SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
17 (2011)

SYNTHESIS AND THERMODYNAMIC PROPERTIES OF AMORPHOUS CALCIUM PHOSPHATE

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Received September 30, 2011

The formation of amorphous calcium phosphate (ACP) was studied in the context of urinary stones formation. Processes of ACP precipitation were observed in simulated medium: in aqueous solutions of potassium chloride and artificial urine (SRAU) at the ionic strength of 0.3 mol dm^{-3}. Amorphous structure of calcium phosphate was verified by X-ray diffraction analysis and Raman spectroscopy. Thermochemical study of ACP precipitation was investigated by using the isoperibolic reaction twin calorimeter. The experiments were carried out at the initial concentrations of ACP in the range from 6 to 14 mmol dm^{-3} for KCl solution and 6-16 mmol dm^{-3} for SRAU. The ACP precipitation was performed by reaction of Ca^{2+} and $H_2PO_4^-$ ions with Ca:P molar ratio close to 1.5 at the temperatures of 298.15 K and 310.15 K. The molar enthalpy of ACP formation was calculated by use of ACP solubility which was determined in this work. Molar enthalpies of ACP formation, ΔH_m, are 29.8 ± 0.9 kJ mol^{-1} at 298.15 K and 36.3 ± 0.9 kJ mol^{-1} at

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310.15 K in KCl solution, and $\Delta H_m^{298.15} = 35.4 \pm 1.3$ *kJ mol*$^{-1}$ *and* $\Delta H_m^{310.15} = 41.6 \pm 1.5$ *kJ mol*$^{-1}$ *in SRAU.*

Introduction

ACP phase is one of the most frequent forms of calcium phosphate minerals (CaP) in biological organisms. They have been found, for example, in the mitochondria of eukaryote and prokaryote cells and ACP is still considered a precursor phase of bone mineral in the form hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) [1]. The ACP transformation to crystalline material has been subjected to numerous investigations for its high bioactivity and excellent biodegradable properties. Different formulas of ACP are presented in the literature, e.g., $Ca_xH_y(PO_4)_z{\cdot}nH_2O$ [1], $Ca_x(PO_4)_y$ [2] or $Ca_3(PO_4)_2$ (am) [3]. The structures and methods for the synthesis of many phosphatic compounds are already well known. But very few experimental thermochemical studies have been undertaken.

ACP is used to preparation of crystalline calcium phosphates with different compositions [4,5]. CaP can be used in various bio-applications, for example: surface coating of replacement protheses, filler [6,7], bone cements [8] and metal implants [9], bio-ceramics preparation, repair of periodontal bone defects [10,11], ridge augmentation [12], ear implants, drug carrier for controlled release of bioactive molecules based on adsorption/desorption properties [13]. Furthermore, calcium phosphates material is used in apparel industry, production of fertilizer, field of catalyst [14,15] and environmentalism [16,17].

Abnormal accumulation of CaP in a body is called pathological calcification, e.g., soft tissue calcification (damaged joints, blood vessels), kidney and urinary stones, dental calculus, gall stones, coronary calcification, atherosclerotic arteries and veins. This work is focused on examination of urinary stones. General conditions that contribute to stone formation are high concentration of salts in urine, retention of these salts and crystals, pH, infection, and a decrease in the body's natural inhibitors of crystal formation. Urinary stones are often heterogeneous, containing mainly oxalates, phosphates and uric acid crystals. Other, minor phases in urolithiasis are, e.g., cystine, xanthine, calcium carbonate, silicon dioxide or calcium sulphate. Calcium phosphates and their precursor ACP often form the nucleus upon which other urinary minerals are deposited, and they tend to be formed in alkaline urine (pH > 7.5).

