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THERMAL BEHAVIOUR OF NEW Ni(II) AND Cu(II) COMPLEXES WITH MACROCYCLIC LIGANDS FUNCTIONALISED WITH 1,2,4-TRIAZOLE

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The investigations concerning the thermal behaviour of a series of Ni(II) and Cu(II) complexes of type $[NiLCl_2]\cdot mH_2O$ ((**1**) L:L_1, m=6; (**3**) L:L_2, m=4) or $[CuLCl]_nCl_n\cdot mnH_2O$ ((**2**) L:L_1, m=6; (**4**) L:L_2, m=4) are presented. The ligands L(1) and L(2) have been synthesised by template condensation of 3,6-diazaoctane-1,8-diamine or 1,2-diaminoethane with formaldehyde and 2-amino-4H-1,2,4-triazole. The bonding and stereochemistry of the complexes have been characterised by IR, electronic and magnetic studies at room temperature. The in vitro qualitative and quantitative antimicrobial activity assays showed that the complexes exhibited variable antimicrobial activity against planktonic as well as biofilm embedded Gram-negative, Gram-positive and fungal strains. The thermal behaviour provided confirmation of the complexes composition as well as the number and nature of water molecules and the intervals of thermal stability.

Keywords: *formaldehyde, macrocyclic ligand, template, thermal stability, 1,2,4-triazole moiety*

Introduction

In the last years much interest was involved in synthesis and characterisation of complexes with heterocyclic ligands with particular attention focused on bioinorganic studies. The chemistry of 1,2,4-triazole derivatives have also received much attention because of their significant biologic activities [1]. It has been reported in the literature that 1,2,4-triazoles exhibit analgesic, anti-inflammatory [2] as well as antibacterial and antifungal activity [3, 4]. It was shown also that the complexes with 1,2,4-triazole derivatives are biologically active having nuclease like activity [5], antiproliferative [6, 7] as well as antibacterial activity [8]. On the other hand, complexes with macrocyclic ligands present a high kinetic as well as thermodynamic stability [9]. These properties are very important for biological active complexes because this kind of compounds can rich easily to a biological 'target site' without metabolisation in biological fluids. Some complexes of this type behave as antifungal, antimicrobials [10, 11] as well as cytostatic compounds [12]. Based on these properties, complexes could be used as drugs in order to prevent or cure the affections generated by pathologic organisms or in some cases, certain cancer type.

In order to study the possibility of complexes derived from 1,2,4-triazole to present biological activity, complexes of Ni(II) and Cu(II) with ligands resulted by 'one pot' condensation of 3,6-diazaoctane-1,8-diamine (L_1) or 1,2-diaminoethane (L_2) with 3-amino-4H-1,2,4-triazole and formaldehyde in alkaline medium were synthesized. Compounds have the general formula $[NiLCl_2]\cdot mH_2O$ ((**1**) L:L_1, m=6; (**3**) L:L_2, m=4) or $[CuLCl]_nCl_n\cdot mnH_2O$ ((**2**) L:L_1, m=6; (**4**) L:L_2, m=4). The 3-amino-4H-1,2,4-triazole was selected for condensation having in view the pharmacological applications of 1,2,4-triazole derivatives. It is to be also mentioned the 3-amino-4H-1,2,4-triazole ability to inhibit catalase activity that confers such unit selectivity in interaction with proteins [13].

Chemical analyses as well as IR, EPR, electronic spectroscopy and magnetic data at room temperature were used in order to characterise the compounds.

The thermal behaviour of these derivatives was investigated in air by thermal analysis (TG, DTG, DTA) in order to evidence the modifications at heating and also the thermodynamics effects that accompany them. It is to be mentioned that are very few study concerning its thermal behaviour of such type of derivatives [14, 15].

Thermal analysis evidenced a similar thermal behaviour and also a remarkable stability for all compounds after water elimination. The water molecules are losing up to 120°C as indicative of their uncoordinated nature. The anhydrous complexes are very stable, thermal degradation starts above 300°C. The oxidative degradation of the organic part occurs in two or

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three exothermal steps beginning with the 1,2,4-triazole moiety elimination. The metallic oxides are the final products as powder XRD indicates.

The biological assays indicated that complexes do not behave as HIV inhibitors but exhibited a good antimicrobial activity. It is to be mentioned that in the most cases Cu(II) complexes present a higher activity by comparison with Ni(II) compounds.

