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THE PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF ZINC(II) COMPLEXES

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Spectroscopic (IR), thermoanalytical (TG/DTG, DTA) and biological methods were applied to investigate physicochemical and biological properties of seven zinc(II) complex compounds of the following formula $Zn(HCOO)_2 \cdot 2H_2O$ (I), $Zn(HCOO)_2 \cdot tph$ (II), $Zn(CH_3COO)_2 \cdot 2H_2O$ (III), $Zn(CH_3COO)_2 \cdot tph$ (IV), $Zn(CH_3COO)_2 \cdot 2phen$ (V), $Zn(CH_3CH_2COO)_2 \cdot 2H_2O$ (VI), $Zn(CH_3CH_2CH_2COO)_2 \cdot 2H_2O$ (VII), where tph=theophylline, phen=phenazone. The formation of various intermediates during thermal decomposition suggests the dependence on the length of aliphatic carboxylic chain and type of N-donor ligand (tph, phen). The final product of the thermal decomposition was ZnO. The antimicrobial activity of these complexes were tested against G^+ and G^- bacteria. Strong inhibitive effect was observed towards *E. coli*, salmonellae and *Staph. aureus*.

Keywords: *antimicrobial activity, complexes, IR spectra, thermal properties, Zn(II)*

Introduction

Zinc is an essential microelement for all living systems including also microorganisms. Zinc is required to maintain normal physiological and biochemical functions in cells. It is a structural and catalytic cofactor in many bacterial metalloproteins, e.g. zinc-dependent proteinase in the cell wall of *Lactobacillus delbrueckii* subsp. *bulgaricus*, phospholipase C in *Bacillus cereus*, alcohol dehydrogenase isolated from *Mycobacterium bovis*, *Bacillus subtilis* and *Helicobacter pylori* [1–3]. On the other hand zinc inhibits the growth of a lot of bacteria, e.g. *Escherichia coli*, *Streptococcus faecalis* and some strains of soil bacteria [4]. This inhibiting effect of zinc has been used successfully in the treatment of *E. coli* diarrhoe in post-weaning piglets [5]. Moreover, zinc is used in prevention and therapy of many illnesses itself or as a component of drugs (e.g. zinc bacitracin) and biopreparations. Despite its antimicrobial effect, influence of zinc on the probiotic lactobacilli is poorly known.

Zinc(II) carboxylates form a part of coordination compounds that are studied from chemical and biological viewpoints. Our scientific research has been focused on the study of syntheses, spectral, thermal, structural, chromatographic and biological properties of zinc(II) carboxylate complex compounds [6–9].

This paper presents results in thermoanalytical study and biological activity of further zinc(II) carboxylate compounds.

Materials and methods

The following chemicals of A.R. grade were used for the synthesis of the compounds: $ZnCO_3$ (Lachema), HCOOH 86% (Lachema), CH_3COOH 98% (Lachema), CH_3CH_2COOH 99% (Lachema), $CH_3CH_2CH_2COOH$ 98% (Merck), theophylline (Aldrich), phenazone (Aldrich).

Preparation of the complexes

Compounds (I, III, VI, VII) were prepared by the gradual addition of 0.01 mole appropriate carboxylic acid to a water solution of 0.005 mole zinc carbonate. Compounds (II, IV, V) were prepared by dissolving phenazone or theophylline in hot water, followed by addition of a water solution 0.01 mole appropriate carboxylato Zn(II) complexes in a molar ratio 4:1. After stirring, the solutions were reduced in volume at 70°C in a water bath and left to crystallize at room temperature. The complexes which formed were fil-

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Table 1 The results of chemical analysis of the synthesized compounds