The structure of ACP was first determined by Eanes and Posner [18-20]. A short range order was evidence in ACP, corresponding to $Ca_9(PO_4)_6$ units with an average diameter of 0.95 nm, referred to as "Posner's clusters". These clusters correspond to a local arrangement of calcium and phosphate ions existing in the structure of apatites. A representation of the Posner's cluster is given in Fig. 1. This arrangement is analogous to that existing in several other crystalline

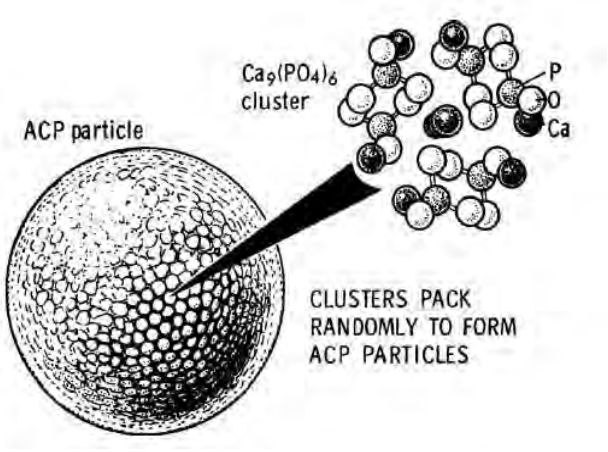

Fig. 1 ACP particle, showing one Posner's cluster

phosphates, such as apatites, hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) or β-tricalcium phosphate (β-TCP, $Ca_3(PO_4)_2$).

The formation of calcium phosphates was studied under different initial conditions, such as molar ratio of Ca/P, reaction time, choice of medium, temperature and the initial pH of the mixture [3,21,22]. Kibalczyc *et al*. investigated the kinetics of formation and conversion of calcium phosphates by calorimetric methods [21,23]. The experimental data presented in Kibalczyc's work show that the formation of calcium phosphates was associated with endothermic effect and the enthalpies of formation ΔH increase with increasing temperature. They also studied the dependence of the heat of formation on the initial pH of the mixtures with different molar ratios of Ca/P. The authors concluded that the best conditions for ACP formation were found at pH ~ 7.5 and the molar ratio Ca/P = 1.5.

The aim of the present study is to examine the solubility of ACP in KCl and SRAU and formation enthalpy of pure ACP under similar conditions, and to study the influence of SRAU components on ACP formation.

Experimental

ACP was prepared by rapid mixing of aqueous solutions of calcium and phosphate salts. Calcium chloride (23.1 g $CaCl_2 \cdot 2H_2O$ dissolved in 200 cm^3 distilled water) was mixed with monobasic potassium phosphate solution (13.6 g KH_2PO_4 dissolved in 200 cm^3 distilled water) at room temperature. Initial pH of the mixed solution was adjusted by adding KOH solution in accordance with Kibalczyc's study [23]. The precipitate was filtered and washed with distilled water and ethanol. The product was dried between two filter papers and this fresh gel was used for solubility determination.

All the dissolution experiments were performed in tempered cells with an ion-selective electrode (ED Ltd. Turnov, the Czech Republic) to determine Ca^{2+} ion concentration. The systems were stirred with magnetic stirrer (speed 500 RPM). The solubility of ACP was measured in distilled water, KCl solution (I = 0.3 mol dm^{-3}) and SRAU at 298.15 ± 0.1 and 310.15 ± 0.1 K. SRAU is aqueous solution of NaCl, Na_3Cit, Na_2SO_4, NaH_2PO_4, $MgSO_4$, KCl, NH_4Cl with ionic strength I = 0.3 mol dm^{-3} [24]. Dissolution of amorphous gel was observed by means of a calcium selective electrode. The quantity of Ca^{2+} ion in saturated solution was determined by atomic absorption spectrometer GBC 906 AA (GBC, Australia). All measurements were repeated 5 times for each temperature and each solution. These solubilities were used for determination of molar enthalpy of formation, ΔH_m.

A calorimetric investigation of ACP precipitation was employed using an isoperibolic twin calorimeter. This instrument was constructed at the Department of Inorganic Technology, and its details were fully described elsewhere [25,26]. The experiments were carried out by mixing of $CaCl_2$ and KH_2PO_4 solutions. The precipitated product was amorphous with molar ratio Ca/P = 1.5. The ACP formation was studied in aqueous KCl solution and artificial urine (ionic strength 0.3 mol dm^{-3}) at the same conditions as those used for disolution of ACP. The precipitation was carried out at the initial concentrations of ACP in the range from 6 to 14 mmol dm^{-3} from KCl solution, and from 6 to 16 mmol dm^{-3} from SRAU at the temperatures of 298.15 K and 310.15 K. The reaction and reference vessels were placed in a water bath at constant temperature before each experiment for at least 15 min. They were subsequently transferred into the calorimeter body and kept at equal temperature for next 15 min. The reaction vessel contained 100 cm^3 of $CaCl_2$/KCl solution, and the syringe contained 7 cm^3 of KH_2PO_4/KOH solution. The reference vessel did not contain any crucial component for precipitation of ACP (Fig.2).

