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CRYSTAL GROWTH

Crystallization of Carbonate Hydroxyapatite in the Presence of Strontium Ranelate

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Abstract—The influence of strontium ranelate on the crystallization of carbonate hydroxyapatite from a prototype of synovial fluid of humans has been investigated. The synthesis products are studied by IR Fourier spectroscopy, X-ray diffraction, and differential thermal analysis. The amount of strontium in the samples is determined by atomic emission analysis. The sizes of crystallites in the synthesized phases are calculated from the Selyakov–Scherrer formula; the lattice parameters are also determined. The phases obtained are found to be species of calcium-deficient strontium-containing carbonate hydroxyapatite of mixed *A* and *B* types. Schemes of chemical reactions occurring during heat treatment are proposed.

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INTRODUCTION

In the last years, the most promising biologically active materials in the group of resorptive calcium–phosphate materials based on hydroxyapatite, which are used in orthopedics and traumatology [1], have been intensively sought after.

The main advantages of these composites are that they possess biocompatibility and osteoinductivity in vivo and that their chemical composition is closest to that of human bone tissue [2].

One of the main structural characteristics of hydroxyapatite is its ability to various isomorphic substitutions with conservation of the hexagonal sp. gr. $P6_3/m$ [3].

These substitutions may change the thermal stability, solubility, texture, and reactivity of the main-component surface [4–15]. Inorganic ions, in particular, sodium [3], strontium [7], zinc [8, 9], iron [10], and magnesium [11] cations and fluorine [7], carbonate [5, 6], and chlorine [7] anions play an important role in the composition of human bone apatite.

Biological effects of inorganic ions and their influence on the processes related to the formation of bone tissue, which is important for therapy of diseases and development of bone mineralization and demineralization models, have been described in the literature [11, 12]. Osteoporosis is known to be one of the most widespread diseases of locomotor apparatus organs. This disease results in a violation of remodeling processes of bone tissue, decrease in its weight, and fracture [14–16].

Based on the results of experimental and clinical studies, we proved that which strontium-based preparations stimulate bone formation and slow down bone resorption. One such preparation is strontium ranelate, which simultaneously stimulates the formation of bone tissue and suppresses its fracture [14]. The medicinal formula of strontium ranelate contains two strontium atoms (in the stable state) and one molecule of ranelic acid. Ranelic acid is an organic part providing desired values of molecular mass, favorable pharmacokinetic properties, and good drug tolerance. Note that the character of influence of strontium ranelate on the crystal structure of human bone apatite has been studied poorly.

In this paper we report the results of studying the crystallization from a prototype of human synovial fluid in the presence of strontium ranelate and the properties of Sr-containing carbonate hydroxyapatite (Sr-**CHA**).

EXPERIMENTAL

Sr-substituted carbonate hydroxyapatite was crystallized from a model medium with ionic electrolyte composition, pH, and ionic strength close to the corresponding characteristics of human synovial fluid, using the technique described in [17, 18]. To obtain Sr-containing materials based on carbonate hydroxyapatite, strontium and calcium ions were introduced into model samples with the conservation of their fixed ratio (the concentrations were, respectively, 10 and 90% of maximally possible concentration of calcium ions (12.1 mmol/L). This concentration range was chosen based on the preliminary experiments aimed at determining the influence of Sr-containing agents on the mineralizing ability of Sr-substituted

