **6**. The values of $\Delta = \nu_{as}(COO) - \nu_{sym}(COO)$, 160, 167 and 150 cm^{-1}, respectively, are in the range of ionic acetate.[32]

Some new bands appearing in the IR spectra of the complexes at low wavenumbers can be assigned to the metal–nitrogen vibrations, ν(M–N) (490–512 cm^{-1}).[33]

The ν(OH) band is observed in the IR spectra of the complexes **1**–**4** in the range 3300–3340 cm^{-1} and is due to the presence of lattice water or ethanol.[28,29] It overlaps with the N–H vibration, leading to the enlargement of the absorption in this region. The presence of lattice water and ethanol was further confirmed by thermal analysis.

Thermal decomposition

The thermograms obtained for the thermal decomposition of complexes **1**–**6** are presented in the Supplementary material to this paper (Figs. S-1–S-6).

Analysis of the thermograms of the Ni(II) complexes indicates that the first mass loss process occurred below 100 °C (80–100 °C) for complexes **1** and **2**, the mass loss corresponding to two crystalline water molecules (complex **1**: Δ_{exp} = 5.03 %, Δ_{calc} = 5.36 %; complex **2**: Δ_{exp} = 5.53 %, Δ_{calc} = 5.97 %) and 60–80 °C for complex **3**, this step corresponding to the loss of one ethanol molecule (Δ_{exp} = = 6.87 %, Δ_{calc} = 6.31 %).

For complexes **1** and **2**, the second step, between 240–270 °C, corresponds to the endothermic removal of two HCl/HBr molecules, provided by anionic chloride and bromide, respectively. The corresponding protons result from deprotonation of the chlorhexidine ligand. The experimental mass losses were 10.85 % (calc. 10.57 %) and 20.75 % (calc. 21.04 %), respectively.[34]

For [Ni(CHX)]$(CH_3COO)_2 \cdot C_2H_5OH$ (**3**), the second decomposition step, with an experimental weight loss of 15.94 %, occurred between 120–210 °C and corresponds to the elimination of two acetate ions (Δ_{calc} = 16.19 %). This low temperature range of degradation indicates the ionic nature of the acetate.[20]

For all three Ni(II) complexes, the last decomposition step (300–680 °C), strongly exothermic, is related to the oxidative degradation of the chlorhexidine ligand. The residue is NiO and the metal content determined is in accordance with the results of the chemical analysis.

[Pd(CHX)][$PdCl_4$]$\cdot 2H_2O$ lost two water molecules above 80 °C. The TG curve indicates a rapid weight loss in two steps, in the ranges 200–320 °C and 330–430 °C. These steps most likely resulted from the overlap of at least two processes, due to the degradation of two complex ions. The final product was PdO below 700 °C and Pd above this temperature.[35] The metal percentage determined from thermal analysis (23.26 %) is in accordance with that resulting from the chemical determination.

[Pd(CHX)]$(CH_3COO)_2$ commenced decomposition at 240 °C, which excludes the presence of any solvent molecules. As with the previous complex, decomposition occurred rapidly and led to metallic palladium as the final residue.

The complex [$Cr(CHX)Cl_2$](CH_3COO) was also stable up 220 °C; the first stage of mass loss occurred in the range 220–260 °C, in a strongly endothermic process. The experimental mass loss in this stage (19.5 %) corresponds to the elimination of coordinated chloride and ionic acetate (Δ_{calc} = 18.90 %). The large, highly exothermic process, occurring between 260 and 650 °C is due to oxidative degradation and elimination of the chlorhexidine ligand, with the final formation of chromium(III) oxide. The metal percentage determined from the residue was 7.52 %.

NMR spectral analysis

A comparative study of the ^{1}H-NMR and ^{13}C-NMR spectra of chlorhexidine diacetate monohydrate and its Ni(II) and Pd(II) complexes provided information about the metal-binding sites of the ligand and the formation of these complexes. Chemical shifts for the ligand and for two representative complexes, [Ni(CHX)]$Br_2 \cdot 2H_2O$ and [Pd(CHX)][$PdCl_4$]$\cdot 2H_2O$, are presented in the Supplementary material.

