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Thermal, spectral and antimicrobial study on some Cu(II) complexes with ligands bearing biguanide moieties

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Abstract New complexes of type $[Cu(HTBG)_2]Cl_2$ (**1**), $[Cu(TBG)_2]\cdot 3H_2O$ (**2**) and $[CuL]\cdot nH_2O$ (**3**) $L{:}L^1$, $n = 2$ and (**4**) $L{:}L^2$, $n = 1$ (HTBG: 2-tolylbiguanide, L^1 and L^2: ligands resulted from 2-tolylbiguanide, ammonia/hydrazine and formaldehyde one pot condensation) were synthesised and characterised. The features of complexes have been assigned from microanalytical, IR and UV–Vis data. Redox behaviour was established by cyclic voltammetry. The in vitro qualitative and quantitative antimicrobial activity assays showed that the complexes exhibited variable antimicrobial activity against Gram-negative and Gram-positive strains isolated from the hospital environment. The thermal analyses have evidenced the thermal intervals of stability and also the thermodynamic effects that accompany them. After water elimination, complexes have a similar thermal behaviour. Processes as water elimination, melting, chloride anion removal as well as oxidative degradation of the organic ligands were observed. The final product of decomposition was copper (II) oxide.

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Keywords Antimicrobial activity · Copper (II) complex · One pot condensation · Thermal behaviour · *o*-Tolylbiguanide

Introduction

The increasing incidence of bacterial drug resistance imposes an improvement of the known antimicrobial drugs and also the development of new ones. In the last years the attention in this field was oriented to inorganic species among the organic ones. Although many complexes showed a good antimicrobial activity so far only a few are used as metalloantibiotics (antiseptics and antimicrobial) or disinfectants [1]. So far a good antimicrobial activity was observed for complexes bearing a biocation [2–9] and a multidentate ligand and/or having a proved antimicrobial activity [3, 9].

Among biocations, copper is preferred having in view: (i) the low human toxicity associated with the both presence of the albumin in the plasma and the metalotionein in the cytosol [10]; (ii) the borderline character and the fact that forms the most stable complexes among the Irving Williams series of cations; (iii) the stereochemical versatility; (iv) the easiness to change its oxidation state and (v) the known biological activity including the ability to inhibit enzyme, one of the mechanisms responsible by the antimicrobial activity [11].

As for ligands those having the ability to coordinate as chelate and/or to generate neutral species are preferred in order to enhance the complex lipophilicity and thus allow the biological membranes across [12]. The presence of some supplementary groups able to interact with biomolecules through hydrogen bond formation or week physical interaction is also important for such ligands. If these

ligands posses itself an antimicrobial activity then this could generate the synergism [13]. Some of these complexes that displays an antimicrobial activity were also thermal characterised [2, 5, 8].

Hawing in view the antimicrobial activity of both biguanide derivatives [14] and complexes [15, 16], we report here the synthesis and characterization of some Cu(II) complexes with 2-tolylbiguanide as well as the ligands obtained by its one pot condensation with ammonia/hydrazine and formaldehyde, in order to evaluate the biological properties and thermal stability of these derivatives. The complexes have been characterized by different analytical and spectral methods. The antimicrobial activity of these derivatives was also assayed against planktonic microbial strains. The thermal behaviour of these derivatives was investigated in synthetic air flow by thermal analysis (TG, DTA).

Experimental

Materials and methods

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 a Perkin Elmer PE 2400 analyzer. Chloride was determined gravimetrically while copper was determined volumetrically using thiosulfate method.

IR spectra were recorded in KBr pellets with a Bruker Tensor 37 spectrometer in the range 400–4,000 cm^{-1}.

Electronic spectra by diffuse reflectance technique, with MgO as standard, were recorded in the range 300–1,500 nm, on a Jasco V670 spectrophotometer.

