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SYNTHESIS AND PROPERTIES OF INORGANIC COMPOUNDS

Hydroxyapatite of Platelet Morphology Synthesized by Ultrasonic Precipitation from Solution

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Abstract—Hydroxyapatite (HA) synthesis by precipitation with urea from aqueous solutions of calcium nitrate and ammonium hydrogenphosphate is studied. Ultrasonication during the synthesis decreases the size of platelet HA crystals from several micrometers to 200–300 nm. At low calcium concentrations in solution, the crystallizing phase is carbonate-hydroxyapatite, whereas at high calcium concentrations, octacalcium phosphate (OCP) precedes hydroxyapatite crystallization.

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Bone tissue is a composite material with a complex architecture in a mineral–biopolymer (collagen) system in which the mineral component is represented by carbonate-hydroxyapatite [1]. Hydroxyapatite (HA) nanocrystals exist in bones mainly in the platelet form with lengths of 40–60 nm, widths of about 20 nm, and thicknesses up to 5 nm, arranged by their long side (axis *c*) along collagen fibers [2]. Biocompatible materials for bone implants are, naturally, designed so that the composition and structure of the synthetic material approach those of the bone tissue [3, 4]. Hydroxyapatite is, as a rule, prepared by precipitation from solution. Its morphology is influenced by various factors, such as the temperature of the reaction mixture, pH, solution concentrations, precipitator, and ultrasonication. The synthesis of acicular and platelet HA crystals is described in [5–7]. This work studies the synthesis of HA with designed particle morphology from aqueous solutions by precipitation with urea (a mild alkaline agent) using ultrasonication, which can influence the particle size and morphology [8].

EXPERIMENTAL

Precipitation from solution through the reaction with urea (a mild alkaline agent) is one of the most promising methods for HA synthesis. When HA is prepared without ultrasonication, to a solution of $Ca(NO_3)_2$ with concentrations ranging from 0.005 to 0.03 mol/L, a solution of $NH_4H_2PO_4$ with concentrations ranging from 0.003 to 0.018 mol/L was added to achieve the Ca/P ratio equal to 1.67. The solutions were heated to 70–75°C; then, a solution of urea $(NH_2)_2CO$ was added with continuous stirring to maintain the synthesis temperature at 70°C. After opalescence appeared, the precipitate was allowed to stand for aging under the mother solution for 1 h; then, it was vacuum sucked on a Buechner funnel. After the urea solution was added, the solution pH was measured before the solution became opalescent and after a precipitate appeared. The urea hydrolysis kinetics can be inferred from the pH versus time dependence.

A set of experiments with varied reagent concentrations was carried in which HA was precipitated from a urea solution with heating and ultrasonication, as follows. A mixture of $Ca(NO_3)_2$ and $NH_4H_2PO_4$ solutions preheated to 60°C was transferred to a flask mounted in an ultrasonic bath, and a urea solution preheated to 40°C was added to the bath. The solution temperature was maintained at 70°C. After opalescence appeared, the precipitate was left to age for 1 h; then, it was separated by filtering followed by air drying for 24 h. To determine intermediates in HA crystallization, syntheses were carried out as described above but with a shorter aging time (15 min).

In addition, synthetic experiments with 1% aqueous ammonia as an alkaline agent were carried out. In these experiments, unlike in those with urea, the solutions were not heated and stirring was stopped 10 min after the solutions were combined. The aging time was 1 h.

The synthesis products and powders after high-temperature treatment (annealing for 1 h at 900°C, which was intended to ascertain the deviation of the Ca/P ratio from the set value), were characterized by X-ray powder diffraction on a DRON-3M diffractometer using

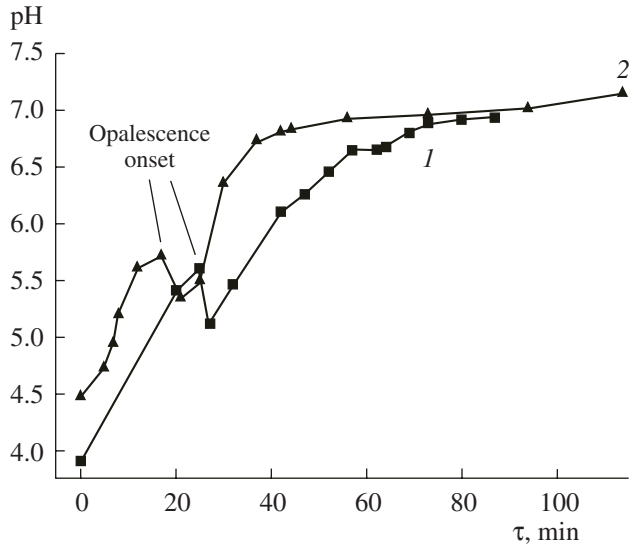

Fig. 1. Solution pH vs. time (T = 70°C) in the absence of ultrasonication with the use of a urea solution with initial temperature of (*1*) 15 and (*2*) 40°C.

