Journal of Thermal Analysis and Calorimetry, Vol. 92 (2008) 1, 239–243

COPPER(II) COMPLEXES WITH N,N-DIMETHYLBIGUANIDE
Thermal, spectroscopic and biological characterization

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The N,N-dimethylbiguanide (HDMBG) complexes $[Cu_2(HDMBG)_2Cl_4]$ (**1**) and respectively $[Cu(HDMBG)_2]Cl_2{\cdot}2H_2O$ (**2**) exhibit *in vitro* antimicrobial activity. The complexes were characterised by IR, electronic as well as EPR spectra. The IR spectra of complexes show the pattern of N,N-dimethylbiguanide coordinated as chelate. The electronic and EPR data are in agreement with a square pyramidal stereochemistry for (**1**) and a square planar one for (**2**). The *in vitro* qualitative and quantitative antimicrobial activity assays showed that the complexes exhibited variable antimicrobial activity against Gram-negative strains (*Escherichia coli*, *Klebsiella spp*. and *Enterobacter sp*.) isolated from the hospital environment.

The thermal analysis has evidenced the thermal intervals of stability and also the thermodynamic effects that accompany them. The thermal behaviour in nitrogen is complex according to TG and DTA curves including melting, dehydration as well as compounds decomposition.

Keywords: *antimicrobial activity, copper(II) complex, N,N-dimethylbiguanide, thermal behaviour*

Introduction

The natural proteins containing copper are involved in metal ions storage, oxygen or electrons transport as well as in enzymatic reactions [1, 2]. These involvements of copper in physiological processes regulation have stimulated the synthesis of some complexes containing this ion in order to develop therapeutics. From these, the compound demonstrating antimicrobial activity could be used as antibiotics or disinfectants. As result some copper(II) complexes with multidentate ligands having nitrogen donor set such as bi- [3], tri- [4, 5] or tetradentate [6] Schiff base have attracted considerable attention due to their remarkable antimicrobial (both antibacterial and antifungal) as well as antitumor activity [5].

As for N,N-dimethylbiguanide, among other biological properties such as glucose lowering agent, analgesic, antimalarial and antimetabolite [7–9], both its derivatives and complexes demonstrate selective and effective antimicrobial activity [10–12].

In order to assay the biological activity of some N,N-dimethylbiguanide (HDMBG) complexes as well as their thermal stability, two species $[Cu_2(HDMBG)_2Cl_4]$ (**1**) and respectively $[Cu(HDMBG)_2]Cl_2{\cdot}2H_2O$ (**2**) have been characterised by IR, EPR and electronic spectra. These complexes were already structurally characterised as dinuclear and mononuclear species respectively [13].

The in vitro antimicrobial activity of complexes was evaluated using qualitative and quantitative assays against different Gram-negative strains recently isolated from different surfaces in hospital environment. The results showed that the tested compounds exhibited significant antimicrobial activity.

The thermal behaviour of these derivatives was investigated by thermal analysis (TG, DTA) in order to evidence the modifications appeared at heating and also the thermodynamics effects that accompany them. It was observed the melting of both complexes before degradation. The two complexes have a similar thermal behaviour except for the water elimination in the case of complex (**2**). The final residue was metallic copper in both cases.

Experimental

All reagents were of commercial analytical quality and have been used without further purification. Chemical analysis of carbon, nitrogen and hydrogen has been performed using an EA 1110 analyzer. Copper was determined using the thiosulfate method.

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1388–6150/$20.00

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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 standard, were recorded in the range 380–1100 nm, on a VSU 2P-Zeiss Jena spectrometer.

The EPR measurements were performed on microcrystalline samples using a JEOL JES-ME upgrade spectrometer equipped with X- and K-band cavities (9.5 and 24 GHz, respectively) and a Jeol system for low- and high-temperature experiments (80–550 K). A modulation frequency of 100 kHz and a modulation amplitude of 0.4 mT were used. The magnetic field was calibrated using diphenylpicrylhydrazyl (DPPH) standard sample (g=2.0036). Microwave frequency was measured by means of an external digital Takeda Riken 5502D frequency counter.

