

# Insight on thermal behaviour of new complexes of Ni(II), Cu(II) and Zn(II) with a bismacrocyclic ligand developed as biologically active species

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Abstract A multi-component reaction involving metal ion, amines and formaldehyde has been used for a series of decaaza bismacrocyclic complexes M<sub>2</sub>L(CH<sub>3</sub>COO)<sub>4</sub>·nH<sub>2</sub>O [(1) M: Ni, n = 2.5; (2) M:Cu, n = 1; (3) M:Zn, n = 10; L:1,3-bis(*N*,*N*-1,3,6,9,12-pentaazacyclotridecane)-benzene] preparation. Elemental analyses, ESI-MS, IR, UV-Vis-NIR, NMR and EPR spectra, magnetic susceptibility at room temperature, molar conductivities, as well as thermogravimetric analysis, provided data concerning complexes features. The macrocyclic ligand behaves as bischelate, resulting in either a square planar or an octahedral stereochemistry. The in vitro screening of the antimicrobial activity was performed against both reference and clinical isolates multi-drug-resistant strains. The overall antimicrobial potency of complexes was enhanced in comparison with the free ligand, against both planktonic and biofilm-embedded pathogenic strains. Complexes exhibit no cytotoxicity on the HCT 8 tumour cells.

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Thermogravimetric curves (TG, DTG and DTA) evidenced in air processes as water elimination, acetate into carbonate transformation as well as oxidative degradation of the bismacrocyclic ligand. The powder X-ray diffraction data indicate MO (M: Ni, Cu, Zn) as final product.

Keywords Complexes · Bismacrocycle · One-pot condensation · Antimicrobial · Thermal behaviour

# Introduction

Data concerning azabismacrocyclic ligands have received much attention since the discovery of paraxylyl bicyclam (AMD3100) as HIV cell entry blocker [1]. Also, the species as Mozobil or Plerixafor are prescribed to mobilise hematopoietic stem cells for collection and subsequent transplantation in patients with non-Hodgkin's lymphoma or multiple myeloma and entered recently in clinical trial phase for the AIDS [2, 3] and pancreatic, ovarian and colorectal advanced cancers treatments [4].

New bis(azamacrocycles) as well as their complexes have been designed and synthesised, proving interesting chemical properties (magnetic, redox or electrochemical) [5, 6] or a large spectrum of biological activity [7–9]. Binuclear complexes with bis(azamacrocycles) were also the subject of extensive investigation concerning their catalytic effects. Thus, it has been found that some dimetal(II) complexes act as efficient catalysts in oxidative [10–13] or reductive [14–16] processes.

Many bismacrocyclic complexes were studied in order to obtain a specific metallocyclam configuration in order to target the CXCR4 co-receptor, thereby interfering with the HIV entrance in the host cell [17, 18]. Such species with an enhanced activity were obtained by incorporation of Cu(II), Ni(II), Zn(II) or VO(IV) in the xylyl bicyclam structure [17–22].

Data regarding one-pot synthesis of some azabismacrocyclic complexes as well as their thermal behaviour and antimicrobial potential for eradication of both planktonic and biofilm-embedded microbial strains were previously reported by our team [23–26].

In order to gain further insight into thermal behaviour of bismacrocyclic complexes, we here report data concerning synthesis and characterisation of a new series of Ni(II), Cu(II) and Zn(II) species with 1,3-bis(*N*,*N*-1,3,6,9,12-pen-taazacyclotridecane)-benzene ligand. Complexes have been characterised by suitable analytical, spectral and magnetic methods. The thermal behaviour of these derivatives was investigated by thermal analysis (TG, DTG and DTA) in order to evidence the modification that appears at heating in synthetic air and also the thermodynamic effects that accompany them. Complexes were screened for their antimicrobial and cytotoxic activity.

# Experimental

### Materials

The high-purity reagents were purchased from Sigma-Aldrich [Ni(CH<sub>3</sub>COO)<sub>2</sub>· $6H_2O$ , Cu(CH<sub>3</sub>COO)<sub>2</sub>· $H_2O$ , Zn(CH<sub>3</sub>COO)<sub>2</sub>· $2H_2O$ ], Merck (1,3-phenylenediamine, 3,6-diazaoctane-1,8-diamine, methanol) and Loba (formalde-hyde, triethylamine) and were used as received without further purification.

### Instruments

Chemical analysis of carbon, nitrogen and hydrogen has been performed using a PerkinElmer PE 2400 analyser.

Metal content was determined with AAS on a Avanta GBC spectrometer by using a stock standard solution (Merck, 1 g mL<sup>-1</sup>), while the working solutions were prepared by a suitable dilution of the sample obtained after complexes calcination at 450 °C and successive treatments with HCl and HNO<sub>3</sub> of the residue.

