

UDC 542.65

Investigation of the Properties of Hydroxyapatite Extracted from the Bone Tissue of Agricultural Animals

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(Received February 10, 2012; revised July 9, 2012)

Abstract

Dependence of the properties of calcium phosphate compounds on the method of their isolation from biological raw material was studied. Physicochemical studies of the properties of resulting materials were carried out. The proposed procedure allows one to obtain ultrafine powders of biological hydroxyapatite with controllable particle size and composition maximally approaching the composition of bone tissue. It was established that the calcium phosphate compound deposited from the solution that was obtained by demineralization with the solution containing hydrogen chloride 1 mol/L is most close to the mineral matrix of bone tissue.

Key words: calcium phosphate compounds, deposition, dispersity, phase composition, stoichiometry

INTRODUCTION

Substitutive reparation of the regions of organ tissues in affected area of an organism is one of the urgent problems of modern medicine. This relates almost to all areas of surgery where it is necessary to reconstruct the lost structure of an organ or tissue and their functional characteristics. Reconstruction should proceed both due to the specific structure and properties of implant and due to the activation of the own cell elements and enhancement of tissue regeneration in general [1]. Calcium phosphate powders, in particular hydroxyapatite (HA) of various stoichiometric and granulometric compositions with different morphologies are used in developing biocompatible materials. Hydroxyapatite species of biological and artificial origin are used at present. Biological hydroxyapatite is obtained mainly by baking cattle bones and subsequent grinding. HA powders are characterized by a broad range of particle

size, they comprise particles 50–100 μm in size. Synthetic HA is obtained mainly using solution-based and solid-phase methods. Solution-based methods include deposition, hydrothermal synthesis and hydrolysis of calcium phosphates. Depending on synthesis methods, HA powders may differ in morphology, specific surface, stoichiometry and crystallinity degree [2].

Stable chemical composition and the presence of microelements characteristic of bone mineral are typical for HA obtained from biological raw material.

The extraction of calcium phosphate compounds (CPC) from bone tissues of agricultural animals using the solutions of hydrochloric acid was described in [3]. Investigations showed that the use of concentrated solutions of hydrochloric acid is accompanied by the loss of such osteotropic elements as phosphorus, magnesium and sulphur, which can have a negative effect on the biological compatibility of calcium phosphate materials for implants. Cal-

cium phosphate compounds extracted from cattle bone tissues using diluted solutions of hydrochloric acid (for demineralisation) and saturated ammonium hydroxide solution (for the precipitation of calcium phosphates) are most close in calcium to phosphorus ratio to the major mineral component of bone tissue (HA).

Obtaining HA from animal bone tissues [4] has a number of advantages in comparison with other methods:

- the valuable microelement composition maximally close to the composition of human bones is conserved;
- it is possible to control the size of hydroxyapatite particles;
- the wastes from the industrial process do not contain chemically active and volatile compounds (the process is ecologically friendly);
- simplicity of the apparatus arrangement of the method).

The goal of the present work was to obtain ultrafine HA powder with the microelement and phase composition identical to native bone.

EXPERIMENTAL

Investigations were carried out with the help of the scanning electron microscope Philips SEM 515 and transmission microscope CM 30/STEM with the resolution 0.24 nm. The phase composition was determined using X-ray diffractometer Shimadzu XRD 6000. Elemental composition was determined with the help of X-ray fluorescence analysis (XFA) using VRA-30 spectrometer and SPECORD M 80 IR spectrophotometer. Specific surface of HA particles was measured with the help of Sorbtometer M ver. 1.0.0.0 by means of BET multipoint procedure.

Calcium phosphate compounds were obtained from cattle bone tissues under laboratory conditions.