The qualitative screening of the susceptibility spectra of different microbial strains to the complexes was performed by adapted diffusion techniques: paper filter disk impregnation with the tested substances solutions, the disposal of tested solutions in agar wells and the spotting of tested solutions on microbial inoculums seeded medium, while the quantitative assay for the establishment of the minimal inhibitory concentration (M.I.C., μg cm^{-3}) value was based on liquid medium serial dilutions [14]. The compounds were dissolved in DMF to a final concentration of 1 mg mL^{-1}. The in vitro biological screening effects were tested against a bacterial inoculum ($1.5 \cdot 10^8$ UFC cm^{-3}) represented by 82 Gram negative, Enterobacteriaceae strains isolated from different surfaces in the hospital environment (*Escherichia coli*, *Klebsiella spp*. and *Enterobacter sp*.). For the M.I.C. assay, stock solutions were prepared by dissolving the compounds in DMF and serial binary dilutions were performed in nutrient both distributed in 96-multiwell plates, further inoculated with a standard inoculum of bacterial strains. The plates were incubated at 37°C for 24 h. The minimal inhibitory concentration was read by wells observation: in the first wells containing high concentrations of compounds the culture growth was not visible, the microbial cells being killed or inhibited by the tested compound. At lower concentrations of the tested compounds, the microbial culture became visible. The lowest concentration, which inhibited the visible microbial growth, was considered the MIC value for the tested compound. In the next wells, including the standard culture growth control wells, the medium become muddy as a result of the microbial growth. In the sterility control wells series the medium had to remain clear. From the last well without any visible microbial growth and from the first one with a microbial growth, Gram stained smears were performed for the results confirmation.

The heating curves (TG, DTA and DTG) were recorded in a nitrogen atmosphere with a Labsys 1200 Setaram thermobalance, with a sample mass between 6–10 mg over the temperature range of 20–800°C, using a heating rate of 10°C min^{-1}. The measurements were carried out in a nitrogen atmosphere (flow rate 20 cm^3 min^{-1}) by using alumina crucibles.

The syntheses and structural data for complexes were reported elsewhere [13]. The composition of complexes has been confirmed by chemical analyses. The complexes were purified by recrystallisation from water and ethanol respectively.

$[Cu_2(HDMBG)_2Cl_4]$ (**1**): IR (KBr pellet), cm^{-1}: $\nu_{as(NH_2)}$, 3354vs; ν_{NH}, 3286s; $\nu_{s(NH_2)}$, 3185s; $\nu_{C=N}$, 1656vs; $\delta_{as(NH_2)}$, 1505m; $\delta_{s(NH_2)}$, 1451m; ν(chelate ring), 1381w; ν_{C-N}, 1230w; $\rho_{(CH_3)}$, 776w.

$[Cu(HDMBG)_2]Cl_2 \cdot 2H_2O$ (**2**): IR (KBr pellet), cm^{-1}: $\nu_{as(NH_2)}$, 3368vs; ν_{OH}, 3310sh; ν_{NH}, 3250s; $\nu_{s(NH_2)}$, 3195s; $\nu_{C=N}$, 1673s, 1624vs; $\delta_{as(NH_2)}$, 1509m; $\delta_{s(NH_2)}$, 1441m; ν(chelate ring), 1323w; ν_{C-N}, 1274m; $\rho_{(CH_3)}$, 719w.

Results and discussions

Physico-chemical characterisation of compounds

In this paper, we report the physico-chemical and biological characterisation of N,N-dimethylbiguanide (HDMBG) complexes $[Cu_2(HDMBG)_2Cl_4]$ (**1**) and $[Cu(HDMBG)_2]Cl_2 \cdot 2H_2O$ (**2**).

The IR spectra of complexes reveal the characteristic bands of N,N-dimethylbiguanide coordinated as chelate through N^2 and N^4 atoms. Comparison with the N,N-dimethylbiguanide hydrochloride spectrum indicated that the bands assigned to $\nu_{as(NH_2)}$ and ν_{NH} are shifted towards lower wavenumbers while the band assigned to $\nu_{s(NH_2)}$ is shifted towards higher wavenumbers [12]. At 1656 and 1673 cm^{-1}, respectively, appears an intense band characteristic to $\nu_{C=N}$ vibration mode, shifted also to higher wavenumbers. The new band at 1381 and 1323 cm^{-1}, respectively, can be associated with the chelate ring formation for the biguanide derivatives [15]. The water presence in complex (**2**) generates a shoulder at 3310 cm^{-1} [16].

Electronic spectra of the complexes are shown in Fig. 1. The two spectra display a single band, as is usually observed for copper(II) in complexes with different ligands [17, 18].