The molar conductance was determined for  $10^{-3}$  M solutions of complexes in DMF with a multi-parameter analyser CONSORT C861.

Mass spectra were recorded by electrospray ionisation tandem mass spectrometry (ESI-MS) technique. The sample (1 mg) was dissolved in acetonitrile/water 1/1 and injected directly in the mass spectrometer LC/MS/MS (Varian triple quadrupole mass spectrometer) electrospray interface. The injection was performed with 0.02 mL min<sup>-1</sup> flow. Air at 250 °C and 18 psi was used for desiccation, and nitrogen at

50 psi was used for dispersion. Molecular ions scanning range (m/z) was 150–1500.

IR spectra were recorded in KBr pellets with a Bruker Tensor 37 spectrometer in the range of 400-4000 cm<sup>-1</sup>.

Electronic spectra by diffuse reflectance technique, with spectralon as standard, were recorded in the range of 200–1500 nm, on a Jasco V670 spectrophotometer.

Magnetic measurements were done at room temperature, on a Lake Shore's fully integrated Vibrating Sample Magnetometer (VSM) system 7404, calibrated with a Ni— 0.126 g sphere—SRM 772a; moreover, VSM was intercalibrated with a absolute calibrated Faraday balance or calibrated with Hg[Co(NCS)<sub>4</sub>] as standard. The molar magnetic susceptibilities were calculated and corrected for the atomic diamagnetism.

The X (9.2 GHz)-band EPR measurements were taken at room temperature with the Adani CMS 4800 spectrometer, employing a field modulation of 100 kHz. The microwave frequency and magnetic field were measured with an accuracy of  $10^{-5}$  with the digital frequency counter Pendulum CNT-60. The magnetic field calibration at the sample was performed with a DPPH (diphenylpicrylhydrazyl) standard marker, exhibiting a narrow EPR line (g = 2.0036).

<sup>1</sup>H NMR spectra were recorded on a Bruker Avance spectrometer (working frequency 200 MHz) at 25 °C. Chemical shifts were measured in parts per million from internal standard TMS.

The heating curves (TG, DTG and DTA) were recorded using a Labsys 1200 SETARAM instrument, with a sample mass of 10–12 mg over the temperature range of 20–900 °C, using a heating rate of 10 K min<sup>-1</sup>. The measurements were taken in synthetic air atmosphere (flow rate  $16.7 \text{ cm}^3 \text{ min}^{-1}$ ) by using alumina crucibles.

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

#### **Biological assays**

The antimicrobial activity of complexes was determined against ATCC reference and clinical microbial strains, i.e. Gram-positive (*Bacillus subtilis* 12488, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* MRSA 1684, *Staphylococcus aureus* ATCC 13294, *Staphylococcus aureus* 0364, *Staphylococcus epidermis* 1736), Gram-negative (*Enterobacter cloacae* 61R, *Escherichia coli* ATCC 25922, *Escherichia coli* 714, *Escherichia coli* 13147, *Escherichia coli* ESBL 1576 (ESBL Extended Spectrum Beta Lactamase), *Klebsiella pneumoniae* 2968, *Klebsiella pneumoniae* 1771, *Klebsiella pneumoniae* 1204, *Proteus mirabilis* 1777, *Pseudomonas*  aeruginosa ATCC 1671, Pseudomonas aeruginosa 1397) and fungal (Candida albicans ATCC 249, Candida albicans 128 şi Candida krusei 963). The qualitative evaluation of the antimicrobial activity was performed by the adapted disc diffusion, as previously reported [23], using Mueller-Hinton agar (MHA) medium for bacteria and yeast peptone glucose (YPG) in case of fungi. The compounds were solubilised in dimethyl sulphoxide (DMSO), and the starting stock solution was of 1000 µg mL<sup>-1</sup> concentration.

The quantitative assay of the antimicrobial activity was performed by the liquid medium microdilution method, in 96-multi-well plates, in order to establish the minimal inhibitory concentration (MIC) and the minimal biofilm eradication concentration (MBEC) values [26]. All biological experiments were performed in triplicate.

