

## Brief notes on previous and recent results of thermoanalytical research of bone

*Csontok termoanalitikai vizsgálatának korábbi és újabb eredményeiről*

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**Abstract** – The understanding of inorganic phases of bone is of high importance for the treatment of bone diseases, and for the development of biocompatible bone replacements as well. This paper gives a brief overview about the thermoanalytical research of bone and bone replacements and shows the results of our research on swine bone. Three calculation models were developed in order to determine the chemical formula of bone apatites. Our results support the theory of the occurrence of distinct calcite phase in bones, along with carbonate containing hydroxylapatite.

**Összefoglalás** – A csontok szeretlen fázisainak ismerete kiemelkedően fontos a csontrendszer érintő megbetegedések kezelése szempontjából, ide értve többek között a biokompatibilis csontpótlások fejlesztését is. Jelen cikkünkben áttekintjük a csontok és csontpótló anyagok termoanalitikai vizsgálatának fontosabb eredményeit, valamint saját, sertéscsontokon végzett kutatásaink eredményeit is bemutatjuk. Háromféle modellszámítást dolgoztunk ki a csontokban előforduló apatitok kémiai képletének meghatározására. Eredményeink alátámasztják azt az elképzelést is, mely szerint a csontokban karbonátos hidroxil-apatit mellett önálló kalcit fázis is előfordul.

**Keywords** – bone, apatite, calcite, thermal analysis, osteoporosis

**Tárgyszavak** – csont, apatit, kalcit, termoanalízis, osteoporózis

### Introduction

The Department of Mineralogy and Geology, University of Debrecen has had significant experience in the thermoanalytical studies of bones and tooth restoration materials for decades by professor emeritus Gyula Szöőr, analyst Éva Balázs and their colleagues. The authors dedicate this paper to the memory of Professor Szöőr.

The results support the treatment, curing and replacement of teeth in a more natural way than today (e.g. PETERS et al. 2000, GUIZZARDI et al. 2000, SARKAR et al. 2001, TADDEI et al. 2005, ODRIUZOLA & MARTÍNEZ-BLANES 2007, OOI et al. 2007, YANG et al. 2008, PEK et al. 2008). In the case of bone replacements the most important problem is the less-known connection between the implants, the organic macromolecules and the inorganic compounds on the surface and inside (e.g. trabecula). If the structure of implants is similar to that of the original bone, the probability of rejections decrease and the period of healing becomes shorter.

The aim of other researches is to study the pathologic formation (osteoblasts) and resorption (osteoclasts) of bone in vivo. The first steps to realise this are in vitro bone and teeth projects of e.g. osteoporosis, calcification of bones, development of bone spurs and their treatment (e.g. MÁNDI et al. 1975, PINTÉR et al. 1975, BOHIC et al. 2000, WANG & FENG 2005, GINEBRA et al. 2006, BENHAMOU 2007).

Remains of bones and teeth are able to give stratigraphic, paleontologic (SZÖÖR 1971, 1979) and archaeological data (e.g. DÁVID 1969, KISZELY 1969, ENZO et al. 2007, PIGA et al. 2008). Minerals in fossil bones can have specific alteration in appropriate circumstances, which qualify it for determination of age, paleoenvironment and sometimes paleoclimate (SZÖÖR & KORDOS 1981, SZÖÖR 1982a,b,c, SZÖÖR et al. 1987).

### Aims

Considering multiple application and importance of bone studies, a well-known bone-structure is expected. However, a lot of studies have been published in the last 50 years, and they contain results inconsistent with each other. Therefore in order to interpret our results reviewing the most remarkable data from these studies is considered to be important.

The most important inorganic components of vertebrate bones are calcium-phosphates with apatite lattice. Despite its physiological relevance, the inorganic structure of bones and teeth are unclear. Modern instrumental analyses and mathematical approximations of the data give only partly consistent results (e.g. RISCHÁK 1982). Under in vivo human conditions this might be the result of perpetual chemical changes in compounds generated by biological activities in quasi-constant temperature, physical-chemical conditions and hormonal effects (MCCONNELL 1979). Consequently researchers would like to describe these changes in components and morphology of bones.

One of the most significant differences between the studies is the summing up of the carbonate content and CO<sub>3</sub><sup>2-</sup> positions in the lattice. Therefore numerous dissimilar, sometimes contradictory methods are developed in bone replacements, preparing, etc.