In the ^{1}H-NMR spectra of these complexes, the signal at 1.71 ppm in the spectrum of CHX, corresponding to the methyl group in the acetate ion, is absent, in accordance with the proposed formulas. The hydrogen atoms in the 4-chlorophenyl moieties appear as one broad signal compared to the starting chlorhexidine acetate for which they appear as two distinct doublets.[36] In addition, the slight shielding of the methylenic groups is also proof of complex formation.

In the ^{13}C-NMR spectra of these metal complexes, the first obvious change is the disappearance of the signals at 24.7 and 175.9 ppm, assigned to the carbon atoms of the acetate group. The carbon atoms in the biguanidine moiety appeared at 153.3, 154.8 and 160.2 ppm for the nickel(II) complex and 148.5, 150.0 and 160.6 ppm for the palladium(II) complex, shifted from their positions in the spectrum of chlorhexidine (155.1 and 159.6 ppm), which is proof of the coordination of chlorhexidine through the imine nitrogen atoms.

Magnetic and electronic spectral data

The electronic spectra were recorded on solid samples, diluted with MgO powder.

Chlorhexidine diacetate exhibits strong absorption bands in the UV region, at 208 (48100 cm^{-1}), 255 (39215 cm^{-1}), 298 (33500 cm^{-1}) and 344 nm (29000 cm^{-1}), which could be assigned to n–σ*, π–π* and n–π* transitions, respectively.[37] The UV–Vis spectra of the metal complexes showed, besides these bands characteristic of the chlorhexidine ligand, additional bands due to d–d transitions of the metal ions.

The electronic d–d transitions observed in the spectra of the complexes with their assignments and the corresponding symmetry are given in Table IV.

TABLE IV. Electronic d–d transitions for the Ni(II), Pd(II) and Cr(III) complexes with chlorhexidine

Complex	Observed bands (λ_{max} / nm) / ($\tilde{\nu}_{max}$ / cm^{-1})	Assignments	Symmetry
$[Ni(CHX)]Cl_2 \cdot 2H_2O$	698 / 14327	$^1A_{1g} \rightarrow {}^1A_{2g}$	D_{4h}
	469 / 21322	$^1A_{1g} \rightarrow {}^1B_{1g}$	
$[Ni(CHX)]Br_2 \cdot C_2H_5OH$	472 / 21186	$^1A_{1g} \rightarrow {}^1B_{1g}$	D_{4h}
$[Ni(CHX)](CH_3COO)_2 \cdot C_2H_5OH$	476 / 21008	$^1A_{1g} \rightarrow {}^1B_{1g}$	D_{4h}
$[Pd(CHX)][PdCl_4] \cdot 2H_2O$	489 / 20450	$^1A_{1g} \rightarrow {}^1A_{2g}$	D_{4h}
	371 / 26954	$^1A_{1g} \rightarrow {}^1B_{1g}$	
$[Pd(CHX)](CH_3COO)_2$	370 / 27027	$^1A_{1g} \rightarrow {}^1B_{1g}$	D_{4h}
$[Cr(CHX)Cl_2](CH_3COO)$	692 / 14451	$^4B_{1g} \rightarrow {}^2A_{1g}, {}^2B_{1g}$	Distorted
	585 / 17094	$^4B_{1g} \rightarrow {}^4E_g{}^a$	O_h
	426 / 23474	$^4B_{1g} \rightarrow {}^4B_{2g}$	
	290 / 34382	$^4B_{1g} \rightarrow {}^4A_{2g}{}^a, {}^4E_g{}^b$	

The magnetic moment of the chromium complex is 3.87 μ_B, implying an octahedral geometry around the metal ion. All Ni(II) and Pd(II) complexes show diamagnetic behavior, in accordance with a square planar geometry, as suggested by their orange color.

The electronic spectra of Ni(II) show no bands below 10000 cm^{-1}, which also suggests a square–planar geometry around the metal ion.[38] The low tran-

sition $^1A_{1g} \rightarrow {}^1A_{2g}$, at 14327 cm^{-1} is observed only in the spectrum of complex **1**.[38–40] The main absorption band for all nickel(II) complexes is observed around 21000 cm^{-1} and may be assigned to the $^1A_{1g} \rightarrow {}^1B_{1g}$ transition.[38]