# Synthesis and spectral data of complexes

To a suspension contained 3,6-diazaoctane-1,8-diamine (20 mmol), hydrated metal (II) acetate (20 mmol), 2 mL triethylamine and 2 mL formaldehyde (37 %) in 150 mL methanol were added drop wise a solution of 1,3-phenylenediamine (10 mmol) in 50 mL methanol. The reaction mixture was refluxed 24 h until a sparingly soluble brown coloured compound was formed. The microcrystalline products were filtered off, washed with methanol and air-dried.

$$\begin{split} & [\mathrm{Ni}_2\mathrm{L}](\mathrm{CH}_3\mathrm{COO})_4\cdot 2.5\mathrm{H}_2\mathrm{O}~(1): \mbox{ Analysis, found (\%): Ni, } \\ & 14.05; \ C, \ 42.33; \ H, \ 7.52; \ N, \ 16.58; \ \mathrm{Ni}_2\mathrm{C}_{30}\mathrm{H}_{61}\mathrm{N}_{10}\mathrm{O}_{10.5} \\ & \mbox{requires (\%): Ni, } 13.79; \ C, \ 42.33; \ H, \ 7.70; \ N, \ 16.45; \ ESI-\\ & \mbox{MS}~(\mathrm{CH}_3\mathrm{CN}:\mathrm{H}_2\mathrm{O}; \ 1:1) \ \textit{m/z:} \ [\mathrm{Ni}_2\mathrm{C}_{30}\mathrm{H}_{56}\mathrm{N}_{10}\mathrm{O}_8]^+, \ 803.60; \\ & [\mathrm{Ni}_2\mathrm{C}_{26}\mathrm{H}_{50}\mathrm{N}_{10}\mathrm{O}_4]^+, \ \ 691.23; \ \ [\mathrm{C}_{22}\mathrm{H}_{50}\mathrm{N}_{10}]^+, \ \ 454.32; \\ & [\mathrm{C}_{20}\mathrm{H}_{39}\mathrm{N}_8]^+, \ 391.32; \ [\mathrm{C}_{14}\mathrm{H}_{22}\mathrm{N}_8]^+, \ 302.40; \ [\mathrm{C}_{10}\mathrm{H}_{21}\mathrm{N}_4]^+, \\ & 197.21; \ \textit{\Lambda}_{\mathrm{M}}~(\mathrm{DMSO}): \ 260\ \ \Omega^{-1}\ \mathrm{cm}^2\ \mathrm{mol}^{-1}. \end{split}$$

$$\begin{split} & [\text{Cu}_2\text{L}(\text{CH}_3\text{COO})_4]\cdot\text{H}_2\text{O}\left(2\right):\text{Analysis, found (\%): Cu, 15.25;}\\ & \text{C, }43.28;\text{ H, }7.34;\text{ N, }16.94;\text{Cu}_2\text{C}_{30}\text{H}_{58}\text{N}_{10}\text{O}_9\text{ requires (\%): Cu,}\\ & 15.24;\text{ C, }43.21;\text{ H, }7.49;\text{ N, }16.80;\text{ESI-MS (CH}_3\text{CN:H}_2\text{O};1:1)\\ & \textit{m/z}: [\text{Cu}_2\text{C}_{30}\text{H}_{53}\text{N}_{12}\text{O}_6]^+, 804.65; [\text{Cu}_2\text{C}_{27}\text{H}_{52}\text{N}_{10}\text{O}_4]^+, 707.32;\\ & [\text{Cu}_2\text{C}_{26}\text{H}_{48}\text{N}_{10}\text{O}_4]^+, \quad 691.23; \quad [\text{Cu}_2\text{C}_{23}\text{H}_{43}\text{N}_{11}]^+, \quad 600.48;\\ & [\text{C}_{22}\text{H}_{50}\text{N}_{10}]^+, \quad 454.31; \quad [\text{C}_{22}\text{H}_{37}\text{N}_8]^+, \quad 413.30; \quad [\text{C}_{20}\text{H}_{39}\text{N}_8]^+,\\ & 391.32; \quad [\text{C}_{14}\text{H}_{22}\text{N}_8]^+, \quad 302.39; \quad [\text{C}_{10}\text{H}_{21}\text{N}_4]^+, \quad 197.28; \quad \textit{A}_{M}\\ & (\text{DMSO): } 8 \ \Omega^{-1} \ \text{cm}^2 \ \text{mol}^{-1}. \end{split}$$

The ligand (1,3-bis(*N*,*N*-1,3,6,9,12-pentaazacyclotridecane)-benzene) was synthesised and characterised as reported [22].

# **Results and discussions**

# Synthesis and physicochemical characterisation of the ligand and its complexes

The one-pot reaction of excess formaldehyde with 2:2:1 molar ratio of nickel (II), copper(II) or zinc (II) acetate, 3,6-diazaoctane-1,8-diamine and 1,3-phenylenediamine in triethylamine presence, produced the species M<sub>2</sub>L(CH<sub>3</sub>. COO)<sub>4</sub>·nH<sub>2</sub>O ((1) M:Ni, n = 2.5; (2) M:Cu, n = 1 and (3) M:Zn, n = 10; L: bismacrocyclic ligand resulted in condensation) (Scheme 1). The chemical analyses are in accord with formulas proposed for complexes (see "Experimental" part). By using the same method, the chloride derivatives with the same ligand M<sub>2</sub>LCl<sub>4</sub>·nH<sub>2</sub>O (M:Ni, n = 4; M:Cu, n = 2.5 and M:Zn, n = 1.5) were obtained for which only the hydration degree is different [25].