Initial raw material was prepared using the following procedures [5]: cattle bones were mechanically purified from paraosseous tissues, washed with warm water, rinsed with distilled water. Then bone demineralisation was carried out with the solution of hydrochloric acid with the concentration of 0.5–4.5 mol/L. After demineralisation, the liquid part of the suspension was separated by filtering. Hydroxyapatite was precipitated from decalcified solutions by adding 20–25 % solution of ammonium hydroxide under intense mixing until pH 7.3–7.5 was achieved. Abundant precipitation was observed. The suspension was filtered. The resulting pasty product was washed with distilled water until pH of washing water became equal to pH 6.0–6.5. To purify the product from bone proteins co-precipitated during the precipitation of inorganic matrix, the material was treated with carbamide solution. Then the precipitate was dried at a temperature of 100 °C and ground. We studied HA samples precipitated from solution that was obtained by bone tissue demineralisation with HCl solution with the concentration of 0.5, 1.0, 2.0, 2.5, 3.5, 4.5 mol/L (samples Nos. 1–6, respectively), and HA sample obtained by baking cattle bones at 1200 °C (sample No. 7).

CPC precipitation was carried out after 2, 5, 9 and 13 days from bone demineralisation. The series of experiments with HCl concentration 7 mol/L was not carried out because in this case coalification and complete dissolution of bone tissue occurred. The product mixture became jelly-like, so it was impossible to separate it from the liquid fraction.

TABLE 1
Results of the elemental analysis of hydroxyapatite powders

Sample No.	HCl concentration, mol/L	Atomic concentrations of elements, %			Ca/P
		O	P	Ca	
1	0.5	67.05	14.26	18.69	1.31
2	2	65.08	13.22	21.70	1.64
5	3.5	61.71	10.78	20.28	1.88
7	0	64.27	13.96	21.77	1.56

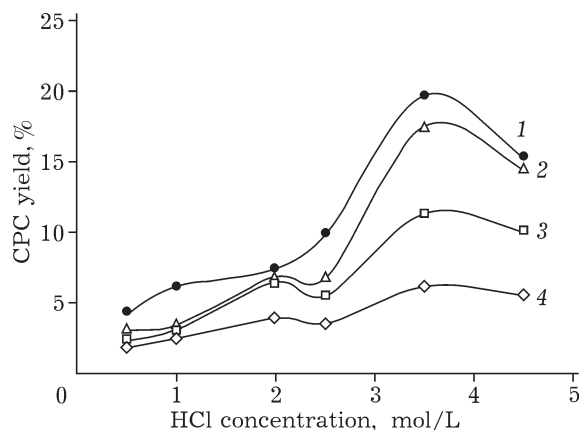


Fig. 1. Dependence of the mass of calcium phosphate compounds (CPC) on the concentration of hydrogen chloride solution. Decalcification time, days: 2 (1), 9 (2), 5 (3), 13 (4).

RESULTS AND DISCUSSION

The results of elemental analysis [6] (Table 1) allow one to assess the stoichiometry of resulting powders. It is evident that Ca/P ratio closest

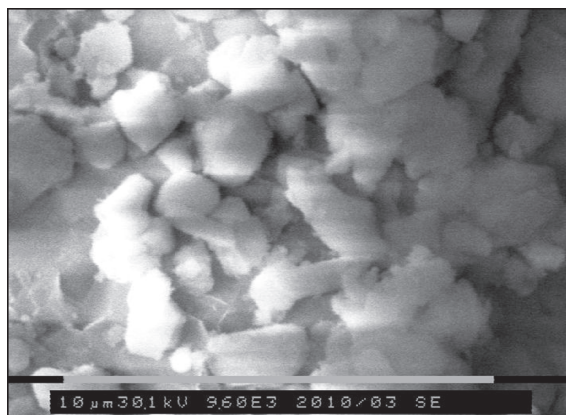


Fig. 3. Micrograph of hydroxyapatite particles obtained through cattle bone baking. Magnification: 9600.

to the stoichiometric one (1.64) is exhibited by HA powder precipitated from the solution that was obtained by demineralisation with the solution having HCl concentration 1 mol/L (sample No. 2).