Cyclic voltammograms were recorded by an electrochemical system (potentiostat/galvanostat) Autolab PGSTAT 12. Electrochemical studies were performed at room temperature under inert atmosphere (Ar 99.9999%) in DMSO containing tetrabutylammonium perchlorate (Bu_4NClO_4) 0.1 M as supporting electrolyte. The reference electrode was Ag/AgCl (LiCl saturated in ethanol). The counter electrode was the platinum wire. The working electrode was a glassy carbon (GC) with the effective aria of electrode 7.065 mm^2.

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 [17]. The compounds were solubilised in DMSO to a final concentration of 1 mg/mL. The in vitro biological screening effects were tested against a microbial inoculum of $\sim 1.5 \times 10^8$ UFC/cm^3, corresponding to 0.5 McFarland density, represented by *Bacillus subtilis*, *Bacillus cereus*, *Salmonella* sp. 361, *Staphylococcus aureus* M., *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* 1443, recently isolated from clinical samples. The evaluation of the influence of different complex concentrations on the ability of the tested bacterial strains to colonize the inert substratum, a very simple microtitre plate method was used. In this purpose, the microplates used for the M.I.C. assay were emptied, washed three times by PBS (phosphate buffered saline). The biofilm formed on the plastic wells wall was fixed for 5 min with cold methanol, coloured for 15 min by violet crystal solution and resuspended by 33% acetic acid solution. Cell density was measured by reading the optical density of the coloured solution at 490 nm using an ELISA reader (Apollo LB 911).

The heating curves (TG and DTA) were recorded using a Labsys 1200 SETARAM instrument, with a sample mass of 5–24 mg over the temperature range of 20–900 °C, using a heating rate of 10 K/min. The measurements were carried out in synthetic air atmosphere (flow rate 16.66 cm^3/min), using alumina crucibles.

The melting was evidenced with Automated Melting Point System (AMPS) MPA 100 OptiMelt Stanford Research System.

The X-ray powder diffraction patterns were collected on a DRON-3 diffractometer with a nickel filtered Cu K_α radiation ($\lambda = 1.5418$ Å) in a 2θ range of 5–70°, a step width of 0.05° and an acquisition time of 2 s on each step.

Synthesis of the complexes

$[Cu(HTBG)_2]Cl_2$ (**1**): To a solution containing 5 mmol (0.853 g) copper(II) chloride dihydrate in 50 mL acetonitrile was added drop wise at 50 °C, under continuous stirring, a solution of 10 mmol (2.365 g) $HTBG{\cdot}HCl{\cdot}0.5H_2O$. The reaction mixture was magnetically stirred at 50 °C temperature for 24 h, until a sparingly soluble species, pink coloured was formed. The precipitate was filtered off, washed with ethanol and air-dried.

$[Cu(TBG)_2]{\cdot}3H_2O$ (**2**): To a solution of copper chloride dihydrate (0.853 g, 5 mmol) and KOH (0.561 g, 10 mmol) in water:ethanol, 3:1 (200 mL) was added a solution of 10 mmol (2.365 g) $HTBG{\cdot}HCl{\cdot}0.5H_2O$ until the solution turns magenta. The reaction mixture was then magnetically stirred at 50 °C for 30 min, until a solution magenta coloured was formed. After slow evaporation for one month, dark violet sparingly soluble specie was formed. The precipitate was filtered off, washed with ethanol and air-dried.

$[Cu(L^1)]{\cdot}2H_2O$ (**3**): To a solution of chloride dihydrate (0.853 g, 5 mmol), $HTBG{\cdot}HCl{\cdot}0.5H_2O$ (2.365 g, 10 mmol) in 50 mL methanol was added drop wise 2 mL formaldehyde (37%) and 5 mL ammonia. The reaction mixture was refluxed 80 h until a brown sparingly soluble compound was

formed. The microcrystalline product was filtered off, washed with MeOH and air-dried.