The goal of this paper is to evidence the thermal behaviour of these complexes which proved *in vitro* antimicrobial activity, the ability to inhibit biofilm formation on inert support and a low cytotoxicity.

# IR, <sup>1</sup>H NMR and ESI-MS spectra

Table 1 summarises the most important bands from IR spectra of ligand and complexes. These bands can be assigned to some characteristic vibration modes for basic moieties provided both by 3,6-diazaoctane-1,8-diamine and 1,3-phenylenediamine for macrocyclic backbone, such as that characteristic for ethylene in the range of 2820–2920 cm<sup>-1</sup> and C=C (aromatic) groups in the range of 1450–1565 cm<sup>-1</sup>, respectively [11]. In the IR spectra, a single broad absorption around 3250 cm<sup>-1</sup> appears, assigned to secondary amine stretching vibration [11, 27]. This band is shifted by 32–116 cm<sup>-1</sup> to lower wavenumbers in comparison with metal-free ligand indicating its coordination through secondary amine nitrogen [11].

In the IR ranges characteristic for carboxyl group, two bands can be noticed for all complexes in the ranges of 1570–1660 and 1390–1460 cm<sup>-1</sup>, respectively, assigned to  $v_{as}(COO)$  and  $v_{s}(COO)$  vibration modes. The difference between these bands of 162 cm<sup>-1</sup> for complex (1) indicates that acetate behaves as free ion while a value of 200 and 186 cm<sup>-1</sup> in the case of complexes (2) and (3) is in agreement with the unidentate nature of acetate in this case [28, 29]. Moreover, the behaviour of compound (1) as 1:4 electrolyte and of compounds (2) and (3) as non-electrolytes in DMSO [30] sustains these observations.

In the characteristic ranges for water, a broadband in the range of  $3330-3350 \text{ cm}^{-1}$  assigned to v(OH) stretching vibrations can be noticed in complexes spectra [31].

Scheme 1 Route for complexes synthesis



 Table 1 IR absorption bands (cm<sup>-1</sup>) for ligand and complexes

L	(1)	(2)	(3)	Assignment
_	3341s	3336s	3350s	v(OH)
3330m	3288s	3214m	3298s	v(NH)
-	3045m	3042m	3066m	$\nu(CH_{aromatic})$
2921w	2938m	2934m	2920w	$v_{as}(CH_2)$
2843w	2847w	2823w	2851w	$v_s(CH_2)$
	1616vs	1656vs	1579vs	$v_{as}(COO)$
1558m	1565vs	1562m	1513m	$v(C=C) + \delta(CH)$
1456m	1514m	1506s	1480m	
	1454s	1456m	1393s	$v_s(COO)$
1390w	1340m	1388m	1290w	$v(C_{aromatic}-N)$
1136vs	1163m	1179m	1177m	$v(C_{aliphatic}-N)$
876w	869w	885w	890w	$\gamma(CH_{aromatic})$
850w	833w	843w	845w	
-	661m	647m	669m	δ(COO)
-	-	516w	547w	v(M–O)
-	406w	438w	422w	v(M–N)

vs very strong, s strong, m medium, w weak

Furthermore, the IR spectra of complexes exhibit new low intensity bands in the ranges of 400–440 and 510–550 cm<sup>-1</sup> tentatively assigned to v(M-N) and v(M-O) [31], respectively, except for Ni(II) complex for which only the band assigned to v(M-N) is observed.

The <sup>1</sup>H NMR spectrum of ligand reveals the pattern characteristic for all functional groups of bismacrocycle as was already reported [25]. The <sup>1</sup>H NMR spectra of diamagnetic complexes (1) and (3) show two singlet signals corresponding to NH and -NCH<sub>2</sub>N- protons, and one triplet arising from -NCH<sub>2</sub>CH<sub>2</sub>N- link (Table 2). The aromatic protons are responsible for the broad signal appeared in 7.00–7.90 ppm range. The amino group coordination is further supported by <sup>1</sup>H NMR spectra of complexes where the resonance assigned to NH group is upfield shifted in comparison with the signal of the free ligand. Supplementary singlet observed at 2.75 and 2.80 ppm in complexes spectra was assigned to acetate group.

