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Synthesis, biological and physicochemical properties of Zinc(II) salicylate and 5-chlorosalicylate complexes with theophylline and urea

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Abstract New zinc(II) salicylate complex compounds of general formula $(X\text{-}C_6H_3\text{-}2\text{-}(OH)COO)_2Zn \cdot L_n \cdot xH_2O$ (where X = H, 5-Cl; L = theophylline, urea; $n = 2, 4$; $x = 1, 2, 4$) were prepared and their thermal, spectral and biological properties were studied. It was found that the thermal decomposition of hydrated compounds starts with the release of water. During the thermal decomposition of anhydrous compounds, the release of salicylic acid, theophylline, urea, CO_2, H_2O and C_6H_5Cl takes place. Zinc oxide was found as the final product of the thermal decomposition heated up to 900 °C. The complexes were tested against bacteria, yeasts and filamentous fungi. The highest biological activity show 5-chlorosalicylate compounds.

Keywords Zinc(II) salicylate · Theophylline · Urea · IR spectra · Thermal properties · Biological properties

Introduction

Zinc is one of the most significant biometals. It is an important component of many proteins. Zn^{2+} ion strongly interacts with electronegative sulphur, nitrogen, oxygen. It does not promote the formation of toxic free radicals [1].

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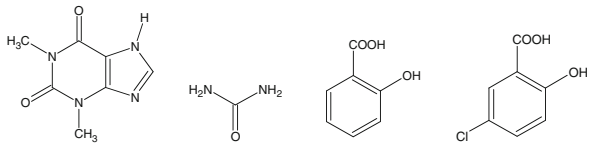
Metal complexes of biologically important ligands are sometimes more effective than the free ligands [2]. Zinc complexes with bioactive ligands catalyze many enzymatic processes in biological systems [3] and they are considered to have pharmaceutical effects against bacteria, fungi and viruses [2]. Kráľová et al. studied from this point of view inhibition of oxygen evolution rate, algal growth and chlorophyll production in Chlorella vulgaris by Cu(II) complexes with biologically active ligands (flufenamate, mefenamate, niflumate, naproxenate, nicotinamide, N,N-diethylnicotinamide, 3-hydroxymethylpyridine, caffeine and methyl-3-pyridylcarbamate) [4].

The study of the effect of zinc(II) on the growth of *E. coli* studied by microcalorimetry was done by Yao et al. [5]. They found out that a low concentration of zinc(II) had a promoting action on the growth of *E. coli*, but a high concentration of zinc(II) had an inhibitory action. Kose studied mixed-ligand m-hydroxybenzoate complexes of Co(II), Ni(II), Cu(II), and Zn(II) with nicotinamide and characterized them by elemental analysis, FT-IR spectrometry, solid state UV–VIS spectrometry and magnetic susceptibility measurements [6]. Czakis-Sulikowska et al. studied mixed-ligand Ni(II) and Zn(II) complexes with bipyridine isomers and bromoacetates [7]. Thermal, spectral and magnetic properties of complexes of 4-chloro-2-methoxybenzoic acid anion with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} were characterized by Ferenc et al. [8].

In continuation to our previous papers which dealt with the study of thermal, spectral, structural and biological properties of aliphatic [9–12] and aromatic zinc(II) carboxylates and halogenocarboxylates [13–15], in this paper the thermal, spectral and biological properties of zinc(II) salicylate complexes and their chloroderivatives with bioactive organic ligands theophylline and urea are reported.

Experimental

The following A. R. grade chemicals were used for the preparation of studied compounds: $ZnCl_2$ (Fluka), $NaHCO_3$ (Centralchem), salicylic acid (Lachema Brno), 5-chlorosalicylic acid (Aldrich), theophylline (Fluka), urea (Lachema Brno).



Synthesis of the compounds

The following compounds were prepared:

$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2{\cdot}2H_2O$ (**I**)

$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**)

$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**)

$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**)

$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**)

$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**)

Syntheses may be expressed by these equations:

$$ZnCl_2 + 2NaHCO_3 \rightarrow ZnCO_3 \downarrow + 2NaCl + H_2CO_3$$

$$ZnCO_3 + 2X\text{-}C_6H_3\text{-}2\text{-}(OH)COOH \rightarrow Zn(X\text{-}C_6H_3\text{-}2\text{-}(OH)COO)_2 + H_2O + CO_2 \uparrow$$

$$Zn(X\text{-}C_6H_3\text{-}2\text{-}(OH)COO)_2 + nL \rightarrow Zn(X\text{-}C_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot L_n \cdot xH_2O$$

$ZnCO_3$ was prepared by the reaction of aqueous solutions of the stoichiometric amounts of $ZnCl_2$ and $NaHCO_3$. Then methanol solution of salicylic or 5-chlorosalicylic acid was added to an aqueous suspension of $ZnCO_3$ under continual stirring in a stoichiometric amount. The reaction mixtures were stirred for 1 h. A methanol/aqueous solution of an organic ligand was added to the filtrate in a molar ratio 1:2 and stirred together for 30 min. Then the reaction mixtures were reduced to ¼ of volume in a water bath at 80 °C and left to crystallize at room temperature. In a few days white crystalline product precipitated.