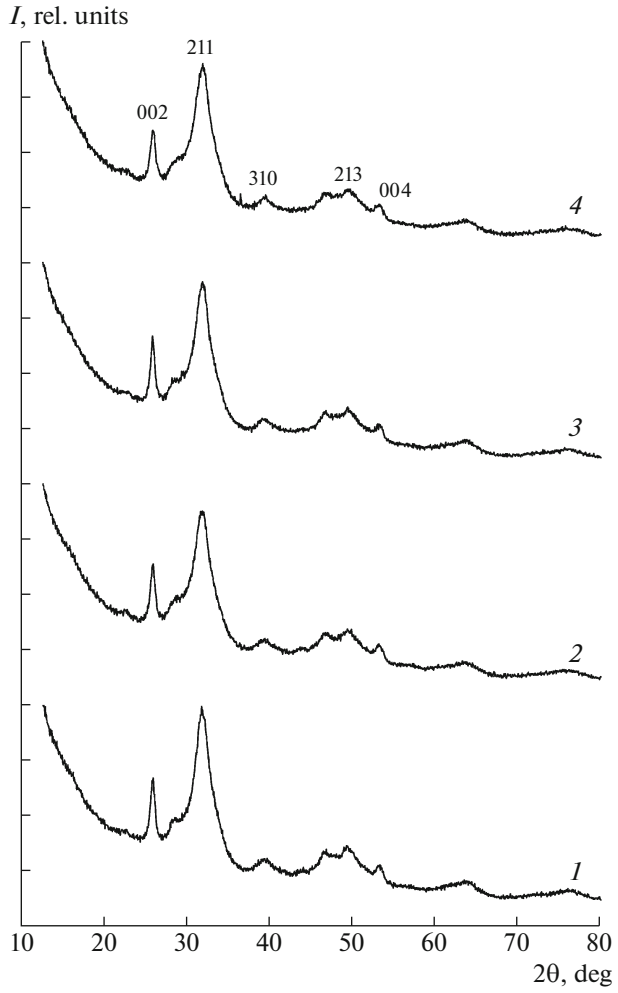

Fig. 1. XRD data on Sr-substituted carbonate hydroxyapatite for 10% Sr and 90% Ca ions in the solution and different synthesis times: (*1*) 7, (*2*) 14, (*3*) 21, and (*4*) 28 days.

carbonate hydroxyapatite from a prototype of human synovial fluid. The source of strontium ions was the drug strontium ranelate $C_{12}H_6N_2O_8SSr_2$ (commercial title of the Bivalos preparation, produced by Les Laboratoires Servier Industrie, France). The concentrations of other mineral components corresponded to those in human synovial fluid [17, 18]. The solid phase was crystallized for different time intervals, chosen based on the pharmacokinetic characteristics, indicating the concentration of equilibrium strontium ions in human blood plasma after 7, 14, 21, and 28 days; this schedule corresponds to the schedule by which patients take this preparation. After ripening a precipitate under the mother solution, the solid phase was filtered off from the solution after certain time intervals, dried at a temperature of ~80°C to a constant weight and complete removal of chemically unbound water, weighted, and analyzed by several physicochemical methods.

Synthetic solid phases were investigated using a complex of physicochemical methods.

An X-ray diffraction (**XRD**) analysis of precipitates was performed by X-ray powder diffraction (D8 Advance, Bruker; TOPAS 3.0 (Bruker) program). Quantitative phase analysis of multicomponent samples, a calculation of crystallite sizes (coherent-scattering regions (**CSRs**)), and phase identification were carried out using the ICDDPDF database for powder diffraction.

IR spectra were recorded on previously pelletized initial solid phases with KBr (IRPrestige-21 IR Fourier spectrometer (Shimadzu)).

The specific surface was analyzed according to the Brunauer–Emmett–Teller (BET) technique on a Katakon sorptometer.

Differential thermal analysis (DTA) was performed in the thermogravimetry–differential thermogravimetry–differential thermal analysis (TG–DTG–DTA) mode (NETZSCH STA-449C). The stage of thermal decomposition onset in air with a flow of 20% O_2 in Ar at a rate of 70 mL/min was analyzed. The heating rate was 10°C/min, and the temperature range under study was 100–900°C.

The elemental composition of the synthesis products was determined by atomic emission analysis on a Varian 710-ES spectrometer (AgilentTechologies).

RESULTS AND DISCUSSION

Based on the XRD data on the precipitates, it was found that the crystalline phase is hydroxyapatite, because the main reflections correspond to interplanar spacings *d* equal to 3.43, 3.08, 2.80, 2.73, 2.27, 1.95, 1.84, and 1.74 Å (Fig. 1).