Experimental

Synthesis of the complexes

Compounds $ML(1)Cl_2 \cdot 6H_2O$

To a suspension of hydrated metal(II) chloride (5 mmol), 3-amino-4H-1,2,4-triazole (T) (5 mmol, 0.42 g) and 3,6-diazaoctane-1,8-diamine (5 mmol, 0.82 mL) in 100 mL ethanol was added dropwise 1 mL triethylamine and 2 mL formaldehyde (37%). The reaction mixture was refluxed 24 h until a sparingly soluble compound was formed. The microcrystalline product was filtered off, washed with EtOH, Et_2O and air-dried.

(**1**) Analysis, found: Ni, 11.52; C, 24.77; Cl, 14.52; H, 6.89; N, 22.82%; $NiC_{10}Cl_2H_{34}N_8O_6$ requires: Ni, 11.93; C, 24.41; Cl, 14.41; H, 6.96; N, 22.77%; IR (KBr pellet), cm^{-1}: ν_{OH}, 3410s, sh; ν_{NH}, 3200–3300m, br; $\nu_{as(CH_2)}$, 2920m; $\nu_{s(CH_2)}$, 2865m; $\nu_{C=N}$, 1630vs,1590vs; δ_{NH}, 1460m, 1430m; δ_{CH}, 1370s, 1350m; $\nu_{C_{heterocyclic}-N}$, 1290s, 1280m, 1220m; $\nu_{C_{aliphatic}-N}$, 1180m, 1175m, 1160m; ν_{C-C}, 1075s, 1050s; ν_{N-N}, 920w, 900w; ρ_{CH_2}, 790w; ν_{Ni-N}, 405w; UV-Vis (MgO pellet), cm^{-1}: $^3A_2 \rightarrow {}^3T_2$: 25640; $^3A_2 \rightarrow {}^3T_1(F)$: 16035; $^3A_2 \rightarrow {}^3T_1(P)$: 10150; B= 749 cm^{-1}; β=0.72; μ_{eff}, μ_B: 3.08.

(**2**) Analysis, found: Cu, 12.69; C, 24.28; Cl, 14.35; H, 6.78; N, 22.62%; $CuC_{10}Cl_2H_{34}N_8O_6$ requires: Cu, 12.79; C, 24.17; Cl, 14.27; H, 6.86; N, 22.55%; IR (KBr pellet), cm^{-1}: ν_{OH}, 3557s, sh; ν_{NH}, 3428vs; $\nu_{as(CH_2)}$, 2940m; $\nu_{s(CH_2)}$, 2830m; $\nu_{C=N}$, 1636m, 1618m; δ_{NH}, 1457m; δ_{CH}, 1384m; $\nu_{C_{heterocyclic}-N}$, 1287m; $\nu_{C_{aliphatic}-N}$, 1140m; ν_{C-C}, 1110s, 1090s; ν_{N-N}, 910w; ρ_{CH_2}, 746w; ν_{Cu-N}, 415w; UV-Vis (MgO pellet), cm^{-1}: CT: 25445; $d_{xz},d_{yz} \rightarrow d_{x2-y2}$: 16670; $d_{xy} \rightarrow d_{x2-y2}$: 13530; μ_{eff}, μ_B: 1.52.

Compounds $ML(2)Cl_2 \cdot 4H_2O$

To a suspension of hydrated metal(II) chloride (5 mmol), T (10 mmol, 0.84 g) and 1,2-diaminoethane (10 mmol, 0.67 mL) in 100 mL ethanol was added dropwise 1 mL triethylamine and 2 mL formaldehyde (37%). The reaction mixture was refluxed 24 h until a sparingly soluble compound was formed. The microcrystalline product was filtered off, washed with EtOH, Et_2O and air-dried.