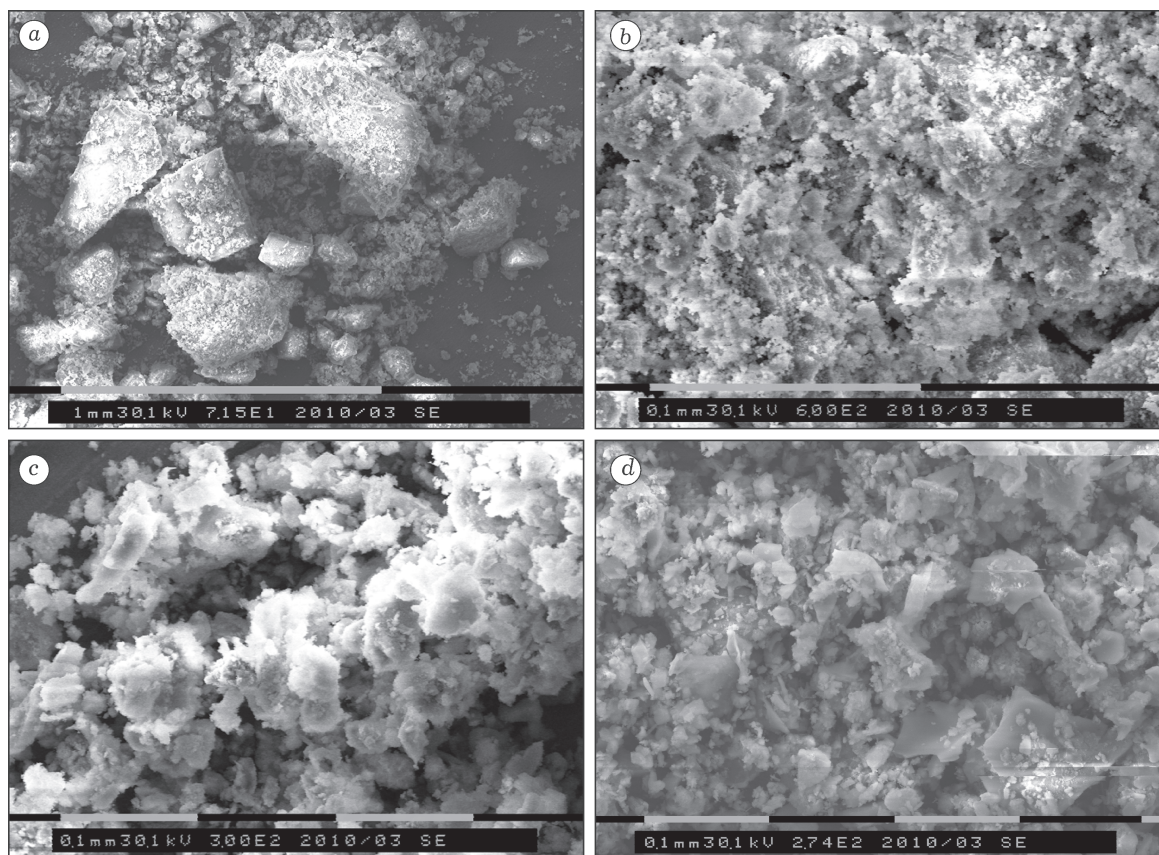


Fig. 2. Micrographs of the particles of phosphate compound obtained using HCl solutions with different concentrations (mol/L): 1 (a), 2.5 (b), 3.5 (c), 4.5 (d); magnification: 71.5 (a), 274 (b), 300 (c), 600 (d).

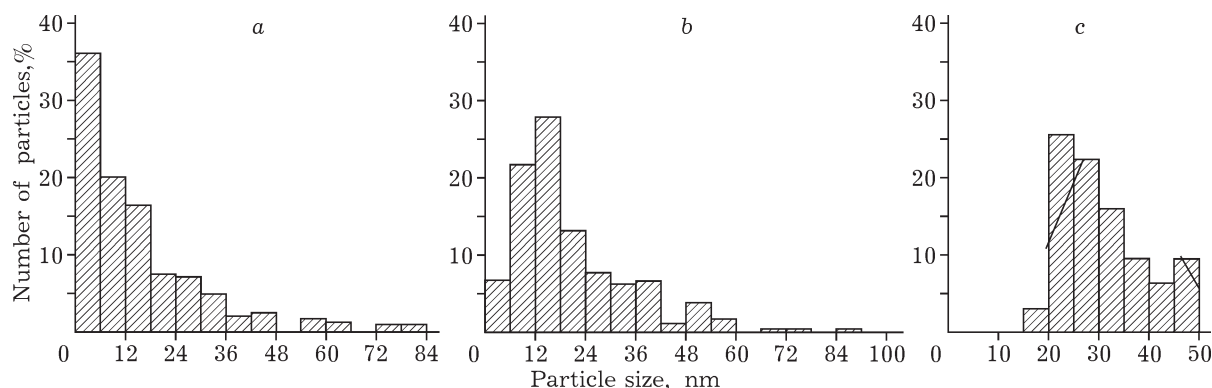


Fig. 4. Histograms of hydroxyapatite particle size distribution: *a-c* – samples Nos. 1, 2, 7, respectively (see Table 1).