Table 1. Crystallographic parameters of solid phases

Synthesis time, days	Hydroxyapatite lattice parameters		*a*/*c*	Hydroxyapatite unit-cell volume *V*, Å^3	Average crystallite sizes, nm
	a, Å	*c*, Å			
7	9.454 ± 0.005	6.869 ± 0.006	1.376	527.987	~4.0
14	9.445 ± 0.005	6.865 ± 0.006	1.376	526.675	~4.0
21	9.437 ± 0.005	6.869 ± 0.006	1.373	526.089	~4.0
28	9.438 ± 0.005	6.865 ± 0.006	1.375	525. 894	~4.0

Table 2. Strontium content in precipitates

Synthesis time, days	Sr content, wt %
7	3.1 ± 0.1
14	3.6 ± 0.1
21	3.4 ± 0.2
28	3.2 ± 0.1

Since carbonate ions enter the initial composition of synovial fluid, [17, 18], one can state that the compound formed is Sr–Ca carbonate phosphate hydrate with possible formula $Ca_{8-n}Sr_nH_2(PO)_4 \cdot 6H_2O \cdot NaHCO_3 \cdot H_2O$ [19].

The XRD data indicate that the replacement of calcium with strontium leads to a shift of the [002] reflections to larger 2θ angles, which is confirmed by the decrease in lattice parameter *a* and insignificant fluctuations of parameter *c*. A comparison of the data obtained (Table 1) with the parameters of hydroxyapatite from human bone tissue ($a = 9.410$ Å, $c = 6.890$ Å, $a/c = 1.366$) [19] shows that parameters *a* and *c* of Sr-containing carbonate hydroxyapatite are, respectively, larger and smaller than the corresponding characteristics of bone apatite. These values of lattice parameters are typical of nonstoichiometric calcium-deficient hydroxyapatites, including carbonate-containing ones [19, 20]. Based on the XRD data, we approximately estimated (using the Selyakov–Scherrer formula) the sizes of crystallites. The crystallite (CSR) size, averaged over all directions, is approximately 4 nm. XRD patterns show that the particle sizes are different in different directions. In particular, the particle size is larger in the [001] direction; the 002 ($2\theta \approx 25.9°$) and 004 ($2\theta \approx 53.2°$) peaks (Fig. 1) are narrower than the reflections from other families of planes. The unit-cell volume of Sr-containing carbonate hydroxyapatite decreases with an increase in the synthesis time, which may indicate possible isomorphic replacements of calcium ions with strontium ions in the hydroxyapatite structure [3]. Thus, we can suggest that crystallites of calcium-deficient Sr-containing carbonate hydroxyapatite were obtained.

Based on the atomic emission data on the synthesized solid phases, we established that they contain strontium ions and determined strontium content in them (Table 2).

The data on the content of strontium ions in solid phases were statistically processed with a probability $P = 0.95$, after which the confidence interval was calculated to be 3.3250 ± 0.3526. The strontium concentrations in the samples were found to belong to one general set [21]. Only insignificant fluctuations of ion concentrations are observed, which are likely caused by the complexity of settling the solid phase–solution dynamic equilibrium.

The IR spectra of the solid phases contain a number of absorption bands (due to the vibrations of inorganic-group bonds) characteristic of hydroxyapatite (Fig. 2). These are (*1*) vibrations of PO_4^{3-} bonds: asymmetric stretching vibration ν_1 P–O (1100–1090 cm^{-1}), symmetric stretching vibration ν_1 P–O (968–962 cm^{-1}), and bending vibration ν_2 O–P–O (471 cm^{-1}); (*2*) vibrations of CO_3^{2-} bonds: asymmetric stretching vibrations ν_3 C–O, corresponding to the *A* and *B* types of substitution in phosphate tetrahedra in the apatite structure (1550 and 1460–1420); and (*3*) vibrations of water bonds: stretching vibrations of OH^- groups (3570–3540 cm^{-1}). The unresolved peaks in the vicinity of 1550 and 1460–1420 cm^{-1} indicate that carbonate ions occupy sites of hydroxyl groups OH^- and PO_4^3 groups in the hydroxyapatite structure, and the synthesis product is carbonate hydroxyapatite of mixed *A* and *B* types. The absence of vibrations characteristic

