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Natural hydroxyapatite—its behaviour during heat treatment

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Abstract

Natural origin hydroxyapatite (HAp) was extracted from animal bones by their treatment with hot NaOH solution. The remaining organic material oxidized in air atmosphere at moderate temperatures. In the material of this kind carbonate groups and small amounts of magnesium were found, usually not present in the synthetic HAp. Ca/P ratio in pig cortical bones was higher than that in the stoichiometric (synthetic) material. Fraction of carbonate groups decreased with temperature and CaO appeared at higher temperatures, but structure of hydroxyapatite became preserved even at 1000 °C, the highest temperature applied in this investigation. At temperatures >700 °C crystallite growth became intensive, specific surface area of the powder decreased and compacts of such powder started to shrink. Biological test showed that CAL-72 (human osteosarcoma) cells growth depended on heat treatment temperature of the natural HAp substrate.

Keywords: Calcination; Biomedical applications; Bone or gin hydroxyapatite

1. Introduction

Hydroxyapatite (HAp) with its high biocompatibility and good bioaffinity, stimulates osteoconduction and is slowly replaced by the host bone after implantation.[1,2] HAp material manufactured from animal bones have the advantage that it inherits some properties of the raw material such as its chemical composition and structure. Therefore, it seems to be an alternative for numerous products based on synthetic HAp. In our previous studies,[3] we focused on the method of extraction of HAp from bovine bones. In the present work pig bones were used as source of the HAp mineral.

Three kinds of hydroxyapatite applications can be pointed out: (i) as a porous or granulated material useful in bone surgery, (ii) as an additive to organic polymers improving their biofunctionality and (iii) as coatings, plasma sprayed[4] or prepared by laser ablation.[5] All these applications involve treatment of the material at elevated temperature. That is why in the present study characteristics of the starting natural origin HAp material of pig origin will be followed by its characterization at elevated temperatures.

2. Experimental procedures

Preliminary experiments have shown that organic part of the system can be nearly totally removed from bovine bones by treating them with hot sodium hydroxide solution.[3] In the present study, we focused on hydroxyapatite extracted from the cortical part of long pig bones.

The following preparation route was applied: about 200 g of clean bones were treated in a poly(tetrafluoroethylene) (Teflon) beaker at 100 °C for 24 h with 1000 cm^3 of NaOH water solution of 4 $\mathrm{mol/dm}^{-3}$ concentration. The treatment was repeated for another 24 h at 100 °C with a new NaOH solution of the same concentration. In order to remove remaining sodium hydroxide the material was washed with distilled water until pH 7 of the filtrate was reached and then dried at 120 °C in air atmosphere.

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Specific surface areas of the starting and calcined powders were determined by nitrogen adsorption (BET isotherm) using a Sorpty 1750 (Carlo Erba) apparatus. A standard technique was applied in preparation transmission infra red spectra (Bio-Rad FTS 60 MV apparatus): a 2 mg sample was pressed with 300 mg of KBr. Number of scans 256 and resolution 4 cm^{-1} characterized these measurements. DTA and TG measurements were made using Derivatograph model C (MOM Budapest). Rate of temperature increase 10 °C/min, 100 mg samples, Pt crucibles and α-Al_2O_3 reference samples were used. Chemical analysis of gases emitted at elevated temperatures by the HAp sample was performed using mass spectrometer QMD 300 Thermostat (Balzers). Rate of temperature increase was 10 °C/min. Philips CM 20 transmission electron microscope allowed us to observe morphology of HAp powder. Linear shrinkage of the HAp compacts were measured using home made dilatometer. Rate of temperature increase 10 °C/min and samples uniaxially pressed under 50 MPa and repressed isostatically under 200 MPa ware applied. Phase compositions of samples were studied using X-ray diffraction (Cu Kα radiation) and Philips X'Pert system. Identification was based on.[6]