The investigation described in this paper is a part of a major project for osteoporosis treatment and focuses on studying the structure and chemical composition of the inorganic component of bones, mainly by thermal analytic methods. Thermal gravimetry is appropriate for determining the CO<sub>3</sub><sup>2-</sup> content of inorganic bones, and if completed by differential thermal analyses it is possible to draw conclusions regarding CO<sub>3</sub><sup>2-</sup> position or the presence of a separate calcite phase.

## Structure and composition of apatites in bones

Relevant studies describe the inorganic part of bones as hydroxylapatite (HA) or carbonate-hydroxylapatite (CHA). HA has apatite lattice, which contains OH<sup>-</sup> ions in the channel like axes between the Ca<sup>2+</sup> ions (Fig. 1).

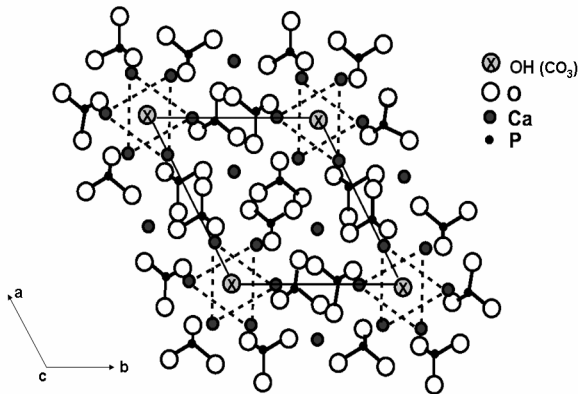


Figure 1 Unit cell of HA or CA viewed from the direction of the *c* axis (after WOPENKA & PASTERIS 2005)

1. ábra A hidroxil-apatit (karbonát-apatit) elemi cellája a kristálytani *c*-tengely irányából nézve (WOPENKA & PASTERIS 2005 alapján)

Composition of crystallographic unit cell of HA is Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>. OH<sup>-</sup> ions in the channel site can be substituted by other anions, principally F<sup>-</sup> and Cl<sup>-</sup>. When substitution is complete, fluorapatite and chlorapatite is formed, respectively. The F<sup>-</sup> ion fits best in the anion channel, and in this case the lattice has a hexagonal symmetry. HA has monoclinic symmetry due to the asymmetry of OH<sup>-</sup> ions. Latest research showed that carbonate-apatite (CA) which has CO<sub>3</sub><sup>2-</sup> ions in the channels has hexagonal lattice as well (SUETSUGU et al. 2000).

Based on TEM researches apatite crystals are plate, tablet or needle-like. Size of crystals is not more than a few nanometers in width and a few tens of nanometers in length (RUBIN et al. 2003, HABERKO et al. 2006). Apatite crystals are located in the collagen fibres, aligned in a fibre, and rotated by 120 degrees around the axis of the fibre. The research of RUBIN et al. (2003) reported differences in the number of trabecula between normal and osteoporotic bones, although the trabecular structure was similar in their bone specimens.

A series of Raman microprobe spectroscopy on cortical bones of mammals shows that the O–H bond is partly or totally absent from the apatites of bones. Since these compounds are not HA (PASTERIS et al. 2004, WOPENKA & PASTERIS 2005), CO<sub>3</sub><sup>2-</sup>-ions are located in the channel with a maximum concentration of about 5–6wt%. LOONG et al. (2000) verifies it and shows the presence of HPO<sub>4</sub><sup>2-</sup>.

CO<sub>3</sub><sup>2-</sup> is an important component of bone due to its ability to occupy the position of PO<sub>4</sub><sup>3-</sup> (B-type substitution, CHA) or the vacancy in the channels (A-type substitution, CA). A-type substitution has been produced only in high pressure or temperature conditions, however, B-type substituted CA has been synthesised below 100 °C. Some

studies detect complex substitution between the two types (SUETSUGU et al. 2000). The size of the crystallographic unit cell reduces, the thermal stability decreases and solubility increases as the CO<sub>3</sub><sup>2-</sup> content increases. The end member of substitution of PO<sub>4</sub><sup>3-</sup> and OH<sup>-</sup> by CO<sub>3</sub><sup>2-</sup> is CaCO<sub>3</sub>.

