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Short communication

Behaviour of bone origin hydroxyapatite at elevated temperatures and in O_2 and CO_2 atmospheres

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Abstract

In the present work the behaviour of HAp extracted from pig bones at elevated temperatures up to 1000 °C in O_2 and CO_2 atmospheres has been studied. It has been found that CO_2 atmosphere arrests HAp decomposition. Chemical analysis and infrared spectroscopy reveal that no free CaO appears and no decrease of CO_3^{-2} group concentration occurs in the material calcined in CO_2 atmosphere. In the O_2 atmosphere at elevated temperatures, CaO and CO_2 are emitted from the samples, although the remaining material retains the HAp structure as indicated by the X-ray diffraction.

Keywords: Bone origin hydroxyapatite; Biomedical applications; Calcination

1. Introduction

Hydroxyapatite (HAp) with its high biocompatibility and bioaffinity, stimulates osteoconduction and is slowly replaced by the host bone after implantation [1,2]. HAp material extracted from animal bones seems to be an alternative for numerous products based on synthetic HAp. Pig bone origin HAp examined in soft tissues of mice [3] shows that it can be safely used as a filling material alone, or as a composite graft. The same material can be colonized by human osteosarcoma cells CAL-72 [4].

In our previous work [5] we elaborated a method of HAp extraction from animal bones. Later the material characteristics and transformation at elevated temperatures in air atmosphere were studied [4]. There is an essential difference between synthetic hydroxyapatite (HAp) and the material which occurs in animal bones. The latter is non-stoichiometric; its Ca/P ratio is higher than 1.67, it contains carbonate groups (CO_3^{-2}) and usually some Mg built into the HAp structure. The application of such material involves subjecting it to the treatment at elevated temperatures in order to prepare useful shapes for biological and medical purposes. However, it was found that in the oxidizing atmosphere the material decomposes starting from about 700 °C; the emission of CO_2 indicates the decrease of the CO_3^{-2} groups content; also some free CaO appears. The remaining material still retains the hydroxyapatite structure, but its composition approaches to the stoichiometric compound. It should be emphasized that nonstoichiometry (surplus Ca content) and the presence of carbonate groups is a feature of the material synthesized in living organisms also in the human bodies. That is why in the present study we are focusing on the experiments which should allow retaining both chemical composition and structure of the natural material at elevated temperatures. The application of such temperatures is important in order to manufacture products and shapes useful for biological and medical applications. The procedure is based on the assumption that heat treatment in the CO_2 atmosphere should arrest or at least slow down emission of carbon dioxide from the material and therefore decrease the CO_3^{-2} groups concentration in the original material. We also hope that heat treatment under such conditions will cease emission of CaO. Its excess in a biomaterial is harmful because it leads to the inflammatory effect in the living tissues. For the comparison purposes heat treatment in oxygen atmosphere was also performed.

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2. Experimental procedure

The following preparation route was applied: about 200 g clean cortical parts of pig long bones were treated with 1000 cm^3 hot (100 °C) NaOH water solution of 4 M concentration. A 48 h period of time with an intermediate exchange of the leaching solution was applied. The operation was performed in a poly(tetrafluoroethylene) (Teflon) beaker. In order to remove remaining sodium hydroxide the material was carefully washed with distilled water until pH7 in the filtrate was reached and then the material was dried at 120 °C in air atmosphere. The material was lightly ground in an alumina mortar.

The calcination of ~5 g samples was performed in the tube furnace equipped with dense alumina tube of 5 cm diameter. Oxygen and carbon dioxide flowing through the tube created selected atmospheres. In each case 1 dm^3/min of O_2 flow was applied up to 400 °C with 30 min soaking in order to burn out traces of the organic matter remaining in the material after the NaOH treatment [4]. Further heat treatment was performed up to the pre-selected temperature using either O_2 or CO_2 atmosphere. A 30 min soaking time at the final temperature was applied. The rate of the temperature increase up to 400 °C and up to the final temperature was 5°/min. Cooling with furnace up to 400 °C in selected atmosphere was used. At this temperature samples were removed from the furnace.