The following procedures were applied to determine Ca(2+), Mg(2+) and $PO_3{}^{3-}$ content: a 0.5 g sample was dissolved in concentrated HCl (0.9 cm^3) and 5 cm^3 of water. After cooling the solution was diluted with water to about 80 cm^3 and pH was fixed to 3 using diluted NH_3 water solution. Phosphate ions, $PO_4{}^{3-}$, ions were separated from Ca(+2) and Mg(+2) in an ion exchanger Dowex 50 W by means of the method applied for cement.[7] The amount of $PO_4{}^{3-}$ was determined indirectly by precipitation as $LaPO_4$ and complexometric titration of the excess of La(3+) ions by EDTA in the acetic acid buffer.[8] The results obtained by complexometric titration were verified by amperometric titration using standard solution of Pb(2+) ions and precipitating $PO_4{}^{3-}$ as $Pb_5(PO_4)_3Cl$.[9] After elutriation from the ion exchanger the amount of Ca(2+) was determined by complexometric titration with EDTA in alkaline solution of NaOH and the amount of Ca(2+) + Mg(2+) in the ammoniacal buffer of pH 10. The amount of Mg(2+) was determined from the difference between the two values. However, in case of small amounts of Mg(2+) this method was found to be not sufficiently accurate and flame atomic absorption spectroscopy (AAS) had to be used.

The amount of $CO_3{}^{2-}$ ions was determined by the method based on the precipitation of $CaCO_3$. The HAp sample (0.1–1.0 g, depending on the expected $CO_3{}^{2-}$ content) was treated with an excess of HCl in a closed vessel. The released CO_2 was carried by a slow stream of argon and absorbed in two washing bottles connected in series, containing the known amount (100 cm^3) of saturated $Ca(OH)_2$ water solution. The mixture was allowed to stand overnight, in order to let $CaCO_3$ to sediment, and concentration of remaining Ca(2+) ions was determined by complexometric titration with EDTA. The amount of $CO_3{}^{2-}$ was calculated, based on the difference between initial concentration of Ca(2+) and its concentration after treating the solution with CO_2.

3. Results and discussion

Specific surface area of the material as measured by nitrogen adsorption (BET) was of 71.4 m^2/g. Its transmission electron micrograph showed anisometric, plate-like particles of nanometric thickness (Fig. 1).

In Table 1, chemical analysis of the studied material is given. For comparison two commercial, synthetic materials are shown as well as the analysis of bovine origin HAp studied in our earlier work.[3] The essential difference between synthetic and animal origin materials was that the latter showed higher Ca/P ratio than the synthetic material, while the former was closer to the stoichiometric HAp (1.67). Other authors (e.g. 10) also observed Ca/P ratio higher than stoichiometric. Natural HAp contains Mg, which is lacking or nearly lacking in the synthetic material. Carbonate groups were found in the bovine origin HAp.[3] It will be shown below that, they also exist in the pig origin HAp. However, they were absent in the tested synthetic materials. It should be pointed out that the methods of synthesis were elaborated which allow incorporation of Mg and carbonate groups into the HAp structure.[11–13]

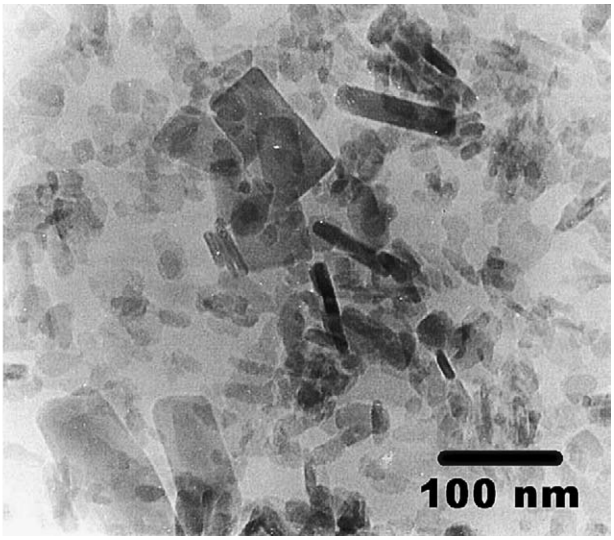

Fig. 1. Transmission electron micrograph of the hydroxyapatite particles extracted from the pig bone.