Instrumentation

C, H, N analyses were performed using a Perkin Elmer 2400 CHN analyser. The content of zinc was determined complexometrically using Complexone III as an agent and Eriochrome black T as an indicator.

Infrared spectra of prepared compounds were recorded on AVATAR 330 FT-IR Thermo Nicolet using KBr pellets (2 mg/200 mg KBr) in the range 4,000–400 cm^{-1}.

The thermal properties (TG/DTG, DTA) were studied in air atmosphere in ceramic crucibles under dynamic conditions on NETZSCH STA 409 PC/PG Thermoanalyzer (heating rate 5–9 °C min^{-1}).

Gas chromatograph coupled with a quadrupole mass spectrometer (HP G1800 A GCD system) was used for the determination of volatile products of thermal decomposition. Every analysed sample was dissolved in solution (chloroform-ethanol, ratio 2:1) and 1 μL was injected. The products were separated on a fused-silica capillary column (30 m × 0.25 mm) with a 0.25 μm thick chemically bonded HP1. The column temperature was kept at 80 °C for 5 min and programmed to 280 °C at the increments of 20 °C with a helium carrier gas (flow rate 0.8 mL/min), after which isothermal conditions were maintained for 10 min. The interface and ion source were kept at 280 °C. The products were ionized at 70 eV electron impact voltages.

Antimicrobial assay

The antimicrobial activity of Zn(II) complexes (**I–VI**), salicylic acid (**VII**), 5-chlorosalicylic acid (**VIII**), urea (**IX**) and theophylline (**X**) was evaluated by a micro-dilution method [16] using G^+ bacteria *Staphylococcus aureus* CCM 3953, G^- bacteria *Escherichia coli* CCM 3988 (both from the *Czech Collection of Microorganisms*, *Masaryk University*, Brno, Czech Republic). The effects of these compounds on the yeasts *Candida albicans* (purchased from the *Laboratory of Medical Mycology*, *Slovak Medical University*, Bratislava, Slovakia) were determined by macro-dilution method in L-shaped tubes adapted for direct measurement of absorbance [17]. The cultures of bacteria (in Mueller–Hinton medium) and yeasts (Sabouraud-glucose medium) were incubated under vigorous shaking. The efficiency of prepared derivatives on the growth of filamentous fungi *Rhizopus oryzae*, *Alternaria alternata* (both from the *Collection of Microorganisms of Department of Biochemistry and Microbiology*, *Faculty of Chemical and Food Technology Slovak University of Technology*, Bratislava, Slovakia), *Aspergillus fumigatus* CCM F-373 and *Microsporum gypseum* (from the *Laboratory of Medical Mycology, Slovak Medical University*, Bratislava, Slovakia) was observed by macro-dilution technique on Sabouraud's (dermatophytes) and malt agar (other fungi) during the static cultivation and the diameters of growing fungal colonies were measured at intervals [18].

The antimicrobial activity of tested compounds was characterized by the IC_{50} values (concentration of a derivative which in comparison to the control inhibits the

Table 1 The results of elemental analysis

Compounds	C%		H%		N%		Zn%	
	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.
$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**) $C_{14}H_{14}O_8Zn$ F.W. = 375.649	44.68	44.76	3.76	3.76	–	–	16.75	17.41
$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**) $C_{16}H_{22}N_4O_{10}Zn$ F.W. = 495.767	40.63	40.22	4.12	4.22	12.64	11.72	13.18	13.19
$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**) $C_{28}H_{30}N_8O_{12}Zn$ F.W. = 735.974	45.52	45.70	4.17	4.11	15.82	15.23	8.86	8.88
$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**) $C_{14}H_{12}O_8 \cdot Cl_2Zn$ F.W. = 444.54	37.85	37.82	2.74	2.72	–	–	14.35	14.71
$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**) $C_{18}H_{26}N_8O_{11}Cl_2Zn$ F.W. = 666.75	32.78	32.42	3.88	3.93	17.32	16.81	8.66	9.81
$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**) $C_{28}H_{32}N_8O_{14}Cl_2Zn$ F.W. = 840.90	40.34	39.99	3.85	3.84	13.87	13.33	7.33	7.78

growth of microorganisms to 50%) and MIC values (minimal inhibitory concentration of a derivative which inhibits microbial growth by 100%). The IC_{50} and MIC values were read from toxicity curves. MIC experiments on subculture dishes were used to assess the minimal microbicidal concentration (MMC). Subcultures were prepared separately in Petri dishes containing competent agar medium and incubated at 30 °C for 48 h (bacteria, yeasts) and at 25 °C for 96 h (filamentous fungi). The MMC value was taken as the lowest concentration, which showed no visible growth of microbial colonies in the subculture dishes.

Chromatographically pure compounds were dissolved in dimethylsulfoxide (DMSO); its final concentration never exceeded 1.0% vol. in either control or treated samples. Concentration of tested compounds was in the range of 0.01–3.00 mmol L^{-1} in all experiments.

