=== **CHEMISTRY** ===

The Influence of NO_3^-, CH_3COO^-, and Cl^- Ions on the Morphology of Calcium Hydroxyapatite Crystals

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Received September 13, 2006

Calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA) and related calcium phosphates are of considerable interest for medicine as biocompatible materials because HA is the main component of bones and teeth [1, 2]. Due to the closeness of the composition of HA to that of the bone mineral and unique physical and chemical properties, materials based on calcium phosphates are widely used as ceramics, cements, and composites. HA powders are also used in many other fields: chromatographic separation of proteins and amino acids, catalysis, sorption of heavy metals, and others.

Crystals of synthetic apatite and its biological analogue differ in shape and size [1]. In particular, bone apatite exists in the form of flat crystals less than 50 nm long. The most typical shape of synthetic apatite crystals is an elongated hexagonal prism. Inasmuch as the surface state and area of HA often affect the effectiveness of a powder in its application (including in medicine and biology), the morphology of HA crystals is of special interest [2].

The nonstoichiometry range of hydroxyapatite $Ca_{10-x}(PO_4)_{6-x}(HPO_4)_x(OH)_{2-x}$ $(0 < x < 10)$ is known to be rather wide (the molar ratio Ca : P = 1.5–1.67). Among the factors potentially important for the morphology of HA crystals synthesized from solutions (the initial solution concentration, pH, synthesis temperature, solution ionic strength, and concentration of an impurity ion (modifier)), we consider in this work the first three of them. These are precisely the factors that should be chosen when planning to obtain HA by precipitation from a solution, whereas the introduction of an impurity ion is not a necessary condition for the synthesis [3–6]. In addition to these factors, there is yet another factor that can influence crystal morphology, namely, the anionic composition of a solution (i.e., the composition of the calcium-containing salt); as a rule,

this factor is ignored in crystal shape analysis. We assumed that the modifying effect of anions on the morphology of HA crystals could be caused by specific interactions of the anions with growing crystals: (i) adsorption of the anion on the apatite surface or (ii) substitution of this anion for the phosphate or hydroxide ion in the HA lattice.

In this work, calcium nitrate, chloride, and acetate were used as calcium-containing salts. These salts were chosen because of their high solubility in water (for example, $S_{Ca(NO_3)_2 \cdot 4H_2O} = 42.8^{20}$ g/100 g H_2O, $S_{Ca(CH_3COO)_2 \cdot H_2O} = 43.6^{20}$ g/100 g H_2O). The nitrate anion can be considered to be a nonmodifying ion because it does not enter the HA structure and is not subject to hydrolysis. The acetate ion, vice versa, is involved in ionic equilibria: it hydrolyzes, decreases the medium acidity, and interacts with a calcium ion to form an ion associate (ion pair) CH_3COOCa^+ ($\log K = 1.18$, where K is the ion pair formation constant). It is unclear whether the acetate ion can enter the apatite structure. It is only known that the acetate ion promotes the formation of octacalcium phosphate (whose structure can, under certain conditions, include dicarboxylic acids) at 40°C in a neutral solution. The chloride ion is considered to be a modifying one because it can be substituted for the HA hydroxyl ions to form $Ca_{10}(PO_4)_6(OH,Cl)$. The water solubility of chlorapatite is less than that of HA [7].

Calcium hydroxyapatite was synthesized by precipitation. A 0.5 M solution of chemically pure (Russian State Standard) potassium hydrogen phosphate K_2HPO_4 (60 mL) was slowly (~2 mL/min) added to 40 mL of a 0.5 M solution of a calcium salt CaX_2 (X = CH_3COO^-, Cl^-, or NO_3^-) under vigorous stirring at room temperature. The amounts of the salts were calculated from the molar ratio Ca : P = 1.67 (to obtain the stoichiometric hydroxyapatite). Potassium hydroxide