Table 1
Chemical analyses of hydroxyapatite samples

Sample	Elektromet (Poland) synthetic	Mitsubishi (Japan) synthetic	HAp (bovine)	HAp (pig)
CaO	55.71	51.65	52.25	51.37
MgO	Traces	0.05	0.41	0.62
P_2O_5	42.74	38.88	38.37	37.76
Ca/P	1.66	1.68	1.73	1.72

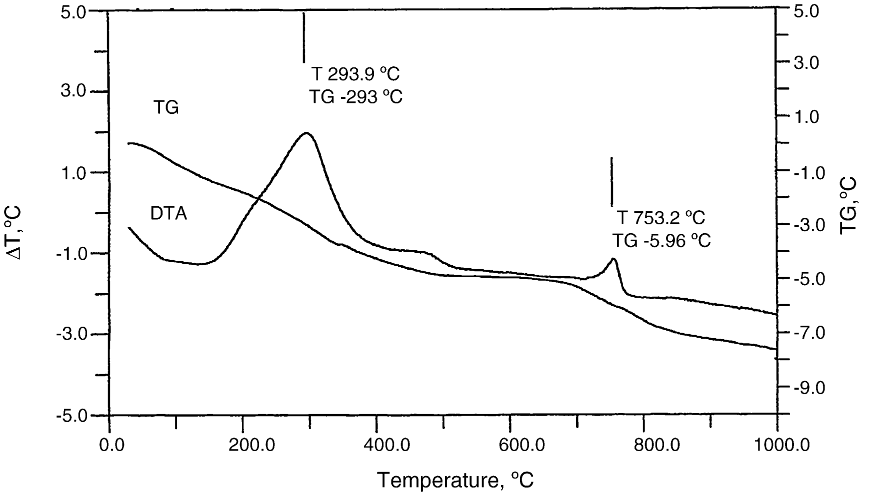

Fig. 2. Pig bone DTA and TG curves. Rate of temperature increase 10 °C/min., air atmosphere.

In Fig. 2 differential thermal analysis (DTA) and thermogravimetric curves (TG) are shown. The low endothermic effect should plausibly be attributed to evaporation of water adsorbed on high surface area of the material. Within the whole temperature range sample lost its mass. At higher temperatures two exothermic effects occurred. In order to recognize their nature two calcinations were performed for 30 min at the end temperatures of these effects, i.e. 350 and 780 °C. Rate of temperature increase was 10 °C/min, i.e. the same as applied during the DTA/TG measurements. Fig. 3 shows infrared absorption spectra of these samples as well as the sample before thermal treatment. Absorption bands were ascribed to particular bonds according to.[14] Vibrations of P–O bonds are assigned to the absorption bands within wave number ranges of 471–603 cm^{-1} and 992–1091 cm^{-1}. Bands in the 1415–1468 cm^{-1} range confirm the above-mentioned existence of carbonate groups in the natural hydroxyapatite. Absorption bands of 2860 and 2929 cm^{-1} are assigned to stretching vibrations of C–H bonds. The remaining bands in the high wave number range result from vibrations of O–H bonds. These bonds occur in water, which is adsorbed on the high surface area of the starting powder and in hydroxyl groups present in the HAp structure.

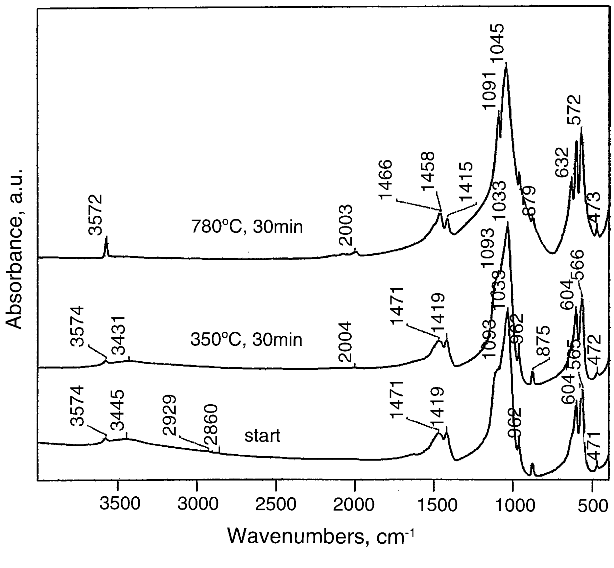

Fig. 3. Transmission infrared spectra of pig origin hydroxyapatite.