UV–Vis spectra of the palladium(II) complexes exhibited a strong absorption band, with a maximum at 26954 cm^{-1} for complex **4** and at 27027 cm^{-1} for complex **5** and a shoulder around 20400 cm^{-1} for both complexes. The shoulder was tentatively assigned to d–d transitions, most probably to the $^1A_{1g} \rightarrow {}^1A_{2g}$ transition in a square–planar geometry.[38,41] The strong band could be attributed to the $^1A_{1g} \rightarrow {}^1B_{1g}$ transition. The high intensity of this band for [Pd(CHX)][PdCl$_4$]·2H$_2$O (Fig. 2) is probably due to a contribution of the ligand to a metal charge transfer in the complex anion $PdCl_4^{2-}$ (Cl → Pd).[38]

The UV–Vis spectrum of the chromium(III) complex could be explained assuming an octahedral symmetry with tetragonal distortion by elongation of the octahedron along the *z*-axis (Fig. 3).

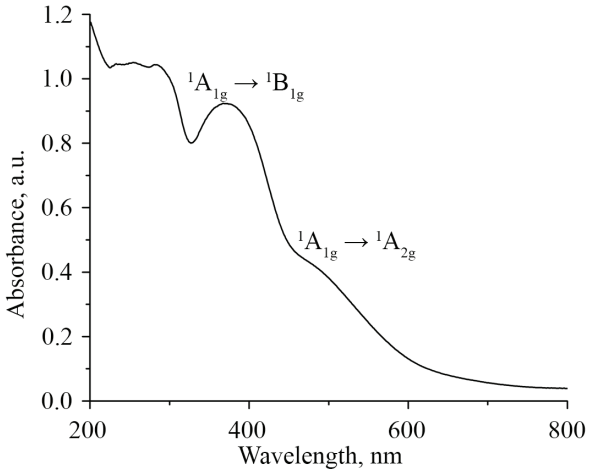

Fig. 2. UV–Vis spectrum of [Pd(CHX)][PdCl$_4$]·2H$_2$O.

The weak absorptions, around 14450 cm^{-1}, are spin-forbidden doublet transitions.[42] A very large splitting is observed for the first "octahedral" band (Fig. 3) and hence, the in-plane ligand field strength Dq_L = 2374 cm^{-1} could be deduced.[41] This high value is in accordance with the strong field generated by chlorhexidine. The second "octahedral" band could be observed only as a shoulder at around 34000 cm^{-1}, while the third band is completely covered by the ligand bands.

Based on the results of analytical and physicochemical analyses, the formula presented in Fig. 4 could be assigned to the complex cation of the Ni(II) and Pd(II) compounds and the formula presented in Fig. 5 to the Cr(III) complex.

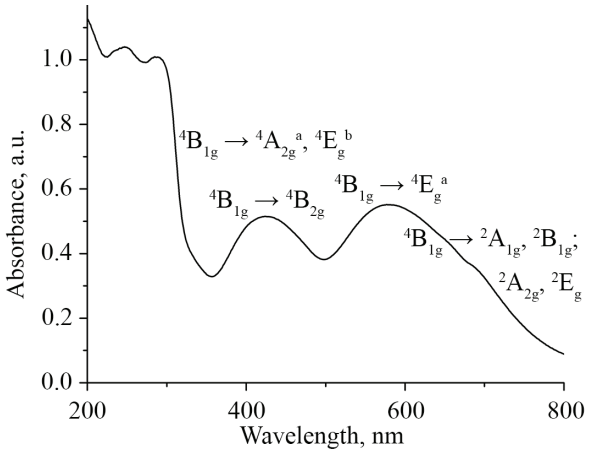

Fig. 3. UV–Vis spectrum of $[Cr(CHX)Cl_2](CH_3COO)$.

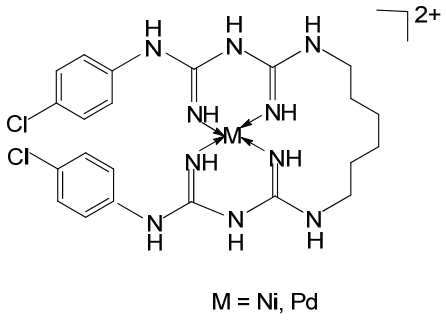

Fig. 4. Proposed structural formula for the complex cation of Ni(II) and Pd(II) coordination compounds.

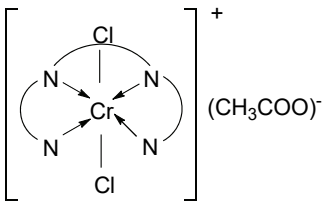

Fig. 5. Proposed structural formula for $[Cr(CHX)Cl_2]$ (CH_3COO).