If an ion of the crystal lattice is substituted by another ion with different charge, the consequent charge imbalance must be compensated. In the case of OH<sup>-</sup> – CO<sub>3</sub><sup>2-</sup> substitution, charge imbalance may be compensated by a PO<sub>4</sub><sup>3-</sup> – CO<sub>3</sub><sup>2-</sup> substitution. Electric neutrality may be maintained by the substitution of cations as well (e.g. Ca<sup>2+</sup> – Na<sup>+</sup> substitution), thus several cations occur in bone apatite in minor quantities. FLEET & LIU (2007) synthesised A–B type CHAs in which 1–10% of the Ca<sup>2+</sup> ions are substituted by Na<sup>+</sup>. The chemical composition and IR spectra of these CHA samples were very similar to that of bovine bones and dental enamel. Ion substitution largely depends on the chemistry of the environment (in vivo cases: the composition of blood and other body fluids) in which the CHAs form. TÖNSUAADU et al. (1995, 2003) carried out in vivo experiments which have shown that CHA precipitates from different solutions incorporated Na<sup>+</sup>, Mg<sup>2+</sup>, NH<sub>4</sub><sup>+</sup> and HPO<sub>4</sub><sup>2-</sup> ion in their lattice. On the basis of the IR spectra of the precipitates, the CO<sub>3</sub><sup>2-</sup> ions substitute the PO<sub>4</sub><sup>3-</sup> in the crystal structure.

Appropriate function groups of the collagen fibres are able to incorporate into the CO<sub>3</sub><sup>2-</sup> and OH<sup>-</sup> positions on the crystal surface especially on edges and corners, forming in this way a strong bond between the organic and inorganic materials. The location of these bonding sites has a major influence on the stability of the system (DEOLIVEIRA & LAURSEN 1997, PASTERIS et al. 2004).

Although nowadays the presence of CO<sub>3</sub><sup>2-</sup> in bone is doubtless, it is not clear whether they appear as CA, CHA or as a separate CaCO<sub>3</sub> phase. It is also uncertain whether CaCO<sub>3</sub> appears – if present in the bone – either as a solid compound or as a discrete crystal.

## Thermal properties of bone

### Thermal properties of organic compounds

Thermal properties of bone are divided into two parts. One of them is the thermal decomposition of organic materials, occurring at temperatures between 200 °C and 550 °C, and usually has two exothermic reactions. Thermal behaviour of collagens is studied for medical purposes (OOI et al. 2007) and developing secondary utilization and disposal of animal wastes (e.g. meat and bone meal, CONESA et al. 2003, SKODRAS et al. 2007). These materials lose 75wt% of their weight in the course of 3 steps during combustion in air atmosphere or 60wt% of their weight in the course of 2 steps during pyrolysis in nitrogen (inert) atmosphere. These two processes are coincident below 300 °C (CONESA et al. 2003), while there is no weight loss above 600–700 °C which can be related to organic decomposition. Contrarily, ROVERI et al. (2003) note that the end of combustion of collagen could reach 900 °C. Same results are discussed by KISZELY (1969) about fossil and subfossil bones and SZŐÖR (1971) about teeth of mammals.

*Thermal properties of inorganic compounds*

Phosphate minerals with OH<sup>-</sup> substitution usually lose the hydroxyl group between 600°C and 800°C (FÖLDVÁRI 1986). Stoichiometric factor of HA is 55.8, consequently end-member HA has maximum OH<sup>-</sup>-lost of 1.79wt%. Dehydroxylation of artificial HA in air occurs between 680–1080°C, while oxyapatite, then tricalcium phosphate is formed (WANG et al. 2004), since in inert atmosphere the reaction occur only above 1100°C (OOI et al. 2007). Electron microscopic images of HA sintered at different temperatures show that the size of these crystals are greater as temperature increases, this recrystallisation process is continuous. A type CO<sub>3</sub><sup>2-</sup> discharges between 210°C and 390°C, B type CO<sub>3</sub><sup>2-</sup> discharges at 733°C from CHA extracted with subcritical hydrolysis from bone. The extent of substitutions is strongly influenced by the extraction methods (BARAKAT et al. 2008).

Loss of CO<sub>3</sub><sup>2-</sup> – having less stoichiometric factor, since it is able to separate easier from the disturbing noise – is detectable if the reaction occurs after the combustion/pyrolysis. TÖNSUAADU et al. (1995) detected multi-stage CO<sub>3</sub><sup>2-</sup> loss with evolved gas analysis on synthetic CA. It can be the result of the thermal dissociation of the present HPO<sub>4</sub><sup>2-</sup> and its reaction with NH<sub>4</sub><sup>+</sup> ions present (max. 0.02wt%), which lead to pyrophosphate. This Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub> is able to decrease the dissociation temperature, while CO<sub>2</sub> and water are formed (KOEL et al. 1998). CHA dissociate solely as well, the average bond energy of different lattice positions results in different dissociation temperatures.

Other volatiles cause significant weight loss as well. NH<sub>4</sub><sup>+</sup> is eliminated between 100°C and 400°C (TÖNSUAADU et al. 1995, VEIDERMA et al. 2005), while sulphates and phosphorus-bearing anions may react with anorganic components of synthetic apatites in solid phase (HSU 2003).