Absorption bands assigned to the C–H vibrations in the starting sample suggest that treatment with NaOH does not result in the total removal of the organic material of bones. These absorption bands disappear in the materials calcined at 350 °C and at higher temperatures. Based on this, we conclude that the exothermic effect observed at the low temperature range (Fig. 2) corresponds to oxidation of the remaining organic materials. This is corroborated by the mass spectrometric analysis of gases emitted from the sample heated in air atmosphere. Fig. 4 shows an increased signal at about 300 °C corresponding to a species of mass/charge quotient

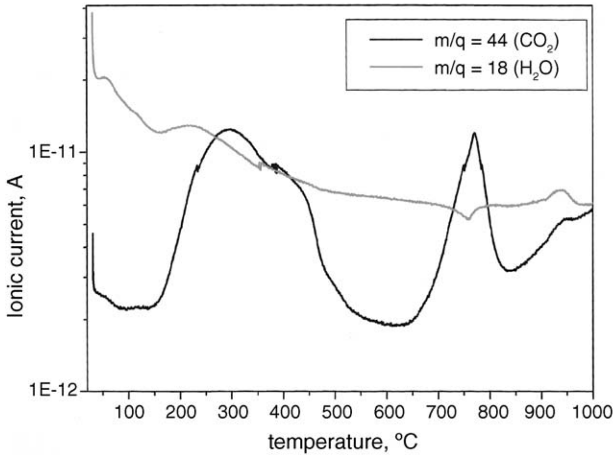

Fig. 4. Mass spectrometric analysis of the gases emitted from the HAp sample heated in flowing air. Rate of temperature increase 10 °C/min.

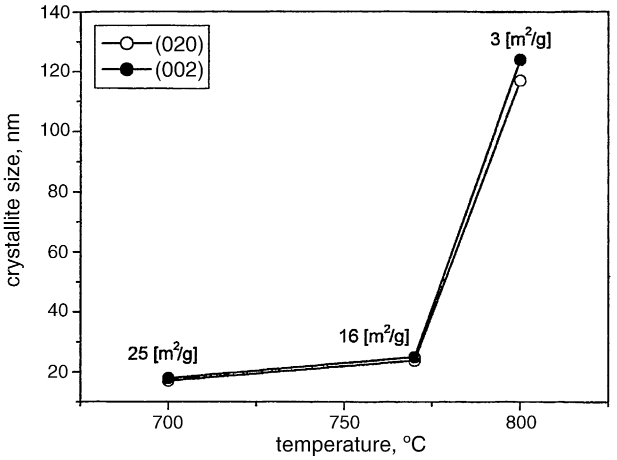

Fig. 5. Changes of specific surface area and crystallite sizes of HAp vs. temperature. Samples prepared under dynamic heating conditions with the rate of temperature increase of 10 °C/min.

(*m*/*q*) of 44 which can be attributed to the singly ionised CO_2 molecules. The pale line of $m/q = 18$ corresponds to the singly ionised water vapour molecules.

Using the DTA equipment the sample was heated (10 °C/min) to the temperature just below the beginning of the second exothermic peak and quickly quenched. The same procedure was repeated at the peak temperature and at temperature slightly higher than the peak temperature. Fig. 5 indicates specific surface area of these three samples and changes of the crystallite sizes assessed based on the X-ray line broadening. We notice that a sudden decrease of the specific surface area and an increase of crystallite sizes accompany the higher temperature exothermic effect. Most probably such phenomena are responsible for this effect. Fig. 4 indicates that simultaneously with the exothermic effect carbon dioxide is secreted from the sample changing its composition. Another coinciding effect was found by the dilatometric measurements (Fig. 6). Close to the beginning of the second exothermic effect, compacts of the HAp powder started to shrink.