A standard technique was applied in preparation of samples for transmission infrared spectra (Bio-Rad FTS 60 MV apparatus); a 2 mg sample was pressed with 300 mg KBr. The number of scans 256 and resolution 4 cm^{-1} characterized these measurements. Identification of the absorption bands was based on the data presented by Farmer [6]. Phase composition of samples was studied using X-ray diffraction analysis (Cu Kα radiation) with Panalytical X′Pert system. The method of chemical analysis of the starting powder is described elsewhere [4]. The amount of free CaO in calcined samples was determined by washing the known mass of the powder with the surplus amount of the de-carbonated distilled water. During this operation CaO forms $Ca(OH)_2$ which is reasonably well soluble in water. The analysis of the filtrate by atomic absorption spectroscopy (AAS, PerkinElmer 3110) allowed us to determine the amount of calcium removed from HAp vs. the calcination temperature and applied gas atmosphere. Specific surface area of the powder was measured by nitrogen adsorption using the BET isotherm (Nova 1200e, Quantachrome Co.)

3. Results and discussion

Table 1 shows chemical composition of the powder calcined at 400 °C in O_2 atmosphere and its specific surface area (S_w).

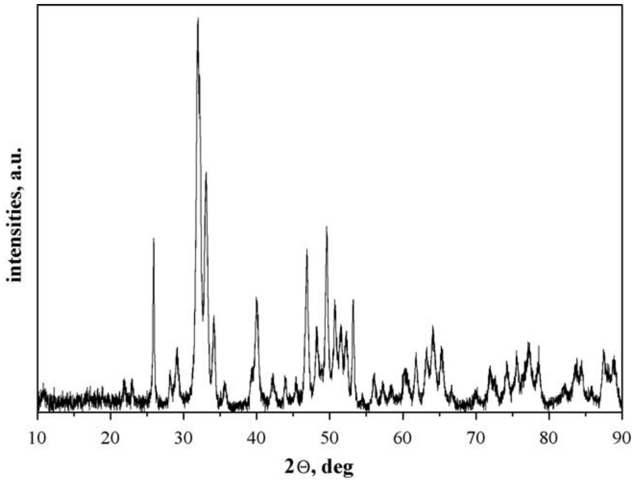

Fig. 1. X-ray diffraction pattern of the HAp sample calcined at 400 °C in O_2 atmosphere.

The characteristic feature of the material is its Ca/P mole ratio higher than in the stoichiometric compound (1.67). Some amount of magnesium built into the HAp structure occurs as is usually observed in the natural origin material. Sodium revealed through the analysis either was present in the original material or was introduced into its structure during treatment with NaOH solution. It is worth noticing that X-ray diffraction reveals HAp as the only phase (Fig. 1).

In Fig. 2 fraction of free CaO emitted by the samples calcined at different temperatures in O_2 or CO_2 atmospheres is shown. Chemical analysis of the starting material (Table 1) and the data of Fig. 2 allowed us to calculate the resulting Ca/P ratio (Fig. 3). We noticed that in the CO_2 atmosphere decomposition of the material, manifested by emission of free CaO, is brought to a stop. Ca/P ratio remains on the level close to the starting material. This is not the case for the material heat treated in oxygen atmosphere. At the highest applied temperature (1000 °C) the Ca/P ratio approaches the value characteristic for the stoichiometric hydroxyapatite (1.67). X-ray diffraction shows that also in this case hydroxyapatite remains the main constituent of the system, although a small amount of rhenanite ($NaCaPO_4$) appears. Its fraction, assessed by the Rietveld method [7] corresponds to 4.8%. Fig. 4 shows two diffraction patterns of the samples calcined at 1000 °C, the one in O_2 atmosphere and another one in CO_2 gas. Rhenanite reflection peaks visible in the former sample are indicated. No such phase occurs in the material heat treated in CO_2 and also in O_2, but at lower temperatures.

Infrared spectroscopy allowed us to distinguish absorption bands corresponding to ${CO_3}^{-2}$ groups and to express their area as a percentage of the area of all absorption bands. Results are

Table 1
Chemical composition of the HAp starting sample.

CaO (wt%)	P_2O_5 (wt%)	MgO (wt%)	Na_2O (wt%)	Ca/P	S_w (m^2/g)
51.91 ± 0.02	38.25 ± 0.01	0.60 ± 0.02	2.84 ± 0.01	1.72 ± 0.02	71.7 ± 0.4

(±) Denotes standard deviation.