(**3**) Analysis, found: Ni, 10.82; C, 26.81; Cl, 13.22; H, 6.03; N, 31.36%; $NiC_{12}Cl_2H_{32}N_{12}O_4$ requires: Ni, 10.91; C, 26.79; Cl, 13.18; H, 5.99; N, 31.24%; IR (KBr pellet), cm^{-1}: ν_{OH}, 3420s, ν_{NH}, 3300m; $\nu_{as(CH_2)}$, 2950m; $\nu_{s(CH_2)}$, 2880m; $\nu_{C=N}$, 1620vs, 1590s; δ_{NH}, 1535m, 1465m; δ_{CH}, 1400m; $\nu_{C_{heterocyclic}-N}$, 1320m, 1295m, 1220m; $\nu_{C_{aliphatic}-N}$, 1180m, 1160m, 1140m; ν_{N-N}, 980w; ρ_{CH_2}, 760m; ν_{Ni-N}, 480w; UV-Vis (MgO pellet), cm^{-1}: $^3A_2 \rightarrow {}^3T_2$: 24390; $^3A_2 \rightarrow {}^3T_1(F)$: 14490; $^3A_2 \rightarrow {}^3T_1(P)$: 12990; B=865 cm^{-1}; β=0.83; μ_{eff}, μ_B: 3.16.

(**4**) Analysis, found: Cu, 11.67; C, 26.46; Cl, 13.07; H, 5.88; N, 30.80%; $CuC_{12}Cl_2H_{32}N_{12}O_4$ requires: Cu, 11.70; C, 26.55; Cl, 13.06; H, 5.94; N, 30.96%; IR (KBr pellet), cm^{-1}: ν_{OH}, 3500s, sh; ν_{NH}, 3300vs; $\nu_{as(CH_2)}$, 2940m; $\nu_{s(CH_2)}$, 2860m; $\nu_{C=N}$, 1620s, 1600vs; δ_{NH}, 1440m; δ_{CH}, 1360s; $\nu_{C_{heterocyclic}-N}$, 1230m; $\nu_{C_{aliphatic}-N}$, 1140m; ν_{C-C}, 1045m; ν_{N-N}, 980s; ρ_{CH_2}, 770m; ν_{Cu-N}, 410w; UV-Vis (MgO pellet), cm^{-1}: CT: 25316; $d_{xz},d_{yz} \rightarrow d_{x2-y2}$: 16800; $d_{xy} \rightarrow d_{x2-y2}$: 14140; μ_{eff}, μ_B: 1.16.

Methods

All reagents were of commercial analytical quality and have been used without further purification. Chemical analysis of carbon, nitrogen, sulphur and hydrogen has been performed using a Perkin Elmer PE 2400 analyzer. Nickel and chloride were determined gravimetrically using dimethylglyoxyme and silver nitrate respectively while copper was determined volumetrically using thiosulfate method in the laboratories of Inorganic Chemistry Department.

IR spectra were recorded in KBr pellets with a BIO-RAD FTIR 135 spectrometer in the range 400–4000 cm^{-1}. Electronic spectra by diffuse reflectance technique, with MgO as standard, were recorded in the range 300–2000 nm, on a Jasco V 670 spectrophotometer. Magnetic measurements were done by Faraday's method, at room temperature, using $Hg[Co(NCS)_4]$ as standard. The molar magnetic susceptibilities were calculated and corrected for the atomic diamagnetism. EPR spectra were recorded on microcrystalline samples at room temperature with a Varian E-9 spectrometer. The field was calibrated using crystalline diphenyylpicrylhydrazyl (g=2.0036).

The qualitative screening of the susceptibility spectra of different microbial strains to the complexes was performed by adapted diffusion techniques, while the quantitative assay of minimal inhibitory concentration (M.I.C., $\mu g\ cm^{-3}$) value was based on liquid medium serial microdilutions [16]. The compounds were solubilised in DMF to a final concentration of 1 mg mL^{-1}. The in vitro biological screening effects were tested against a microbial inoculum of

~$1.5 \cdot 10^8$ UFC cm^{-3}, corresponding to 0.5 McFarland density, represented by *Enterobacteriaceae* (*E. coli, Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella pneumoniae*) *Pseudomonadaceae* (*Pseudomonas aeruginosa*, *Acinetobacter boumani*), *Micrococcaceae* (*Staphylococcus aureus*), *Bacillaceae* (*Bacillus* sp.) and *Candida* strains, reference ones and recently isolated from clinical samples, respectively. The assessment of anti-biofilm activity of the tested compounds was performed using a simple method for microbial biofilm development in 96 multiwell plates for 24 h; after incubation, the microwells content was discarded, the planktonic microbial cells being removed by three washing steps and the microbial cells immobilized in biofilms formed on the plastic walls were fixed with cold methanol, coloured by violet crystal and resuspended with acetic acid 33%. The absorbance of coloured solution was measured at 490 nm by an ELISA reader, the results being proportional with the number of microbial cells adhered to the plastic wells.

