# Mechanochemical Synthesis of Nonstoichiometric and Substituted Apatites with Nanosized Particles for Use as Biologically Compatible Materials

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## Abstract

Isomorphic varieties of substituted apatite have been synthesized mechanochemically for evaluating the applicability of the synthesized samples as biologically compatible materials. Mechanical treatment of reaction mixtures in a planetary apparatus for 5–30 min gave the final nanosized crystalline products synthesized directly in an activator. We have synthesized calcium- and phosphorus-deficient nonstoichiometric apatites, stoichiometric hydroxylapatite, hydroxylapatite with superstoichiometric potassium, zinc, and copper ions introduced in its structure, as well as apatites with potassium partially replaced by magnesium and barium. The kinetics of mechanochemical synthesis is affected by water, which stabilizes the structure of apatite. The results of bioactivity and cytotoxicity tests are given. Apatites synthesized mechanochemically may be used as biologically compatible materials.

## INTRODUCTION

In recent decades, apatites and calcium phosphates have been comprehensively studied worldwide as promising materials for bioceramics and metallic implantate coatings [1]. Calcium phosphates are bioactive substances which have found increasingly wide application in traumatology and orthopedy due to their high ability to be integrated with bone tissue [2].

This paper gives the results of our studies on mechanochemical synthesis of apatite samples with different calcium to phosphorus ratios and with calcium partially substituted by other cations. The aim of this study is to seek an optimal composition of apatite leading to biologically compatible samples. The concept of biological compatibility states that biodegradation of an artificial material liberates into environment biologically active (primarily, calcium and phosphate) ions used by the organism to form a layer of biological apatite on the surface of an implantate [3, 4] to construct a new bone-implantate functional system. Sanitary regulations require preliminary cytotoxicity analysis of the material before application of calcium phosphates to metallic implantates or formation of a 3D article [5]. Therefore we performed biological (*in vitro*) analysis of apatite samples obtained by mechanochemical synthesis.

Substituted apatite samples may be prepared by hydrothermal, thermal, or mechanochemical methods, or by precipitation from solution [6–9].

Mechanochemical activation of a reaction mixture for a few minutes leads to samples of various isomorphic varieties of substituted apatite of given composition, unobtainable by other methods [10]. Apart from the simple procedure, this method permits one to synthesize specifically substituted samples; however, the sample designed for use as an implantate material should possess the property of being biodegradable. The term "biodegradable" implies that an artificial material can be gradually decomposed or dissolved by the biological substance, liberating the components (in particular, calcium and phosphate) used by the organism for generating new bone tissue with a layer of biological apatite on the implantate [11]. Thus it is important, one the one hand, that the material should be a crystalline sub-

stance promoting fast nucleation of biological apatite and, on the other, that it should possess the property of being readily biodegradable. Apatites synthesized mechanochemically satisfy these requirements. Activation of a reaction mixture forms crystalline apatites directly in the mill [10]; the particles have small dimensions and are substantially defective, and are therefore more liable to be readily assimilated by the organism than apatites obtained thermally or by precipitation from solution with subsequent annealing.

In recent years, a number of publications discussed variations of the mechanochemical procedure for the synthesis of apatite [12–17]. However, none of these procedures have led to a perfect solid crystalline product formed directly in the mill. In some procedures, synthesis was carried out in the liquid phase [14, 15]; in others, $Ca(OH)_2$ and $P_2O_5$ were used as the starting materials [16], but in this case, synthesis is actually liquid-state because of fast hydration of phosphorus oxide, and mechanical activation is reduced to stirring the components. Therefore, these variations can hardly be called mechanochemical methods. As reported in [14], after mechanical activation, the reaction mixture was subjected to hydrothermal treatment; in [17], after prolonged mechanical activation, the reaction mixture was annealed at 1373 K.

We have first synthesized (directly in an activator) isomorphic varieties of substituted apatite from solid precursors (salts, oxides, and other compounds) [9, 10].

The aim of the present work is to study the phases formed by mechanochemical activation of binary or multicomponent phosphate-containing mixtures using different substrates and different synthetic conditions in order to obtain isomorphic varieties of apatite of specified structure and composition to be further analyzed from the viewpoint of their applicability as biocompatible materials.