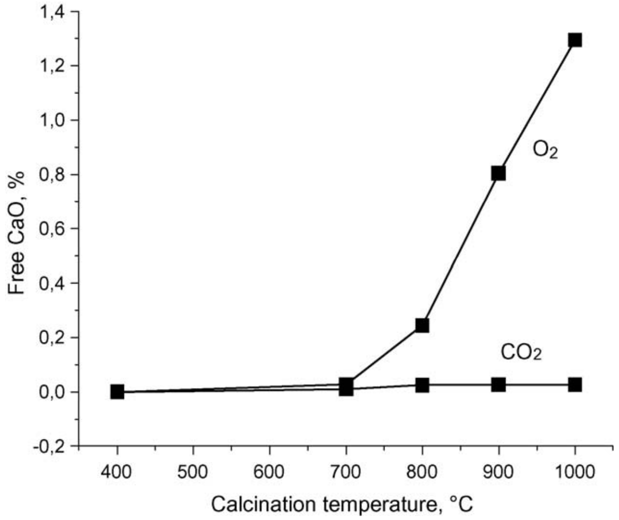

Fig. 2. Free CaO vs. calcination temperature in indicated atmospheres. Standard deviation of the measurements is within the markers.

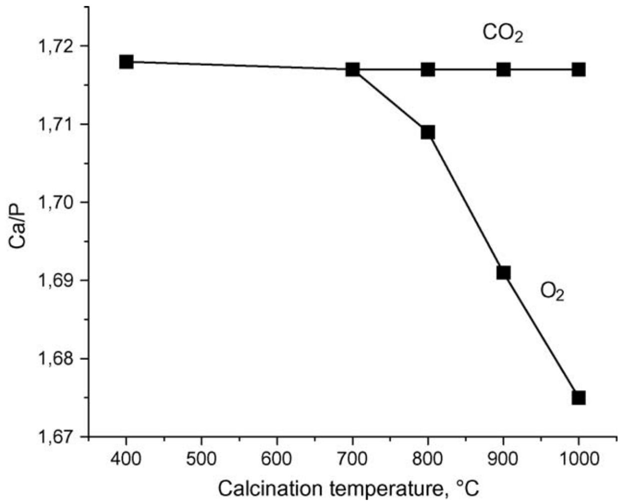

Fig. 3. Ca/P vs. calcination temperature in indicated atmospheres.

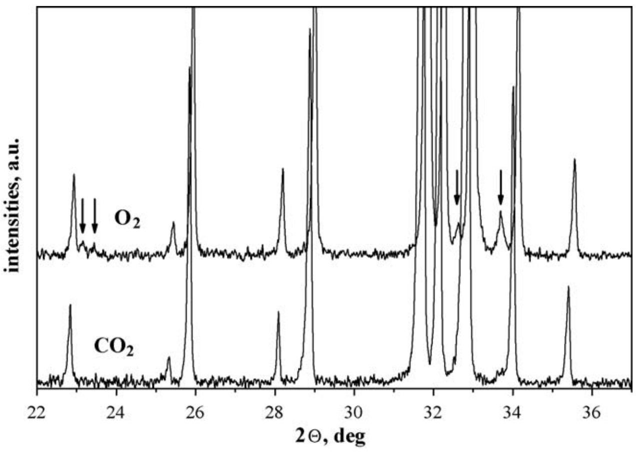

Fig. 4. X-ray diffraction patterns of the samples calcined at 1000 °C in O_2 and CO_2 atmospheres. Reflections corresponding to rhenanite ($NaCaPO_4$) indicated.

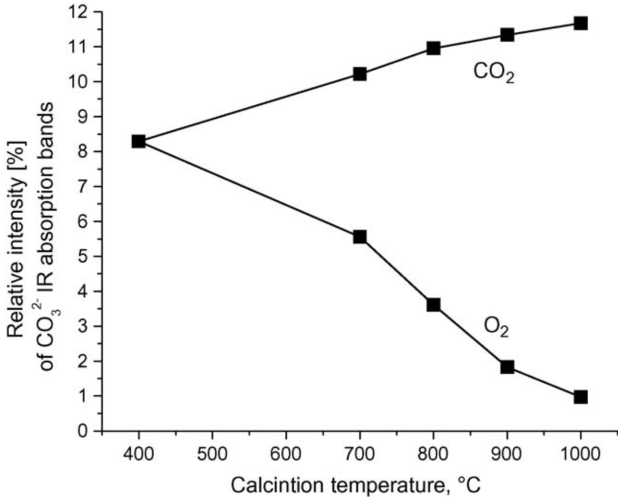

Fig. 5. Relative intensity of CO_3^{-2} IR absorption bands in indicated atmospheres vs. calcination temperature in indicated atmospheres.

presented in Fig. 5. They indicate that O_2 atmosphere leads to the decreased content of the carbonate groups in the natural origin HAp at elevated temperatures. On the contrary, in CO_2 atmosphere concentration of these groups even increases. These facts can be visualized by the IR spectra shown in Fig. 6. Absorption bands characteristic for the CO_3^{-2} groups are clearly visible in the material calcined at 1000 °C in CO_2 atmosphere. The same material heat treated in O_2 atmosphere shows traces of these bands only.