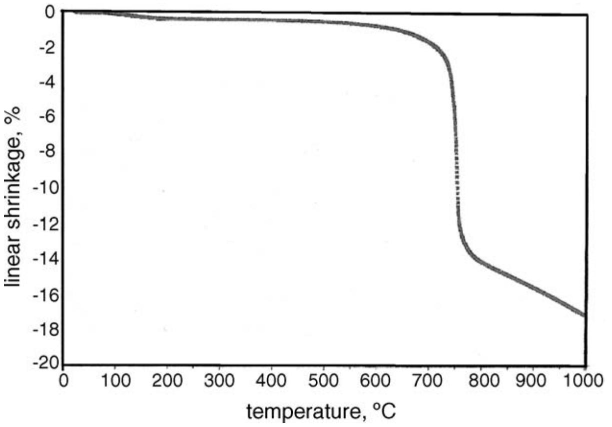

Fig. 6. Linear shrinkage of the HAp powder compact vs. temperature. Rate of temperature increase 10 °C/min.

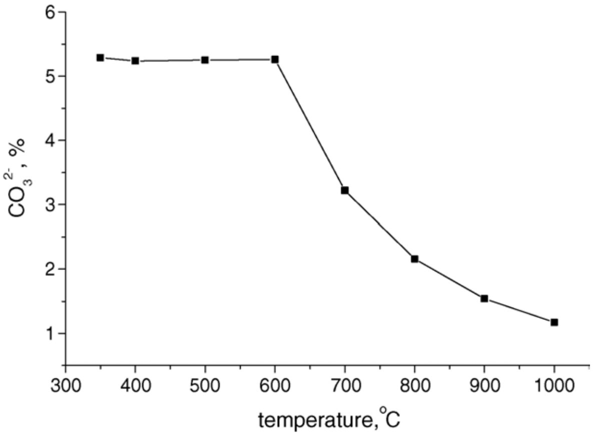

Fig. 7. Content of CO_3^{2-} groups vs. calcination temperature.

To study in detail chemical changes in HAp taking place with temperature a series of samples were manufactured by heating them at temperatures in the range from 350 °C to 1000 °C. Rate of temperature increase was 5 °C/min, soaking time 30 min at the pre-selected temperature and cooling with the furnace. Fig. 7 shows content of CO_3^{2-} groups determined by the chemical analysis. We notice that under static heat treatment conditions concentration of carbonate groups decreases starting from calcination temperature of 700 °C, in agreement with the phenomena above described. In samples calcined at this temperature and higher, X-ray diffraction (Fig. 8) reveals one reflection corresponding to CaO. It could easily be removed from the system by washing the sample with distilled and de-carbonated water. A reaction of CaO with water results in $Ca(OH)_2$ formation, which is reasonably well soluble in water. Washing was continued until pH of the filtrate reached the value of 7. Chemical analysis of the filtrate allowed us to determine the amount of calcia removed from HAp versus calcinations temperature (Fig. 9). We notice again that this phenomenon starts at temperature at which deep changes of the material begin. Nevertheless, X-

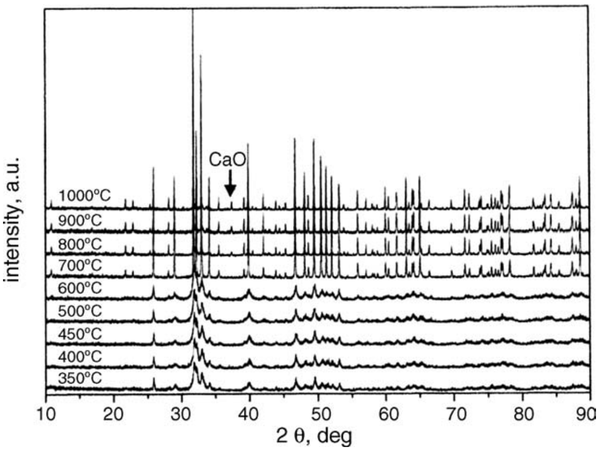

Fig. 8. X-ray diffraction patterns of the HAp samples calcined at indicated temperatures.