The absorption maximum for the complex (**1**) is found at 680 nm and one shoulder can be observed at lower values. The position of the absorption maxima and the general aspect of this spectrum are in agreement with a square-pyramidal coordination. The electronic spectrum of complex (**2**) displays a narrow

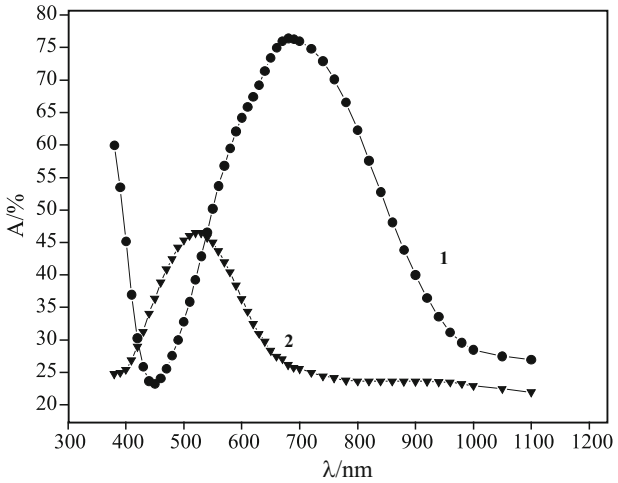

Fig. 1 Electronic spectra of complexes; ● – **1**, ▼ – **2**

band with the absorption maximum at 525 nm that indicates a square-planar stereochemistry.

The complex (**1**) shows an axial EPR spectrum at room temperature. The simulated spectrum yields $g_{\|}$=2.126, $g_{\perp}$=2.070 (g_{iso}=2.088), $A_{\|}$= 58·10^{-4} cm^{-1}, $A_{\perp}$=6·10^{-4} cm^{-1} (A_{iso}=23 cm^{-1}) and P=2·10^{-4} cm^{-1} (P represent the quadrupolar interaction).

The room temperature EPR spectrum of (**2**) consists of a narrow line centred at g=2.04, and a broad shoulder at lower fields. It was simulated as an orthorhombic spectrum with the parameters: g_1=2.19, g_2=2.040, g_3=2.044 (g_{iso}=2.09), A_1≅12.0 mT, A_2≅1.3 mT, A_3≅0.4 mT (A_{iso}=4.5 mT). This aspect could be generated by the fact that in the coordination polyhedron the two methyl groups of the ligands are in a trans-configuration.

Biological activity

Because of the permeability barrier provided by the outer membrane (OM), Gram-negative bacteria are inherently resistant to many hydrophobic antibiotics including erythromycin, fusidic acid, novobiocin and rifampin. Such intrinsic resistance in Gram-negative bacteria is largely attributed to the activity of multidrug resistance (MDR) efflux pumps. Moreover, these pumps also play a significant role in acquired clinical resistance, which limits the arsenal of antibiotics that are effective in treating Gram-negative bacterial infections. The incidence of Gram-negative pathogens resistant to multiple classes of antibiotics is increasing and the resultant deficit in effective therapeutic agents emphasizes the urgent need for new agents and new therapeutic approaches for the treatment of Gram-negative infectious disease [19].

The qualitative screening of the antimicrobial activity performed by adapted diffusion techniques revealed that the tested complexes inhibited the growth of the tested Gram-negative microbial strains.

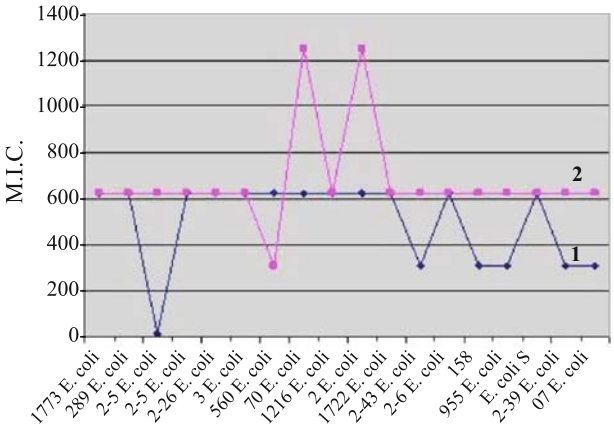

Fig. 2 The antimicrobial activity of the tested compounds *vs*. *E. coli* strains; ◆ – **1**, ■ – **2**