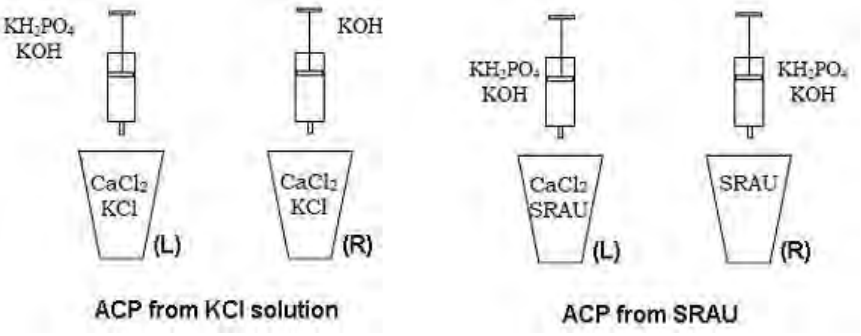

Fig. 2 ACP precipitation from solutions: reaction (L) and reference systems (R)

The precipitation of ACP from artificial urine was disposed in a different way from that used in previous setting in KCl solution. The reaction vessel contained 100 cm^3 $CaCl_2$/SRAU solution and the syringe contained 7 cm^3 KH_2PO_4

solution with KOH. The reference vessel only contained SRAU, while the syringe was filled up with 7 cm^3 KH_2PO_4/KOH solution. This arrangement prevented premature precipitation. All the measurements were repeated 5 times for each tested concentration and temperature.

The precipitation of ACP was characterized by X-ray diffraction analysis (XRD), Raman spectroscopy (RS) and determination of particle size using a Mastersizer 2000 MU apparatus (Malvern Instruments Ltd., United Kingdom).

Results and Discussion

Amorphous calcium phosphate was obtained by rapid precipitation from solutions of $CaCl_2$ and KH_2PO_4 with addition of KOH. Structures of ACP were verified by XRD, as shown in Fig. 3. This figure shows a broad amorphous peak characteristic of the amorphous phase without diffractive lines. The shape of diffractogram is in good accordance with the results previously published by Bienstock and Posner [27]. In contrast, the commercially available cHA (99.999%) reveals well-formed peaks, indicative of diffraction from crystal planes in the randomly oriented grains. The diffraction patterns identify the two materials — ACP, HA — and provide a crossreference to Raman spectra. The particles of ACP obtained by precipitation from KCl solution had a size of 11.55 μm, while the precipitation from SRAU solution provided the particle size of 9.36 μm. Differences in the size and shape of X-ray diffractograms of acquired ACP are caused by the presence of certain components in SRAU, resulting in noticeable decrease of agglomeration of primary ACP particles (Fig. 3).

