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THERMAL BEHAVIOR, SPECTROSCOPIC AND BIOLOGICAL CHARACTERIZATION OF Co(II), Zn(II), Pd(II) AND Pt(II) COMPLEXES WITH N,N-DIMETHYLBIGUANIDE

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The complexes of the type $[M(HDMBG)Cl_3]$ ((**1**) M:Co; (**2**) M:Zn;) and $[M(DMBG)Cl_2]$ ((**3**) M:Pd; (**4**) M:Pt; DMBG: N,N-dimethylbiguanide) present in vitro antimicrobial activity. The modification evidenced in IR and ^{1}H NMR spectra (in the case of complex (**2**)) was correlated with the presence of N,N-dimethylbiguanide ion as unidentate, coordinated through N^3 and of N,N-dimethylbiguanide as chelate, coordinated through N^1 and N^4 respectively. The electronic reflectance spectrum showed the d–d transition for complex (**1**) characteristic for the tetrahedral surrounding while the spectra for complexes (**3**) and (**4**) have the characteristic pattern for square-planar stereochemistry. The cyclic voltammetric data show the characteristic waves for mononuclear complexes of the metallic ions presented below. The thermal analysis has evidenced the thermal intervals of stability and also the thermodynamics effects that accompany them. The different nature of the ligands generates a different thermal behaviour for complexes.

Keywords: *complexes, Gram-negative strain, Gram-positive strain, N,N-dimethylbiguanide, thermal behaviour*

Introduction

There is a much interest in biguanides ligands and their transition metal complexes with particular attention focused on structural studies [1–7] and to evidence the ability of these species to generate hydrogen-bonded supramolecular assemblies [8, 9]. It is also well-known that the biguanides have biological properties. Among this derivatives N,N-dimethylbiguanide is known as a glucose lowering agent [10], analgesic, antimalarial [11] and antimetabolite for organisms that inhibit the metabolism of folic acid [12]. Recently it was shown that vanadyl–biguanide complexes are potential synergistic insulin mimics [13] while the complexes of technetium isotope (^{99}Tc) with biguanide derivatives could be used as renal imaging agents [14]. Regarding the thermal behaviour of these derivatives there is only one study concerning the iron(III) complexes [15].

In order to modulate the biological activity of N,N-dimethylbiguanide (DMBG) and to correlate this activity with thermal stability, a series of complexes with general formula $[M(HDMBG)Cl_3]$ ((**1**) M:Co; (**2**) M:Zn) and respectively $[M(DMBG)Cl_2]$ ((**3**) M:Pd; (**4**) M:Pt) have been characterised as mononuclear species by elemental analysis, IR, ^{1}H NMR and electronic spectra. The redox behaviour of complexes has been investigated by cyclic voltammetry.

The in vitro antimicrobial activity of the investigated compounds was tested against Gram-positive and Gram-negative strains (*Bacillus subtilis*, *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*).

The most biological active compounds will be incorporate in polymeric matrix. Having in view that the polymerisation processes usually occur at higher temperature it was also investigated the thermal behaviour of these derivatives in order to evidence the thermal intervals of stability and also the thermodynamics effects that accompany them.

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. Cobalt, zinc, palladium, platinum and chloride were determined gravimetrically 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 380–1100 nm, on a VSU 2P-Zeiss

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Jena spectrometer. The ^{1}H NMR spectra (300 MHz) were recorded on a Bruker AM 300 spectrometer with tetramethylsilane as internal reference. The voltammetric measurements were accomplished with a polarographic and voltammetric ensemble Trace Master 5 and POL 150 Polarographic Analyzer (Radiometer Copenhagen). Cyclic voltammetry was carried out in DMF containing 0.1 M $NaClO_4$. The working electrode was HMDE (hanging mercury drop electrode), the auxiliary electrode was a coiled platinum wire, and the reference electrode was Ag/AgCl. The dissolved oxygen from the analyzed solution was eliminated by bubble a pure argon stream.

The in vitro biological screening effects of the complexes and dimethylbiguanide hydrochloride were tested against a bacterial inoculum ($1.5\cdot10^8$ UFC/cm^3) represented by Gram-positive and Gram-negative, reference and environmental (river water) strains (*Bacillus subtilis*, *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*). The antibiogram technique, namely the liquid medium dilution method was used for determining minimum inhibitory concentration (M.I.C., μg cm^{-3}). Stock solutions were prepared by dissolving the compounds in DMF. The standard inoculum of bacterial strain was inseminated in a discontinuous gradient of concentration, represented by the tested compounds, in tubs containing nutritive bullion Mueller Hinton. This mixture was incubated at 37°C for 24 h.