Our previous studies [10] on synthesis of apatite indicated that the structure and composition of the starting compounds, as well as those of intermediates, produce a substantial effect on the mechanism of interaction, kinetics of synthesis, and the state of the end product. Water formed by interaction of acid phosphates and oxides [9] and the hydration water of the starting substances play an essential role in mechanochemical synthesis. Mechanochemical interaction of oxides and acid salts liberating water is called "soft mechanochemical synthesis" [18].

## EXPERIMENTAL

### *Mechanochemical synthesis of apatites*

To reveal an optimal calcium to phosphorus ratio in apatite designed for biocompatibility analysis we synthesized nonstoichiometric hydroxylapatites with atomic ratios of $(Ca/P)_{at} = 1.5$ and 1.6 (calcium-deficient samples [19]) and a sample with a ratio of $(Ca/P)_{at} = 1.8$ (phosphorus-deficient sample [10]); we also obtained stoichiometric hydroxylapatite with $(Ca/P)_{at} = 1.666$. The apatite of bone tissue always contains small amounts of substituent atoms in the cation and anion sublattices [20]. We have synthesized apatites with minor inclusions of potassium and copper with zinc, playing the role of microelements, as well as samples with potassium partially replaced by other cations, stimulating or inhibiting bioactivity [21].

Mechanochemical synthesis of apatite was conducted in an EI-2×150 type laboratory planetary mill with rotating titanium drums (rate 850 rpm, which corresponds to ~40 *g*) using titanium carbonitride balls to prevent iron from penetrating into the activated samples. As titanium implantates are biologically inert [21], a small amount of titanium appearing during synthesis will not affect the data obtained by testing the synthesized apatite samples.

As the starting components for the synthesis we employed monosubstituted calcium, barium, and magnesium phosphates $Ca(H_2PO_4)_2 \cdot H_2O$, $Ba(H_2PO_4)_2$, $Mg(H_2PO_4)_2$, as well as hydroxides and oxides of these elements; the reactions liberated crystallization water, but are conventionally (at least, outwardly) considered to be solid-phase reactions. The composition of the resulting phases was investigated by X-ray phase analysis (XRPA) and IR spectroscopy. We also performed thermal analysis, and several samples were subjected to transmission electron microscopy.

TABLE 1

Mechanochemical synthesis of hydroxylapatite with substituted atoms

| Sample No. | Reactions for mechanochemical synthesis of apatites | Activation time, min | Phase composition (XRPA and IR spectroscopy data) |
|---|---|---|---|
| 1 | $3Ca(H_2PO_4)_2 \cdot H_2O + 6Ca(OH)_2 = Ca_9HPO_4(PO_4)_5(OH) + 14H_2O$; $(Ca/P)_{at} = 1.5$ | 30 | Apatite |
| 2 | $3Ca(H_2PO_4)_2 \cdot H_2O + 6.6Ca(OH)_2 = Ca_{9.6}(PO_4)_6(OH)_{1.2} + 15H_2O$; $(Ca/P)_{at} = 1.6$ | 30 | » |
| 3 | $3Ca(H_2PO_4)_2 \cdot H_2O + 7Ca(OH)_2 = Ca_{10}(PO_4)_6(OH)_2 + 15H_2O$; $(Ca/P)_{at} = 1.67$ | 30 | » |
| 4 | $3Ca(H_2PO_4)_2 \cdot H_2O + 7.8Ca(OH)_2 = Ca_{10.8}(PO_4)_6(OH)_{3.6} + 15H_2O$; $(Ca/P)_{at} = 1.8$ | 30 | » |
| 5 | $3Ca(H_2PO_4)_2 \cdot H_2O + 7Ca(OH)_2 + KH_2PO_4$ (0.04 %) $= Ca_{10}K_{0.04}(PO_4)_{6.04}(OH)_2 + 15H_2O$ | 30 | » |
| 6 | $3Ca(H_2PO_4)_2 \cdot H_2O + 7Ca(OH)_2 + CuO$ (0.04 at. %) + ZnO (0.04 at. %) $= Ca_{10}(Cu,Zn)_{0.08}(PO_4)_6(OH)_{2.16}$ | 30 | » |
| 7 | $3Ca(H_2PO_4)_2 \cdot H_2O + 6Ca(OH)_2 + Ba(OH)_2 \cdot 8H_2O = Ca_9Ba(PO_4)_6(OH)_2 + 23H_2O$ | 10<br>30 | »<br>» |
| 8 | $2Ca(H_2PO_4)_2 \cdot H_2O + 7Ca(OH)_2 + Mg(H_2PO_4)_2 = Ca_9Mg(PO_4)_6(OH)_2 + 14H_2O$ | 10<br>30 | »<br>» |
| 9 | $3Ca(H_2PO_4)_2 \cdot H_2O + 5Ca(OH)_2 + 2Ba(OH)_2 \cdot 8H_2O$ = amorphous phase + unidentified product | 30 | Amorphous phase |
| 10 | $3Ba(H_2PO_4)_2 + 7Ba(OH)_2 \cdot 8H_2O = Ba_{10}(PO_4)_6(OH)_2 + 68H_2O$ | 5<br>10<br>30 | Apatite<br>»<br>$Ba_3(PO_4)_2$ |
| 11 | $3Ba(H_2PO_4)_2 + 7BaO = Ba_{10}(PO_4)_6(OH)_2 + 5H_2O$ | 5<br>10<br>30 | Apatite<br>$Ba_3(PO_4)_2$<br>» |