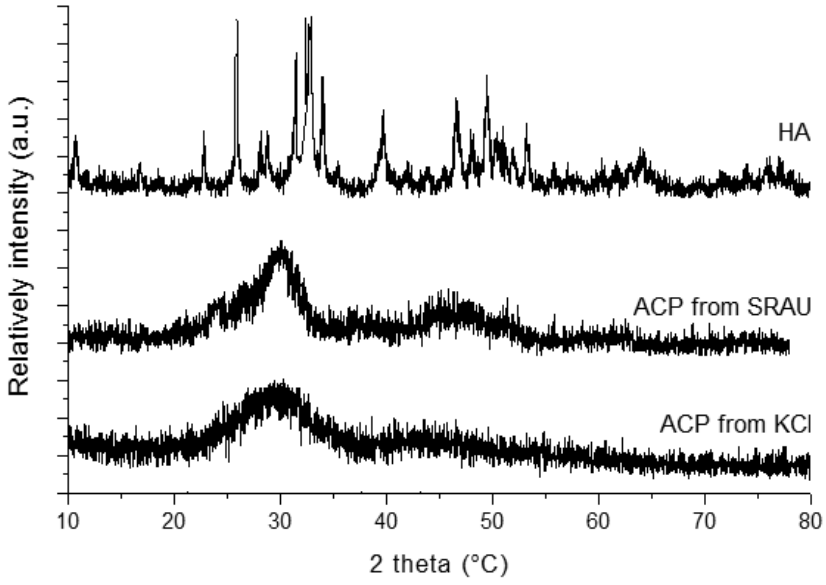

Fig. 3 Diffractograms of ACP and HA

RS produces a broad peak at 950 cm^{-1} for the ACP and a sharp peak at 960 cm^{-1} for the sintered HA, published by S. Saber-Samandari and Gross [28]. The broad peak thus serves as an identifier for amorphous calcium phosphate. The results reveal a characteristic peak shift. A peak at 960.2 cm^{-1} is obtained for HA, but the broad peak is displaced to a lower wavenumber of 954.9 cm^{-1} for the ACP (see Fig. 4).

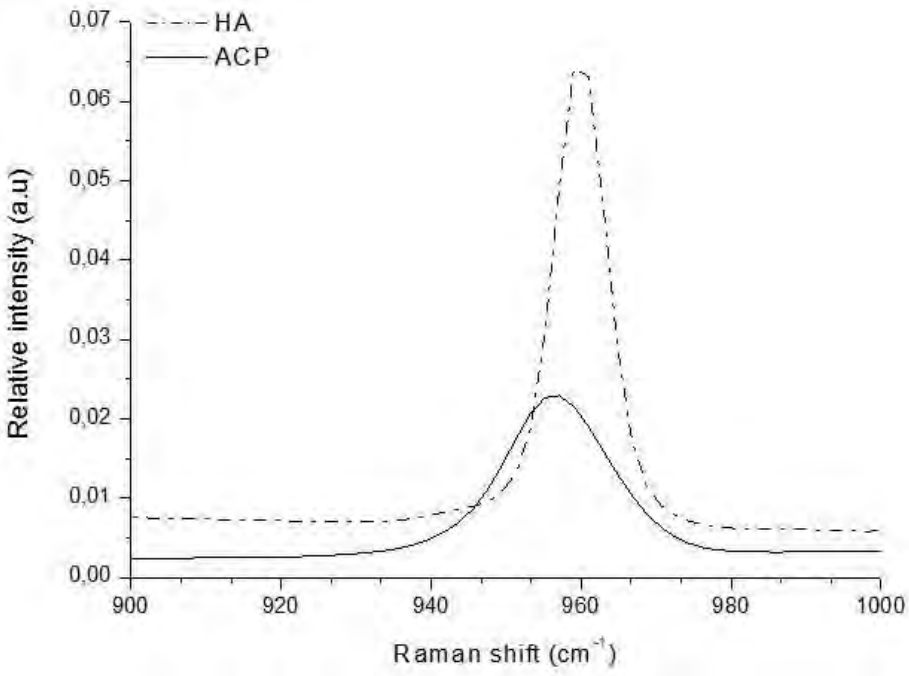

Fig. 4 Characteristic Raman shift between ACP and HA

The solubility of ACP was studied in distilled water and simulated solutions. Simulated solutions were as follows: Aqueous solution of KCl as the simplest simulated physiological medium and SRAU solution of ionic strength 0.3 mol dm^{-3} [24]. Solubility of ACP was determined in these solutions and is reported in Table I. Lower ACP solubility in SRAU can be due to the presence of inhibitors such as citrate and Mg^{2+} ions.

Table I ACP solubility in simulated solutions at the temperatures of 298.15 and 310.15 K

T, K	$m \times 10^3$, g per 100 g H_2O	
	KCl	SRAU
298.15	3.42 ± 0.04	2.98 ± 0.02
310.15	3.47 ± 0.03	2.28 ± 0.03

Solubility of ACP in distilled water was determined (Table II). The higher solubility of ACP in artificial solutions than in distilled water is probably due to

Table II ACP solubility in distilled water at the temperatures of 298.15 and 310.15 K

T, K	$m\times10^3$, g per 100 g H_2O
298.15	1.53 ± 0.01
310.15	1.47 ± 0.02

Table III Solubility of related phosphates

T, K	$m\times10^6$, g per 100 g H_2O	
	HA[1)]	TCP[2)]
298.15	4.49	20.1
310.15	4.38	15.0

[1)] Data by McDowell *et al*. [29]; [2)] Data by Gregory *et al*. [30]

the presence of Cl^-, citrate etc. ions that favour formation of Ca-Cl or Ca-Citrate complexes in the solution. The ACP solubility was compared with related calcium phosphates such as HA and TCP. Solubilities of HA and TCP are shown in Table III. As it can be seen, ACP solubility is remarkably higher than solubilities of HA and TCP.