CoK_α radiation. WinXPOW software was used to analyze X-ray diffraction patterns with reference to the JCPDS file.

Quantitative X-ray diffraction analysis of the products was carried out using the reference intensity ratio (RIR) method, as described in [9]. The reference intensity ratio is I/I_c, the ratio of the intensities of the 100% line of the test compound and that of corundum (α-Al_2O_3) in a 50/50 (wt/wt) mixture. In two-component mixtures with known RIRs, $\Sigma X_k = 1$, where $k = 1$ or 2 and X_k stands for the weight fractions of components (in our case, HA and tricalcium phosphate $Ca_3(PO_4)_2$ (hereafter, β-TCP)). The weight fraction of component a (X_a) is calculated from

$$X_a = I_{ia}/(\mathrm{RIR}_a \cdot I_{ia}^{rel}) \cdot [\Sigma_k(I_{jk}/(\mathrm{RIR}_k \cdot I_{jk}^{rel}))]^{-1}, \quad (1)$$

where I_{ia} is the intensity measured for the ith reflection; I_{ia}^{rel} is the relative intensity of this reflection (borrowed from the PDF-2 File); RIR_a is RIR for the phase to be determined; and I_{jk}, I_{jk}^{rel}, and RIR_k refer to all components, including component a. The nonstoichiometry of precipitation products (formation of calcium-deficient hydroxyapatite $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ is a problem for all wet methods of HA synthesis. Inasmuch as nonstoichiometric HA degrades upon calcining to produce β-TCP and stoichiometric HA, the nonstoichiometry of the starting powders can be determined by quantitative analysis of the product ratio in the calcined mixture.

The degradation reaction is

$$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x} \xrightarrow{t > 850^\circ C} (3xCa_3(PO_4)_2 + (1-x)Ca_{10})(PO_4)_6(OH)_2 + xH_2O. \quad (2)$$

The weight ratio between HA and β-TCP in each case can be determined from RIRs for HA and β-TCP; once the formula weights are known, the calcium nonstoichiometry x and the actual Ca/P ratio can be recalculated. The 100% peaks of HA and β-TCP, lying at 2θ 37.1° and 36.2°, respectively, were used in the calculations. In our case, RIR(HA) = 1.05, RIR(β-TCP) = 1.25, and I^{rel}(HA) = I^{rel}(β-TCP) = 100%. Then, x can be estimated from the relationship

$$x = \left(1 + \frac{3\mathrm{FW(TCP)} \cdot X(\mathrm{HA})}{\mathrm{FW(HA)} \cdot X(\mathrm{TCP})}\right)^{-1}, \quad (3)$$

where FW(TCP) and FW(HA) are the formula weights of TCP and HA, equal to 310 and 1004 g/mol, respectively; X(HA)/X(TCP) = 1.25I(HA)/1.05I(TCP); and Ca/P = $(10 - x)/6$.

The IR absorption spectra of intact samples were recorded as potassium bromide disks on a Perkin-Elmer PE-1600 FTIR spectrometer in the range 400–4000 cm^{-1} with increments of 4 cm^{-1}. Ion activities in solutions were studied on an Expert 001-1 single-channel pH meter equipped with a combination glass pH electrode. Particle morphology was studied using a Carl Zeiss LEO Supra 59VP scanning electron microscope.

RESULTS AND DISCUSSION

Figure 1 displays the pH versus time for the reaction without ultrasonication with the use of a urea solution of room temperature (15°C) or preheated to 40°C. The pH at which opalescence appears is virtually unchanged. The curves are symbatic; some delay in achieving the set pH is observed for room-temperature urea. Likely, temperature is the factor controlling urea hydrolysis; therefore, in further experiments we used a preheated urea solution to shorten the synthesis duration.