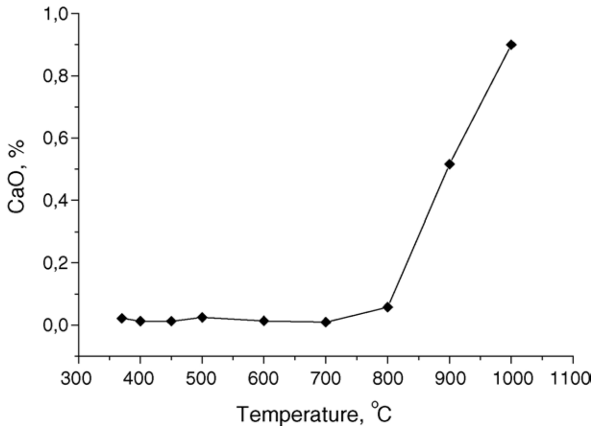

Fig. 9. Content of free CaO vs. calcination temperature.

ray diffraction pattern (Fig. 8) and infra red spectra (Fig. 10) indicate that animal origin HAp preserves its structure even at calcination temperature as high as 1000 °C.

Preliminary experiments allowed us to check hydroxyapatite originating from pig bones for biocompatibility with CAL-72 (human osteosarcoma). Discs of diameter fitting the well of 12-well cell culture micro-plate were formed by uniaxial pressing (50 MPa). Half of the plates were calcined at 350 °C and another half at 550 °C. Then the discs were washed with distilled water followed by conditioning in growth medium prior to cell seeding. It was found that samples calcined at 550 °C were colonized more effectively than those fired at 350 °C. It indicates that the procedure of the HAp thermal treatment affects the biocompatibility of the material.

HAp nanoparticles of pig bone origin were used to modify surface of carbon fibres.[15] It was found that this modification stimulated biomimic growth of apatite from the simulated body fluid (SBF). In vivo no toxic reactions were observed.

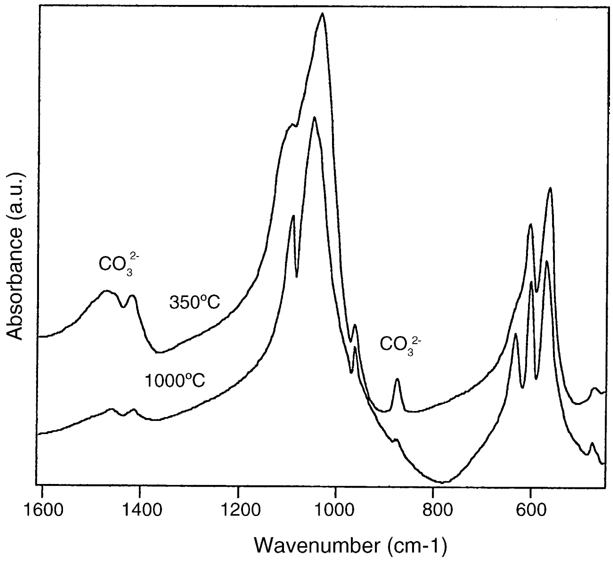

Fig. 10. Transmission infrared spectra of HAp calcined at (a) 350 °C and (b) at 1000 °C. Measuring conditions and equipment as indicated in Fig. 3.

4. Summary and conclusions

The presented chemical procedure allowed us to extract hydroxyapatite from pig bones. The rest of organic material disappeared at 350 °C. Crystallite sizes of the natural HAp classify it as a nanometric material. In contrast to synthetic hydroxyapatite, natural HAp contained carbonate groups and small amounts of Mg. The Ca/P ratio was higher than that in the stoichiometric (synthetic) material. At temperatures >700 °C the material starts to decompose; CaO and carbonate groups were partially removed from the system. Simultaneously compacts of the HAp powder shrink, specific surface area decreased and crystallites grew effectively. In spite of the indicated chemical changes pig origin hydroxyapatite calcined at a temperature as high as 1000 °C preserved it structure.

Preliminary biological tests have showed that rate of growth of the human osteosarcoma cells was faster on the powder compacts calcined at 550 °C than those which had been heat treated at 350 °C. In vitro nanometric HAp particles modifying surface of carbon fibres stimulate biomimic growth of apatite from the SBF. In vivo no toxic reactions were observed.

Acknowledgment

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