The dependence of the mass of precipitate on the concentration of hydrochloric acid is shown in Fig. 1. One can see that the mass of precipitate increases with an increase in decalcification time in all the cases. The maximal yield is observed when HCl solution with the concentration of 3.5 mol/L is used.

The micrographs of HA powders are presented in Figs 2, 3. According to the results of scanning electron microscopy, the synthesized HA samples are powdered mixtures of agglomerates up to 400 μm in size.

The histograms of HA particle size distribution obtained through secant line over STEM images are shown in Fig. 4. For sample No. 1 (0.5 mol/L HCl) the peak is localized within the range 0–6 nm. For sample No. 2 (1 mol/L HCl), the peak is localized within the range 12–18 nm. In both cases we observe extended tails toward larger values. For sample No. 7 the distribution is localized within a narrow range (20–50 nm).

According to the diagram shown in Fig. 5, the smallest specific surface area is that of HA sample obtained by baking cattle bones (sam-

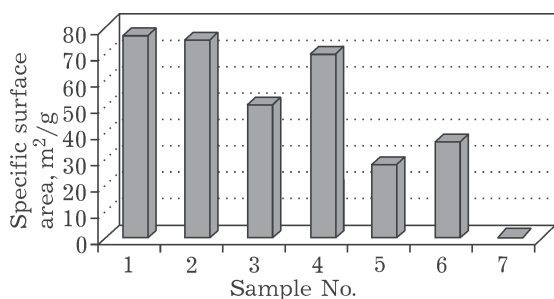


Fig. 5. Specific surface area of HA particles for all the samples resented.

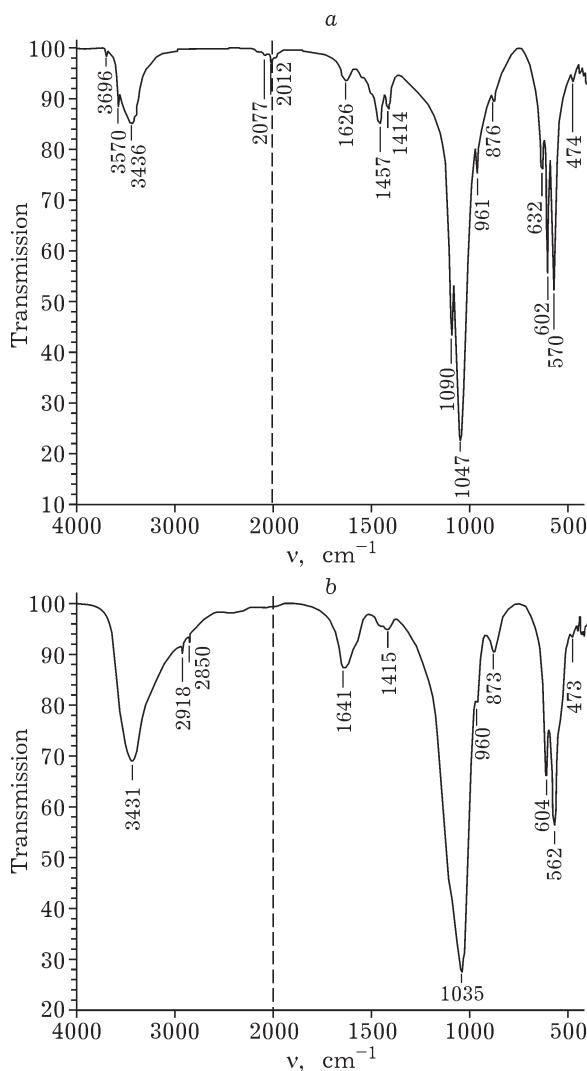


Fig. 6. IR spectra of HA samples: *a* – precipitated from the solution obtained by demineralization of bone tissue by hydrogen chloride solution; *b* – obtained by cattle bone baking.

ple No. 7). Powders precipitated from the solutions obtained by demineralisation with HCl solutions having concentrations 0.5 and 1 mol/L have the maximal specific surface area.