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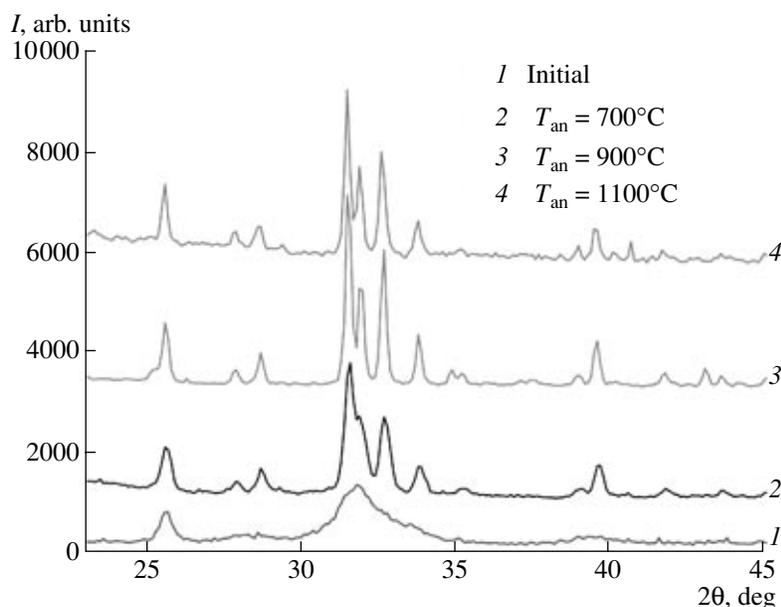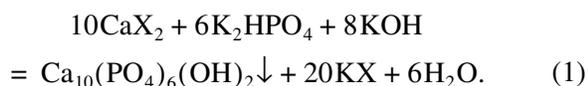   Moscow, 119991 Russia

Fig. 1. X-ray powder diffraction pattern of the HA sample obtained by reaction (1) from calcium acetate.

KOH (chemically pure) was used to maintain the target pH at 10:

$$10CaX_2 + 6K_2HPO_4 + 8KOH$$

$$= Ca_{10}(PO_4)_6(OH)_2\downarrow + 20KX + 6H_2O. \qquad (1)$$

The resulting precipitate was aged for 4 days and then filtered off (filter paper), washed with distilled water, and dried in air for 24 h. The dried powders were ground in an agate mortar.

To analyze the influence of annealing conditions on coarsening of HA crystallites and their thermal stability, powders were annealed in a muffle furnace at 500, 700, 900, or 1100°C for 2 h.

X-ray powder diffraction was performed on a DRON-3M diffractometer with CuK_α radiation ($\lambda = $ 1.54 Å, a nickel β filter). Data were collected over the 2θ range 20°–60° with a step size of 0.1° at a counting time of 2 s per point for the phase analysis and over the 2θ range 24°–27° with a step size of 0.03° at a counting time of 6 s per point for studying the profiles of X-ray diffraction maxima. The microstructure of the samples was studied using a Carl Zeiss LEO SUPRA 50VP scanning electron microscope with a field emission source. IR spectra were recorded as KBr pellets on a Perkin Elmer Spectrum One spectrophotometer in the range 400–4000 cm^{-1} with a step size of 4 cm^{-1}. The pellets, 13 mm in diameter, were formed by compression of a mixture of 1 mg of powder and 150 mg of KBr (pure for analysis) in a manual press at a pressure of 7 t.

According to the X-ray powder diffraction data (Fig. 1), hydroxyapatite was obtained as a major product by reaction (1) from various calcium salts ($Ca(NO_3)_2$, $Ca(CH_3COO)_2$, $CaCl_2$).

The considerable line widths observed in the X-ray diffraction patterns are indicative of a small particle size (Table 1). According to the literature data, this is characteristic of the synthesis procedure chosen. The average particle size was evaluated by the Debye–Scherrer formula.

The IR spectra (Fig. 2, Table 2) showed a small amount of carbonate ions present in all samples, which was caused by interaction between the reaction system and carbon dioxide of the air (the system was not specially isolated from the air).

The carbonate ion content was virtually the same in all samples. The positions of the absorption bands due to carbonate groups indicated that heterovalent substitution of carbonate ions for phosphate groups of the apatite occurred to form $Ca_{10-y+u}(PO_4)_{6-y}(CO_3)_y(OH)_{2-y+2u}$,

Table 1. Crystal sizes of the samples obtained from various calcium salts at different temperatures