The heating curves (TG, *T*, DTA and DTG) were recorded in a static air atmosphere using a Shimadzu DTG-TA-51H thermogravimetric analyzer with a sample mass between 6–13 mg over the temperature range of 20–1000°C, using a heating rate of 10 K min^{-1}.

Synthesis of the complexes

The syntheses and structural data for complexes [M(HDMBG)Cl_3] ((**1**) M:Co; (**2**) M:Zn)) were reported elsewhere [2, 3]. The composition of complexes has been confirmed by chemical analyses.

[Co(DMBG)Cl_3] (**1**): IR (KBr pellet), cm^{-1}: $\nu_s(NH_2)$, 3414vs; $\nu_s(NH_2)$, 3322vs; ν(NH), 3222m; ν(C=N), 1639vs; $\delta_{as}(NH_2)$, 1520vs; $\delta_s(NH_2)$, 1459m; ν(C–N), 1277w, 1235w; $\rho(CH_3)$, 720w.

[Zn(DMBG)Cl_3] (**2**): IR (KBr pellet), cm^{-1}: $\nu_{as}(NH_2)$, 3412vs; $\nu_s(NH_2)$, 3320vs; ν(NH), 3225m; ν(C=N), 1638vs; $\delta_{as}(NH_2)$, 1526vs; $\delta_s(NH_2)$, 1460m; ν(C–N), 1275w, 1239w; $\rho(CH_3)$, 721w; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.923 (s, 6H, CH_3), 6.554 (s, 4H, NH_2), 7.192 (s, 2H, NH).

The complexes, [M(DMBG)Cl_2] ((**3**) M:Pd; (**4**) M:Pt)), were obtained following the general procedure: to a suspension of 2 mmole of MCl_2 in 50 cm^3 ethanol was added dropwise at 50ºC, under continuous stirring, a solution of 2 mmol dimethylbiguanide hydrochloride in 20 cm^3 ethanol. The brown, sparingly soluble compound formed after one hour was filtered out and washed with ethanol and air-dried.

[Pd(DMBG)Cl_2] (**3**): analysis found: Pd, 34.68; C, 15.61; Cl, 23.16; H, 3.68; N, 22.86%; calculated for $PdC_4Cl_2H_{11}N_5$: Pd, 34.72; C, 15.68; Cl, 23.14; H, 3.62; N, 22.85%; yield (reported to palladium chloride) 95%; IR (KBr pellet), cm^{-1}: $\nu_{as}(NH_2)$, 3400vs; ν(NH), 3361m; $\nu_s(NH_2)$, 3297m; ν(C=N), 1654vs, 1622vs; $\delta_{as}(NH_2)$, 1542vs; $\delta_s(NH_2)$, 1466m; ν(C–N), 1250w; $\rho(CH_3)$, 723w.

[Pt(DMBG)Cl_2] (**4**): analysis found: Pt, 49.30; C, 12.14; Cl, 17.98; H, 2.71; N, 17.60%; calculated for $PtC_4Cl_2H_{11}N_5$: Pt, 49.37; C, 12.16; Cl, 17.94; H, 2.80; N, 17.72%; yield (reported to platinum chloride) 91%; IR (KBr pellet), cm^{-1}: $\nu_{as}(NH_2)$, 3378vs; ν(NH), 3309m; $\nu_s(NH_2)$, 3200m; ν(C=N), 1652vs, 1623vs; $\delta_{as}(NH_2)$, 1542vs; $\delta_s(NH_2)$, 1467m; ν(C–N), 1253w; $\rho(CH_3)$, 722w.

DMBG·HCl: IR (KBr pellet), cm^{-1}: $\nu_{as}(NH_2)$, 3375vs; ν(NH), 3297m; $\nu_s(NH_2)$, 3175vs; ν(C=N), 1629vs; $\delta_{as}(NH_2)$, 1578vs; $\delta_s(NH_2)$, 1478m; ν(C–N), 1280w, 1240w; $\rho(CH_3)$, 729w; ^{1}H NMR (DMSO–d_6, 300 MHz): δ 2.923 (s, 6H, CH_3), 6.803 (s, 4H, NH_2), 7.219 (s, 2H, NH).