The IR spectra of the samples of biological HA are presented in Fig. 6. In the case of the sample of biological HA obtained by cattle bone baking, a peak at 3436 cm^{-1} is observed, which corresponds to the vibrations of OH groups in adsorbed water. Absorption bands with wavenumbers 2012, $1047\text{--}1090$, 961 cm^{-1} belong to the vibrations of P–O bonds in HA, while the band at 632 cm^{-1} relates to the bending vibration of OH group in HA. Absorption bands with wavenumbers 570 and 602 cm^{-1} correspond to the bending vibrations of phosphate groups.

The IR spectra of the samples of biological HA obtained by demineralisation of bone tissue with the solutions containing different concentrations of hydrochloric acid (0.5–4.5 mol/L) contain a broad absorption band at 3431 cm^{-1} , which corresponds to the stretching vibrations of OH groups in adsorbed water. Peaks at 1035 , 960 cm^{-1} relate to the vibrations of P–O

in HA, the peaks at $562\text{--}604\text{ cm}^{-1}$ belong to the bending vibrations of phosphate groups. The bands at 1515 , 852 , 880 cm^{-1} correspond to vibration modes ν_3 , ν_2 and ν_4 of carbonate groups, respectively. The presence of carbonate ion in HA structure is a characteristic feature of biological HA comprising bone tissues of organisms. Absorption spectra of samples Nos. 1–6 are identical.

According to the results of X-ray phase analysis (Table 2), synthesized HA powders are heterophase mixtures of HA of hexagonal and monoclinic modifications and are represented by the crystal phase accounting for 99 %; HA powder obtained by cattle bone baking is represented by the crystal phase by 100 %. Moreover, it is evident that the phase composition of HA can be varied.

SUMMARY

1. The proposed method of obtaining biological calcium phosphates from the bone tis-

TABLE 2
Results of X-ray phase analysis of hydroxyapatite powder

Sample No. (HCl concentration)	Phases detected	Volume fraction of phases, %	Lattice parameters, Å
1 (0.5 mol/L)	$\text{Ca}_5(\text{PO}_4)_3(\text{OH})$	76	$a = 9.522$ $c = 6.875$
	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	17	$a = 9.519$ $b = 18.748$ $c = 6.850$ $\beta = 120.66$
	$\text{Ca}(\text{HPO}_4)(\text{H}_2\text{O})$ – brushite	6	$a = 6.389$ $b = 15.227$ $\beta = 118.95$
	Amorphous	1	–
2 (1 mol/L)	$\text{Ca}_5(\text{PO}_4)_3(\text{OH})$	89.4	$a = 9.522$ $c = 6.875$
	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	10.5	$a = 9.519$ $b = 18.748$ $c = 6.850$ $\beta = 120.66$
	$\text{Ca}(\text{HPO}_4)(\text{H}_2\text{O})$ – brushite	0.1	$a = 6.389$ $b = 15.227$ $\beta = 118.95$
	Amorphous	1	–
7 (0)	$\text{Ca}_5(\text{PO}_4)_3(\text{OH})$	99.7	$a = 9.4016$ $c = 6.8797$
	$\text{Ca}_{0.5}(\text{Ti}_2\text{P}_3\text{O}_{12})$	0.3	$a = 8.375$ $c = 22.01$

sue of vertebrates using the solutions of hydrochloric acid allows obtaining biological material close in phase composition to hydroxyapatite formed after cattle bone baking.

2. It was established that the powder precipitated from the solution obtained by bone tissue demineralisation using the solution with hydrogen chloride content 1 mol/L is the closest in composition to stoichiometric HA ($\text{Ca/P} = 1.64$).

3. The proposed method of hydroxyapatite synthesis allows obtaining ultrafine powders with controllable particle size (within the range 10 to 500 nm).

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