The question arises about the place of the extra CO_3^{-2} groups situation. There are two known possibilities [8–10]: either CO_3^{-2} ions can substitute in the HAp structure for OH^{-1} ions (type A) or for PO_4^{-3} ions (type B). Relative intensity of the IR absorption bands corresponding to hydroxyl groups in the starting material amounts to 9.4%. It drops down to the

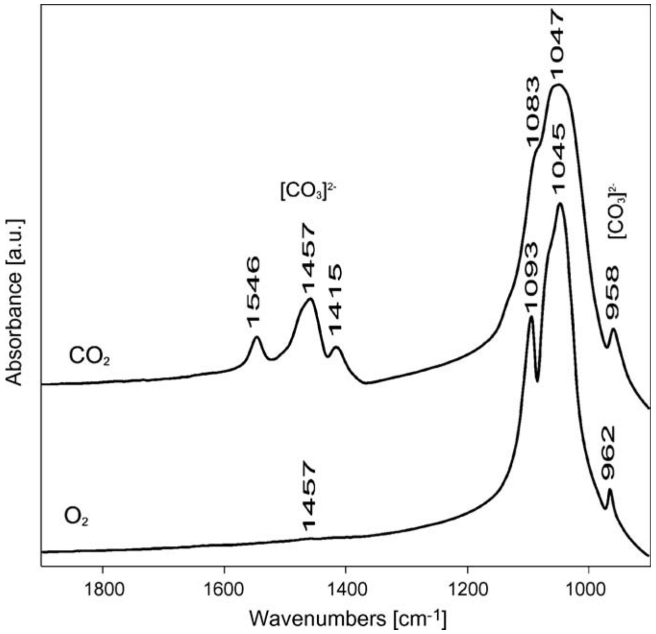

Fig. 6. IR spectra of the samples calcined at 1000 °C in O_2 and CO_2 atmospheres. Absorption bands of CO_3^{-2} groups indicated.

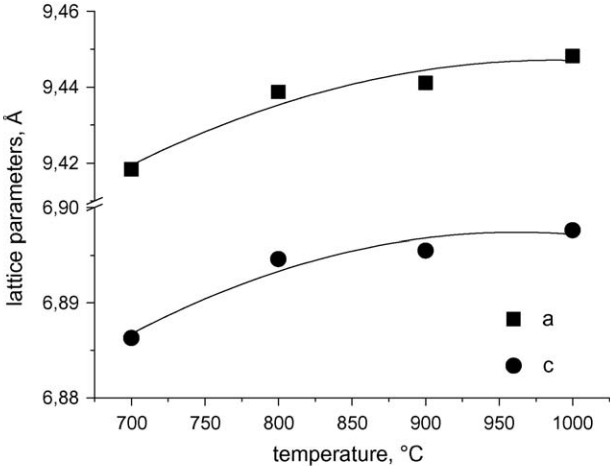

Fig. 7. Lattice parameter changes vs. temperature of the material calcined in CO_2 atmosphere. a and c correspond to the HAp lattice parameters

value of 3.1% in the material heat treated in CO_2 atmosphere at 1000 °C; it suggest substitution of the extra CO_3^{-2} groups for hydroxyl ions. This conclusion seems to be corroborated by the lattice parameters increase vs. calcination temperature in CO_2 atmosphere (Fig. 7). Lattice expansion should be expected when larger groups (CO_3^{-2}) substitute for the smaller ones (OH^{-1}).

4. Conclusions

Hydroxyapatite extracted from the cortical part of long pig bones is a non-stoichiometric material. Its Ca/P mol ratio is higher than in the stoichiometric compound and it contains Mg, Na, and carbonate groups. Heat treatment in oxygen atmosphere leads to a decreased concentration of carbonate groups. Simultaneously free CaO appears. The remaining material approaches chemical composition of the stoichiometric compound. A small fraction of rhenanite ($NaCaPO_4$) was found in the sample calcined at 1000 °C in O_2, although the basic material still retains the hydroxyapatite structure.

In carbon dioxide atmosphere no such phenomena occur. In this case the amount of carbonate groups built into the HAp structure increases. They, most probably, substitute for the hydroxyl groups. But the material retains hydroxyapatite structure up to 1000 °C.

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