In experiments with urea as the precipitator and ultrasonic treatment, the time elapsed from urea addition until the opalescence onset decreased with rising urea concentration (Fig. 2). The time until opalescence onset decreased considerably with rising Ca^{2+} concentration in solution: from 40 min for 0.005 mol/L Ca^{2+} to 13 min for 0.03 mol/L Ca^{2+}. This can be due to the fact that higher pHs were required for nucleation (opalescence onset) at lower concentrations because of the constancy of the solubility product.

Ultrasonication increased the urea-hydrolysis rate on account of the higher efficiency of carbon dioxide

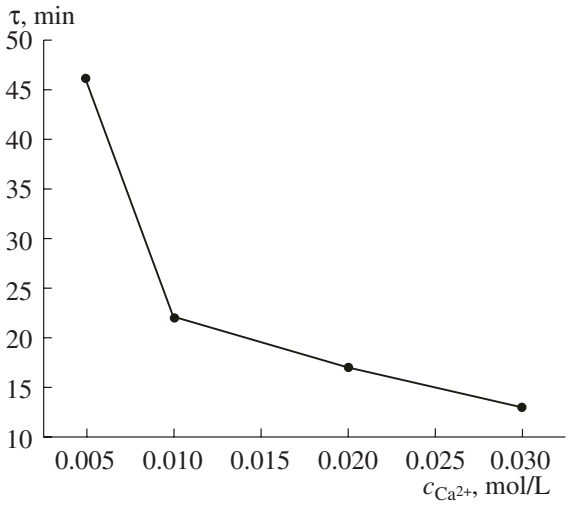

Fig. 2. Opalescence onset time vs. solution concentration during synthesis with urea under heating to 70°C with ultrasonic treatment.

elimination, thus shifting equilibrium (1) toward products:

$$NH_2C(O)NH_2 + H_2O \longrightarrow 2NH_3 + CO_2. \qquad (4)$$

Hydroxyapatite powders were synthesized by precipitation from aqueous solutions of various concentrations using urea and ultrasonic treatment. X-ray powder diffraction patterns for samples aged for 1 h (Fig. 3) show that the composition of these powders mostly corresponded to HA, except for the sample with the high $Ca(NO_3)_2$ concentration (0.03 mol/L): in this case, the major phase after heat treatment was metastable octacalcium phosphate (OCP, $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$), which is prone to hydrolyze to HA [10]. The considerable broadening of diffraction liens, especially for powders synthesized from solutions with low $Ca(NO_3)_2$ concentrations, signifies the nanorystalline state of the product. Quantitative X-ray powder diffraction analysis of samples precipitated in the presence of urea under ultrasonication and heat-treated at 900°C according to [9] showed that the Ca/P ratio was virtually unaffected by the calcium concentration in solution and equaled 1.55.

The samples after 15 min of aging contained OCP; for the minimum $Ca(NO_3)_2$ concentration (0.005 mol/L), partial conversion to HA was observed: X-ray powder diffraction showed a mixture of OCP and HA. Thus, in this case OCP is the precursor for HA.