$[Cu(L^2)]\cdot H_2O$ (**4**): To a solution of chloride dihydrate (0.853 g, 5 mmol), HTBG·HCl·0.5H_2O (2.365 g, 10 mmol) in 50 mL methanol was added drop wise 2 mL formaldehyde (37%) and 5 mL hydrazine. The reaction mixture was refluxed 20 h until a dark brown sparingly soluble compound was formed. The microcrystalline product was filtered off, washed with MeOH and air-dried.

Results and discussions

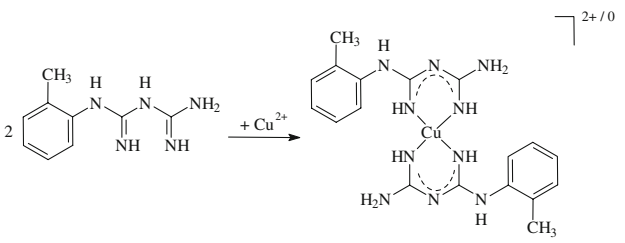
Synthesis and physico-chemical characterisation of complexes

The complexes $[Cu(HTBG)_2]Cl_2$ (**1**) and $[Cu(TBG)_2]\cdot 3H_2O$ (**2**) were synthesized by the reaction of copper(II) chloride with 2-tolylbiguanide hydrochloride semihydrate (HTBG·HCl·0.5H_2O) in neutral and basic condition respectively (Scheme 1).

Condensation of $[Cu(TBG)_2]\cdot 3H_2O$ with ammonia or hydrazine and formaldehyde resulted in neutral new complexes $[CuL]\cdot nH_2O$ (**3**) L:L_1, $n = 2$ and (**4**) L:L_2, $n = 1$ (L^1 and L^2: ligands resulted from ammonia or hydrazine system) formation (Scheme 2).

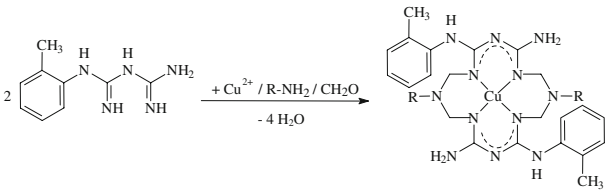
The elemental analyses show 1:2 and 1:1 stoichiometry respectively for compounds (Table 1).

The IR spectra of complexes reveal the characteristic bands of biguanide and 2-substituted benzene moieties respectively. The intense band characteristic to ν(C=N) vibration mode in the 1,650–1,680 cm^{-1} range, appears shifted to higher wavenumbers in the complexes spectra as result of coordination. The new band at about 1,330 cm^{-1} can be assigned to chelate ring formation by the biguanide derivatives [18]. For complex (**4**) in the characteristic range of $\delta(NH_2)$ vibrations more bands appear in accord with the supplementary amine groups inserted in the macrocycle structure by the hydrazine. The water presence in complexes (**2**)–(**4**) generates a broad band around 3,650 cm^{-1} [19].



(**1**) $[Cu(HTBG)_2]Cl_2$
(**2**) $[Cu(TBG)_2]3H_2O$

Scheme 1 Synthesis of complexes (**1**) and (**2**)



(**3**) $[Cu(L^1)]2H_2O$ (R: H)
(**4**) $[Cu(L^2)]H_2O$ ($R: NH_2$)

Scheme 2 Synthesis of complexes (**3**) and (**4**)

Table 1 Elemental analysis for complexes

Compound	%Cu		%C		%H		%N	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
(**1**)	12.23	12.29	41.93	41.82	5.06	5.07	27.17	27.10
(**2**)	12.68	12.76	43.38	43.41	6.05	6.07	28.17	28.12
(**3**)	11.19	11.26	46.87	46.84	6.31	6.43	29.85	29.79
(**4**)	9.98	10.03	45.78	45.86	6.28	6.30	34.12	34.04

Electronic spectra of the complexes (**1**) and (**2**) show a single narrow band at high energy, as is usually observed for Cu(II) complexes with a square planar stereochemistry [20]. For complex (**3**) and (**4**), the broad bands that appear have a splitting tendency in two or three components. The absorption maxima is shifted to higher values for Cu(II) series of complexes as an indicative of the crystalline field increasing in the ligand series: HTBG → TBG → L^1 → L^2.