### *Biological testing procedure*

Biodegradation of an implantate can be simulated *in vivo* and *in vitro* by means of physicochemical and biochemical solution of the material in aggressive liquids or using bioresorption by cellular systems of the organism (microphages, osteoblasts), or by both methods. However, analyses *in vitro* are more sensitive than those *in vivo* [5]. The bioactivity concept is applicable to both 3D ceramic implantates and metal-supported coatings [11]. Biodegradation of 3D calcium phosphate materials is well known [22]. For testing apatites synthesized mechanochemically, from the thigh bone of rats we isolated the marrow and grew myelokaryocytes on a 24-hole plastic plane table at a concentration of $8/5 \cdot 10^6$/hole for 1 h at 37 °C in 1 ml of a liquid culture medium consisting of *L*-glutamine (Sigma) (280 mg), gentamycin sulfate (80 mg/l), 5 % embryonal calf serum (ICN), and 95 % Dulbekko balanced phosphate buffer (BPB) having no calcium and magnesium ions ("Vector" Concern).

Cytotoxicity of the apatite samples (1 mg/ml of cell culture) and their seven-day extracts was examined on nonadhesive marrow cells of rats using 0.4 % trypan blue. Sterile extracts from the samples were prepared according to ISO 10993–5 (1992) requirements by growing them in a 0.9 % NaCl solution at 37 °C at a concentration of 0.1 mg/ml.

The amount of cells that stuck (after 1 h) to the plastic plate was determined by measuring

their optical characteristics after they had been fixed in methanol and colored with azure II eosine. The density of the object (in arbitrary optical density units, aodu) was calculated with the Adobe PhotoShop 7.0 program based on the statistics of gray levels, isolating the region of interests (ROI) consisting of units with a fixed area [23].

Statistical processing of the results was conducted using Wilcoxon – Mann – Whitney's nonparametric $U$-criterion ($P_U$).

Details of the testing procedure are given in [24].

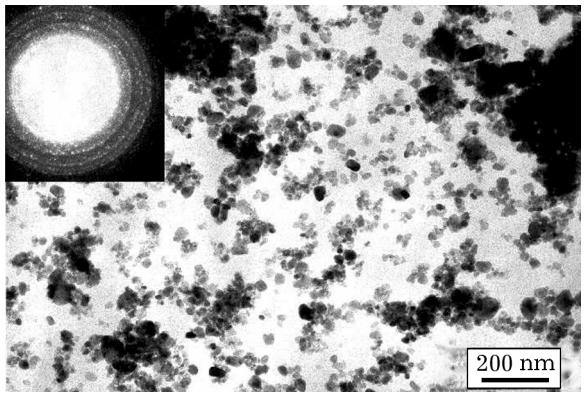


Fig. 2. TEM photograph showing the particles of hydroxylapatite synthesized mechanochemically, where $(Ca/P)_{at}$ = 1.67.