Results and discussion

The prepared compounds $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**), $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**), $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**), $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**), $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**) and $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**) are white in colour, stable on air and light. They are: soluble in H_2O, CH_3OH, C_2H_5OH, DMFA; insoluble in CCl_4; a little soluble in $CHCl_3$, $(C_2H_5)_2O$, $(CH_3)_2CO$, DMSO.

The results of elemental analysis (Table 1) are in a good agreement with calculated ones. The presence of individual functional groups was confirmed by IR spectra (Table 2). The difference between asymmetric and symmetric carboxylate vibration was calculated ($\Delta = \nu_{as}(COO^-) - \nu_s(COO^-)$) and this value was used as a criterion of carboxylate anion coordination to metal ions. It was found for sodium salts of formiate and acetate, that if the Δ value is less than 200 cm^{-1}, it indicates bidentate coordination of carboxylate group. If it is more than 200 cm^{-1} carboxylate group is monodentately coordinated [19]. According to the literature data we propose monodentate binding mode of carboxylate anion (Δ in the range 208–212 cm^{-1}) in prepared compounds (**I**, **II**, **IV**, **V**) and bidentate binding mode (Δ in the range 170–185 cm^{-1}) in compounds (**III**, **VI**) (Table 2).

Thermal behaviour

$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**)

The thermal decomposition of compound (**I**) (Fig. 1), starts with the release of two moles of water in the temperature range 90–170 °C shown on the DTA curve as an endothermic effect at 112 °C (the experimental mass loss 9.77%; the theoretical mass loss 9.59%). In the next step of the thermal decomposition in the temperature range 170–345 °C salicylic acid (m/z = 138) is released.

Table 2 IR spectroscopic data of the prepared compounds $\nu[cm^{-1}]$

Assignment/compound	(I)	(II)	(III)	(IV)	(V)	(VI)
ν(O–H) H_2O	3,313 s	3,411 s	3,456 m	3,358 s	3,424 s	3,454 m
$\nu(C–H)_{ph}$	3,072 s	3,068 m	3,007 m	–	–	–
$\nu(C–H)_{alif}$	–	–	2,922 m	–	–	28,54 m
$\nu(C{=}O)_{u,\ tph}$	–	1,660 s	1,701 vs	–	1,660 s	1,686 s
δ(O–H) H_2O	1,625 s	–	1,647 vs	1,628 s	1,628 s	1,635 s
δ(O–H)ph	1,337 s	1,346 s	1,331 m	1,338 s	1,327 m	1,325 m
$\nu(C–OH)_{ph}$	1,239 s	1,242 s	1,252 s	1,246 vs	1,245 s	1,246 m
$\nu_{as}(COO^-)$	1,597 s	1,594 s	1,562 s	1,589 s	1,590 s	1,558 s
$\nu_s(COO^-)$	1,385 s	1,382 s	1,392 s	1,379 m	1,382 m	1,373 m
Δ_{COO}	212	212	170	210	208	185
$\nu(C–C)_{arom}$	1,487 s; 1,467 s	1,484 s; 1,467 s	1,485 s; 1,448 s	1,477 s, 1,441 vs	1,473 s; 1,444 s	1,471 s;1,431 s
$\nu(N–H)_{tph,\ u}$	–	3,338 s	3,350 m	–	3,341 m	3,400 m
$\delta(COO^-)$	685 m	672 m	671 m	652 m	652 w	681 w
$\gamma(C–H)_{ph}$	764 s	748 s	762 s	752 m	776 w	768 m
ν(C–Cl)	–	–	–	727 s	719 m	714 m

Ph phenyl, *tph* theophylline, *u* urea

$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**); $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**); $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**); $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**); $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**); $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**)

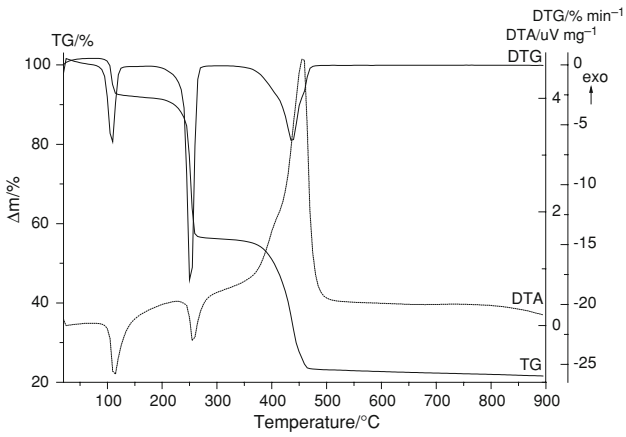


Fig. 1 TG/DTG and DTA curves of $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**)