Dissociation of CO<sub>3</sub><sup>2-</sup> in Israeli phosphorites takes place in two steps (first between 720°C and 740°C, second 765°C and 800°C). The first step may be associated with the decomposition of B-type CA, and the dissociation of calcite in the samples may be associated with the second reaction (TÖNSUAADU et al. 2001). Hydrous apatite precipitates with different compositions dissociate during a weight-losing reaction between 330°C and 340°C due to the elimination of H<sub>2</sub>O and CO<sub>2</sub> from the lattice. The observed exothermic reaction in this temperature range implies the rearrangement of the lattice structure. The structural change is affected by minor cations (TÖNSUAADU et al. 2003). Thermal dissociation of carbonate-bearing apatites occurs above 600°C in 2 or 3 steps. One of them is always in the range of 770–780 °C. XRD and IR analyses of the samples suggest that the decomposition destabilizes the lattice and decreases the CO<sub>3</sub><sup>2-</sup> dissociation temperature. Researchers note that CO<sub>3</sub><sup>2-</sup> in position B is able to move into position A.

*Thermal properties of bone*

In the Department of Mineralogy and Geology of the former Kossuth Lajos University (now University of Debrecen) Gyula Szöőr had studied bone and tooth samples by thermal analysis since the 1970s (SZÖÖR 1971, 1975, 1979, 1982a,b,c, SZÖÖR & KORDOS 1982). He started

his research with fossil and recent vertebrate tooth samples and pointed out that there is a high temperature exothermic peak on the differential thermoanalytical (DTA) curve which disappears following NaOH treatment, which refers to organic residues above 600°C. CO<sub>3</sub><sup>2-</sup> dissociation in NaOH treated samples occurs in two steps, interpreted by the author as the presence of Mg<sup>2+</sup> in the position of Ca<sup>2+</sup>. He proved the catalytic role of platinum crucible on the combustion.

In later bone studies SZÖÖR used thermal analysis for facies analysis and palaeoclimatic research on the vertebrae of snakes. The exothermic reactions between 200 °C and 550 °C are related to the formation of hetero-polyaromatic compounds during the combustion of organic matter. Endothermic reactions above that range are referred as dissociation of CO<sub>3</sub><sup>2-</sup> of CHA and calcite formed during fossilisation. CO<sub>3</sub><sup>2-</sup> dissociation with two steps occurred only in one sample and was interpreted as the presence of Mg<sup>2+</sup> and Fe<sup>2+</sup>. Twin peaks on the curves are relatively sharp, while a drag reaction is identified as dissociation of CHA. In some fossil samples the weight loss between 600–1000°C reaches 10wt% in one step.

The evolved gases were examined by using a quadrupole mass spectrometer coupled to the thermoanalytical instrument. This method proved the elimination of CO<sub>2</sub> and CH to be radical between 220°C and 600°C. H<sub>2</sub>O elimination was not detected above 220°C in inert atmosphere (SZÖÖR et al. 1987).

Heating bovine bone in inert atmosphere, OOI et al. (2007) did not detect weight loss between 700°C and 1000°C, while the traces of CO<sub>3</sub><sup>2-</sup> in the sample above 1000°C was proven by FTIR method. However, the results of FTIR and thermoanalytical measurements are not consistent with each other. HABERKO et al. (2006) used IR spectroscopy to examine the exothermic peaks of DTA curves of swine bones, and proved the dissociation of organic compounds below 350°C during CO<sub>2</sub> elimination. The second endothermic peak at 750°C seems to relate to the recrystallisation of CHA that increases the size and decreased the specific surface and length of crystallites. In this range significant amount of CO<sub>2</sub> evolve as well. This study note the presence of CO<sub>3</sub><sup>2-</sup> in natural bone, therefore the Ca/P ratio is always higher than in HA prosthesis.

PETERS et al. (2000) divide the thermal properties of natural and artificial bones to three parts. Adsorbed water evaporate below 260°C, organic materials burn in the range of 260–600°C, while the weight loss between 650°C and 850°C relate to the dissociation of CHA.

In archaeological remains of human bones the CO<sub>3</sub><sup>2-</sup> content usually increases, although the decrease in carbonate content is possible as well (PIGA et al. 2008), which may be the result of acidic soil chemistry. Post mortem fire increases the size of apatite crystallites (ENZO et al. 2007, PIGA et al. 2008).