## RESULTS AND DISCUSSION

### *Mechanochemical synthesis of apatites*

Table 1 presents the compositions of the reaction mixtures and apatite and phosphate products for different activation times.

Previously, based on the kinetic energy diagram for the $Ca(H_2PO_4)_2 \cdot H_2O$ – CaO system it was shown [10] that the higher the Ca/P ratio in a mechanically activated mixture, the lower the time of synthesis of compounds with an apatite structure. This is explained by the fact that the limiting stage of the reaction is deprotonation of the $HPO_4^{2-}$ group. Therefore, the rate of formation of the crystalline hydroxylapatite product depends on the substitution $Ca^{2+} \rightarrow 2H^+$, increasing with the CaO content in the mixture. For most samples, mechanical activation was carried out for 30 min (see Table 1) to achieve the same synthetic conditions for further biological testing, although 10 min is sufficient for sample 4, and 5 min for sample 11 to form in a mill from the mixture.

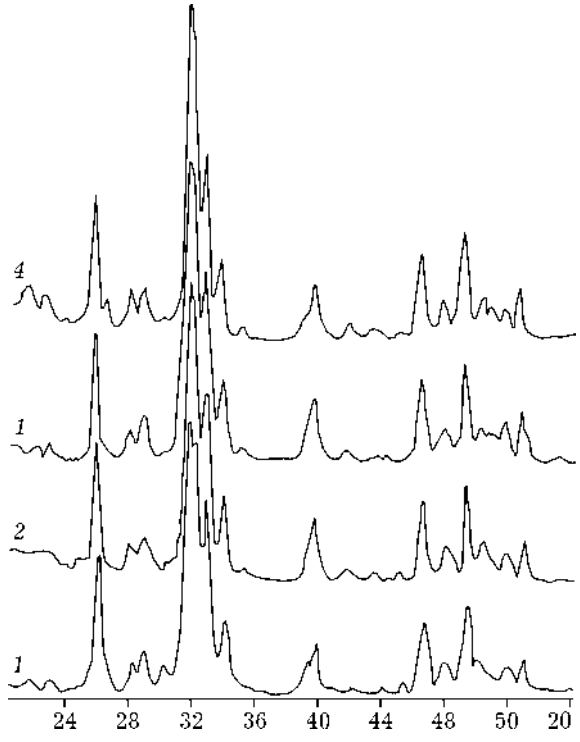


Fig. 1. Diffractograms of hydroxylapatites synthesized mechanochemically. Curve numbers correspond to sample numbers from Table 1.

After mechanical activation for 30 min, the diffractograms of apatite samples with different Ca/P ratios (Table 1, No. 1–4) acquired characteristic reflections of the crystalline lattice of apatite [25] (Fig. 1). The reflections on the diffractograms of these samples were broader than those of thermally synthesized apatites [25] because of the nanometer size of particles and the presence of structurally bonded water.

Figure 2 shows a TEM photo of mechanochemically synthesized stoichiometric hydroxylapatite with a ratio of $(Ca/P)_{at}$ = 1.67. This shape of particles is also characteristic of other apatite samples synthesized mechanochemically. Along with particles 15–20 nm in diameter, aggregates of particles sized 100 nm or more were present.