The electrochemical investigation of the complexes was studied using cyclic voltammetry at different scan rates (10–400 mV s^{-1}) in the potential range from +1.0 to −1.0 V (Fig. 1). Cyclic voltammograms for all the complexes show one or two quasireversible redox processes (Table 2) due to copper ion only, more or less pronounced, in concordance with both ligand nature and scan rate [21]. It was observed that 2-tolylbiguanide (HTBG) is redox silent. In comparison with the reference complex $[Cu(DMSO)_4]Cl_2$ that in the working conditions is reversible

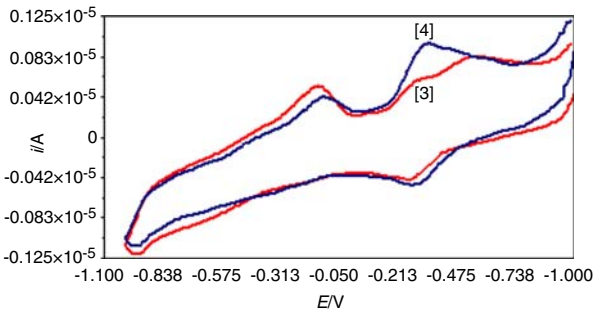


Fig. 1 Cyclic voltammograms for (**3**) and (**4**) recorded in DMSO at GC electrode (scan rate 50 mV s^{-1}, complex concentration 2×10^{-4} M, supporting electrolyte $[Bu_4N]ClO_4$ 0.1 M)

Table 2 Peak potentials for complexes in DMSO solution containing 0.1 M $[Bu_4N]ClO_4$ (scan rate 50 mV s^{-1})

Complex	(**1**)	(**2**)	(**3**)	(**4**)
E_{pc1} (V)	+0.325	–	+0.285	+0.295
E_{pc2} (V)	−0.108	−0.339	–	–
E_{pc3} (V)	−0.541	–	−0.636	–
E_{pa1} (V)	−0.279	−0.219	−0.155	−0.128
E_{pa2} (V)	+0.557	+0.739	+0.520	+0.335

reducible on the glassy carbon electrode (GC) (E_{pc1} = +0.275 V), the complexes displays more positive values for the first reduction potential, excepting complex (**2**) that follows a single reduction step. This behaviour can be correlated with the supplementary stability induced by the chelate rings formation.

Biological activity

The antimicrobial activity of the tested compounds was performed against microbial strains, the majority of them being isolated from different clinical samples and exhibiting different resistance patterns.

All tested compound exhibit a good antimicrobial activity (with MIC values ranging from 1.953 to 1,000 μg/ml). The 2-tolylbiguanide hydrochloride semihydrate presents the widest activity spectrum against most of the tested strains, being active both on Gram positive and Gram negative ones, being particularly efficient against *Staphylococcus aureus* strains. Complex (**3**) exhibits a 31.25 μg/ml MIC against *Bacillus cereus* strains, demonstrating a very good antimicrobial activity against these sporulating bacteria.

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

Concerning the influence of different concentrations of the tested compounds, either MIC or subinhibitory concentrations, on the bacterial adherence to the inert substratum, the results were different, depending on the tested strains and complex. The compound (**1**) inhibits the adherence of *Pseudomonas aeruginosa* while HTBG and complexes (**1**) and (**3**) inhibited that of *S. aureus* strains to the inert substratum, even at very low concentrations. The *S. aureus* reference strain was very susceptible to all tested compounds.