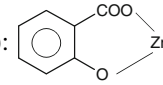
There were found other mass fragments *m/z* for salicylic acid: $C_5H_4^+$ (64), $C_6H_4O^+$ (92), $C_7H_4O_2^+$ (120). The proposed mass fragmentation of salicylic acid is shown in Scheme 1 and it is in a good agreement with the results of Smith [20]. The release of salicylic acid is accompained by endothermic effect on the DTA curve at 256 °C (the experimental mass loss 36.09%; the theoretical mass loss 36.77%) and solid intermediate $Zn(C_6H_4\text{-}2\text{-}(O)COO)$ is formed. The absorption band of phenolic group δ(OH) at 1,337 cm^{-1} in the solid intermediate product is missing. In the temperature range 345–520 °C solid intermediate decomposes and CO_2 ($m/z = 44$) and H_2O ($m/z = 18$) are released with an exothermic effect at 456 °C. The final solid product of the thermal decomposition is ZnO (the final experimental mass value 21.53%; the theoretical mass value 21.67%) (Table 3). The mechanism of the thermal decomposition of (**I**) is similar to the thermal decomposition of compound with four molecules of water [13]. The following mechanism is proposed for the thermal decomposition:

$$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O \xrightarrow{90-170\,^\circ C} Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 + 2H_2O$$

$$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \xrightarrow{170-345\,^\circ C} Zn(C_6H_4\text{-}2\text{-}(O)COO) + C_6H_4\text{-}2\text{-}(OH)COOH$$

$$Zn(C_6H_4\text{-}2\text{-}(O)COO) \xrightarrow[345-520\,^\circ C]{7O_2} ZnO + 7CO_2 + 2H_2O$$

$Zn(C_6H_4\text{-}2\text{-}(O)COO)$:



$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**)

The thermal decomposition (Fig. 2) of compound (**II**) starts with the release of water, urea, salicylic acid ($m/z = 138$), CO_2 ($m/z = 44$) and H_2O ($m/z = 18$) with endothermic and exothermic effect at 102 and 533 °C in the temperature range 75–564 °C (the experimental mass loss 83.21%; the theoretical mass loss 83.58%). ZnO was found as the final product of the thermal decomposition (the final experimental mass value 16.79%; the theoretical mass value 16.42%) (Table 3).

Table 3 Thermal decomposition of the prepared compounds

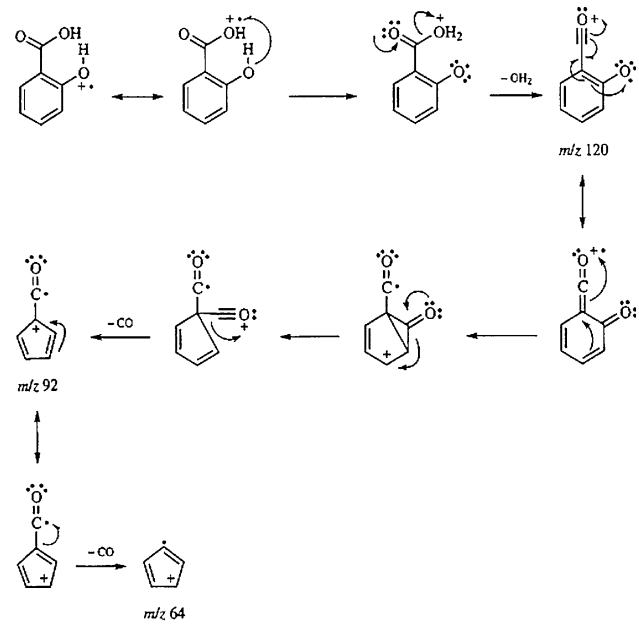
Compounds	DTA T/°C	Products of the thermal decomposition	Mass loss/%	
			Exp.	Theor.
$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**)	112/endo	$2H_2O$	9.77	9.59
	256/endo	Sal.acid	36.09	36.77
	456/exo	CO_2, H_2O	32.61	31.97
	520	ZnO	21.53	21.67
$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**)	102, 223/endo; 535/exo	$2H_2O$ + 2u + sal.acid + CO_2 + H_2O	83.21	83.58
	580	ZnO	16.79	16.42
$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**)	133/endo	$2H_2O$	5.36	4.90
	212/endo; 469, 540, 599/exo	Sal.acid + 2 theophylline + CO_2 + H_2O	81.53	84.04
	640	ZnO	13.11	11.06
$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**)	151/endo	$2H_2O$	8.03	8.10
	256/endo; 489/exo	5-Clsal.acid + C_6H_5Cl + CO_2	72.78	74.04
	600	ZnO	19.19	18.30
$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**)	116, 154/endo	H_2O	2.68	2.70
	215, 233/endo;462,502,589/exo	4u + 5-Clsal.acid + C_6H_5Cl + CO_2	84.63	85.09
	640	ZnO	12.96	12.21
$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**)	135/endo	$4H_2O$	8.58	8.57
	272/endo; 469/endo	5-Clsal.acid + 2tph	20.46 + 42.85	20.52 + 42.85
	691/exo	C_6H_5Cl, CO_2	19.68	18.62
	750	ZnO	8.25	9.68

Endo endothermic effect, *exo* exothermic effect

$$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O \xrightarrow[75-564\,^{\circ}C]{7O_2} 2H_2O + 2u + C_6H_4\text{-}2\text{-}(OH)COOH + ZnO + 7CO_2 + 2H_2O$$

$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**)