In the region of P–O bond vibrations characteristic of apatite, the IR spectra [25] were almost identical for samples No. 1–6. All samples except calcium-deficient apatite (sample No. 1, see Table 1) contained a small amount of the carbonate ion in the apatite lattice. The

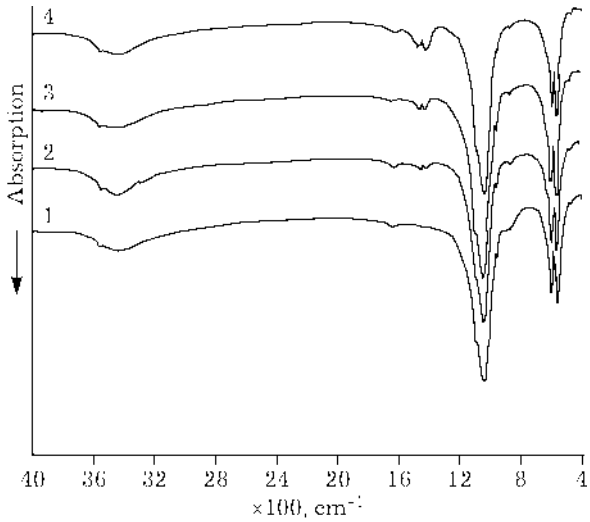


Fig. 3. IR spectra of hydroxylapatites synthesized mechanochemically with different Ca/P ratios. Spectrum numbers correspond to samples No. 1–4 from Table 1.

IR spectra of the samples contained weak absorption bands around 885 $cm^{-1}$ (due to the deformation vibrations of the C–O bonds) and bands at 1420–1460 $cm^{-1}$ (due to the stretching vibrations of these bonds [26]) (Fig. 3). This is explained by the fact that mechanochemical synthesis was conducted in atmospheric conditions, in which the formation of carbonate apatite is thermodynamically preferable [27]. Judging from the intensity of the absorption bands, the content of the carbonate ion in the lattice of the synthesized apatite slightly increases with the calcium to phosphorus ratio (see Fig. 3).

For apatite samples synthesized mechanochemically and containing small amounts of superstoichiometric potassium and copper ions with zinc (see Table 1, samples No. 5 and 6), diffraction reflections were similar in intensity (Fig. 4, samples No. 5 and 6). The IR spectra of these samples also indicated that the synthesis formed crystalline apatite; the spectra differed in band intensity in the region of the O–H bond stretching vibrations of structurally bound water [28]. As mentioned above, the limiting stage of the reaction between hydrophosphate and calcium oxide is deprotonation of the $HPO_4^{2-}$ group. If the reaction mixture has potassium ions, the reaction is enhanced, forming apatite in 10 min. The synthesized sample contains an insignificant amount of water. The IR spectrum contains a weak broad band of O–H bond stretching vibrations in the region 3400–3500 $cm^{-1}$ (Fig. 5, spectrum *1*).

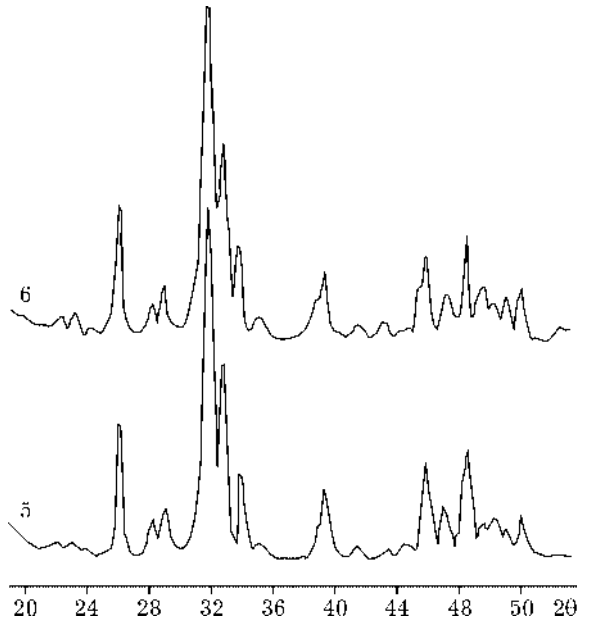


Fig. 4. Diffractograms of hydroxylapatites synthesized mechanochemically and having potassium ions (sample No. 5) and copper and zinc (sample No. 6) introduced into the lattice. Curve numbers correspond to sample numbers from Table 1.