Results and discussion

Physico-chemical characterisation of complexes

In this paper, we report the preparation, physico-chemical and biological characterisation of some complexes with N,N-dimethylbiguanide of type [M(HDMBG)Cl_3] ((**1**) M:Co; (**2**) M:Zn) and respectively [M(DMBG)Cl_2] ((**3**) M:Pd; (**4**) M:Pt). The major goal of this paper was to evidence the thermal behaviour of these complexes that also present in vitro an antibacterial activity.

It is to be mentioned that several study have evidenced that the coordination mode of N,N-dimethylbiguanide strongly depends by the preference of the metallic ion for tetrahedral or square planar stereochemistry, that influences the bond strength and, consequently, the thermal behaviour. The cation that forms easily tetrahedral species leads to species that contain the unidentate N,N-dimethylbiguanidium ion [2, 3] while those that prefer the square planar stereochemistry generated species with chelate N,N-dimethylbiguanide [1, 5].

Moreover, the coordination mode of the N,N-dimethylbiguanide in complexes it is possible now to achieve without no doubt, on the basis of the structural X-ray determination. We have been corre-

lated the spectral data with the known structural data for the complexes (**1**) and (**2**) and for similar species.

The major IR spectral features of complexes presented at experimental part indicate that in the spectra of complexes appear the characteristic bands of biguanide moiety [16].

In the range characteristic for the NH vibrations, the spectrum of the N,N-dimethylbiguanide hydrochloride display three components, which could be associated with the presence of primary amine and imine groups. The bands corresponding to the vibration modes of the NH_2 group are significantly shifted towards higher wavenumbers in the spectra of the complexes (**1**) and (**2**), moreover those assigned to NH vibration mode is shifted towards lower wavenumbers with 80 cm^{-1}. This information suggests that the coordination through N^3 decreases the charge density on this atom and also generates the destruction of the hydrogen bonds network, evidenced in the lattice of the dimethylbiguanide hydrochloride by the X-ray diffraction [17]. For these complexes in the range characteristic for the ν(C=N), $\delta_{as}(NH_2)$, and respectively $\delta_s(NH_2)$, vibrations [18], the spectra of the complexes display three very strong bands. The presence of unidentate N,N-dimethylbiguanidium leads to the shift of the band associated with ν(C=N) towards higher wavenumbers while the bands assigned to $\delta(NH_2)$ are both shifted at lower wavenumbers.

For complexes (**3**) and (**4**) the different spectral features indicate a chelate coordination mode for the ligand. In the spectra of these compounds, the bands corresponding to NH stretching vibrations preserve the aspect and intensity ratio as in the spectrum of dimethylbiguanide hydrochloride but all are shifted significantly toward higher wavenumbers. Moreover in range characteristic for ν(C=N) vibration there appear two bands, one being shifted to higher wavenumbers. At 1384 cm^{-1} appears for both complexes a new band. This band was observed in the spectra of some complexes with biguanide derivatives [15, 16] and can be associated either with the formation of the chelate ring or with the activation of an IR band as a result of the ligand symmetry decrease upon coordination.

The ^1H NMR spectrum of complex (**2**) shows modification in the amine region, namely both signals characteristic to DMBG·HCl are shifted to lower values.

The electronic spectrum of (**1**) is characterized by the presence of a broad band of medium intensity at 14705 cm^{-1} and a second one situated in the near IR range, which exceeds the spectrometer recording limits. These bands can be associated with the spin allowed d–d transitions $^4A_2 \rightarrow {}^4T_1(P)$ and $^4A_2 \rightarrow {}^4T_1(F)$ which are characteristic for Co(II) in tetrahedral surrounding. The spectra of compounds (**3**) and (**4**) show the characteristic pattern of the square-planar stereochemistry of d^8 ions. The intense bands about 26315 cm^{-1} are assigned to $^1A_1 \rightarrow {}^1E$ transition while the shoulder at 20 000 and the band at 16 950 cm^{-1} are associated with the spin forbidden transition $^1A_1 \rightarrow {}^3E$.