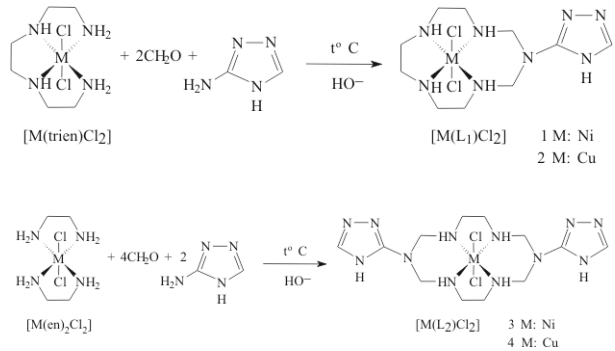
Anti-HIV tests were realised by adding the DMSO solutions with various concentrations of complexes to cell culture medium, T4 lymphocytes and then HIV-1. Cultures were incubated at 37° in a 5% carbon dioxide atmosphere for six days. To the incubated cultures was then added the tetrazolium salt and the formazan color development was analysed spectrophotometrically. Complexes-treated virus-infected cells were compared with complexes-treated noninfected cells as well as with other appropriate controls (untreated infected and untreated non-infected cells, complexes-containing wells without cells) on the same plate.

The heating curves (TG, *T*, DTA and DTG) were recorded in a static air atmosphere using a MOM Hungary, Paulik–Paulik–Erdey derivatograph type with a sample mass of ca. 30–91 mg over the temperature range 20–1000°C, using a heating rate of 10 K min^{-1}.

Results and discussion

Synthesis and physico-chemical characterisation of the complexes

The template condensation reaction of aliphatic polyamines with various nucleophiles and formaldehyde generally leads to unsaturated polyaza macrocyclic compounds containing –N–CH_2–N– linkages. The one-pot reactions (Scheme 1) of excess formaldehyde with 1:1/2:1/2 molar mixture of nickel(II) or copper(II) chloride, 3,6-diazaoctane- 1,8-diamine or 1,2-diaminoethane and 3-amino-4H- 1,2,4-triazole in an alkaline medium produced the species $[NiLCl_2]\cdot mH_2O$ ((**1**) *L*:L_1, *m*=6; (**3**) *L*:L_2, *m*=4) or



Scheme 1

$[CuLCl]_nCl_n\cdot mnH_2O$ ((**2**) *L*:L_1, *m*=6; (**4**) *L*:L_2, *m*=4) (Scheme 1).

All the complexes are polycrystalline solids soluble in dimethyl sulfoxide and N,N-dimethylformamide.

In the IR spectra of complexes are viewed the characteristic patterns of 3-amino-4H-1,2,4-triazole (experimental part). In the range 1590–1640 cm^{-1} two very strong bands for all complexes are assigned to $\nu_{C=N}$ vibrations while the band that appears in the range 910–980 cm^{-1} is assigned to ν_{N-N} vibration mode. The spectra of the compounds exhibit vibration only for secondary and not primary amine groups in the range 3000–3400 cm^{-1}. In the characteristic ranges for water a large band about 3500 cm^{-1} can be assigned to ν_{OH} stretching vibrations. The new bands, observed in 400–480 region are assignable to ν_{M-N} vibrations [17].

The diffuse-reflectance spectra of complexes (**1**) and (**3**) in the Vis-near-IR region show the bands characteristic for Ni(II) complexes with pseudo-octahedral surrounding [18]. The three absorption bands spin allowed together with the corresponding assignments and the crystal field parameters are presented at experimental part. The distortion from a regular octahedron, associated with the different nature of ligands, generated the broad aspect of these bands. The average values of crystal field parameter are consistent with the presence of chlorine as donor beside nitrogen, atoms that generate a weak field.

The electronic spectra of Cu(II) complexes show a broad band centred at 13530/14140 cm^{-1} and a shoulder at higher energies, which also agree with an octahedral geometry [17].

The Ni(II) complexes exhibit at room temperature magnetic moments close to the spin only value indicating the absence of magnetic interaction between paramagnetic ions in this condition [19]. As for the Cu(II) complexes these display small value of the magnetic moment at room temperature indicating an antiferromagnetic coupling between paramagnetic ions.

EPR spectra of Cu(II) complexes display an isotropic signal with g_i value 2.099 and 2.095 and a_i value of 15.75 and 19.20 mT, respectively (Fig. 1).