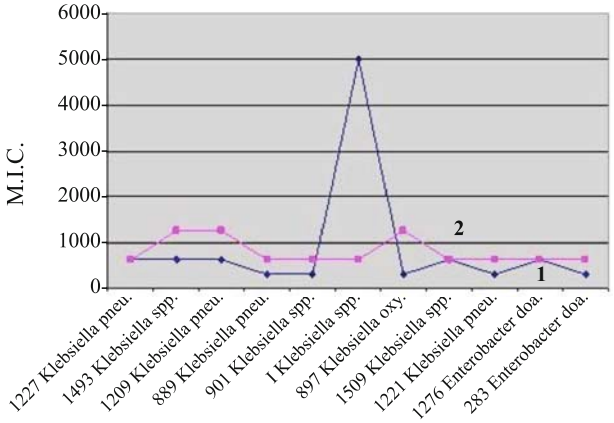

Fig. 3 The antimicrobial activity of the tested compounds *vs*. *Klebsiella sp*. and *Enterobacter cloacae* strains; ◆ – **1**, ■ – **2**

It is to be mentioned that DMF, used in the study to dissolve the complexes does not exhibit any traceable antimicrobial activity at the working concentrations, thus we could conclude that the solvent did not influence the biological activity of the tested compounds. The results of the quantitative assay showed that the compound (**1**) exhibited a slight higher antimicrobial activity than (**2**), showing a good antimicrobial activity and correspondent lower M.I.C. values against *Escherichia coli* (M.I.C. from 18 to 1250 μg cm^{-3}) (Fig. 2) followed by *Klebsiella sp*. and *Enterobacter spp*. strains (M.I.C. from 312.5 to 1250 μg cm^{-3}) (Fig. 3).

Thermal behaviour of compounds

The results concerning the thermal degradation in nitrogen atmosphere of the compounds revealed difference in their thermal behaviour and the general aspect of the TG and DTA curves.

The complex (**1**) is anhydrous so there is no detectable change in TG curve up to 230°C. Also,

Table 1 Thermal behaviour of the N,N-dimethylbiguanide and complexes in nitrogen atmosphere

Compound	Step	Thermal effect	Temperature range/°C	Δm_{exp}/%	Δm_{calc}/%
$[Cu_2(HDMBG)_2Cl_4]$ (**1**)	1.	Endothermic	230 (*m. p.*)*	0	0
	2.	Endothermic	230–375	29.54	29.40
	3.	Miscellaneous	375–495	19.69	19.60
	4.	Exothermic	495–750	26.60	26.89
$[Cu(HDMBG)_2]Cl_2 \cdot 2H_2O$ (**2**)	1.	Endothermic	75–150	8.64	8.40
	2.	Endothermic	206	0	0
	3.	Endothermic	225 (*m. p.*)*	0	0
	4.	Endothermic	225–355	36.14	36.15
	5.	Miscellaneous	355–630	23.71	24.10
	6.	Exothermic	630–800	16.96	16.53

*melting point

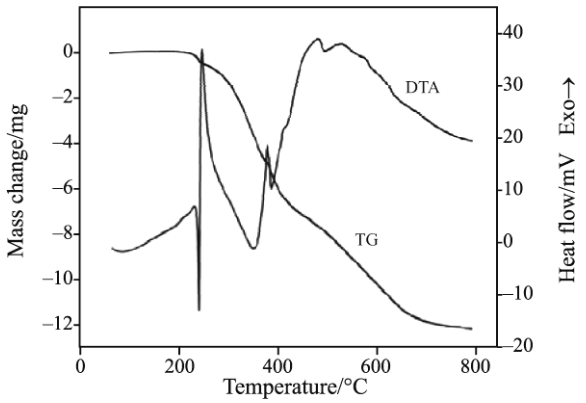

Fig. 4 TG and DTA curves of $[Cu_2(HDMBG)_2Cl_4]$

before the thermal decomposition it was observed the compound (**1**) melting at 230°C, process accompanied by an endothermic effect. This process is immediately followed by the thermal decomposition that occurs in three steps (Fig. 4, Table 1).

The first decomposition step is accompanied also by an endothermic effect and a mass variation of 29.54% that corresponds to a part of ligand (60%) loss. The next step is an overlap of two processes (one endo- and one exothermic) as DTA indicates. The corresponding mass variation is assigned to the remaining ligand elimination. The IR spectrum of the reaction mixture isolated at 400°C display bands characteristic for HDMBG (3324 ($\nu_{as(NH_2)}$), 3177 (ν_{NH}, $\nu_{s(NH_2)}$), 1543 ($\delta_{as(NH_2)}$) and 1403 ($\delta_{as(NH_2)}$ cm^{-1})). However new bands at 1606 ($\nu_{C=N}$), 1338 ($\nu_{C=C}$) and 791 cm^{-1} (ν_{C-C}) can be assigned to the paracyanide species [10, 11]. This indicates that the ligand turns in paracyanide, which is then eliminated from the system up to 500°C. The copper(II) chloride formed after this step decomposes in other two endothermic processes generating metallic copper as final product (found/calcd. overall mass loss: 75.83/75.89).