Thermal behaviour of compounds

The results concerning the thermal decomposition/degradation of the 2-tolylbiguanide hydrochloride semihydrate and complexes are presented in Table 3.

Table 3 Thermal behaviour data (in synthetic air flow) for *o*-tolylbiguanide hydrochloride and complexes

Compound	Step	Thermal effect	Temperature range/°C	Δm_{exp}/%	Δm_{calc}/%
$HTBG{\cdot}HCl{\cdot}0.5H_2O$	1.	Endothermic	80–110	3.7	3.8
	2.	Endothermic	126[a]	0	0
	3.	Exothermic	168–240	15.3	15.4
	4.	Exothermic	240–410	39.0	38.9
	5.	Exothermic	410–790	41.9	41.9
$[Cu(HTBG)_2]Cl_2$	1.	Endothermic	220[a]	0	0
	2.	Exothermic	220–254	7.0	7.1
	3.	Exothermic	254–390	36.0	35.6
	4.	Exothermic	390–810	41.5	41.9
	Residue CuO			15.5	15.4
$[Cu(TBG)_2]{\cdot}3H_2O$	1.	Endothermic	60–165	10.7	10.8
	2.	Exothermic	190–410	36.8	37.0
	3.	Exothermic	410–900	36.6	36.2
	Residue CuO			15.9	16.0
$[Cu(L^1)]{\cdot}2H_2O$	1.	Endothermic	50–125	6.3	6.4
	2.	Exothermic	125–373	32.2	32.7
	3.	Exothermic	373–640	46.7	46.8
	Residue CuO			14.8	14.1
$[Cu(L^2)]{\cdot}H_2O$	1.	Endothermic	64–110	2.9	3.1
	2.	Exothermic	110–435	31.6	32.0
	3.	Exothermic	435–775	51.4	51.1
	Residue CuO			14.1	13.8

[a] Melting point

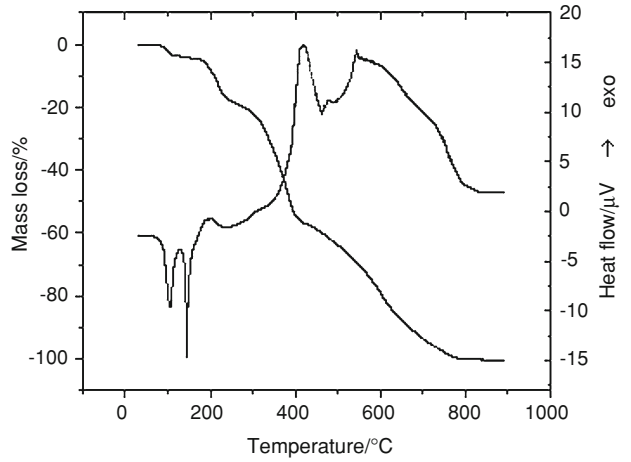


Fig. 2 TG and DTA curves of HTBG·HCl·0.5H_2O

The 2-tolylbiguanide hydrochloride decomposition starts with water elimination (Fig. 2) follows by the hydrochloride specie melting at 126 °C, process evidenced with the AMPS also.

Thermal decomposition follows then in three exothermic steps. After melting, the degradation starts with HCl elimination. The following steps are an overlapping of at least three processes as DTA indicates and corresponds with the gradually oxidative degradation of the 2-tolylbiguanide.

Thermal decomposition of $[Cu(HTBG)_2]Cl_2$

The TG and DTA curves corresponding to the complex (**1**) indicate that after melting, decomposition follows also three steps (Fig. 3).

The compound is anhydrous and it is stable up to 220 °C, where the compound melts, the melting being confirmed with the AMPS instrument. The higher molar weight corresponding to complex leads to a higher melting point in comparison with 2-tolylbiguanide hydrochloride semihydrate.