Precipitation of ACP in the isoperibolic calorimeter was realized by the procedure described in experimental part. Voltage output of the differential resistance bridge, as is reported by Söhnel *at al*. [26], was converted through calibration to the integral heat. The calorimeter was standardized by the endothermic process of solid KCl in distilled water by National Bureau of Standards [31], and the accuracy of isoperibolic calorimeter was determined to be 2.98 %.

Experimental data of ACP precipitation at the temperatures of 298.15 and 310.15 K are presented in Tables IV and V. The molar precipitation enthalpy ΔH_m was derived from our experimentally determined solubility of ACP (Table I) and initial concentration of ACP.

The enthalpy of ACP precipitate increases with increasing initial concentration of ACP in the range of 6-10 mmol dm^{-3}. Higher initial concentration of ACP did not lead to a further increase in ΔH_m value; graphic account of the precipitation of ACP from SRAU is better shown in Fig. 5.

The diverse value of molar enthalpy ΔH_m is probably caused by variable molar ratios of Ca^{2+} ion and components of SRAU, primarily Mg^{2+} and citrate. Decreased values of initial concentration of ACP resulted in decreased molar ratios Mg^{2+}:Ca^{2+}, Citrate:Ca^{2+} and consequent decrease in the formation ACP precipitate (Table VI). Thus, these components are considered as inhibitors of this reaction, which is in accordance with Kibalczyc [32]. The inhibition effect may be neglected for the values of initial concentration above 10 mmol dm^{-3}. Obviously, ΔH_m is

relatively constant for the concentration range of 10-16 mmol dm^{-3}, which is apparent from Fig. 4.