Additional signals observed in <sup>13</sup>C NMR spectra of complexes were assigned to acetate group carbon atoms. Two signals that can be assigned to COO group can be noticed in complex (**3**) spectrum, difference assigned to this group coordination (Table 3).

Except the acetate characteristic signal both in IR and NMR spectra, the same pattern was observed for chloride derivatives of the series [25].

The composition of ligand [25] and complexes was also evidenced by ESI-MS spectra. The pseudomolecular ions for complexes were found as  $[M-2.5H_2O+H]^+$  for (1),  $[M-H_2O+H]^+$  for (2) and  $[M-10H_2O-2CH_3COO]^+$  for (3), respectively. Moreover, other fragments with or without metal ion that can be related to the ligand or complexes structure such as  $[C_{22}H_{44}N_{10}+6H]^+$  (*m/z*: 454.31),  $[C_{20}H_{39}N_8]^+$  (*m/z*: 391.32),  $[C_{14}H_{22}N_8]^+$  (*m/z*: 302.39),  $[C_{10}H_{21}N_4]^+$  (*m/z*: 197.28) were identified in all complexes spectra.

## Electronic, EPR spectra and magnetic data

Electronic spectra correlated with magnetic data at room temperature provided useful information concerning the oxidation state of the metallic ion, stereochemistry and the ligand field nature. Table 4 summarises the electronic absorption bands.

The intraligand  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions that appear in the range of 26,670–46,520 cm<sup>-1</sup> in the ligand spectrum are different shifted in complexes spectra as a result of coordination.

The electronic spectrum of complex (1) (Fig. 1) exhibits a broadband at 24,690 cm<sup>-1</sup>, pattern characteristic for a square planar stereochemistry of Ni(II) [32], the diamagnetic behaviour furthermore sustaining the proposed stereochemistry [33].

A broadband with tendency to split into two components can be noticed in the visible region of the electronic spectrum of compound (2). The maximum at 15,625 cm<sup>-1</sup> and a shoulder at lower energy (Fig. 1) are features that indicate an octahedral distorted stereochemistry [32]. Considering that macrocycle unit is coordinated in equatorial positions around the metal ion as observed for other saturated polyazamacrocyclic derivatives, then the acetate anions in apical position generate a tetragonal elongated distortion around the Cu(II) ion. The additional band at 19,420 is tentatively assigned to the ligand to metal charge transfer transition. The experimental  $(\chi T)_{HT}$  value of 0.73 cm<sup>3</sup> K mol<sup>-1</sup> close to calculated one (0.75 cm<sup>3</sup> K mol<sup>-1</sup>) indicates the absence of any interaction between the metallic centres at room temperature [33].

Compound	δ/ppm						
	NH	CH <sub>3</sub>	(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>2</sub>	Ar–H		
L	2.35	_	3.38	3.91	7.98-8.44		
[Ni <sub>2</sub> L](CH <sub>3</sub> COO) <sub>4</sub> ·2.5H <sub>2</sub> O (1)	2.16	2.75	3.18	3.94	7.11–7.90		
$[Zn_2L(CH_3COO)_4] \cdot 10H_2O$ (3)	2.19	2.80	3.16	3.56	7.03-7.28		

Table 2  ${}^{1}$ H RMN spectroscopic data for ligand and complexes (1) and (3)

Table 3 <sup>13</sup>C RMN spectroscopic data for ligand and complexes (1) and (3)

Compound	$\delta$ /ppm	δ/ppm					
	CH <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub>	СН	CH <sub>3</sub>	<b>C</b> 00		
L	39.90	43.50	114.64	-	_		
			117.53				
			120.42				
[Ni <sub>2</sub> L](CH <sub>3</sub> COO) <sub>4</sub> ·2.5H <sub>2</sub> O (1)	36.26	43.13	114.46	162.16	176.55		
			117.75				
			120.32				
[Zn <sub>2</sub> L(CH <sub>3</sub> COO) <sub>4</sub> ]·10H <sub>2</sub> O ( <b>3</b> )	39.52	42.82	114.13	158.39	171.41		
			117.01	158.76	174.67		
			119.90				

Table 4 Absorption maxima (cm<sup>-1</sup>) from electronic spectra of ligand and complexes (1)-(3) and assignments