According to the TG profile the decomposition of complex (**2**) occurs in four steps with metallic copper as final product (found/calcd. overall mass loss: 85.95/85.18) (Fig. 5).

The observed mass variation during the first step corresponds to the loss of two water molecules. In the IR spectrum of anhydrous complex isolated at 200° the shoulder at 3310 cm^{-1} assigned to ν_{OH} disappears (Fig. 6b). At 206°C a phase transition can be observed for anhydrous complex. After melting at 225°C, the decomposition starts immediately. In the 225–355°C range one endothermic process is observed accompanied by a mass loss of 36.14% assigned to a part of ligand releasing. The next step is an overlap of two processes, one endo- and one exothermic, and the mass variation corresponds to the remaining ligand loss. The ligand degradation occurs also in this step with paracyanide generation as the IR spectrum

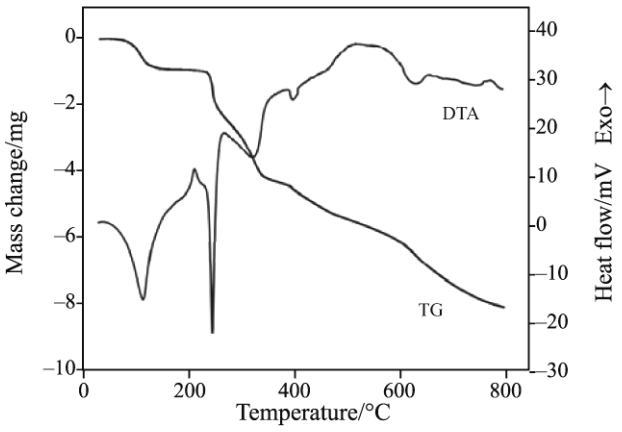

Fig. 5 TG and DTA curves of $[Cu(HDMBG)_2Cl_2] \cdot 2H_2O$

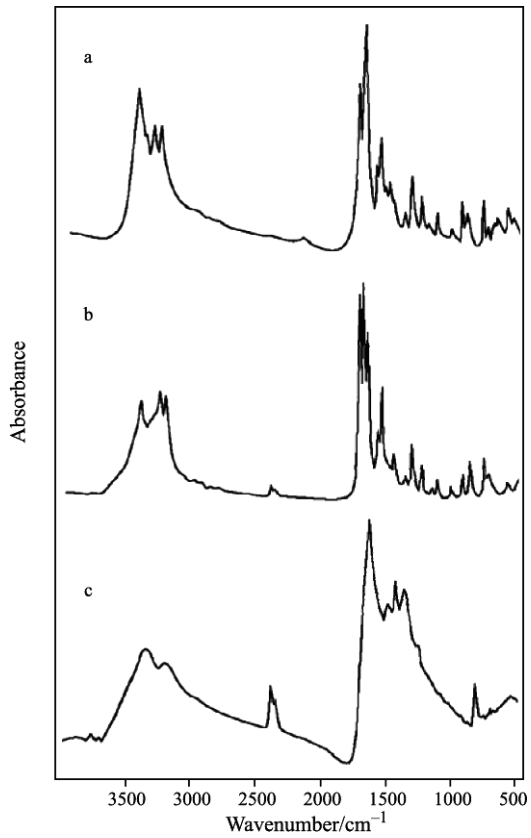

Fig. 6 IR spectra of the a – $[Cu(HDMBG)_2Cl_2]\cdot 2H_2O$ and intermediates formed at b – 200°C and c – 400°C

indicates (Fig. 6c). The last step is assigned also to copper (II) chloride decomposition.

Conclusions

Two N,N-dimethylbiguanide complexes of copper(II) were characterised in order to obtain new effective antibacterial agents.

The electronic and EPR spectra are consistent with a square-pyramidal stereochemistry for complex (**1**) and a square-planar one for complex (**2**).

The in vitro antimicrobial activity assays showed that the complexes exhibited variable antimicrobial activity against Gram-negative strains representing potential candidates in order to design new therapeutic agents for the therapy of bacterial infections with Gram-negative, multiresistant strains.

The compounds melt before thermal transformation that occurs in three and four steps respectively and comprise water elimination, N,N-dimethylbiguanide transformation as well as copper(II) chloride decomposition. The final residue is metallic copper in both cases.

Acknowledgements

This work was supported by the VIASAN grant no. 142/2006 of the Romanian Ministry of Education and Research.

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DOI: 10.1007/s10973-007-8767-3