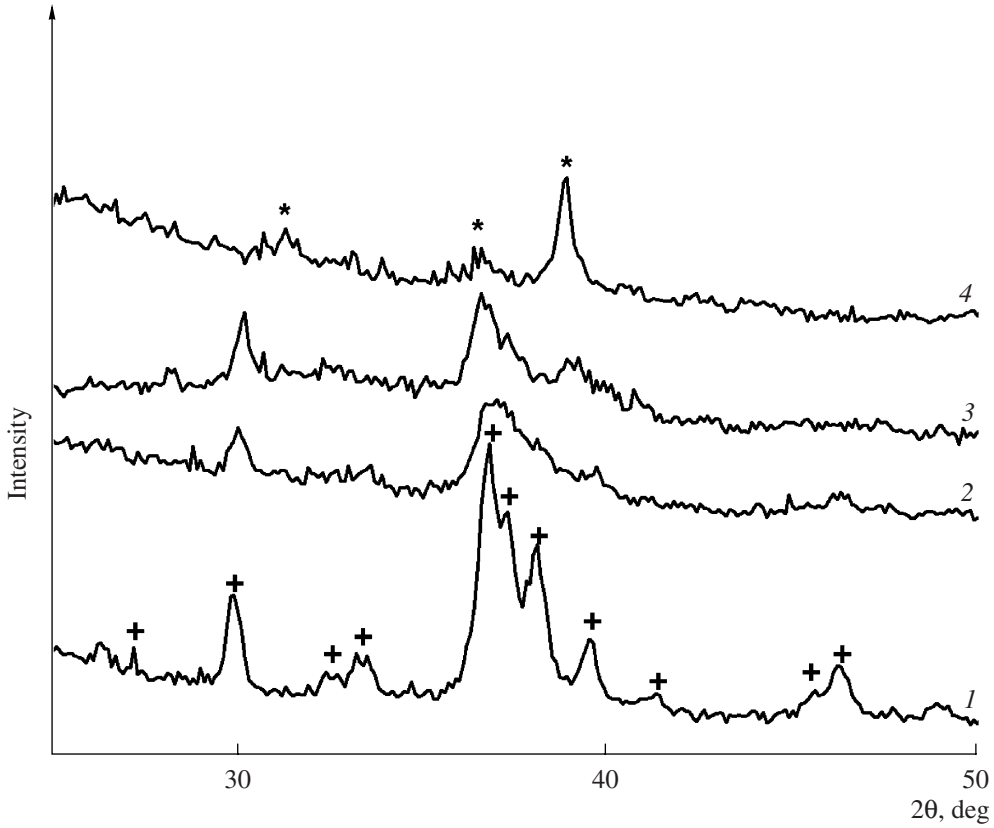

Fig. 3. X-ray diffraction patterns of powders prepared without thermal treatment by the reaction with various calcium nitrate concentrations : (*1*) 0.005 mol/L, 1 h; (*2*) 0.01 mol/L, 1 h; (*3*) 0.02 mol/L, 1 h; and (*4*) 0.03 mol/L, 1 h. Phase notation: (+) HA and (*) OCP.

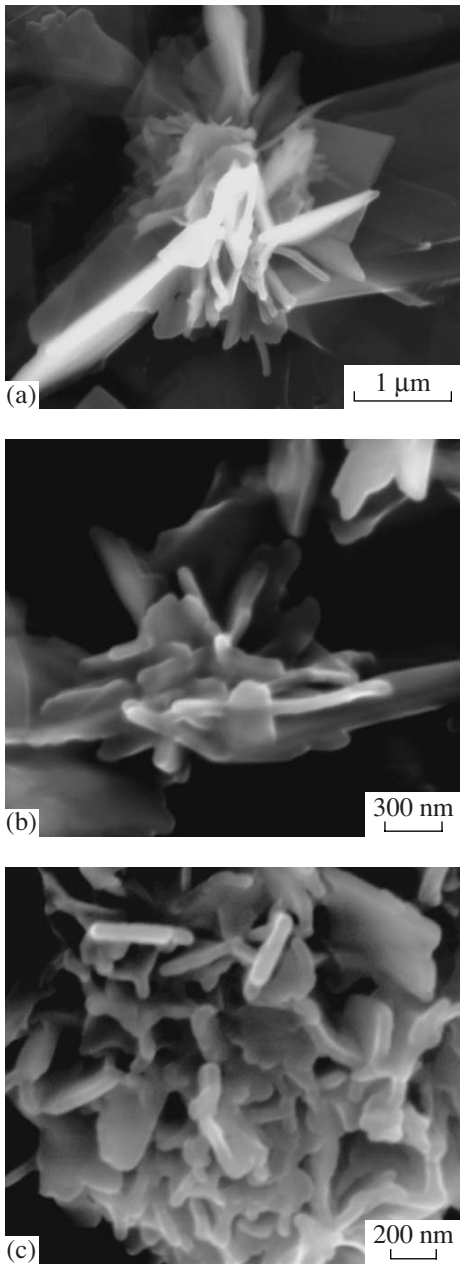

Fig. 4. Micrographs of samples prepared by the reaction with urea (a) from a solution containing 0.02 mol/L Ca under heating without ultrasonication and (b, c) from solutions containing (b) 0.01 and (c) 0.03 mol/L Ca with ultrasonication.

Electron microscopy (Fig. 4) showed platelet morphology for all products; ultrasound-assisted synthesis produced more elongated particles. Samples prepared without ultrasonication had far larger particle sizes (about 1 μm), whereas for samples prepared with ultrasonication, the average size (length) of platelet crystals was 200–300 nm. Powders synthesized with 1% aqueous ammonia as a precipitator had isometric morphology.