Two moles of water are released in the temperature range 80–165 °C with an endothermic effect on DTA at 133 °C (the experimental mass loss 5.36%, the theoretical mass loss 4.90%) shown in Fig. 3. Then the thermal decomposition continues with the release of salicylic acid ($m/z = 138$), theophylline ($m/z = 180$), CO_2 ($m/z = 44$) and H_2O ($m/z = 18$) in the temperature range 165–650 °C (the experimental mass loss 81.53%, the theoretical mass loss 84.04%) with an endothermic effect at 212 °C and exothermic effects at 469, 540 and 599 °C. The final solid product of the thermal decomposition is ZnO (the final experimental mass value 13.11%, the theoretical mass value 11.06%) (Table 3). The following mechanism is proposed for the thermal decomposition:



Scheme 1 Mass fragmentations of salicylic acid

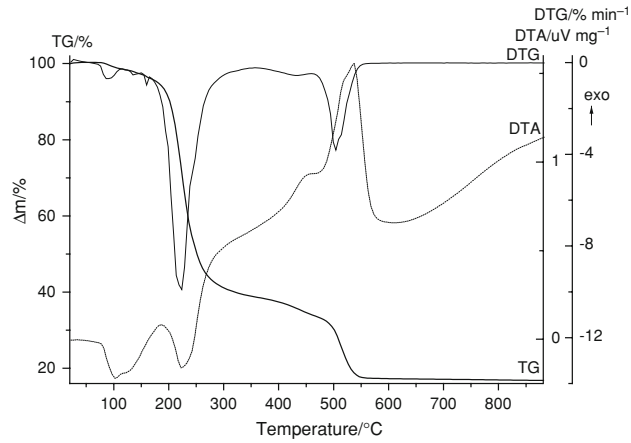


Fig. 2 TG/DTG and DTA curves of $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**)

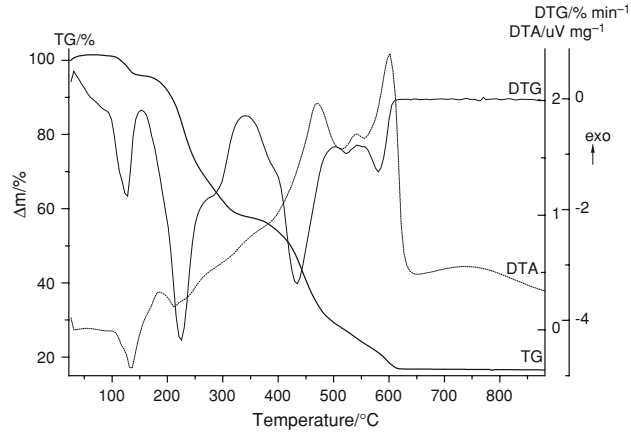


Fig. 3 TG/DTG and DTA curves of $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**)

$$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O \xrightarrow{80-165\,^{\circ}C}$$

$$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph + 2H_2O$$

$$Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \xrightarrow[165-650\,^{\circ}C]{7O_2}$$

$$C_6H_4\text{-}2\text{-}(OH)COOH + 2tph + ZnO + 7CO_2 + 2H_2O$$

$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**)

Compound (**IV**) starts to decompose at 100 °C with the release of two moles of water (Fig. 4) with minimum on the DTA curve at 151 °C (the experimental mass loss 8.03%, the theoretical mass loss 8.10%). The next step of the thermal decomposition is the release of 5-chlorosalicylic acid, C_6H_5Cl and CO_2 ($m/z = 44$) in the temperature range 185–600 °C, with endothermic effect on DTA at 256 °C and exothermic effect at 489 °C (the experimental mass loss 72.78%, the theoretical mass loss 74.04%). ZnO was found as a final product of the thermal decomposition (the final experimental mass value 19.19%, the theoretical mass value 18.30%) (Table 3). The mechanism of the

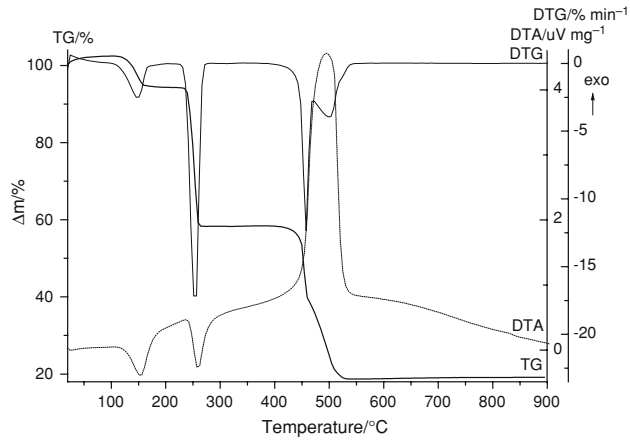


Fig. 4 TG/DTG and DTA curves of $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**)

thermal decomposition is in accordance with results in literature [15]. The thermal decomposition is expressed by the following equation:

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O \xrightarrow{100-185\,^{\circ}C}$$

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 + 2H_2O$$

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \xrightarrow{185-600\,^{\circ}C}$$

$$5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COOH + C_6H_5Cl + CO_2 + ZnO$$