Compound	Absorption maxima/cm <sup>-1</sup>	Assignment
L	46,520	$\pi \rightarrow \pi^*; n \rightarrow \pi^*$
	37,040	
	28,170	
	26,670	
[Ni <sub>2</sub> L](CH <sub>3</sub> COO) <sub>4</sub> ·2.5H <sub>2</sub> O (1)	41,670	$\pi \rightarrow \pi^*; n \rightarrow \pi^*$
	36,360	
	30,960	
	24,690	$^{1}A_{1 g} \rightarrow ^{1}A_{2 g}$
$[Cu_2L(CH_3COO)_4]\cdot H_2O~(\textbf{2})$	41,670	$\pi \rightarrow \pi^*; n \rightarrow \pi^*$
	36,360	
	30,770	
	19,420	LMCT
	15,625	${ m d}_{ m xz}, d_{ m yz}  ightarrow d_{ m x^2-y^2}$
	11,430	$d_{ m z^2}  ightarrow d_{ m x^2-y^2}$
$[Zn_{2}L(CH_{3}COO)_{4}] \cdot 10H_{2}O(3)$	36,360	$\pi \rightarrow \pi^*; n \rightarrow \pi^*$
	31,060	
	30,770	

LMCT ligand to metal charge transfer

It was assumed that diamagnetic Zn(II) complex adopts also an octahedral stereochemistry having in view that a square planar one is rarely found for this ion in compounds with saturated azamacrocyclic ligands. IR spectrum is in accordance with unidentate nature of acetate, and moreover, complex behaves as non-electrolyte in DMF.



Fig. 1 Electronic spectra of ligand and complexes (1) and (2)



Fig. 2 X-band EPR spectrum of [Cu<sub>2</sub>L(CH<sub>3</sub>COO)<sub>4</sub>]·H<sub>2</sub>O (2)

The same pattern was observed in the electronic spectra of chloride derivatives as result of the same stereochemistry [25]. Despite the enhanced coordinative



ability of the acetate anion in comparison with chloride. the Ni(II) complexes adopt the same square planar stereochemistry. As a result, this behaviour could be due to nickel ionic radius, which has the smallest value for the studied cations and fits best with the azamacrocycle hole. Moreover, the strong field created by pentaazamacrocycle imposes this stereochemistry for Ni(II) indifferently by the anion nature.

Room-temperature powder EPR spectrum of complex (2) in X-band (Fig. 2) is characteristic for Cu(II) species with isolated centres and an axial symmetry [34] with  $g_{\rm ll} = 2.175, g_{\perp} = 2.054$ , and hyperfine splitting parameters  $A_{\rm ll} = 16.8$  mT and  $A_{\perp} = 7.2$  mT, respectively. These parameters are in accordance with  $d_{x^2-y^2}$  as ground state. The value of molecular orbital coefficient  $\alpha^2$  of 0.675 indicates a high degree of co-valency for the in-plane Cu-N (macrocycle)  $\sigma$  bonds [35].

Unlike the chloride derivative, in the EPR spectrum of acetate species the hyperfine structure is well solved as a result of a higher degree of packing that allows the alignment of the molecular axes [34].

## Thermal behaviour of ligand and complexes

As shown in Fig. 3, bismacrocycle ligand is stable up to 206 °C and decomposes in two steps. First step, accompanied by a strong exothermic effect, corresponds to 65.7 % mass loss and can be assigned to oxidative degradation of a part of azamacrocycle moiety leading to a species with formula C<sub>10</sub>H<sub>8</sub>N<sub>2</sub>. It is worth mentioning that such a fragment was identified also in ESI-MS spectra of ligand and complexes. The second step is not a single one, but a superposition of at least three processes as both DTG and DTA curves indicate. This behaviour is the result of oxidative degradation of the rest of organic part.



The TG, DTG and DTA curves corresponding to complex (1) indicate that decomposition follows two steps (Fig. 4). The first step of compound thermal transformation consists in an endothermic elimination of water molecules up to 130 °C (Table 5). Such behaviour was observed for other complexes, which contain crystallisation water [36, 37]. The anhydrous species is stable over a 30°C temperature range. The second step is not a single one being an overlap of at least three processes according to both DTG and DTA curves profile and corresponds to decomposition and oxidative degradation of organic components, acetate and bismacrocycle species. All these transformations end with stoichiometric nickel (II) oxide formation (found/calcd. overall mass loss: 82.3/82.5 %). The nature of final product was confirmed by both IR and powder X-ray diffraction data (ASTM 78-0429).