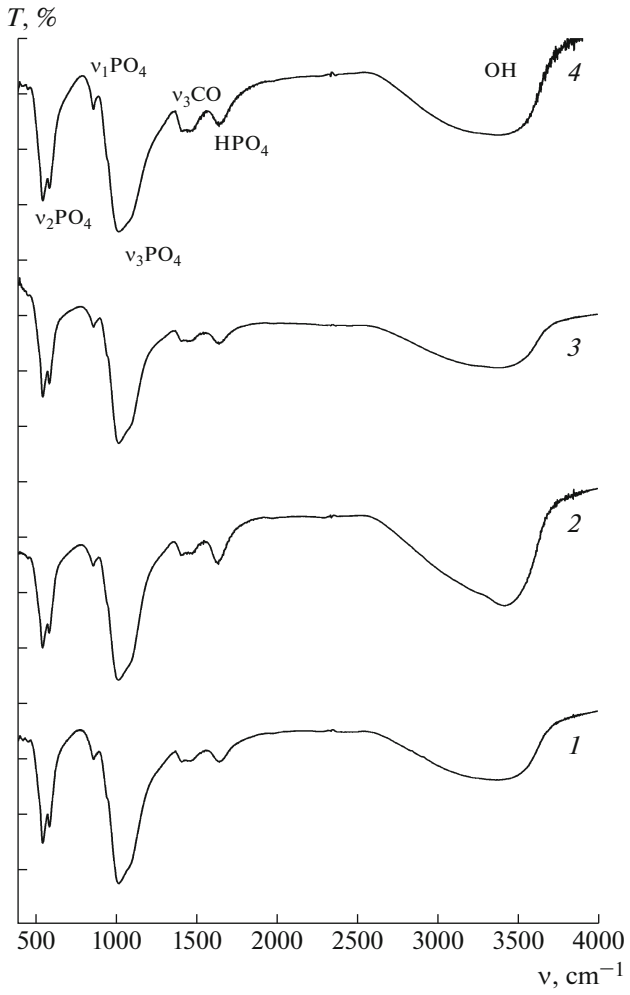

Fig. 2. IR spectra of Sr-substituted carbonate hydroxyapatite for 10% Sr and 90% Ca ions in the solution and different synthesis times: (*1*) 7, (*2*) 14, (*3*) 21, and (*4*) 28 days (4).

Table 3. Specific surface area S_{sp} of samples

Synthesis time, days	S_{sp}, m^2/g
7	79 ± 4
14	113 ± 6
21	118 ± 6
28	94 ± 5

of the organic component of the preparation (ranelic acid) in the IR spectra is indicative of good dissociation of the salt, high bioavailability of strontium ions, and indifference of ranelic acid in minor complexing and adsorption processes on the surface of crystals formed.

Specific surface areas were determined for the samples obtained (Table 3). The specific surface area was found to change nonmonotonically with time (Table 3): it increased for the first three weeks and then decreased. This behavior is related to the complexity of settling the precipitate–solution dynamic equilibrium and to the small differences in the concentrations of strontium ions in the precipitates. A comparison of the specific surface of carbonate hydroxyapatite obtained from a model solution of human synovial fluid ($S_{sp} = 231 \pm 12$ m^2/g) with the specific surface of Sr-carbonate hydroxyapatite revealed that it significantly decreases (by a factor of almost 2) in the presence of strontium ions. This fact is in good agreement with the data in the literature on the influence of Sr-containing components on the specific surface area of carbonate hydroxyapatite. In particular, the bone tissue of patients taking Sr-containing drugs is characterized by lower porosity and higher density [14], which is due to the reduced fraction of bone inner surface. Each sample of Sr-substituted carbonate hydroxyapatite was investigated by thermal analysis (Table 4). It was found that the curves are located identically, the regularity (TG, DTG, DTA) is not violated, and only quantitative effects differ. Curves for the sample obtained by 2-week synthesis are shown as an example in Fig. 3.

Based on the differential thermal curves, it was found that crystalline phases undergo the following