Compound	C/%		H/%		N/%		Zn/%	
	theor.	exp.	theor.	exp.	theor.	exp.	theor.	exp.
$Zn(HCOO)_2{\cdot}2H_2O$	15.44	15.01	3.81	3.05	0.00	0.00	34.15	33.92
$Zn(HCOO)_2{\cdot}tph$	32.18	31.97	3.00	2.98	16.69	16.52	19.48	18.50
$Zn(CH_3COO)_2{\cdot}2H_2O$	21.87	21.73	4.59	4.40	0.00	0.00	29.79	29.20
$Zn(CH_3COO)_2{\cdot}tph$	36.30	36.25	3.88	3.71	15.41	15.38	17.98	16.60
$Zn(CH_3COO)_2{\cdot}2phen$	55.80	55.54	5.40	5.22	10.01	9.92	11.68	10.70
$Zn(CH_3CH_2COO)_2{\cdot}2H_2O$	29.11	29.01	5.70	5.65	0.00	0.00	26.43	26.25
$Zn(CH_3CH_2CH_2COO)_2{\cdot}2H_2O$	34.90	34.76	6.59	6.42	0.00	0.00	23.70	23.58

tered off, washed with diethyl ether and dried at room temperature.

Instrumental measurements

The content of C, H and N was determined by CHN analyser PERKIN ELMER 2400 and zinc content was determined complexometrically using Complexone III as an agent and eriochrome black *T* as an indicator.

IR spectra were recorded with EXCALIBUR FTS 3000 MX FTIR spectrophotometer in the region 4000–400 cm^{-1} using the diffusive reflection method.

Thermal decomposition studies were carried out on Paulik–Paulik–Erdey Derivatograph (OD 102, MOM Budapest) in the air atmosphere under dynamic conditions (heating rate 9°C min^{-1}) using platinum crucibles with a sample mass of 100 mg in the temperature range 20–900°C.

The presence of gaseous intermediates was confirmed by IR spectra measured in gaseous cells on SPECORD spectrophotometer and final solid products were identified by X-ray analysis using X-ray powder diffraction analyser MIKROMETA 2 (Czech Republic).

Antimicrobial activity

The antibacterial activity of Zn(II) complexes (I–VII) and $ZnSO_4{\cdot}7H_2O$ in concentration 0.01 mol dm^{-3} were tested against G^- bacteria – *Escherichia coli* $K88^+ent^+$, *Salmonella enterica* Serovar Düsseldorf, *Salmonella enterica* and G^+ bacteria – *Staphylococcus aureus*, *Lactobacillus plantarum* CCM 7102 and *Lactobacillus fermentum* CCM 7158. Pathogenic bacteria were cultivated in PYG broth medium (5 g peptone for bacteriology, 5 g enzymatic caseine hydrolyzate, 10 g yeast extract, 10 g D(+)glucose/1000 mL, pH 7) and counts of cfu (colony forming units) were evaluated on PYG agar (composition as PYG broth+18 g agar/1000 mL, pH 7) after 24 h cultivation at 37°C. Lactobacilli were grown under anaerobic conditions in MRS broth resp. agar (Merck, Germany) at 37°C for 18–24 h resp. 48 h. The strains were also tested in the presence of sodium(I) formate, acetate, propionate and butyrate in a concentration

Table 2 IR spectroscopic data of the prepared compounds/cm^{-1}

Assigment/Compound	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)
$\nu(O–H)_{H_2O}$	3500	–	3550	–	–	3500	3500
$\delta(O–H)_{H_2O}$	1650	–	1680	–	–	1650	1650
$\nu(C=O)$	–	1660	–	1700	1700	–	–
$\nu_{as}(COO^-)$	1590	1620	1580	1650	1600	1540	1530
$\nu_s(COO^-)$	1330	1380	1320	1410	1400	1400	1380
Δ_{COO}	70	240	150	240	200	220	310
$\nu(C–H)_{ph}$	–	–	–	–	3080	–	–
$\delta(C–H)_{ph}$	–	–	–	–	1400–1000	–	–
$\gamma(C–H)_{ph}$	–	–	–	–	720–580	–	–
$\nu(N–CH_3)$	–	–	–	1150	–	–	–
$\nu(N–H)_{pyr}$	–	–	–	3200	–	–	–

ph = phenyl, pyr = pyridine
(I) $Zn(HCOO)_2{\cdot}2H_2O$; (II) $Zn(HCOO)_2{\cdot}tph$; (III) $Zn(CH_3COO)_2{\cdot}2H_2O$; (IV) $Zn(CH_3COO)_2{\cdot}tph$; (V) $Zn(CH_3COO)_2{\cdot}2phen$; (VI) $Zn(CH_3CH_2COO)_2{\cdot}2H_2O$; (VII) $Zn(CH_3CH_2CH_2COO)_2{\cdot}2H_2O$