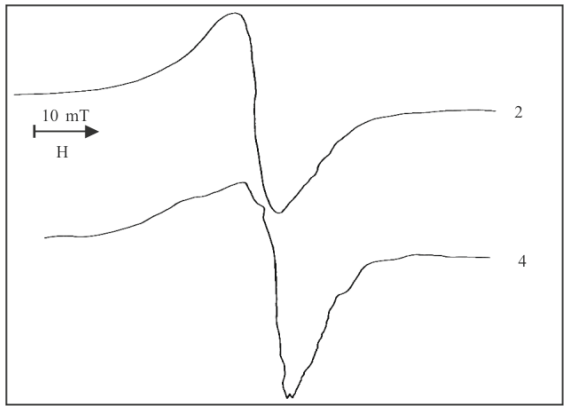


Fig. 1 X-band EPR spectra of complexes (**2**) and (**4**) on polycrystalline samples at room temperature

This aspect of spectra is characteristic for the octahedral species containing misaligned axis [20].

Biological activity

The antimicrobial activity of the tested compounds was performed against 15 microbial strains, the majority of them being recently isolated from different clinical samples and exhibiting different resistance patterns, i.e. *Enterobacteriaceae* (*E. coli* – enteropathogenic (EPEC) strains, as well as strains producing extended spectrum β-lactamases (ESBL) rendering them resistant to all β-lactams, (*Salmonella* sp., *Shigella flexneri* and *Shigella* sp., *Proteus* sp., *Klebsiella pneumoniae*) *Pseudomonadaceae* (*Pseudomonas aeruginosa*, *Acinetobacter boumani*) – both microorganisms being known for their high natural resistance to antibiotics), *Micrococcaceae* (*Staphylococcus aureus – methicilin resistant* (MRSA)), *Bacillaceae* (*Bacillus* sp.) and *Candida albicans* strains. All tested compounds exhibited good antimicrobial activity (with low MIC values ranging from 32 to 128 μg cm^{-3}).

It is to be noticed that all complexes exhibited an improved antimicrobial activity comparing to the ligand used for condensation, in case of *Enterobacteriacae* and fungal strains (Fig. 2).

When referring to *Pseudomonadaceae* and *Micrococcaceae* strains, the activity of the four complexes was similar between them and to that of the ligand, excepting the complex (**3**) that exhibited very good activity against *Acinetobacter boumani* strain (MIC value of 16 μg cm^{-3}) (Fig. 2).

It must to be noticed the very good antifungal activity exhibited by complexes (**1**) and (**2**) against *Candida albicans* strains with a MIC value of 64 μg cm^{-3}.

It is also remarkable that all tested compounds, including the ligand exhibited low MIC values against ESBL producing *E. coli* strains, which are raising at present a real therapy problem, being resistant to almost all β-lactams.

The comparative analysis of the microbial spectrum and MIC values revealed that the most active antimicrobial compound is (**2**) that exhibited good antifungal activity and also antibacterial activity, specifically directed against Gram-negative, *Enterobacteriaceae* strains (Fig. 2).

It must be mentioned that the used solvent, DMF, did not influence the antimicrobial activity of the tested compounds at the working concentrations.

Concerning the influence of the tested compounds on the ability of microbial strains to colonize the inert substratum, thus to interfere with this dual microbial strategy used for survival in the external environment and to initiate infections associated to prosthetic devices, our results showed that different compounds exhibited different efficiencies depending on the microbial tested strains.

The complex (**2**) proved to be the most efficient in combating the biofilms formed by *Escherichia coli* (Fig. 3), *Klebsiella pneumoniae* and *Proteus* sp. from *Enterobacteriaceae*, *Acinetobacter boumani* (Fig. 4) from *Pseudomonadaceae* as well as *Candida albicans* strains (Fig. 6).

All four complexes exhibited very good anti-biofilm properties in case of *Salmonella* (Fig. 5), *Shigella* and *Staphylococcus aureus* strains.