Biological activity

The *in vitro* antibacterial and antifungal activities of the ligand and its metal complexes were determined against three bacterial and one fungal culture by the cup-plate agar diffusion method.

The disk diffusion method was applied according to the standard protocol.[43–45] The standard strains used were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231).

The stock solution of each tested substance contained 0.050 g powder dissolved in 25 mL DMSO *p.a.*; the working solution volume was 5 μL (the amount of compound/disk was 10 μg). A filter disk (Φ = 6 mm) was impregnated with a working solution volume of the ligand or complex and then placed on the agar surface (Mueller–Hinton agar medium for the bacteria and Sabouraud dextrose for fungi) inoculated with bacterial or fungal cultures. The plates were incubated at 37 °C for 24 h in the case of the bacteria and 72 h for the fungi.

The diameter of the zone of inhibition was then measured in mm (including the diameter of the disk) and compared with that of standard antibiotics (ampicillin and ciprofloxacin for the bacteria and miconazole for the fungi). The results are presented in Table V.

TABLE V. Antimicrobial activity for chlorhexidine diacetate and its metal complexes (10 μg/disc for each compound)

Compound	Microbial culture inhibition diameter, mm			
	Gram+ bacteria	Gram– bacteria		Fungi
	S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*
Ampicillin[a]	13	15	15	–
Ciprofloxacin[a]	18	22	22	–
Miconazole[b]	–	–	–	21
Chlorhexidine diacetate	19	16	12	10
$[Ni(CHX)]Cl_2 \cdot 2H_2O$	11	10	10	5
$[Ni(CHX)]Br_2 \cdot 2H_2O$	16	10	10	5
$[Ni(CHX)](CH_3COO)_2 \cdot C_2H_5OH$	18	8	19	4
$[Pd(CHX)][PdCl_4] \cdot 2H_2O$	21	17	14	9
$[Pd(CHX)](CH_3COO)_2$	18	15	14	8
$[Cr(CHX)Cl_2](CH_3COO)$	11	5	8	3

[a]10 μg disc^{-1}; [b]30 μg disc^{-1}

Among the seven complexes, those of Pd(II) showed a high antibacterial activity, especially against *S. aureus*. The complex $[Pd(CHX)][PdCl_4] \cdot 2H_2O$ was the most active, presenting an increase in antibacterial activity as compared to chlorhexidine diacetate against all three tested microbial agents. This complex was more active against *S. aureus* than ampicillin and ciprofloxacin and showed a comparable activity with these antibiotics against the gram-negative bacteria. The screened antifungal activities showed that the present complexes and even chlorhexidine diacetate are less active against *C. albicans* as compared with miconazole.

The observed differences between the antimicrobial activities of the complexes indicate that this activity is influenced by the metal ion, the anion and the metal:chlorhexidine molar ratio. Depending on the metal ion, the antimicrobial activity of the coordination compounds decreases in the order: palladium(II)> >nickel(II)>chromium(III). Of the two palladium complexes, the most active cor-

responds to a 2:1 metal:chlorhexidine mole ratio. The influence of the anion could be observed for the Ni(II) complexes, the antibacterial activity corresponding to the order: acetate >bromide >chloride.

The antioxidant activity of the metal complexes was evaluated by the photochemiluminescence method and quantified by comparison with trolox, as equivalent units of the standard substance, according to the recommended protocols.[46] The results are given in Table VI. The comparative determinations of the total antioxidant capacity show that the Pd(II) complexes presented the highest antioxidant capacity, while the chromium(III) complex was a poor antioxidant agent.

TABLE VI. Total antioxidant capacity of the metal complexes, nmol equiv. trolox / vol. sample

Complex	*V* / μL	
	5	10
$[Ni(CHX)]Cl_2 \cdot 2H_2O$	0.277	0.491
$[Ni(CHX)]Br_2 \cdot 2H_2O$	0.398	0.483
$[Ni(CHX)](CH_3COO)_2 \cdot C_2H_5OH$	0.530	0.739
$[Pd(CHX)][PdCl_4] \cdot 2H_2O$	1.062	1.665
$[Pd(CHX)](CH_3COO)_2$	0.437	1.039
$[Cr(CHX)Cl_2](CH_3COO)$	0.475	0.221

CONCLUSIONS

Six new coordination compounds of Ni(II), Pd(II) and Cr(III) with chlorhexidine diacetate, using the following salts: nickel(II) chloride, bromide and acetate, palladium(II) chloride and chromium(III) chloride, were prepared and characterized.