$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**)

The thermal decomposition of compound (**V**) starts in the temperature range 95–163 °C (Fig. 5) with the release of water and with minimum on the DTA curve at 116 and 154 °C (the experimental mass loss 2.68%, the theoretical mass loss 2.70%). In the next step the release of four moles of urea, a mole of 5-chlorosalicylic acid, C_6H_5Cl and CO_2 ($m/z = 44$) takes place, shown on DTA curve at 215 and 233 °C as endothermic effects and at 462, 502, 589 °C as

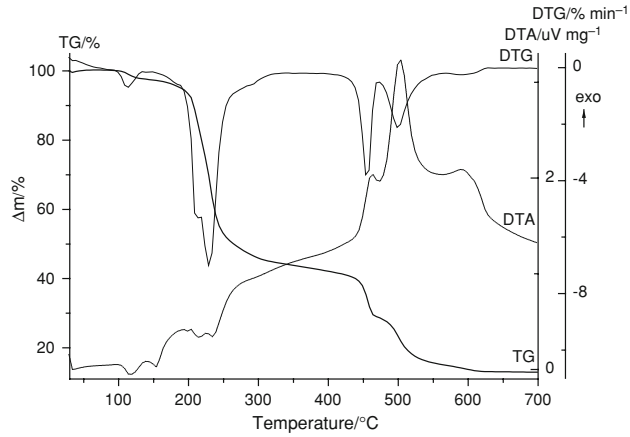


Fig. 5 TG/DTG and DTA curves of $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**)

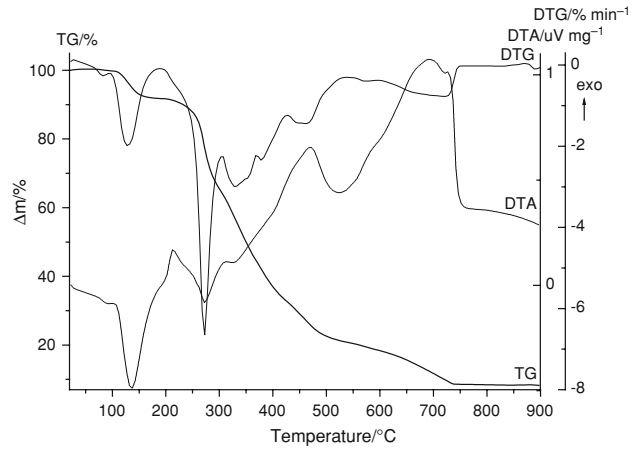


Fig. 6 TG/DTG and DTA curves of $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**)

exothermic effects in the temperature range 163–640 °C (the experimental mass loss 84.63%, the theoretical mass loss 85.09%). ZnO was found as the final product of the thermal decomposition (the final experimental mass value 12.96%, the theoretical mass value 12.21%) (Table 3).

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O \xrightarrow{95-163\,^\circ C} Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u + H_2O$$

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \xrightarrow{163-640\,^\circ C} 4u + 5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COOH + C_6H_5Cl + CO_2 + ZnO$$

$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**)

Compound (**VI**) starts to decompose at 80 °C with the release of four moles of water in the temperature range 80–205 °C. It is shown on the DTA curve (Fig. 6) as an endothermic effect at 135 °C (the experimental mass loss 8.58%, the theoretical mass loss 8.57%). The next step of the thermal decomposition in the temperature range 205–455 °C is the release of 5-chlorosalicylic acid ($m/z = 172$) and two moles of theophylline ($m/z = 180$) (the experimental mass loss 20.46% + 42.85%, the theoretical mass loss 20.52% + 42.85%) with an endothermic effect at 272 and 469 °C on the DTA curve and an intermediate product Zn(5-ClC$_6$H$_3$-2-(O)-COO) is formed. In the temperature range 455–740 °C Zn(5-ClC$_6$H$_3$-2-(O)-COO) decomposes and C_6H_5Cl and CO_2 ($m/z = 44$) are released. The final solid product of the thermal decomposition is ZnO (the final experimental mass value 8.25%, the theoretical mass value 9.68%) (Table 3). The following mechanism is proposed for the thermal decomposition:

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O \xrightarrow{80-205\,^\circ C} Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph + 4H_2O$$

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \xrightarrow{205-455\,^\circ C} Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO) + 5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COOH + 2tph$$

$$Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(O)COO) \xrightarrow{455-740\,^\circ C} C_6H_5Cl + CO_2 + ZnO$$

Table 4 Antimicrobial activity of Zn(II) complexes characterized by numerical values of IC_{50} and MIC (mmol L^{-1})