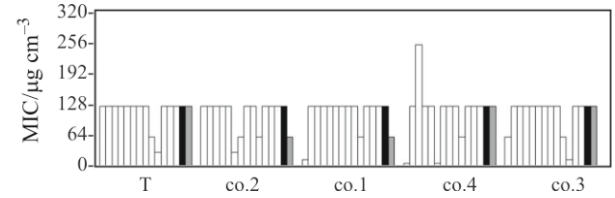


Fig. 2 The representation of the antimicrobial activity (MIC values) of the tested compounds (white columns – Gram negative bacterial strains, black columns – Gram-positive bacterial strains, grey columns – *Candida albicans*)

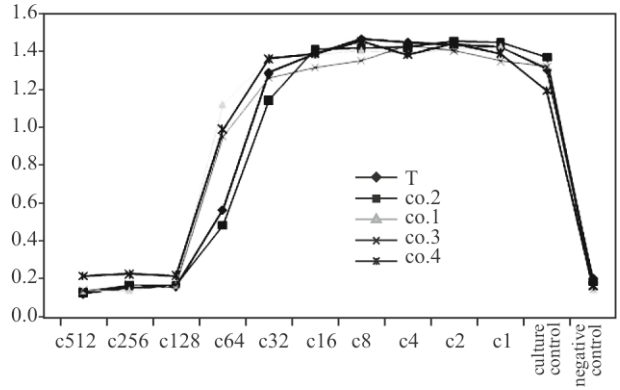


Fig. 3 Influence of binary concentrations of tested compounds (512–1 μg cm^{-3}) on the adherence ability of *E. coli* strains

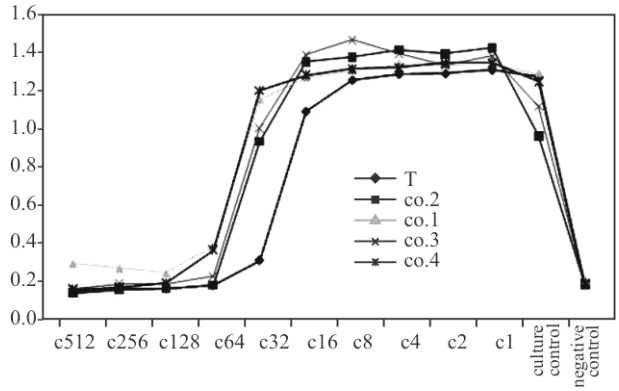


Fig. 4 Influence of binary concentrations of tested compounds (512–1 μg cm^{-3}) on the adherence ability of *Acinetobacter boumani* strain

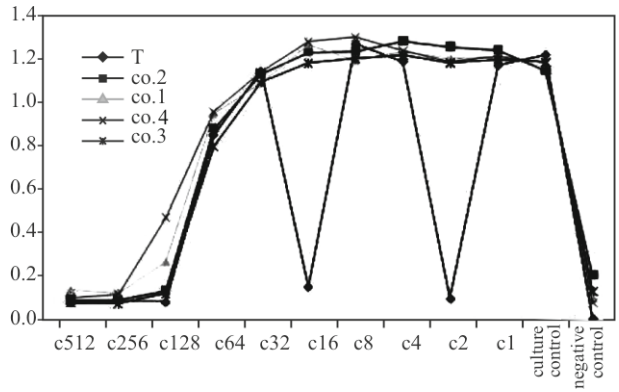


Fig. 5 Influence of binary concentrations of tested compounds (512-1 μg cm^{-3}) on the adherence ability of *Salmonella* sp. strains

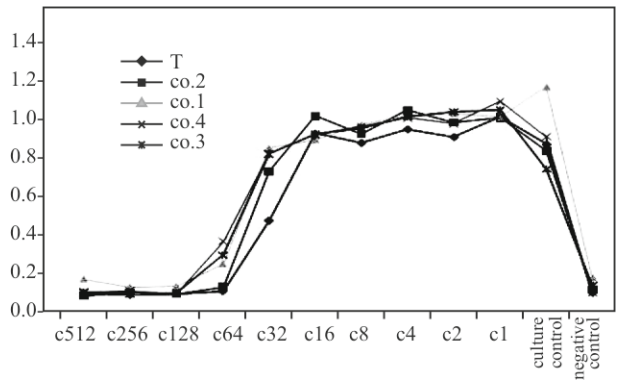


Fig. 6 Influence of binary concentrations of tested compounds (512–1 μg cm^{-3}) on the adherence ability of *Candida albicans* strain

The complex (**3**) exhibited by far the most efficient inhibitory activity against biofilm developing capacity of *Ps. aeruginosa* and *Bacillus subtilis* strains.