0.002 mol dm^{-3}. The viable counts of bacteria are expressed as the log 10 of cfu mL^{-1} of growth media.

Results and discussion

The results of chemical analysis are reported in Table 1. The analytical data reveal a good agreement with calculated data.

Characteristic vibrations of the prepared compounds are collected in Table 2 and are in a good accordance with literature data [10].

The presence of water in compounds (I, III, VI, VII) was confirmed by stretching vibration ν(O–H) occurred in the range 3500–3000 cm^{-1} and deformation vibration δ(O–H) occured in the range 1680–1650 cm^{-1}. The parameter Δ_{COO} was determined by analysis of COO^- group band frequencies, where $\Delta_{COO}=\nu_{as}(COO^-)-\nu_s(COO^-)$ and was used as a criterion of carboxylate anion coordination to metal ions. Values of Δ_{COO} were calculated from IR spectra and are less than 200 cm^{-1} for compounds (I, III) that shows bidentately bounded formiate and acetate group and for compounds (II, IV–VII) are in the range 200–310 cm^{-1} that is in a good agreement with literature data for unidentately bounded carboxylate structures [11] along with the three absorption bands of COO deformation at 920–720 cm^{-1} and a strong absorption near 540 cm^{-1}.

Thermal behaviour was studied on the serie of zinc(II) formiate, acetate, propionate, butyrate and thermal decomposition data are reported in Table 3.

The TG/DTG and DTA curves of complex (I) are given in Fig. 1. The compound is stable up to 70°C

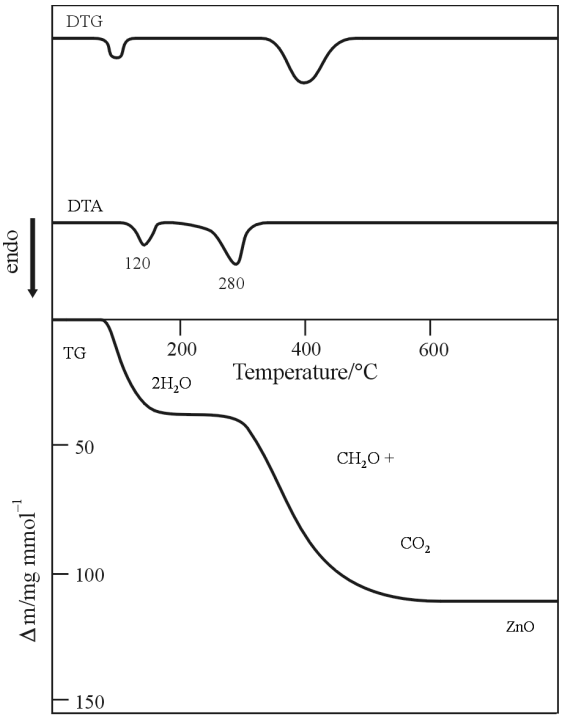

Fig. 1 TG/DTG and DTA curves of $Zn(HCOO)_2{\cdot}2H_2O$

Table 3 Intermediates and final products of the thermal decomposition of the prepared compounds