Zinc complex compound[a]	Bacteria				Yeasts				Filamentous fungi					
	S. aureus		*E. coli*		*C. albicans*		*R. oryzae*		*A. alternata*		*A. fumigatus*		*M. gypseum*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
(**I**)	0.63	2[b]	1.20	3[b]	2.70	>3	>3	>3	2	>3	>3	>3	1.20	2[c]
(**II**)	0.61	>3	1	3[b]	2.30	>3	>3	>3	1.80	3[c]	1.60	>3	2.10	>3
(**III**)	0.61	2[b]	2.10	3[b]	2.50	>3	>3	>3	2.20	>3	>3	>3	1.30	3[c]
(**IV**)	0.51	2[b]	0.70	3[b]	2.30	>3	>3	>3	1	>3	1.70	>3	0.60	2[c]
(**V**)	0.66	2[b]	1.50	>3	3	>3	>3	>3	2.50	>3	2.20	>3	1.20	2[c]
(**VI**)	0.56	2[b]	0.60	3[b]	2.30	>3	2.40	>3	1.80	>3	2.30	>3	1	3[c]
(**VII**)	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	2.50	3[c]
(**VIII**)	>3	>3	>3	>3	>3	>3	>3	>3	2.40	>3	>3	>3	>3	>3
(**IX**)	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
(**X**)	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	2.30	>3

[a] $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**); $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**); $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**); $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**); $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**); $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**); salicylic acid (**VII**); 5-chlorosalicylic acid (**VIII**); urea (**IX**); theophylline (**X**)

[b] Microbistatical effect

[c] Microbicidal effect

Antimicrobial activities

Results of the quantitative determination of antimicrobial activity, characterized by IC_{50} and MIC values, are presented in Table 4. The compounds tested differ in their bioactivities against bacteria, yeasts and filamentous fungi; the bioactivities decrease in the sequence bacteria > filamentous fungi > yeasts.

Comparing the inhibition of growth of *S. aureus* in concentration range 3–0.01 mmol L^{-1} and then comparing the IC_{50} and MIC values it can be stated, that the compounds (**IV**) and (**VI**) inhibited the growth of these bacteria most effectively, $IC_{50} = 0.51$ and 0.56 mmol L^{-1}, respectively; MIC = 2 mmol L^{-1} with bacteristatic effects. As can be seen, the inhibition activity of Zn(II) compounds decreases in the sequences: $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**) > $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 4H_2O$ (**VI**) > $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph \cdot 2H_2O$ (**III**) > $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u \cdot 2H_2O$ (**II**) > $Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**I**) > $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u \cdot H_2O$ (**V**). The effect of the compounds against G^- *E. coli* was considerable lower. Compounds (**IV**) and (**VI**) have shown the highest antibacterial activity, their $IC_{50} = 0.70$ and 0.60 mmol L^{-1}, respectively. Total growth inhibition of G^- *E. coli* was obtained by both compounds at the concentration of 3 mmol L^{-1} with bacteristatic effect on the bacteria cells. All Zn(II) complexes influenced the growth of pathogenic yeasts *C. albicans* weakly ($IC_{50} = 2.30$–3 mmol L^{-1}, MIC >3 mmol L^{-1}). Antifungal efficiency of the tested compounds against filamentous fungi decreased in the order: *M. gypseum* > *A. alternata* > *A. fumigatus* > *R. oryzae*. According to the data presented in Table 4, it can be stated, that the highest antifungal activity shows compound (**IV**) against dermatopathogen *M. gypseum* ($IC_{50} =$ 0.60 mmol L^{-1}). Total growth inhibition was observed at concentration 2 mmol L^{-1} with fungicidal effects on the spores. The maximum inhibitory effect on the growth of phytopathogen *A. alternata* and pathogen *A. fumigatus* was magnifested also in the presence of the compound (**IV**). Tested Zn(II)complexes not influenced the growth of *R. oryzae* ($IC_{50} > 3$ mmol L^{-1}).

Free salicylic acid (**VII**), 5-chlorosalicylic acid (**VIII**), heterocyclic ligands urea (**IX**) and theophylline (**X**), did not affect the growth of model bacteria, yeasts and filamentous fungi ($IC_{50} > 3$ mmol L^{-1}), except *M. gypseum* (**VII**)—$IC_{50} = 2.50$ and MIC = 3 mmol L^{-1} with fungicidal effect on the spores and (**X**)—$IC_{50} = 2.30$ mmol L^{-1}. From the tested compounds compound $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2H_2O$ (**IV**) has shown the highest effect against all model microorganisms (except *R. oryzae*). We have also found that the nitration of N-donor ligands of Zn(II) complexes, in general, not affected positively antimicrobial activities.

Conclusions

After dehydration of compounds (**I**) and (**IV**), salicylic acid or 5-chlorosalicylic acid is released and intermediate products $Zn(C_6H_4\text{-}2\text{-}(O)COO)$ and $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(O)COO)$ are formed. These intermediates result during the thermal decomposition of all prepared compounds. If $Zn(C_6H_4\text{-}2\text{-}(O)COO)$ decomposes, CO_2 and H_2O are released. In the case of intermediate $Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(O)COO)$, C_6H_5Cl and CO_2 are liberated. Compounds with urea (**II**, **V**) after dehydration lose first urea. On the other hand during the thermal decomposition of theophylline compounds (**III**, **VI**), organic ligand theophylline is released after salicylic or 5-chlorosalicylic acid, respectively. The final product of the thermal decomposition in all studied compounds is ZnO.