Thermal behaviour of the complexes

Thermal behaviour of complexes was investigated by thermogravimetric analysis (TG) while the final residues were examined by X-ray diffraction on powder. The intermediate products formed during thermolysis were not possible to identify because the steps were not distinct, except for the anhydrous complexes formed after the first decomposition step.

According to the observed mass losses (Table 1), the following degradation scheme might be proposed for complex (**1**):

$$[Ni(C_{10}H_{22}N_8)Cl_2]\cdot 6H_2O \rightarrow [Ni(C_8H_{21}N_3)Cl_2] \rightarrow NiO$$

The first step in the TG curve (Fig. 7) occurred in the temperature range 50–170°C (the maximum decomposition rate corresponds to 110°C according to DTG curve) with an endothermic effect. This step corresponds to distinct loss of six water molecules. The anhydrous complex suffers at least two degradation processes that are not well separated. The empirical formula $[Ni(C_8H_{20}N_3)Cl_2]$ was tentatively proposed for the intermediate resulted at 330°C according to the mass variation (Table 1) that corresponds to the triazolyl moiety loss. The formed product, after this step is involved in at least 2 oxidative degradation processes (according to both DTA and DTG), total transformation in oxide being made at 770°C.

The thermal degradation of the compound (**2**) is similar and water elimination begins at 60°C. Within the temperature interval 60–160°C the water molecules are lost in a well definite, endothermic step; the temperature range indicates their nature as crystallisation water. The next exothermic step of the thermal degradation that occurs between 160–285°C corresponds to minimum two processes, as the DTG and DTA curves indicate. According to the mass loss (Table 1) in this step the triazole ring is also eliminated from the compound. In the next step accompanied by an exothermic effect, until 790°C, occurs the oxidation of the remaining organic part together with chloride elimination. The final residue is copper(II) oxide.

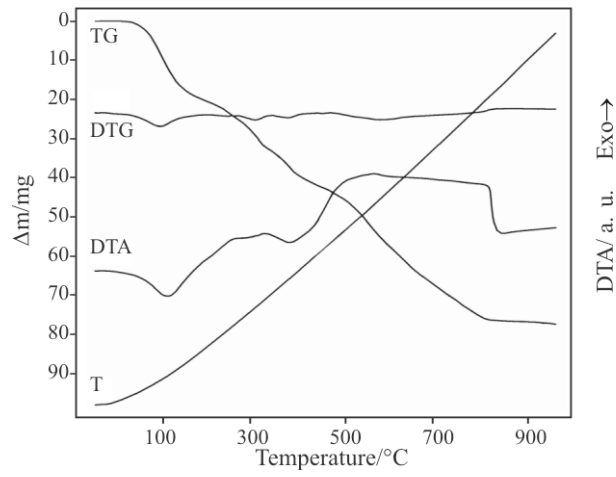


Fig. 7 TG, DTG and DTA curves of $[NiL(1)Cl_2]\cdot 6H_2O$

Table 1 Thermal behaviour data (in static air atmosphere) for the complexes

Complex	Step	Thermal effect	Temperature interval/°C	Δm_{exp}/ %	Δm_{calc}/ %
$[NiL(1)Cl_2]\cdot 6H_2O$ (**1**)	1	endothermic	50–170	21.53	21.97
	2	exothermic	170–330	13.35	13.83
	3	exothermic	330–770	49.57	49.02
			Residue (NiO)	15.53	15.18
$[CuL(1)Cl]_nCl_n\cdot 6nH_2O$ (**2**)	1	endothermic	50–160	18.76	18.81
	2	exothermic	160–310	14.16	14.21
	3	exothermic	310–790	50.51	50.36
			Residue (CuO)	16.56	16.61
$[NiL(2)Cl_2]\cdot 4H_2O$ (**3**)	1	endothermic	50–150	13.49	13.39
	2	exothermic	150–255	12.97	12.65
	3	exothermic	255–320	12.70	12.65
	4	exothermic	320–570	46.97	47.43
			Residue (NiO)	13.87	13.88
$[CuL(2)Cl]_nCl_n\cdot 4nH_2O$ (**4**)	1	endothermic	50–160	13.08	13.27
	2	exothermic	160–250	12.69	12.54
	3	exothermic	250–450	12.31	12.54
	4	exothermic	450–730	47.27	47.00
		Residue (CuO)		14.64	14.65