The screening data for the inhibition diameters of microbial cultures show that all complexes are active against the tested Gram-positive and Gram-negative bacteria, while their antifungal activity is weak. Among the six complexes, those of Pd(II) show good antibacterial activity, which would allow their use for the disinfection of metallic surfaces and medical instruments.

The comparative determinations of the total antioxidant capacity also showed the highest values for the palladium(II) complexes.

SUPPLEMENTARY MATERIAL

TG, DTA and NMR data are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И БИОЛОШКА АКТИВНОСТ НОВИХ Ni(II), Pd(II) И Cr(III) КОМПЛЕКСА СА ХЛОРХЕКСИДИНОМ

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Описана је синтеза, структурна карактеризација, антимикробна и антиоксидативна активност шест нових комплекса Ni(II), Pd(II) и Cr(III) са хлорхексидином као лигандом. Општа формула добијених комплекса је: $[Ni(CHX)]Cl_2 \cdot 2H_2O$, $[Ni(CHX)]Br_2 \cdot 2H_2O$, $[Ni(CHX)](CH_3COO)_2 \cdot C_2H_5OH$, $[Pd(CHX)][PdCl_4] \cdot 2H_2O$, $[Pd(CHX)](CH3COO)_2$ и $[Cr(CHX)Cl_2](CH_3COO)$, где је CHX = хлорхексидин. *In vitro* испитивања антимикробне активности су показала да сви комплекси имају велику активност према тестираним сојевима бактерија и мању активност према гљивама. Од свих испитиваних комплекса, два Pd(II) комплекса су показала најбољу антибактеријску активност, при чему је $[Pd(CHX)][PdCl_4] \cdot 2H_2O$ показао бољу активност према Грам-позитивним и Грам-негативним бактеријама у односу на хлорхексидин-диацетат. Резултати испитивања антиоксидативне активности испитиваних комплекса методом фотохемилуминисценције су показали да комплекси Pd(II) имају највећу антиоксидативну активност.

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REFERENCES

1. D. Sweeney, M. L. Raymer, T. D. Lockwood, *Biochem. Pharmacol.* **66** (2003) 663
2. A. R. Katritzky, S. R. Tala, A. Singh, *ARKIVOC* **2010** (2010) 76
3. C. J. Bailey, *Diabetes Care* **15** (1992) 755
4. B. Villet, B. Guigas, N. Sanz Garcia, J. Leclerc, M. Foretz, F. Andreelli, *Clin. Sci. (Lond.)* **122** (2012) 253
5. P. Ray, *Chem. Rev*. **61** (1961) 313
6. S. M. El-Megharbel, *J. Microb. Biochem. Technol.* **7** (2015) 65
7. M. Zhu, L. Lu, P. Yang, X. Jin, *Acta Crystallogr., E* **58** (2002) 272
8. L. C. Woo, V. G. Yuen, K. H. Thompson, J. H. McNeill, C. Orvig, *J. Inorg. Biochem.* **76** (1999) 251
9. L. Patron, M. Giurginca, G. M. Pătrînoiu, N. Iftimie, A. Meghea, *Rev. Roum. Chim*. **50** (2005) 457
10. T. M. Karpinski, A. K. Szkaradkiewicz, *Eur. Rev. Med. Pharmacol. Sci.* **19** (2015) 1321
11. G. McDonnell, A. D. Russell, *Clin. Microbiol. Rev.* **12** (1999) 147
12. M. C. Kudiyirickal, R. Ivancakova, *Acta Med.* **51** (2008) 3
13. T. Kuyyakanond, L. B. Quesnel, *FEMS Microbiol. Lett*, **100** (1992) 211
14. Q. Zhang, J. Mulder, G. J. Truin, W. H. van Palestein Helderman, *J. Dent. (Oxford, U.K.)* **35** (2007) 588
15. D. E. Slot, N. C. Vaandrager, C. V. Loveren, W. H. van Palenstein Helderman, G. A. van der Weijden, *Caries Res.* **45** (2011) 162
16. E. Varoni, M. Trace, G. Lodi, A. Carrassi, *Minerva Stomatol*. **61** (2012) 399
17. Z. Mohammadi, P. V. Abbott, *Int. Endod. J.* **42** (2009) 288
18. A. Young, G. Jonski, G. Rölla, *Int. Dent. J.* **53** (2003) 237
19. P. S. Thrane, A. Young, G. Jonski, G. Rölla, *J. Clin. Dent*. **18** (2007) 82