**Materials and methods**

Commercial chicken and pig bones were studied following preparation: meat was removed mechanically from the surface of bone, additional cleaning occurred by ultrasound, while samples were dried at 105°C. Before and after grinding samples were washed with acetone. Finally

samples were pulverised to 100 $\mu$ m in agate mortar.

Inorganic samples were analysed as well. HA was produced from solutions of CaCl<sub>2</sub> and Na<sub>2</sub>HPO<sub>4</sub> after HUTCHENS et al. (2006), dried to reach constant weight at room temperature. Analytical grade CaHPO<sub>4</sub> was purchased by trade (Reanal).

Thermal analyses (TG, DTG, and DTA) were performed by Derivatograph-C (MOM, Budapest). Based on earlier measurements heating was usually linear at 10 °C/min. Samples were heated in a small cylindrical Pt crucible in static air atmosphere. The weight of measured samples were 100 $\pm$ 5mg or 50 $\pm$ 2.5mg. Inorganic compounds of bone were removed by acetic acid of 10wt%, in order to separate the organic compounds of bone.

Most of referred studies determined the CO<sub>3</sub><sup>2-</sup> content based on weight loss above 600°C, however, in some of our samples organic materials oxidised in this temperature range, since we determined the evolved CO<sub>2</sub> (henceforth  $\Delta$ m) based on the weight loss associated with CO<sub>3</sub><sup>2-</sup> dissociation peaks on DTG curves.

Endothermic weight loss below 220°C is identified as evaporation of adsorbed water. The end point of oxidation of organic compounds (T<sub>eo</sub>) in each sample is determined. Inorganic content is determined as wt% at T<sub>eo</sub>, while organic content is determined as a weight loss between 220°C–T<sub>eo</sub>. We ascertained the weight losses between T<sub>eo</sub>–1000°C, which are similar to  $\Delta$ m in each sample.

Three models were used to calculate the mineral content of bones:

I.  $\Delta$ m related to „CaCO<sub>3</sub>” („CaCO<sub>3</sub> content”=2.27\* $\Delta$ m), the rest part of the heated sample is HA (as inert).

II.  $\Delta$ m related to CO<sub>3</sub><sup>2-</sup> dissociation in type B CHA, while HA and HPO<sub>4</sub><sup>2-</sup> are absent, its chemical formula is Ca<sub>10-x</sub>[(PO<sub>4</sub>)<sub>6-2x</sub>(CO<sub>3</sub>)<sub>2x</sub>](OH)<sub>2</sub>, where 0 $\leq$ x $\leq$ 3.

III.  $\Delta$ m related to CO<sub>3</sub><sup>2-</sup> dissociation in types A and B CA (or so-called calcium-deficient CA) HA and HPO<sub>4</sub><sup>2-</sup> are absent, its chemical formula is Ca<sub>10-y</sub>[(PO<sub>4</sub>)<sub>6-2y</sub>(CO<sub>3</sub>)<sub>2y</sub>]CO<sub>3</sub>, where 0 $\leq$ y $\leq$ 3. (Results of TÖNSUAADU et al. (1995) and BARAKAT et al. (2008) cited above are not considered in this model.).

The applied models are not sensitive for the step number of CO<sub>2</sub> loss and the proportion of one step to another.

The volume of the HCl-evolved gases was determined by a Scheibler calcimeter. In the course of the measurements 10wt% analytical grade HCl solution was used as a reagent, in order to free the carbonate content of the bone samples. The weight of the samples was about 200mg, measured on an analytical balance with a precision of 0.1mg.

## Results

Numerous measurements are included in the study presenting the results derived from curves of three inorganic minerals, five pig cortical bones and one collagen sample using linear heating (10 °C/min). One of them (Sk2b) weighs 47.6mg and this sample was measured after 1 year standing in closed jar and derived from the same

bone as Sk2. Others weigh about 100mg and were measured immediately after preparation. Most important results of the research are presented in the following sections, relevant data are in Table 1–3, while thermal analytical curves are given in Figure 2.

### *Inorganic samples (HA, CaHPO<sub>4</sub>)*

Weight of HA decreased during the whole range of heating. One significant peak of DTG curve is notable at 91 °C, while two weak peaks are detectable at 685 °C and 790 °C, latter is more distinct. This weight loss with two peaks showed CO<sub>3</sub><sup>2-</sup> in the lattice of HA, according to TÖNSUAADU et al. (2003).

Dissociation of CaHPO<sub>4</sub> occurs between 300°C and 450 °C forming water and calcium-pyrophosphate. Above this there was no detectable reaction. Mixture of 90wt% Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub> and 10wt% CaCO<sub>3</sub> was measured as well. We were not able to demonstrate the reaction between the two combustions referred in KOEL et al. (1998).