Compound	DTA peak *T*/°C	Products of the thermal decomposition	Mass loss/mg $mmol^{-1}$	
			exp.	theor.
$Zn(HCOO)_2{\cdot}2H_2O$	120 / endo	$2H_2O$	36.39	36.02
	280 / endo	CH_2O, CO_2	72.79	74.03
	600	ZnO	82.37	81.38
$Zn(HCOO)_2{\cdot}tph$	170 / endo	tph	182.48	180.17
	280 / endo	CH_2O, CO_2	72.99	74.03
	600	ZnO	82.40	81.38
$Zn(CH_3COO)_2{\cdot}2H_2O$	90 / endo	$2H_2O$	35.16	36.02
	130 / endo	$(CH_3)_2CO$, CO_2	105.50	102.00
	600	ZnO	79.12	81.38
$Zn(CH_3COO)_2{\cdot}tph$	180 / endo	tph	179.50	180.17
	300 / endo	$(CH_3)_2CO$, CO_2	103.40	102.00
	600	ZnO	78.80	81.38
$Zn(CH_3COO)_2{\cdot}2phen$	230 / endo	2phen	375.40	376.46
	310 / endo	$(CH_3)_2CO$, CO_2	104.80	102.00
	600	ZnO	78.20	81.38
$Zn(CH_3CH_2COO)_2{\cdot}2H_2O$	220 / endo	H_2O	19.80	18.01
	380, 620 / endo	H_2O, $(C_2H_5)_2CO$, CO_2	143.50	148.00
	900	ZnO	84.16	81.38
$Zn(CH_3CH_2CH_2COO)_2{\cdot}2H_2O$	220, 470 / endo	$2H_2O$, $(C_3H_7)_2CO$, CO_2	192.84	194.00
	900	ZnO	82.64	81.38