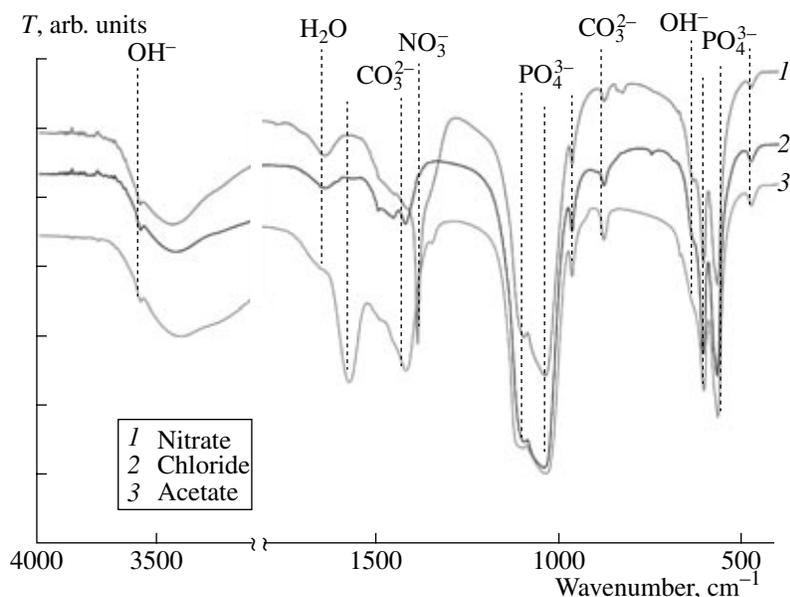| CaX$_2$	Initial sample, nm	$T_{an} = 500°C$	$T_{an} = 700°C$	$T_{an} = 900°C$	$T_{an} = 1100°C$
Chloride	25	25	40	85	60
Nitrate	15	30	40	50	70
Acetate	25	25	40	55	80

Fig. 2. IR spectra of the HA powders.

where $0 \le y \le 2$, $0 \le 2u \le y$. Note that bone apatite also contains carbonate groups (up to 4 wt %) [3].

The presence of the absorption bands of nitrate and acetate groups can be explained by adsorption of the corresponding ions on the surface of HA crystals. Further annealing will lead to decomposition of the corresponding calcium salt, i.e., to the removal of the impurity. Based on the analysis of the pairwise interactions Ca^{2+}–NO_3^- and Ca^{2+}–CH_3COO^- (the ion pair formation constants), we expect a stronger adsorption for the second pair.

The X-ray diffraction data show that the crystal size increases with temperature (500–1100°C), which is indicated by a decrease in diffraction peak widths (Table 1). The crystal size growth is a natural consequence of improvement of the mass transfer efficiency at high temperatures. A powder system tends to minimize the excess surface energy through coarsening of grains. As a result of recrystallization, large particles grow by devouring smaller ones.

Note that the acetate ion exerts the strongest inhibitory effect on the crystal growth. This is in good agreement with the above assumption on the strongest adsorption of this anion on the HA surface. At high temperatures (about 1000°C), hydroxyapatite can decompose, which, as a rule, correlates with the Ca : P molar ratio of the precipitate obtained

$$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$$
$$= 3xCa_3(PO_4)_2 + (1-x)Ca_{10}(PO_4)_6(OH)_2 + xH_2O. \quad (2)$$
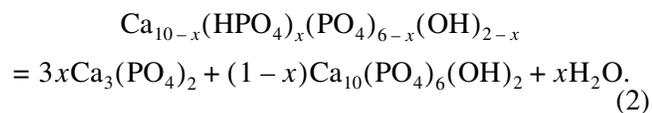
Thus, analysis of the phase composition of the samples annealed at $T = 500$–1100°C allowed us not only

to evaluate the thermal stability of the samples but also to indirectly estimate the deviation of their composition from stoichiometry. As shown by X-ray powder diffraction, all single-phase HA samples were thermally stable at 500–1100°C; this means that the HA powders obtained had the stoichiometry of ideal apatite with Ca : P = 1.67.

The micrographs of the initial HA samples confirm the above conclusion that their particles are small in size. As is seen (Fig. 3), nanosized particles form large aggregates a few micrometers in size. Such a strong aggregation of the primary crystals is characteristic of highly dispersed powder systems. Unfortunately, the possibilities of the microscope used did not allow us to analyze the shape of primary crystals due to their small size.