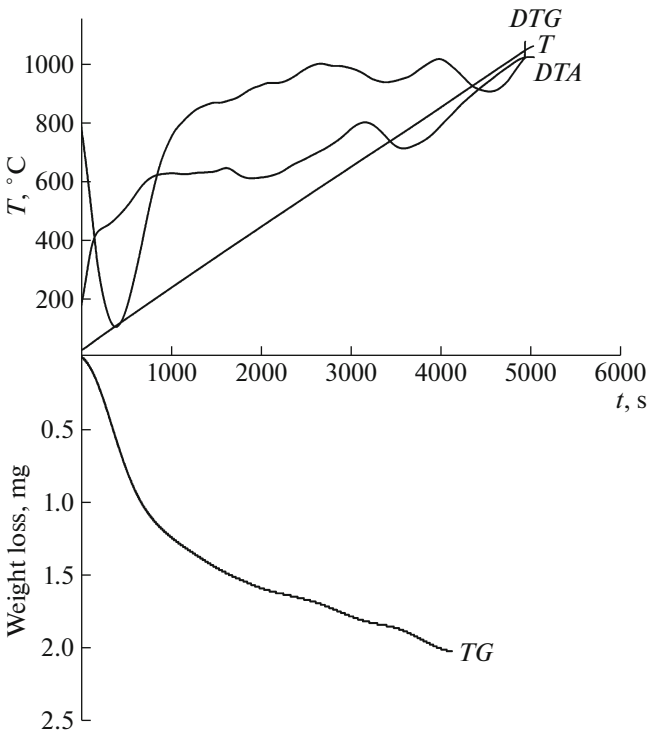

Fig. 3. Derivatogram of Sr-substituted carbonate hydroxyapatite synthesized for 2 weeks: TG, DTG, and DTA curves.

thermal transformations: the removal of chemically unbound water at 100–280°C; the removal of weakly bound (adsorbed) and crystallization water from precipitate at 280–550°C; and the removal of carbonate-ions from the hydroxyapatite structure at 500–900°C, with the formation of mixed Sr-containing β-tricalcium phosphate phase. The latter stage is accompanied by an endothermic effect.

The results obtained (Table 5) indicate that the total weight loss changes nonmonotonically with an increase in the sample synthesis time. This is likely due to the presence of inclusions of trace impurity phases and to the difference in the amounts of crystallization and chemically unbound water.

Table 4. Schemes of possible reactions during thermal transformations

	ΔT, °C	Schemes of possible reactions
I	100–280	removal of chemically unbound water: $[Sr\text{-}CHA \cdot H_2O] \cdot H_2O_{(s)} = Sr\text{-}CHA \cdot H_2O_{(s)} + H_2O_{(v)} + Q$
II	280–550	removal of weakly bound (adsorbed) and crystallization water from precipitate: $Sr\text{-}CHA \cdot H_2O_{(s)} = Sr\text{-}CHA_{(s)} + H_2O_{(v)} + Q$
III	550–900	removal of carbonate ions from the Sr-CHA structure and decomposition of Sr-CHA with formation of β-tricalcium phosphate phase: $Ca_{9-z}Sr_z(PO_4)_{6-x-y}(HPO_4)_y(CO_3)_x(OH)_{2-y(s)} = 3\beta\text{-}Ca_{3-z}Sr_z(PO_4)_{2(s)} + xCO_{2(v)} + 2H_2O_{(v)} - Q$

Table 5. Strontium weight loss (Δm) at different synthesis temperatures and times, wt %

Synthesis time, days	Δm(100–280°C), %	Δm(280–550°C), %	Δm(550–900°), %	$\Sigma\Delta m$, %
7	10.00	2.94	3.46	16.40
14	11.69	0.00	3.31	15.00
21	10.85	1.56	1.14	13.55
28	12.56	3.51	3.40	19.47

The experimental results of this study can be used in the development of new forms of medical preparations facilitating the target delivery of drugs and in traumatology and orthopedics for patients having osteoporosis and taking Sr-containing preparations.

CONCLUSIONS

Sr-containing carbonate hydroxyapatite was obtained by synthesis from a prototype of human synovial fluid in the presence of strontium ranelate.

It was found that lattice parameters a and c of Sr-containing carbonate hydroxyapatite are, respectively, larger and smaller than the corresponding parameters of bone apatite. These lattice parameters are typical of nonstoichiometric calcium-deficient hydroxyapatites, including carbonate-containing ones.

It was revealed that carbonate ions occupy sites of hydroxyl groups OH^- and PO_4^3 groups in the hydroxyapatite structure; this fact indicates that the synthesis product is carbonate hydroxyapatite of a mixed type.

It was determined that the content of strontium ions in solid phases statistically reliably indicates that they belong to one general set ($P = 0.95$; 3.3250 ± 0.3526); only insignificant fluctuations of ion concentration are observed, which are likely caused by the complexity of settling the phase–solution dynamic equilibrium.

The DTA data made it possible to build up schemes of chemical reactions of thermal transformations and indicate the thermal effects of some reactions.

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