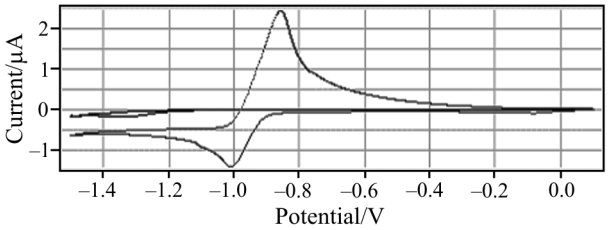

Fig. 1 Cyclic voltammogram of complex (**2**) at a platinum electrode in DMF measured under argon (HMDE, working electrode; concentration of complex, $2 \cdot 10^{-4}$ M; electrolyte, 0.1 M $NaClO_4$; scan rate, 50 mV/s^{-1}).

Cyclic voltammogram of (**1**) shows a catodic peak at –1.260 V *vs.* Ag/AgCl that is assigned to Co(II) reduction process. For complex (**2**) it was observed a well defined reversible peak at $E_0^{'}$= –0.933 V ($E_{1/2red}$= –1.008 V and $E_{1/2ox}$= –0.858 V) assigned to redox couple Zn(II)/Zn (Fig. 1). The voltammogram for complex (**3**) shows a weak-defined peak at –1.220 V characteristic for Pd(II) reduction in 0.1 M ammoniacal buffer. For the complex (**4**) the voltammogram exhibits two weak-defined peaks at 0.172 and 0.372 V that can be assigned to the irreversible oxidations Pt(II)→Pt(III)→Pt(IV).

Biological activity

Antibacterial activity of the dimethylbiguanide hydrochloride and complexes have been carried out against Gram positive and Gram negative, reference and clinical strains (*Bacillus subtilis*, *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*) using liquid medium dilution method. The values of minimum inhibitory concentration (M.I.C., μg cm^{-3}) are presented in Table 1.

It was observed that the DMF does not influence the antimicrobial activity of the tested compounds at the working concentrations. The M.I.C. values indicate a better activity against the Gram-negative strains except that of *Pseudomonas aeruginosa* that usually is very resistant at antibiotics, including carbapenems that are β-lactame derivatives. Beside this, the microorganism expresses an antibiotic resistance mechanism of efflux pump type, known to be involved in the resistance to heavy metals [20]. The most effective antibacterial activity was manifested against *Bacillus subtilis*. In this case, except the compound (**3**), the activity of the complexes is higher than the ligand. Moreover, the complex (**2**) presents constantly the most effective activity against all bacterial strains tested being so a potential antimicrobial agent with a large spectrum of action. It is

Table 1 The minimum inhibitory concentration (M.I.C., μg cm^{-3}) for dimethylbiguanide hydrochloride and complexes (**1**)–(**4**)

Compounds	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
DMBG·HCl	128	256	128	128	>1024
(**1**)	512	256	512	64	128
(**2**)	32	32	128	32	64
(**3**)	512	512	512	512	256
(**4**)	1024	512	512	64	1024

also to be pointed that, complexes (**1**) and (**2**) inhibit at a very low M.I.C. the *Staphylococcus aureus* grow, a bacteria resistant at methicillin. This indicates that the two compounds could be used in the therapy of MRSA (methicillin-resistant *Staphylococcus aureus*) infections.

Thermal behaviour of complexes

The results concerning the thermal decomposition/degradation of the complexes are presented in the following.

Complexes (**1**) and (**2**) have the same thermal behaviour and the general aspect of the DTG, DTA and TG curves as those corresponding to DMBG·HCl. Unlike DMBG·HCl, which melts at 180ºC and starts decomposition itself at 200ºC, the complexes are more stable. The decomposition of the complexes starts at a higher temperature that being a clue of the ligand stabilisation upon coordination. Before the thermal decomposition it was observed the melting of complexes about at the same temperatures (246 and 240ºC) (Fig. 2, Table 2). The melting is immediately followed by the thermal decomposition that starts in the first step with HCl elimination and partial oxidative degradation of DMBG with generation of a stable intermediate until 430ºC. According to the mass loss this compound is a complex of the metallic ions with paracyanide. The nature of this compound has been confirmed by chemical analysis and IR spectra where appears a strong band at 1623 cm^{-1} that could be assigned to ν(C=N) vibration. This step is an overlapping of at least four processes as DTG indicates. The second step corresponds to the transformation of the paracyanide complex into the most stable oxides, respectively Co_3O_4 and ZnO, as XRD indicate. Two processes corresponding to the stepwise depolymerisation of the paracyanide and chlorine elimination form this last step.