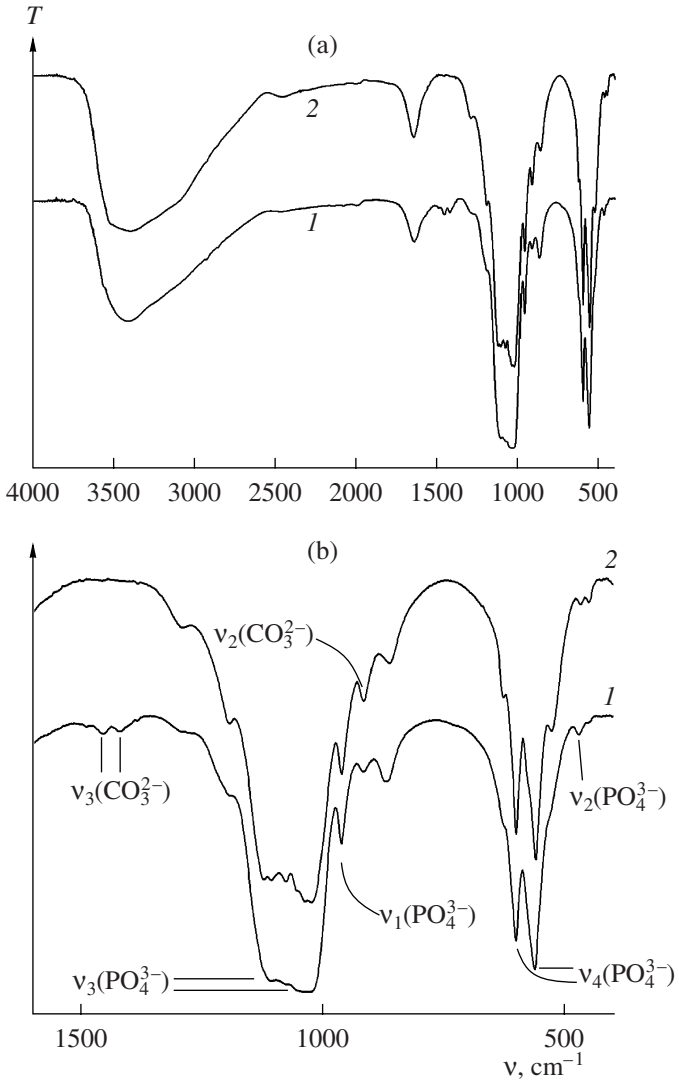

Fig. 5. (a) Full IR spectra and (b) fragments of these spectra for HA samples prepared using urea as a precipitator with the aging time equal to 1 h. Calcium nitrate concentration (mol/L): (*1*) 0.01 and (*2*) 0.03.

Platelet morphology is characteristic of both OCP and carbonate-hydroxyapatite [11]. In our case, apparently, both compounds are formed. The formation of carbonate-hydroxyapatite can be due to the high fraction of CO_3^{2-} ions in solution. IR spectra (Fig. 5) confirm that at least samples synthesized from low-concentration solutions (0.01 mol/L $Ca(NO_3)_2$) were carbonate-hydroxyapatite. Both samples display the band due to the vibrations of OH^- groups at 630 cm^{-1}; for sample 1 (synthesized at a low $Ca(NO_3)_2$ concentration), a shoulder near 3570 cm^{-1} can be seen on the background of a broad absorption band associated with adsorbate water, which is indicative of a possible existence of OH^- groups in the structure of the product. The spectra also display bands of the ν_3 mode of CO_3^{2-} at 1420 and 1450 cm^{-1} and the ν_2 mode at 873 cm^{-1}. For the sample

prepared from the solution with containing 0.03 mol/L $Ca(NO_3)_2$, however, the bands at 1420 and 1450 cm^{-1} are not found; the absorption band at 1540 cm^{-1}, intrinsic to A-type (carbonate-for-hydroxide) substitutions, also does not appear. The absence of A-type substitutions is likely because of the specifics of isomorphic substitutions in OCP, which is the precursor for HA.

The results of the above study imply the following.

Precipitation from solution with urea as a precipitator is a way to synthesize platelet carbonate-hydroxyapatite crystals whose size can be decreased to 200–300 nm by ultrasonication. The structure of the product is influenced by the concentrations of the reagents. At high $Ca(NO_3)_2$ concentrations (0.03 mol/L), the precipitate does not fully convert to HA. In this case, octacalcium phosphate $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$ is the precursor for HA. For the aging time equal to 15 min, full conversion to HA is not observed even at moderate $Ca(NO_3)_2$ concentrations. Hydroxyapatite prepared in this way inherits both the morphology and the stoichiometry of the initially formed octacalcium phosphate.

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