CO<sub>2</sub> analysis was not performed from inorganic samples.

### *Bone samples*

Below 220°C 9.0–11.4% weight loss of adsorbed water was measured, which was followed by the combustion of collagen. It means a strongly exothermic reaction first, in addition to an endothermic one with peak temperatures in the range of 400–415°C. The last reaction related to organic materials is a long reaction with a sharp end at T<sub>eo</sub> different in each sample (between 570°C and 745°C). In the case of sample Sk1 and Sk2 high T<sub>eo</sub> did not allowed the examination of the inorganic phase, consequently T<sub>eo</sub> of Sk1 was assigned on the basis of DTG curves for comparability, however, CO<sub>2</sub> content of Sk2 was unknown.

	H <sub>2</sub> O%	T <sub>eo</sub> (°C)	Org%	Anorg%	CO <sub>2</sub> %
HA	10.6 %	-	-	89.4 %	0.6 %
Sk1	9.0 %	700	28.5 %	62.5 %	1.5 %
Sk2b	9.9 %	570	29.2 %	60.9 %	0.9 %
Sk3	9.4 %	664	25.0 %	65.5 %	4.7 %
Sk4	11.4 %	643	23.2 %	65.4 %	6.3 %

Table 1 Composition of samples and terminal temperature of combustion derived from thermal analyses

### 1. táblázat A minták összetétele a termoanalitikai vizsgálatok alapján a szervesanyag kiégésének véghőmérsékletével

T<sub>eo</sub> of samples Sk2b, Sk3 and Sk4 is less than the peak temperature of CO<sub>3</sub><sup>2-</sup> dissociation. DTG curves of all three samples have two CO<sub>2</sub> loss peaks, Sk2b has similar weight loss in the steps, while in the case of Sk3 and Sk4 the second step is multiple than the first one (Table 3). According to DTA curves of Sk4 the second dissociation could be two reactions at the same temperature.

CO<sub>2</sub> contents derived from Scheibler calcimeter were higher with 2.5–3 % than those from thermal analysis in the case of Sk1 and Sk2b, possibly due to the presence of A-type carbonate with decomposition temperature between 350–370 °C (TÖNSUAADU et al. (1995), BARAKAT et al. (2008), while these two values are approximately equal in the case of Sk3 and Sk4.