The different nature of the ligand and coordination mode generate the different thermal behaviour for complexes type [M(DMBG)Cl_2] (Fig. 3). Excepting this, the two complexes have a similar behaviour.

Table 2 Thermal behaviour of the complexes

Complex	Step	Thermal effect	Temperature interval/°C	Δm_{exp}/%	Δm_{calc}/%
[Co(HDMBG)Cl_3] (**1**)	1	Endothermic	246 (*m. p.*)*	0	0
	2	Exothermic	250–426	39.06	38.41
	3	Exothermic	426–650	33.75	34.41
		Residue (Co_3O_4)		27.19	27.18
[Zn(HDMBG)Cl_3] (**2**)	1	Endothermic	240 (*m. p.*)*		
	2	Exothermic	250–430	38.10	37.72
	3	Exothermic	430–600	35.23	35.56
		Residue (ZnO)		26.67	26.72
[Pd(DMBG)Cl_2] (**3**)	1	Exothermic	225–400	42.75	42.10
	2	Exothermic	400–486	11.42	11.59
	3	Exothermic	486–630	11.10	11.59
		Residue (Pd)		34.73	34.72
[Pt(DMBG)Cl_2] (**4**)	1	Exothermic	190–399	32.17	32.66
	2	Exothermic	399–505	8.81	8.99
	3	Exothermic	505–653	9.06	8.99
		Residue (Pt)		49.96	49.36

* melting point

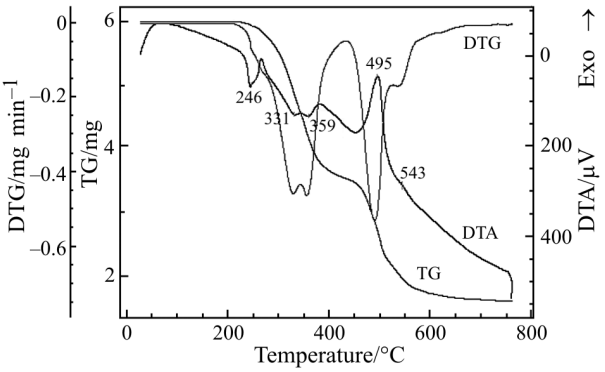

Fig. 2 TG, DTG and DTA curves of [Co(HDMBG)Cl_3]

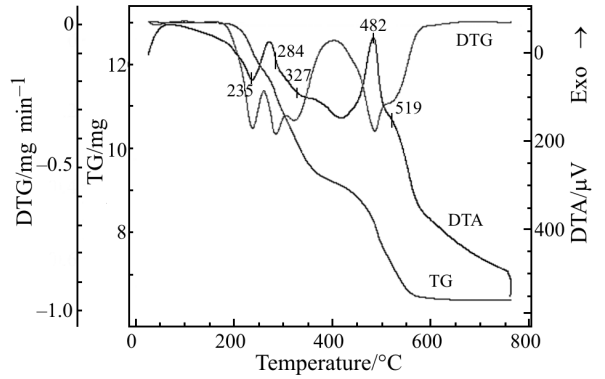

Fig. 3 TG, DTG and DTA curves of [Pt(DMBG)Cl_2]

The two complexes are very stable and the thermal decomposition begins at 225 and 190ºC, respectively. The first step is the result of overlapping of three exothermic processes, as DTG indicates. The next two steps, exothermic also and successive, corresponds to the graduated elimination of chlorine leading to powder metals as final products.

Conclusions

A series of complexes with N,N-dimethylbiguanidium and N,N-dimethybiguanide were characterised in order to obtain new effective antibacterial agents with a large spectrum of biological activity.

The spectroscopic studies revealed two coordination modes unidentate, through N^3 in the case of N,N-dimethylbiguanidium ion and chelate for N,N-dimethylbiguanide, through N^1 and N^4 atoms.

The electronic data indicate the tetrahedral surroundings for Co(II) while the spectra of Pt(II) and Pd(II) complexes are consistent with the square-planar stereochemistry.

The minimum inhibitory concentration (M.I.C.) indicates that some of the complexes are antimicrobial active and present higher activity than free ligand against *Bacillus subtilis*.

The compounds with low molecular masses melt before decomposition, leading in two steps to metallic oxides. This behaviour could arise from lower lattice energy and a higher degree of covalence. The complexes with the higher molecular masses display a thermal behaviour more complex, the steps of the degradation being not well delimited. In these cases the final products being the metals.

Acknowledgements

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