It is notable that the morphology of the HA crystals obtained from calcium nitrate differs from that of the other two samples. The nitrate ion is considered to be nonmodifying: it is poorly adsorbed on the HA crystal faces and is not prone to intercalation into the HA crystal structure. Therefore, one should expect the growth

Table 2. Analysis of the impurity ions in HA powders

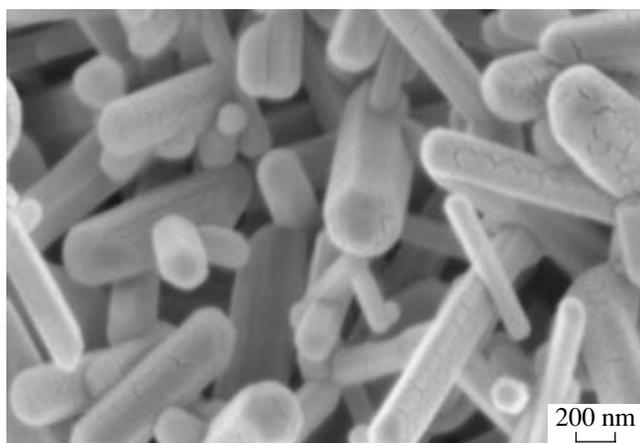| X for HA samples obtained from CaX_2	Wavenumbers of impurity ions, cm^{-1}
Chloride	CO_3^{2-}: 873, 1419, 1454
Nitrate	CO_3^{2-}; NO_3^-: 1383
Acetate	CO_3^{2-}; CH_3COO^-: 1415, 1573

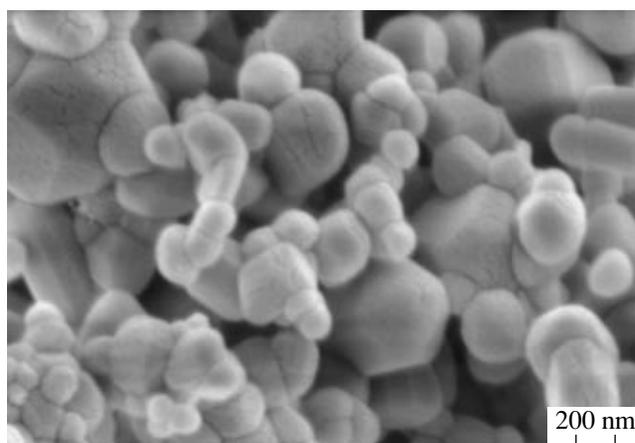**Fig. 3.** SEM image of HA crystals synthesized from calcium nitrate at 900°C.

Fig. 4. SEM image of HA crystals synthesized from calcium acetate at 900°C.

of the equilibrium shape of apatite crystals synthesized from $Ca(NO_3)_2$ (especially with increasing temperature, when mass transfer processes are accelerated).

Hydroxyapatite crystals are hexagonal with $a = 0.942$ nm, $c = 0.687$ nm, and space group $P6_3/m$ [7]. Large HA crystals obtained under conditions close to equilibrium are hexagonal prisms elongated along the [001] direction. In the electron microscopy images of the samples synthesized from calcium nitrate (Fig. 3), rodlike particles up to 2 μm long were observed (after annealing at 900°C for 2 h). Such anisotropic crystal growth at high temperatures is undesirable for formation of dense ceramics. The synthesis of HA from calcium chloride or acetate at 900°C yielded isotropic particles up to 600 and 400 nm in size, respectively (Fig. 4). At the same time, the fraction of small particles (less than 200 nm in size) was considerably larger for the acetate (a bimodal size distribution), which was also shown when calculating the crystal size (Table 2) from the X-ray diffraction data.

Thus, hydroxyapatite powders consisting of particles 25 nm in size with a high degree of agglomeration were obtained by precipitation from aqueous solutions of calcium salts. The micromorphology of the samples obtained changed with changing nature of the initial anions (chloride, nitrate, acetate): plates, needles, or equiaxed particles were obtained. Different sizes and

shapes of the crystals can be explained by different interactions of hydroxyapatite with the anions of the initial salts: adsorption of acetate anions on the HA surface, substitution of chloride ions for the hydroxyl groups of HA, and "inertness" of nitrate anions. The thermal treatment of the samples at 500–1100°C for 2 h led to the formation of faceted crystallites and an increase in their size to 2 μm. The shape of the final particles was substantially determined by the structure of the primary aggregates.

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