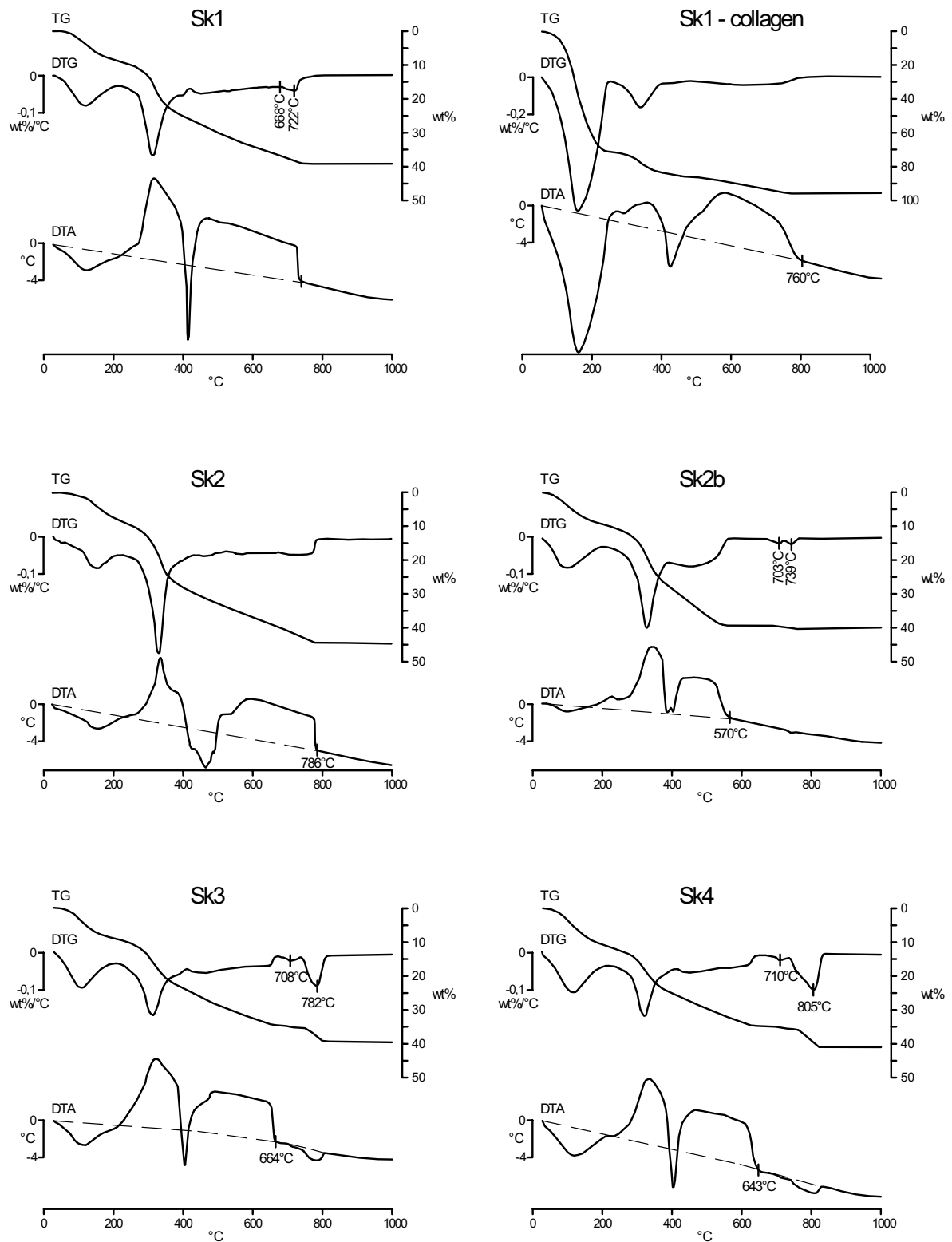


Figure 2 Thermoanalytical curves of samples. In the case of Sk1 both  $T_{eo}$  and temperature of  $CO_3^{2-}$  dissociation are marked on DTG curve, in others  $T_{eo}$  is marked on DTA curve and temperature of  $CO_3^{2-}$  dissociation is marked on DTG curve. DTA baseline is dashed

2. ábra A vizsgált minták termoanalitikai görbéi. Az Sk1 esetében a  $T_{eo}$  és a  $CO_3^{2-}$ -disszociáció a DTG-görbén jelölve, a többi esetben a  $T_{eo}$  a DTA-görbén és a  $CO_3^{2-}$ -disszociációk a DTG-görbén jelölve. A DTA alapvonal szaggatott vonallal jelölve

	model I			model II		model III	
	CaCO <sub>3</sub> / inorg	HA/ inorg	HA/ CaCO <sub>3</sub>	x	chemical formula	y	chemical formula
HA	1.5 %	98.5 %	64.5	0.08	Ca <sub>9,92</sub> [(PO <sub>4</sub> ) <sub>5.84</sub> (CO <sub>3</sub> ) <sub>0.16</sub> ](OH) <sub>2</sub>	-0.42	-
Sk1	5.5 %	94.5 %	17.3	0.27	Ca <sub>9,73</sub> [(PO <sub>4</sub> ) <sub>5.46</sub> (CO <sub>3</sub> ) <sub>0.54</sub> ](OH) <sub>2</sub>	-0.21	-
Sk2b	3.4 %	96.6 %	28.8	0.17	Ca <sub>9,83</sub> [(PO <sub>4</sub> ) <sub>5.66</sub> (CO <sub>3</sub> ) <sub>0.34</sub> ](OH) <sub>2</sub>	-0.32	-
Sk3	16.3 %	83.7 %	5.1	0.75	Ca <sub>9,25</sub> [(PO <sub>4</sub> ) <sub>4.50</sub> (CO <sub>3</sub> ) <sub>1.50</sub> ](OH) <sub>2</sub>	0.31	Ca <sub>9,69</sub> [(PO <sub>4</sub> ) <sub>5.38</sub> (CO <sub>3</sub> ) <sub>0.62</sub> ]CO <sub>3</sub>
Sk4	21.9 %	78.1 %	3.6	0.98	Ca <sub>9,02</sub> [(PO <sub>4</sub> ) <sub>4.04</sub> (CO <sub>3</sub> ) <sub>1.96</sub> ](OH) <sub>2</sub>	0.56	Ca <sub>9,44</sub> [(PO <sub>4</sub> ) <sub>4.88</sub> (CO <sub>3</sub> ) <sub>1.12</sub> ]CO <sub>3</sub>