20. M. Călinescu, T. Negreanu-Pîrjol, R. Georgescu, O. Călinescu, *Cent. Eur. J. Chem*. **8** (2010) 543
21. M. Călinescu, T. Negreanu-Pîrjol, R. Georgescu, O. Călinescu, *Rev. Chim. (Bucharest)* **63** (2012) 682
22. M. Badea, R. Olar, M. Iliş, R. Georgescu, M. Călinescu, *J. Therm. Anal. Calorim.* **111** (2013) 1763
23. N. Puviarasan, V. Arjunan, S. Mohan, *Turk. J. Chem.* **26** (2002) 323
24. P.C. Dhar, *Int. J. Chem. Appl*. **6** (2014) 115
25. C. Dongli, J. Handong, Z. Hongyun, *Polyhedron* **13** (1994) 57
26. B. I. Al-Abdali, I. M. A. Shakir, H. M. Nafea, *Iraqi J. Sci*. **56** (2015) 3036
27. P. V. Bharatam, D. S. Patel, P. Iqbal, *J. Med. Chem*. **48** (2005) 7615
28. P. V. Babykutty, C. P. Prabhakaran, R. Anantaraman, C. G. R. Nair, *J. Inorg. Nucl. Chem*. **36** (1974) 3685
29. S. Singh, R. Malhotra, K. S. Dhindsa, *Proc. Natt. Acad. Sci. India, A* **68** (1998) 217
30. M. Zhu, L. Lu, P. Yang, X. Jin, *Acta Crystallogr., E* **58** (2002) 217
31. M. Zhu, L. Lu, P. Yang, X. Jin, *Acta Crystallogr., E* **58** (2002) 272
32. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 3rd ed., Wiley, New York, 1978, p. 231
33. S. K. Talwar, V. K. Rastogi, R. C. Saxena, *J. Indian Chem. Soc*. **68** (1991) 402
34. D. Z. Obadovic, D. M. Petrovic, V. M. Leovac, S. Caric, *J. Therm. Anal*. **36** (1990) 99
35. S. C. Lemos, S. J. S. Franchi, A. V. G. Netto, A. E. Mauro, O. Treu-Filho, R. C. G. Frem, E. T. Almeida, C. Torres, *J. Therm. Anal. Calorim.* **106** (2011) 391
36. A. T. Balaban, M. Banciu, L. Pogany, *Applications of Physical Methods to Organic Chemistry*, Ed. Ştiinţifică şi Enciclopedică, Bucharest, 1983, p. 95 (in Romanian)
37. D. Sen, *J. Chem. Soc., A* (1969) 2900
38. A. B. P. Lever, *Inorganic Electronic Spectroscopy*, 2nd ed., Elsevier, Amsterdam, 1984, pp. 417, 507
39. S. Goyal, K. Lal, *Acta Chim. Hung.* **127** (1990) 353
40. B. Tiwari, N. K. Sharma, *Asian J. Chem.* **21** (2009) 4209
41. N. Rathee, K. K. Verma, *J. Serb. Chem. Soc.* **77** (2012) 325
42. A. B. P. Lever, *Coordin. Chem. Rev.* **3** (1968) 119
43. J. H. Jorgensen, J. D. Turnidge, J. A. Washington, in *Manual of Clinical Microbiology*, 7th ed., P. R. Murray, M. A. Pfaller, F. C. Tenover, E. J. Baron, R. H. Yolken, Eds., AM Press, Washington DC, 1999, pp. 1526–1543
44. M. F. Hassan, R. Das, A. Khan, M. S. Hossain, M. Rahman, *Adv. Biol. Res*. **3** (2009) 53
45. R. R. Thanigaiarassu, K. Kannabiran, V. G. Khanna, *J. Pharm. Res*. **2** (2009) 273
46. R. Re, N. Pellegrini, A. Pannala, M. Yang, C. Rice-Evans, *Free Radical Biol. Med.* **26** (1999) 1231.