endo=endothermic effect

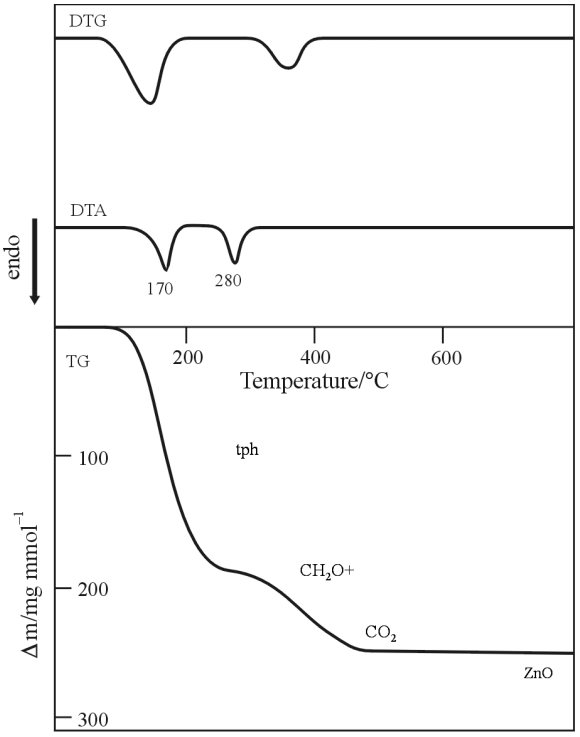

Fig. 2 TG/DTG and DTA curves of $Zn(HCOO)_2 \cdot tph$

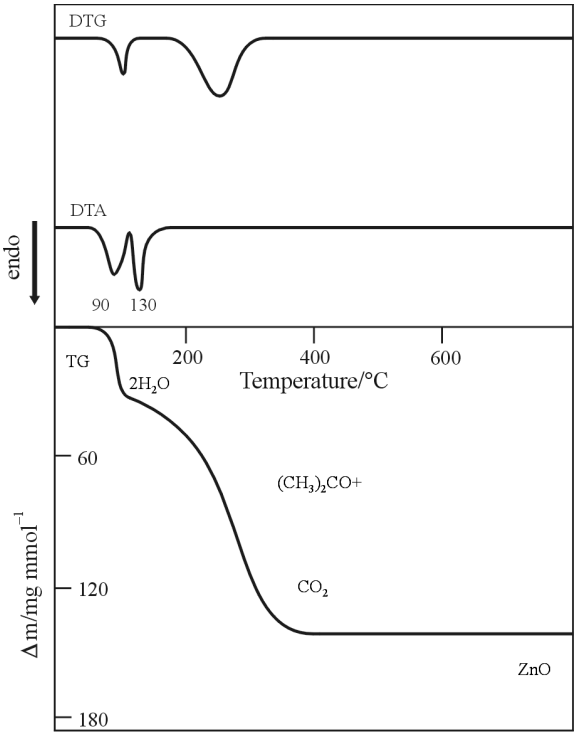

Fig. 3 TG/DTG and DTA curves of $Zn(CH_3COO)_2 \cdot 2H_2O$

where the process of dehydration begins. This is followed by one mass loss step attributed to the release of formaldehyde and carbon dioxide, while the final solid product was ZnO. The following mechanism of the thermal decomposition was proposed:

$$Zn(HCOO)_2 \cdot 2H_2O \rightarrow 2H_2O + CH_2O + CO_2 + ZnO$$

The thermal decomposition of compound (II) starts with the release of theophylline with minimum on the DTA curve at 170°C (Fig. 2). In the next step of the thermal decomposition molecules of formaldehyde and carbon dioxide are released. ZnO was found as a final product. The following reaction was proposed for the mechanism of thermal decomposition:

$$Zn(HCOO)_2 \cdot tph \rightarrow tph + CH_2O + CO_2 + ZnO$$

By the release of two mol of water as depicted in Fig. 3 the thermal decomposition of compound (III) starts. Then, the next step corresponds to the mass loss of acetone and carbon dioxide, on the DTA curve at 120°C. The final product was ZnO. The thermal decomposition can be expressed by the following equation:

$$Zn(CH_3COO)_2 \cdot 2H_2O \rightarrow 2H_2O + (CH_3)_2CO + CO_2 + ZnO$$

Compound (IV) starts to decompose with the elimination of theophylline in one step with minimum on the DTA curve at 180°C (Fig. 4). In next step the release of acetone and carbon dioxide took place and ZnO was found as a final product. The following mechanism of the thermal decomposotion was proposed:

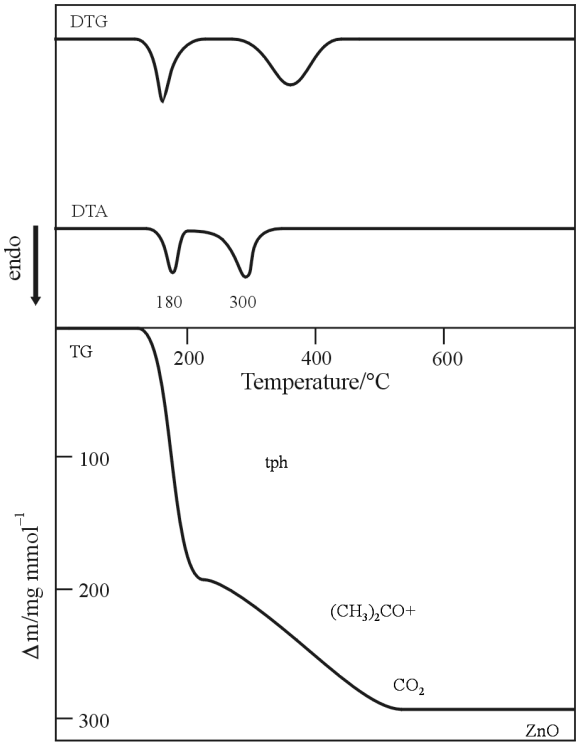

Fig. 4 TG/DTG and DTA curves of $Zn(CH_3COO)_2 \cdot tph$

$$Zn(CH_3COO)_2 \cdot tph \rightarrow tph + (CH_3)_2CO + CO_2 + ZnO$$

The TG curve of compound (V) indicates that the complex is stable up to 160°C as given in Fig. 5. Then two molecules of phenazone are released in one step followed by elimination of acetone and carbon dioxide in next step. The final product was ZnO. The most probable scheme of the thermal decomposition is here:

$$Zn(CH_3COO)_2{\cdot}2phen \rightarrow 2phen+(CH_3)_2CO+CO_2+ZnO$$

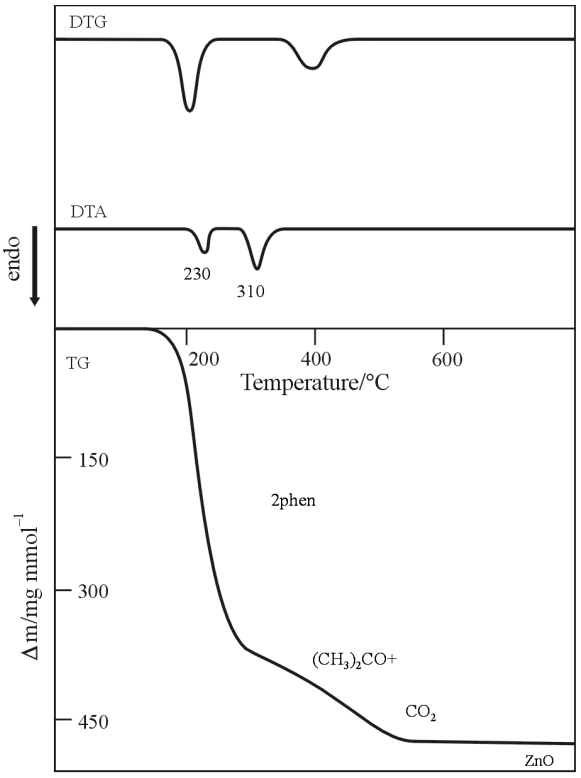

Fig. 5 TG/DTG and DTA curves of $Zn(CH_3COO)_2{\cdot}2phen$

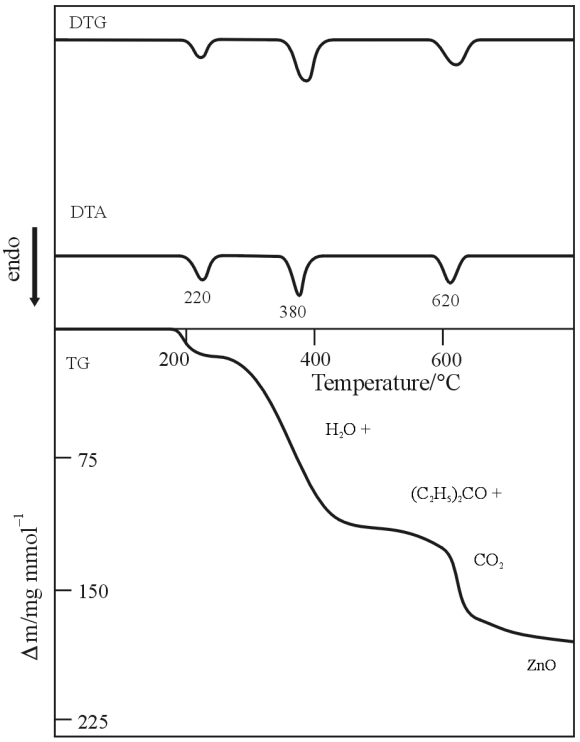

Fig. 6 TG/DTG and DTA curves of $Zn(CH_3CH_2COO)_2{\cdot}2H_2O$

The TG/DTG and DTA curves of compound (VI) are depicted in Fig. 6. The first step of the thermal decompostion begins with the release of water (DTA curve 220°C). Afterwards the DTA curve displays two endothermic peaks with minima at 380 and 620°C that corresponds to the elimination of the second water molecule, diethyl ketone and carbon dioxide. ZnO was found as a final product. The following reaction was proposed for the mechanism of the thermal decomposition:

$$Zn(CH_3CH_2COO)_2{\cdot}2H_2O \rightarrow 2H_2O+(C_2H_5)_2CO+CO_2+ZnO$$

As it is displayed on the DTA curve of compound (VII) (Fig. 7) in two endothermic effects with minima at 220 and 470°C two mol of water, dipropyl ketone and carbon dioxide are released in one step and ZnO was the final product. The mechanism of the thermal decomposition can be expressed by the following equation:

$$Zn(CH_3CH_2CH_2COO)_2{\cdot}2H_2O \rightarrow 2H_2O+(C_3H_7)_2CO+CO_2+ZnO$$

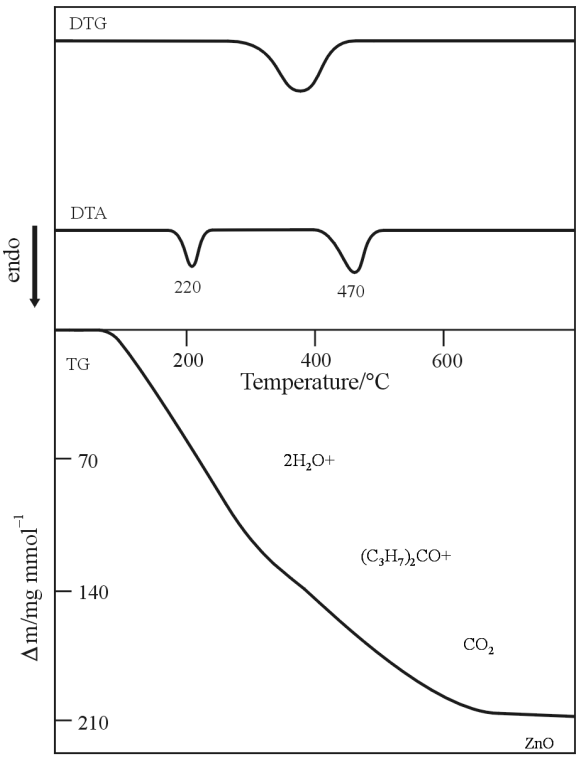

Fig. 7 TG/DTG and DTA curves of $Zn(CH_3CH_2CH_2COO)_2{\cdot}2H_2O$

Table 4 Antibacterial activity of Zn(II) complexes *vs.* G^- bacteria

Compound	*E. coli*		*S. Düsseldorf*		*S. enterica*	
	log cfu	Δ log	log cfu	Δ log	log cfu	Δ log
Control	8.79	–	8.83	–	8.87	
$Zn(HCOO)_2{\cdot}2H_2O$	5.81	3.0	5.76	3.2	4.85	4.0
$Zn(HCOO)_2{\cdot}$tph	2.90	5.9	0.00	8.8	3.81	5.1
$Zn(CH_3COO)_2{\cdot}2H_2O$	6.20	2.6	6.20	2.6	7.00	1.9
$Zn(CH_3COO)_2{\cdot}$tph	3.38	5.4	4.18	4.7	4.04	4.8
$Zn(CH_3COO)_2{\cdot}$2phen	5.62	3.2	4.90	3.9	6.00	2.9
$Zn(CH_3CH_2COO)_2{\cdot}2H_2O$	5.91	2.9	6.20	2.6	5.92	2.9
$Zn(CH_3CH_2CH_2COO)_2{\cdot}2H_2O$	5.57	3.2	6.08	2.8	–	–
$ZnSO_4$	5.85	2.9	6.46	2.4	6.96	2.2

Table 5 Antibacterial and probiotic activity of Zn(II) complexes to G^+ bacteria

Compound	*Staph. aureus*		*L. plantarum*		*L. fermentum*	
	log cfu	Δ log	log cfu	Δ log	log cfu	Δ log
Control	8.57	–	8.82	–	9.35	–
$Zn(HCOO)_2{\cdot}2H_2O$	8.28	0.3	8.43	0.4	9.56	–0.2
$Zn(HCOO)_2{\cdot}$tph	6.67	1.9	6.20	2.6	8.32	1.0
$Zn(CH_3COO)_2{\cdot}2H_2O$	8.63	–0.1	8.86	0.0	9.67	–0.3
$Zn(CH_3COO)_2{\cdot}$tph	6.63	1.9	8.28	0.5	9.04	0.3
$Zn(CH_3COO)_2{\cdot}$2phen	5.72	2.8	8.76	0.1	9.13	0.2
$Zn(CH_3CH_2COO)_2{\cdot}2H_2O$	7.94	0.6	8.61	0.2	9.37	0.0
$Zn(CH_3CH_2CH_2COO)_2{\cdot}2H_2O$	7.28	1.3	8.28	0.5	9.13	0.2
$ZnSO_4$	8.54	0.0	8.57	0.3	9.35	0.0