The thermal stability of dehydrated compounds decreases in the following order:

$$\begin{aligned} &Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 2tph\ (205\,^\circ C) \\ &> Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2\ (185\,^\circ C) \\ &> Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2\ (170\,^\circ C) \\ &> Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2tph\ (165\,^\circ C) \\ &> Zn(5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO)_2 \cdot 4u\ (163\,^\circ C) \\ &> Zn(C_6H_4\text{-}2\text{-}(OH)COO)_2 \cdot 2u\ (150\,^\circ C) \end{aligned}$$

The studied compounds show a stronger antimicrobial activity as the organic ligands (urea, theophylline). Zinc complexes based on 5-chlorosalicylic acid have higher antimicrobial activity than salicylic acid based complexes. It is probably caused by the presence of chlorine atom. The presence of ligands (urea, theophylline) in the tested compounds either did not influence or rarely increased, but mostly decreased the activity of Zn(II) complexes. Compounds (**IV**) and (**VI**) show the highest activity against G^+ bacteria *S. aureus*, G^- bacteria *E. coli* and also against dermatopathogenic fungi *M. gypseum.* N-donor ligands urea and theophylline did not influence the growth of microorganisms.

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References

1. Berg JM, Shi Y. The galvanization of biology: a growing appreciation for the roles of zinc. Science. 1996;271:1081–5.
2. Brewer JG. In: Sigel H, editor. Metal ions in biological systems, vol. 14. New York: Marcel Dekker; 1982. p. 57
3. Körös E. Bioinorganic chemistry. Budapest: Gondolat; 1992.
4. Král'ová K, Šeršeň F, Melník M. Inhibition of photosynthesis in *Chlorella vulgaris* by Cu(II) complexes with biologically active ligands. J Trace Microprobe Tech. 1998;16:491–500.

5. Yao J, Liu Y, Liang HG, et al. The effect of zinc(II) on the growth of *E-coli* studied by microcalorimetry. J Therm Anal Calorim. 2005;79:39–43.
6. Kose AD. Synthesis and characterization of bis(nicotinamide) m-hydroxybenzoate complexes of Co(II), Ni(II), Cu(II) and Zn(II). Russ J Inorg Chem. 2007;52:1384–90.
7. Czakis-Sulikowska D, Czylkowska A, Malinowska A. Mixed-ligand Ni(II) and Zn(II)complexes with bipyridine isomers and bromoacetates. Pol J Chem. 2005;79:1635–43.
8. Ferenc W, Cristovao B, Sarzynksi J. Complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) with 4-chloro-2-methoxybenzoic acid anion—Physico-chemical properties. J Therm Anal Calorim. 2006;86:783–9.
9. Győryová K, Szunyogová E, Kovářová J, Hudecová D, Mudroňová D. Biological and physicochemical study of zinc(II) propionate complexes with N-donor heterocyclic ligands. J Therm Anal Calorim. 2003;72:587–96.
10. Győryová K, Kovářová J, Andogová E, Zeleňák V, Nour El-Dien FA. Thermal, spectral and antimicrobial properties of new zinc(II) isobutyrate compounds. J Therm Anal Calorim. 2002;67: 119–28.
11. Szunyogová E, Mudroňová D, Győryová K, Nemcová R, Kovářová J, Piknová-Findoráková L. The physicochemical and biological properties of zinc(II) complexes. J Therm Anal Calorim. 2007; 88:355–61.
12. Szunyogová E, Győryová K, Hudecová D, Piknová L, Chomič J, Vargová Z, et al. Thermal, spectral and biological properties of Zn(II) complex compounds with phenazone. J Therm Anal Calorim. 2007;88:219–23.
13. Chomič J, Győryová K, Szunyogová E, Kovářová J. Thermal study of zinc(II) salicylate complex compounds with bioactive ligands.J Therm Anal Calorim. 2004;76:33–41.
14. Győryová K, Chomič J, Szunyogová E, Piknová L, Zeleňák V, Vargová Z. Thermal study of zinc(II) 4-chlorosalicylate complex compounds with bioactive ligands. J Therm Anal Calorim. 2006; 84:727–32.
15. Győryová K, Chomič J, Kovářová J. Thermal behaviour of zinc(II) 5-chlorosalicylate complex compounds. J Therm Anal Calorim. 2005;80:375–80.
16. Jantová S, Hudecová D, Stankovský Š, Špirková K, Ružeková L'. Antibacterial effect of substituted 4-quinazolylhydrazines and their arylhydrazones determined by a modified microdilution method. Folia Microbiol. 1995;40:611–4.
17. Dudová B, Hudecová D, Pokorný R, Mikulášová KM, Palicová M, Segľa P, et al. Copper complexes with bioactive ligands. Folia Microbiol. 2001;46:379–84.
18. Hudecová D, Jantová S, Melník M, Uher M. New azidometalkojates and their biological activity. Folia Microbiol. 1996;41:473–6.
19. Nakamoto K. Infrared spectra of inorganic and coordination compounds. 5th ed. New York: Wiley; 1997.
20. Smith RM. Understanding mass spectra. 2nd ed. New Jersey: Wiley; 2004.