The first step of compound thermal transformation consists in an exothermic elimination of hydrochloric acid (Table 3), moreover the chemical analysis of intermediate isolated at 270 °C confirm the chloride absence. The second step, exothermic also, is not a single one being an overlapping of at least three processes as DTA curve indicates. This step corresponds to the partial oxidative degradation of 2-tolylbiguanide according to the mass lost. Next step, exothermic also, consists in at least five processes (according to DTA curves profile) and leads at CuO (found/calcd. overall mass loss: 84.5/84.6%). The residue nature was confirmed with powder X-ray diffraction (ASTM 5-661).

Thermal decomposition of $[Cu(TBG)_2]\cdot 3H_2O$

The decomposition of complex (**2**) comprises also three steps and starts with water elimination, process that occurs at low temperatures (Fig. 4). This indicates the crystallisation nature of the water molecules, behaviour observed for other complexes having this type of water in composition [22–25].

The anhydrous specie is then stable over a 25 °C temperature range. The thermal degradation of this intermediate starts at 170 °C and comprises two steps. The second step occurs in two exothermic processes as both TG and DTA profile indicate while at least four processes (according to both TG and DTA) can be noticed in the last step. Finally, the oxidative degradation leads to CuO in at least five processes (overall mass loss found/calcd: 84.1/84.4%). The IR spectrum of the residue displays a band at 472 cm^{-1} assigned to ν(Cu–O) vibration mode [19].