Table 2 Theoretical chemical compositions derived from the applied models

2. táblázat Az alkalmazott modellszámítások eredményei

	thermal analysis				CO <sub>2</sub> /Anorg Gas volumetry
	1 <sup>st</sup> peak	2 <sup>nd</sup> peak	sum	CO <sub>2</sub> /Anorg	
Sk1	n.d.	1.5 %	1.5 %	2.4 %	4.16 %
Sk2b	0.5 %	0.4 %	0.9 %	1.5 %	3.93 %
Sk3	0.9 %	3.8 %	4.7 %	7.2 %	4.57 %
Sk4	0.7 %	5.6 %	6.3 %	9.6 %	6.91 %

Table 3 CO<sub>2</sub> content of the samples based on thermal analysis and gas-volumetric method

3. táblázat A csontminták CO<sub>2</sub>-tartalma termoanalitikai és gázvolumetrikus meghatározás alapján

Collagen

Sk1-collagen was prepared from Sk1. Its thermal properties are similar to that of the bone, however, reactions were less sharp, the first exothermic reaction of combustion were ancillary to the second long-term reaction. One sixth part of the sample weight without adsorbed water remained at 1000 °C, while this value was approximately four sixth parts in the case of bones.

Discussion

By the amount of CO<sub>2</sub>, models I and II can be applied for every sample, while model III cannot be applied for samples Sk1, Sk2b and HA of course (y<0). CO<sub>3</sub><sup>2-</sup> dissociation appeared as two weight loss reactions in case of most of the samples which is interpreted by only the third model. The rate of CO<sub>3</sub><sup>2-</sup> in position A to position B derived from the model was different from the rate given by thermal analysis. Consequently, neither model can explain the chemical compounds alone.

By the shape and number of peaks related to the CO<sub>3</sub><sup>2-</sup> dissociation we think that calcite is probably present in some bones. Further measurements are required with which the characteristic bounds of calcite can be detected (e.g. IR-spectroscopy) or new preparation will be developed to remove calcite from the samples.

Values of CO<sub>2</sub> content derived from different methods are similar in two samples, however, in the others they were significantly different. It is interpretable by the possible dissociation of CO<sub>3</sub><sup>2-</sup> from position A parallel with the combustion of organic materials (TÖNSUAADU et al. 1995, BARAKAT et al. 2008), which is undetectable by our instruments, as the applied calculations cannot include it. In this case at least three steps of weight loss relate to CO<sub>3</sub><sup>2-</sup> dissociation, in addition Sk3 and Sk4 contain no CO<sub>3</sub><sup>2-</sup> in position A (CHA and calcite), while Sk1 and Sk2b have 3.5 and 4.1 wt% CO<sub>3</sub><sup>2-</sup> respectively (probably mixed type CA).

Another possibility is the presence of unknown compounds in some bone samples which evolve gas during

HCl treatment. EGA combined Scheibler calcimeter is required to clarify the problem with samples heated to different temperatures. The differences between the results of different methods could also be explained by sample preparation, since pulverizing and homogenization of bone samples were difficult. Thus the results from the small amount of samples may be characterised by significant statistical uncertainties. Since measurements did not detect dehydroxilation and reaction between P<sub>2</sub>O<sub>7</sub><sup>4-</sup> and CO<sub>3</sub><sup>2-</sup> the presence of OH<sup>-</sup> and HPO<sub>4</sub><sup>2-</sup> in our samples is uncertain.

Terminal temperature of combustion of collagen exceeded 600°C in some sample, consequently the rate of organic and inorganic parts of bone cannot be determined by weight loss until 600°C. If some amount of CO<sub>3</sub><sup>2-</sup> from inorganic materials evolves below T<sub>co</sub>, the ratio can be distorted as well.

Conclusion

Our research confirms that CO<sub>3</sub><sup>2-</sup> – an important factor regarding solubility and thus biocompatibility – dissociates during and following the combustion of collagen, and explains the experience of better properties (faster bone forming and collagen connection) of prosthesis sintered in low temperature.

On the basis of our results we suggest the presence of independent calcite phase or crystallites in some bones beside CA or CHA. Therefore calcite in fossilised bones is not only syndiagenetic, however, acidic treatment might damage the original structure of bone. Different CO<sub>3</sub><sup>2-</sup> content of fresh bones disrupts the age determination based on calcite or CO<sub>3</sub><sup>2-</sup> content.

Weight loss steps related to the dissociation of CO<sub>3</sub><sup>2-</sup> are able to help to determine the position of CO<sub>3</sub><sup>2-</sup> in the lattice. New methods of preparation (removing the organic part or calcite, etc.) and other instrumental and chemical measurements (e.g. FTIR, TA-MS) may clarify this problem.

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