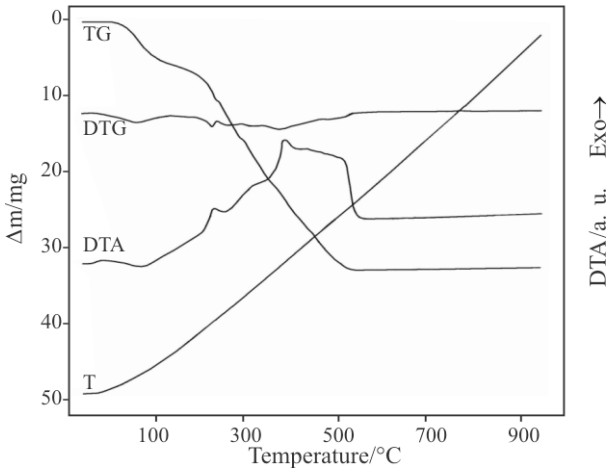


Fig. 8 TG, DTG and DTA curves of $[NiL(2)Cl_2]\cdot 4H_2O$

The TG, DTG and DTA curves corresponding to the complex (**3**) in the 20–1000°C temperature range are presented in Fig. 8. The complex (**3**) loses the four water molecules in the 50–150°C range. The thermal degradation of organic part occurs in three steps in the temperature range 150–570°C. In the first two exothermic steps the triazole moieties are gradually lost. The third step, an exothermic and complex one being composed by two processes, corresponds to the organic residue pyrolise and nickel(II) oxide generation.

On the basis of TG curve, the following degradation scheme might be proposed for complex (**4**):

$$[Cu(C_{12}H_{24}N_{12})Cl_2]\cdot 4H_2O \rightarrow [Cu(C_{10}H_{23}N_9)Cl_2] \rightarrow [Cu(C_8H_{22}N_6)Cl_2] \rightarrow CuO$$

Within the temperature range 50–160°C the crystallisation water molecules are lost in a well definite, endothermic step. In the next exothermic step, between 160–250°C, the thermal degradation of anhydrous complex occurs. The second triazole fragment is then lost in minimum two processes, as the DTG and DTA curves indicate. The empirical formula $[Cu(C_8H_{22}N_6)Cl_2]$ proposed for the intermediates resulted at 450°C according to the mass loss (Table 1) corresponds to the oxidative degradation of the two triazole residues. In the next step accompanied by a strong exothermic effect, until 730°C, occurs the oxidation of the residual macrocyclic ligand together with chloride elimination leading finally to copper(II) oxide.

Conclusions

The nickel(II) and copper(II) complexes with ligands resulted in one-pot condensation of 3,6-diazaoctane-1,8-diamine or 1,2-diaminoethane, formaldehyde and 3-amino-4H-1,2,4-triazole (T) have been synthesised.

The bond and stereochemistry were characterised by means of IR, electronic and EPR spectroscopy. Electronic and EPR spectra of complexes are

characteristic for an octahedral stereochemistry. The modifications in the IR spectra of complexes are in accord with the condensation process. The low values of magnetic moments observed for Cu(II) complexes are an indicative of interaction between paramagnetic ions at room temperature.

The tested compounds exhibited specific antimicrobial activity against different bacterial and fungal strains, recently isolated from clinical samples and exhibiting resistance features to conventional antibiotics, with low MIC values ranging from 32 to 128 μg cm^{-3}. The antimicrobial activity of all four complexes was improved as comparing to the 3-amino-4H-1,2,4-triazole in case of *Enterobacteriacae* and fungal strains, but remained similar in case of *Pseudomonadaceae* and *Micrococcaceae* strains.

Taken together, the antimicrobial testing data concluded that the most active antimicrobial compound is (**2**), that exhibited good antibacterial (against Gram-negative, *Enterobacteriaceae* strains) and antifungal activity against planktonic cells, but also successfully inhibited the biofilm development of the the Gram-positive, Gram-negaive and fungal tested strains.

It is also remarkable that all tested compounds, including the 3-amino-4H-1,2,4-triazole exhibited low MIC values against ESBL producing *E. coli* strains, which are raising at present a real therapy problem, being resistant to almost all β-lactams.

Thermal decomposition of complexes afforded to establish the number and nature of water molecule, the composition of complexes and also the intervals of thermal stability. During the thermal degradation the triazole moiety is first eliminated for all anhydrous complexes. The complexes with the same ligand display a similar thermal behaviour leading to metal oxide as final residues.

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