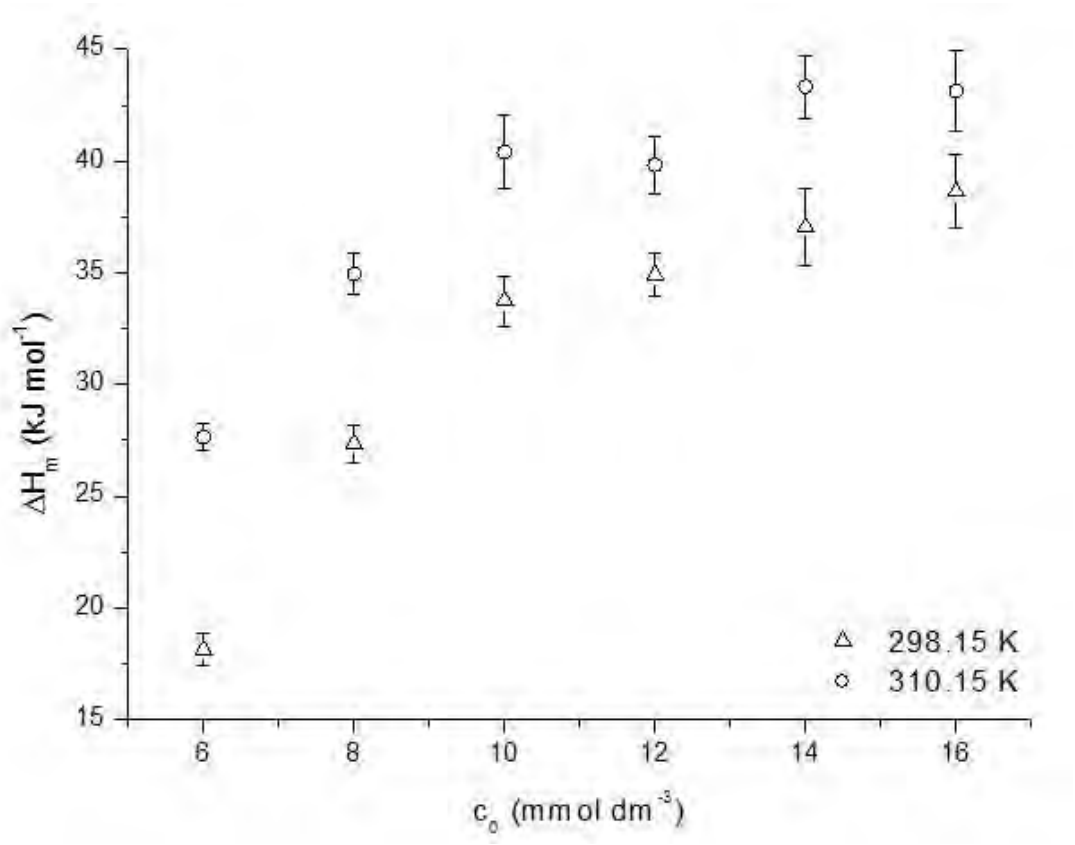

Fig. 5 Precipitation of ACP from SRAU

Table IV Precipitation of ACP from KCl solution

c_0 mmol dm^{-3}	298.15 K		310.15 K	
	ΔH, kJ	ΔH_m, kJ mol^{-1}	ΔH, kJ	ΔH_m, kJ mol^{-1}
6	19.7	29.6	23.8	35.9
8	25.4	28.6	31.1	35.1
10	33.0	29.8	41.3	37.3
12	41.2	31.0	47.5	35.8
14	46.1	29.8	57.7	37.3

$\Delta H_m^{298.15}$ = 29.8 ± 0.9 kJ mol^{-1}

$\Delta H_m^{310.15}$ = 36.3 ± 0.9 kJ mol^{-1}

Table V Precipitation of ACP from SRAU

c_0 mmol dm^{-3}	298.15 K		310.15 K	
	ΔH, kJ	ΔH_m, kJ mol^{-1}	ΔH, kJ	ΔH_m, kJ mol^{-1}
6	11.6	18.1*	17.8	27.6*
8	23.4	27.3*	30.0	34.9*
10	36.0	33.7	43.3	40.4
12	44.8	34.9	51.2	39.8
14	55.4	37.0	64.9	43.3
16	61.9	36.1	73.8	43.1

* – excluded from calculation of molar precipitation enthalpy ΔH_m

$\Delta H_m^{298.15} = 35.4 \pm 1.3$ kJ mol^{-1}

$\Delta H_m^{310.15} = 41.6 \pm 1.5$ kJ mol^{-1}

Table VI Influence of initial concentration on the molar enthalpy of ACP precipitate

c_0, mmol dm^{-3}	$\Delta H_m^{298.15}$, kJ mol^{-1}	$\Delta H_m^{310.15}$, kJ mol^{-1}	Mg^{2+} : Ca^{2+}	Citrate : Ca^{2+}
6	18.1*	27.6*	0.19	0.16
8	27.3*	34.9*	0.14	0.12
10	33.7	40.4	0.11	0.09
12	34.9	39.8	0.09	0.08
14	37.0	43.3	0.08	0.07
16	36.1	43.1	0.08	0.06

* – excluded from calculation of molar precipitation enthalpy ΔH_m

Conclusion

The isoperibolic twin calorimeter represents a reliable and convenient device for the determination of the heat of chemical reaction with accuracy ~ 3 %. In the present study, ACP formation was studied in various simulated solutions — KCl solution as the simplest model of human urine and standard artificial urine (SRAU) — at laboratory and physiological temperature. The precipitation of amorphous calcium phosphate was a quick endothermic reaction that was reached within 10 sec, thus avoiding protection transformation into crystal form. The enthalpies of precipitation were calculated by using ACP solubilities which were published in

this study. In summary, the enthalpy of precipitation values from KCl solution are 29.8 ± 0.9 kJ mol^{-1} at 298.15 K and 36.3 ± 0.9 kJ mol^{-1} at 310.15 K, and for formation from SRAU solution $\Delta H_m^{298.15} = 35.4 \pm 1.3$ kJ mol^{-1} and $\Delta H_m^{310.15} = 41.6$ ± 1.5 kJ mol^{-1}.

Acknowledgements

This study was supported by the Czech Ministry of Education, Youth and Sports under the project MSM 0021627501, and IGA University of Pardubice (SGFChT04). Special thanks to prof. Ing. Miroslav Vlček, CSc.

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