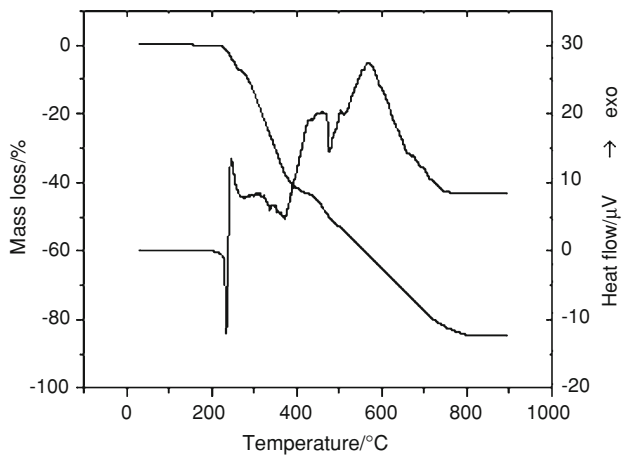


Fig. 3 TG and DTA curves of $[Cu(HTBG_2)]Cl_2$

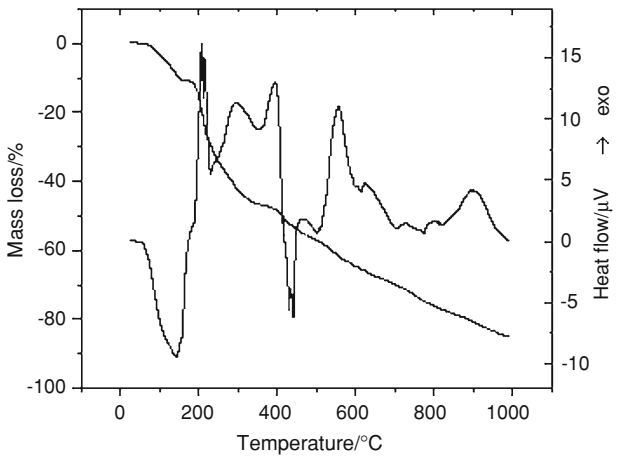


Fig. 4 TG and DTA curves of $[Cu(TBG)_2]\cdot 3H_2O$

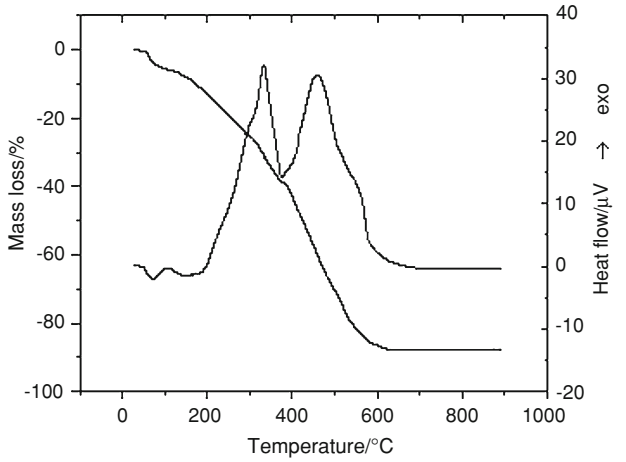


Fig. 5 TG and DTA curves of $[Cu(L^1)]\cdot 2H_2O$

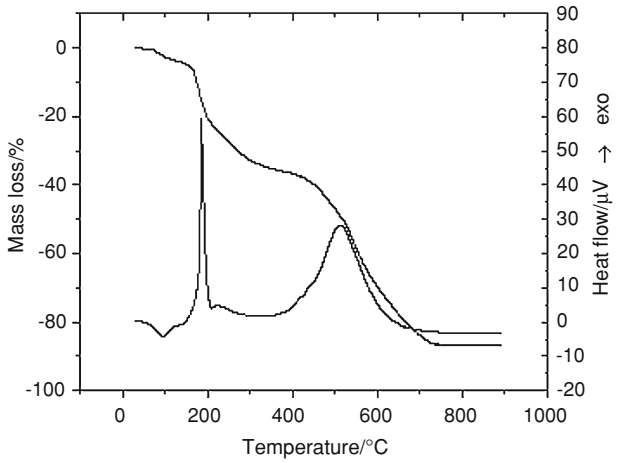


Fig. 6 TG and DTA curves of $[Cu(L^2)]\cdot H_2O$

Thermal decomposition of $[Cu(L^1)]\cdot 2H_2O$

According to the TG profile the decomposition of $[Cu(L^1)]\cdot 2H_2O$ (**3**) occurs in three, well-defined steps (found/calcd. overall mass loss: 85.2/85.9%) (Fig. 5).

After water loss also at low temperatures (50–125 °C range), the partial oxidative degradation starts with tolyle moieties as IR spectrum indicate. In the last step, the paracyanide oxidative degradation leads to CuO.

Thermal decomposition of $[Cu(L^2)]\cdot H_2O$

The water elimination can be observed for complex (**4**) also up to 110 °C (Fig. 6). According with the mass loss and IR spectrum, the oxidative degradation starts also with o-tolyl moieties removal in two interfering processes (as both TG and DTA curves indicate). Next step comprises also at least two processes (according to DTA) and consists in the remaining fragment oxidative elimination. The final product is CuO (found/calcd. overall mass loss: 85.9/86.2%).

Conclusions

Complexes of Cu(II) with tolylbiguanide moieties were characterised in order to obtain new effective antibacterial agents.

Electronic spectra of complexes are characteristic for a square planar stereochemistry, while the modifications in the IR spectra of complexes indicate a chelate coordination of the biguanide moieties.

The cyclic voltammograms indicates for all complexes that the quasireversibility of the Cu(II)/Cu(I) and Cu(I)/Cu(0) reduction steps is influenced by the nature of ligand.

The in vitro antimicrobial activity assays showed that the complexes exhibit a good antimicrobial activity against Gram-negative and Gram-positive strains. Moreover, some compounds inhibit the ability of *Pseudomonas aeruginosa* and *S. aureus* to colonize the inert substratum.

Thermal decomposition of complexes allowed us to establish the number and nature of water molecule, the composition and also the intervals of thermal stability. The thermal degradation occurs in four steps for the organic derivative and the 2-tolyl hydrochloride melting was observed before decomposition. After water elimination up to 160 °C, the complexes decompose in two steps leading to copper(II) oxide as final product. The results are in good concordance with the complexes composition.

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