The results of antimicrobial activity are listed in Tables 4–5. Microbiological studies confirmed a strong antibacterial activity of Zn(II) complexes (I–VII) against G^- pathogens. If Zn^{2+} ion in alkylcarboxylates (I, III, VI, VII) was substituted by Na^+ ion, no antimicrobial activity was found as it is known from literature [12]. Based on these results, it is supposed that zinc is a 'carrier' of an antibacterial activity in the tested zinc compounds without ligands. Compound (II) is the strongest inhibitor of G^- bacteria. This compound absolutely inhibited the growth of *S. enterica* Serovar Düsseldorf (cfu=0) and suppressed the growth of *S. enterica* and *E. coli* by 5–6 log. Similar results were achieved by testing compound (IV) that made counts of G^- strains lower by cca 5 log. In comparison to compounds (I, III, VI, VII) which inhibited the growth of G^- bacteria by 2–4 log, it was confirmed that the main antibacterial component of all compounds was theophylline and G^- bacteria were sensitive to this heterocyclic ligand. The antibacterial activity of theophylline is well known, especially as one of alkaloids contained in the tea leaves [13]. Compound (V) was more active *vs.* pathogens by 3–4 log than compound (III) that inhibited the growth of G^- strains by 2–2.5 log. This confirms that antibacterial effect of zinc is enhanced also by phenazone.

The sensitivity of G^+ bacteria to the tested compounds (I–VII) was higher than that of G^- ones that corresponds with literature data [12]. The counts of *Staph. aureus* were mostly inhibited by compound (V) (2.9 log) and compounds (II, IV) containing theophylline (cca 2 log). It can be supposed that the growth of *Staph. aureus* was inhibited especially by theophylline and phenazone.

The counts of *L. plantarum* that is used as a probioticum for piglets was the most strongly inhibited by compound (II) (2.6 log). The other compounds (I, III–VII) inhibited its growth more weakly or nowise. The counts were lowered in the range 0.1–0.5 log and compound (III) had no effect on the growth of bacteria. The most resistant to the tested zinc compounds was *L. fermentum*. This strain is used in the probiotic preparations for poultry and rabbits. Its growth was inhibited only by compound (II) and weakly by compounds (IV, V, VII). Compound (VI) and $ZnSO_4$ have not influenced the numbers of this strain. On the other hand, the compounds (I, III) had weak stimulative effect on its growth.

Conclusions

The thermal behaviour of studied compounds depends on the length of aliphatic chain. Thermal decomposition of hydrated compounds starts with the release of water in the temperature range 70–220°C. After dehydration, organic ligand take place and carboxylate anion decomposes and CO_2 and ketone are liberated. Volatile intermediates and solid final product were confirmed by X-ray diffraction method and methods of qualitative chemical analysis.

Microbiological studies confirmed a strong antibacterial activity of tested complexes. The strongest antagonistic activity against G^+ and G^- bacteria was found in compound (II). Compounds (II, IV, V) showed a stronger antibacterial activity than basic compounds (I, III, VI, VII). It confirms that theophylline and phenazone enhance inhibitory activity of zinc. The weakest compound of all tested complexes was compound (III). The most resistant to the tested zinc carboxylate complexes were both probiotic strains of lactobacilli. These strains can be used in the feedstuffs containing higher amount of zinc without inhibition of their growth activity.

The achieved results can be used in selective inhibition of the growth of G^- and G^+ bacteria and in development of new preparations that can suppress the growth of some pathogenic bacteria in a digestive tract and at the same time it can be a zinc source for a macroorganism.

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