Water molecules are loss in one well definite, endothermic step as it can see in the DTA curve of compound (2) (Fig. 5). This step occurred in the temperature range of 55-120 °C, respectively, as an indicative of water molecules involved in weak interactions. Next step corresponds to acetate into carbonate transformation and phenyl nucleus elimination, according to mass loss. Such behaviour was observed for other complexes with mixed ligands



Table 5 Thermal behaviour data (in air) for complexes and processes proposed for thermal decomposition

Compound	Step	Thermal effect	Temperature range/°C	$\frac{\Delta m_{\mathrm{exp}}}{\%}$	$\frac{\Delta m_{ m calc}}{\%}$	Process
L	1.	Exothermic	206–612	6 65.7	65.2	Oxidative degradation with two units C <sub>6</sub> H <sub>18</sub> N <sub>4</sub> elimination from bis(azamacrocycle) moieties
	2.	Exothermic	616–796	3 34.3	34.8	Oxidative degradation of the rest of organic intermediate
[Ni <sub>2</sub> L](CH <sub>3</sub> COO) <sub>4</sub> ·	1.	Endothermic	54-130	5.3	5.3	Water elimination
2.5H <sub>2</sub> O (1)	2.	Exothermic	160–525	77.0	77.2	Oxidative degradation of both acetate and bis(azamacrocycle) ligand
$ \begin{matrix} [Cu_2L(CH_3COO)_4] \cdot \\ H_2O~(\textbf{2}) \end{matrix} $	1.	Endothermic	55-120	2.3	2.2	Water elimination
	2.	Exothermic	120–317	23.6	23.2	Acetate into carbonate transformation and C <sub>6</sub> H <sub>4</sub> unit elimination from bis(azamacrocycle) ligand
	3.	Exothermic	317-675	54.9	55.4	Oxidative degradation of the rest of organic matrix and carbonate decomposition
$[Zn_{2}L(CH_{3}COO)_{4}]\cdot$	1.	Endothermic	45-140	17.8	18.1	Water elimination
10H <sub>2</sub> O ( <b>3</b> )	2.	Exothermic	140–470	48.0	47.6	Acetate into carbonate transformation and $C_{16}H_{39}N_9$ unit elimination from bis(azamacrocycle) ligand
	3.	Exothermic	470–580	17.6	17.9	Oxidative degradation of the rest of organic matrix and carbonate decomposition

complex one, resulting from overlapping of at least three

processes as DTG curve profile indicates. According to

mass loss, this step corresponds to acetate into carbonate

transformation and 81 % of bismacrocycle pyrolysis.

According to mass loss only the aromatic unit together with

a nitrogen atom remains from the organic ligand during this

transformation. The carbonate decomposition and the rest

of organic part oxidative degradation can be noticed in the

third step, ending with zinc (II) oxide generation at 580 °C

(ASTM 00-036-1451) (found/calcd. overall mass loss:

was observed that water is eliminated at low temperatures

a similar series of compounds with chloride ion were

as result of its crystallisation nature.

For both series of derivatives (chloride and acetates), it

In our former publication [25], the thermal properties of

120

83.4/83.6 %).

[38, 39], where the acetate decomposition starts before of other organic derivatives. The small exothermic peaks observed on DTA are probably due to both endothermic and exothermic reactions that occur simultaneously, such as cleavage and rearrangement of the bonds, as well as some moieties oxidative degradation. Otherwise, at least three processes occur according to DTG curve. Third step is an overlapping of at least two processes according to both DTG and DTA curves profile and corresponds to carbonate decomposition and oxidative degradation of the rest of bismacrocycle derivative. All these transformations generate finally CuO as powder X-ray diffraction indicates (ASTM 5-661) (found/calcd. overall mass loss: 80.8/80.8 %).

Complex (3) losses the crystallisation water up to  $140 \,^{\circ}$ C (Fig. 6). The second step is an exothermic and

**Fig. 5** TG, DTG and DTA curves for  $[Cu_2L(CH_3COO)_4] \cdot H_2O$  (2)

ΤG 0.2 0 100 0.1 БXО 80 -20 DTG 1 0.0 Mass variation/% 60 DTG/mg min--0 1 -40 Heat flow/µV 40 -0.2 20 -60 -0.3 DTA 0 -0.4 -80 -20 -0.5 ò 200 400 600 800 1000 Temperature/°C 80 0.4 ТG 0.3 0 0.2 60 БXО 0.1 -20 DTG 0.0 1 Mass variation/% 40 DTG/mg min -0 1 -40 -0.2 Heat flow/µV 20 -0.3 -60 -0.4 DTA 0 -0.5 -80 -0.6 -20 -0.7 -100 -0.8 0 200 400 600 800 1000 Temperature/°C

Fig. 6 TG, DTG and DTA curves for  $[Zn_2L(CH3COO)4] \cdot 10H2O$  (3)

determined. Due to the anion change, the decomposition pattern of acetates is different, as the acetate decomposes immediately after water elimination. Despite the different decomposition routes, in acetates the same order of thermal stability Ni > Zn > Cu was observed as in chlorides.

Having in view all above data, complexes were formulated as  $[Ni_2L](CH_3COO)_4.2.5H_2O$ ,  $[Cu_2L](CH_3COO)_4.H_2O$ and  $[Zn_2L(CH_3COO)_4].10H_2O$ , respectively. The coordination proposed for the new complexes is presented in Fig. 7.

### **Biological activity**

The antimicrobial activity of complexes was determined on ATCC reference strains and clinical, resistant isolates. Complexes activity was assayed in comparison with that of the ligand and intermediate species  $Mtrien(CH_3COO)_2$  (M:Ni, Cu, Zn). It is to be pointed out that ligand and  $MtrienCl_2$  did not exhibit a significant antimicrobial activity, the diameter of the growth inhibition zone being below 5 mm for all tested strains, reason for which these two compounds have not been tested by quantitative assays.



Fig. 7 Coordination proposed for complexes (crystallisation water molecules were omitted)

Table 6 MIC ( $\mu g m L^{-1}$ ) values of complexes

Strain	(1)	(2)	(3)
E. coli 714	>1000	>1000	62.50
E. coli 13147	1000	>1000	3.90
S. aureus 13294	62.50	>1000	62.50
C. krusei 963	>1000	>1000	31.25
C. albicans 249	>1000	7.81	250

In the quantitative assay, the active compounds exhibited an improved antimicrobial activity against the planktonic pathogenic strains, in comparison with the ligand (Table 6). Complexes (1) and (3) exhibited a good activity on *S. aureus* 13294 strain (MIC 31.25  $\mu$ g mL<sup>-1</sup>), complex (3) being also very active on both *E. coli* strains (MIC of 3.9-62.5  $\mu$ g mL<sup>-1</sup>) and *C. krusei* 963 strain (MIC 31.25  $\mu$ g mL<sup>-1</sup>). Complex (2) inhibited singularly the *C. albicans* strain up to a very low concentration of 7.81  $\mu$ g mL<sup>-1</sup>.

Overall, the results indicate that complex (3) exhibited the largest spectrum of antimicrobial activity with good results both in the case of bacterial and fungal strains.

Concerning the antibiofilm activity, compound (1) inhibited the biofilm formed by *C. albicans* 249 strain (MBEC of 15.62  $\mu$ g mL<sup>-1</sup>), while compound (3) inhibited the biofilm formed by *C. krusei* 963 up to 62.50  $\mu$ g mL<sup>-1</sup>. In the case of the other strains, the tested complexes were inactive or even stimulated the biofilm adherence.

Taking into account that complex (3) was the most active from this series and having in view the low kinetic stability of Zn(II) complexes, we could hypothesise that after acetate substitution, complex cation could establish electrostatic interactions with negatively charged functional groups of the microbial wall, interfering with complex physiological roles of these structures and thus with microbial cell viability and pathogenicity.

By comparison with Cu(II) and Zn(II) chloride complexes of the series [25], the acetate derivatives that have the same non-electrolytic behaviour were more active against planktonic strains. This behaviour could be the result of an enhanced lipophilicity induced by methyl group that control thus a better penetration through the pathogenic microorganisms' membrane. Instead, for both series of derivatives the Ni(II) species characterised as ionic compounds exhibit an overall lower activity.

The effect of ligand and complexes on HCT 8 tumour cells was assayed by flow cytometry method, the apoptosis being evidenced by double staining with annexin V/propidium iodide method [25]. After 24 h, no cytotoxic effect was observed for the tested complexes.

### Conclusions

One-pot condensation of 1,3-phenylenediamine, 3,6-diazaoctane-1,8-diamine and formaldehyde generated a series of nickel (II), copper (II) and zinc (II) complexes with an azabismacrocycle ligand.

The ligands nature and stereochemistry were characterised by means of IR, NMR and electronic spectroscopy. The modifications in the IR spectra of complexes are in accord with the condensation process and secondary amine group coordination. As for acetate anion, this can be found either as free ion or as unidentate species in these compounds. Electronic spectrum of Ni(II) is characteristic for the square planar stereochemistry while that of Cu(II) displays the pattern of octahedral one.

The number and role of water molecules, the decomposition pattern of complexes and ligand, as well as the intervals of thermal stability, were established from TG experiments. After water elimination up to 140 °C, complexes decompose in one or two steps, leading to metal (II) oxides as final product. The results are in good concordance with complexes composition. The thermal stability of the anhydrous compounds according to TG/DTG/DTA curves depends on the nature of the metal ion in the following order: Ni > Zn > Cu.

The good antimicrobial activity against Gram-positive, Gram-negative bacteria and fungal strains, as well as the inhibitory activity of fungal biofilms development, correlated with the low cytotoxicity on mammalian cells recommends these compounds for further studies aiming to obtain novel antimicrobial agents.

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