The IR spectrum of the apatite sample containing copper and zinc ions had a relatively intense band of O–H bond vibrations with a maximum at 3430 $cm^{-1}$. It is believed that in this region, the absorption bands are due to the presence of structurally bound water coordinated by the copper and zinc cations in the apatite samples (Fig. 5, spectrum *6*). The samples partially lose water during storage. According to thermal analysis data, after they had been stored for half a year, samples No. 5

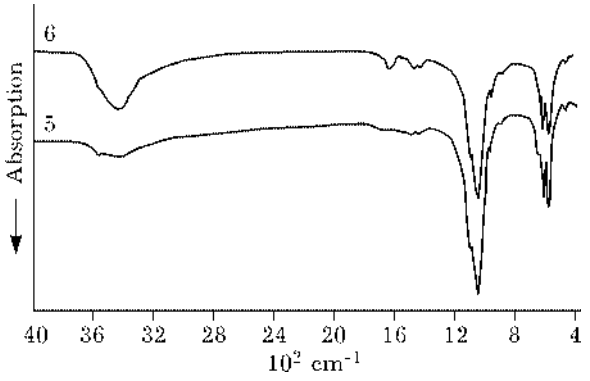


Fig 5. IR spectra of hydroxylapatites synthesized mechanochemically and having potassium ions (sample No. 5) and copper and zinc (sample No. 6) introduced into the lattice. Curve numbers correspond to sample numbers from Table 1.

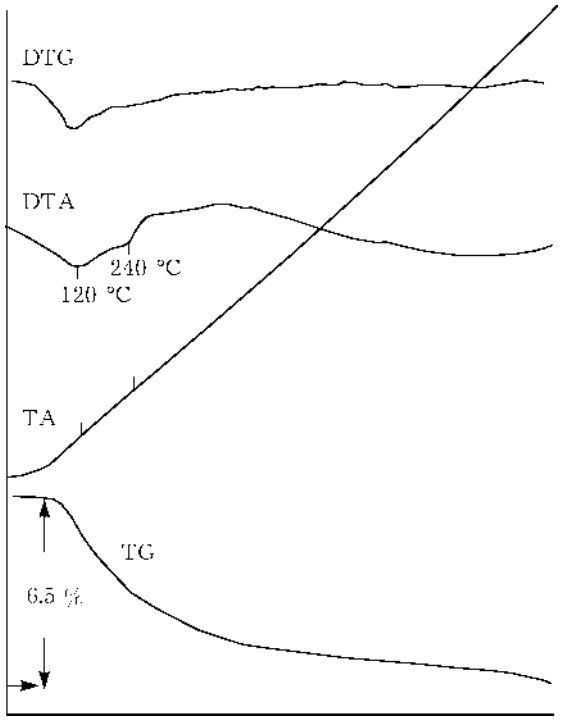


Fig. 6. Thermogravimetric pattern of a hydroxylapatite sample synthesized mechanochemically and having copper and zinc (sample No. 6, see Table 1) introduced into its lattice after having been stored for 6 mos.

and 6 had nearly the same content of structurally bound water (~6.5 %) and started to lose water at 120 °C (Fig. 6).

Investigation of mechanochemical synthesis of apatite with magnesium partially substituted for calcium revealed the stabilizing role of structurally bound water in apatite formation. According to the data of [29], apatite synthesis from solutions of calcium and magnesium nitrates and a solution of disubstituted ammonium phosphate with further annealing led to monophase apatite only if 0.1 mole of calcium was replaced by magnesium. Whitlockite ($Ca_3(PO_4)_2$) formed when 0.25 and 0.5 moles of calcium ions were replaced by magnesium after annealing at 500 °C or when 1.0 mole was replaced at 300 °C [29].

In mechanochemically substituted apatite, 1.0 mole of calcium can be replaced by magnesium (see Table 1, sample No. 8), and the apatite structure does not change when the sample is annealed to 500 °C (Fig. 7). The IR spectrum of the mechanochemically synthesized sample and the spectrum of the same sample after annealing to 500 °C preserve the absorption bands of the O–H bond deformation vibrations around 1640 $cm^{-1}$ and the broad band at 3400–3600 $cm^{-1}$, which is due to the stretching vibrations of the O–H bonds of water coordinated by cations in the apatite lattice (Fig. 8, spectrum *1*). Water stabilizes the apatite

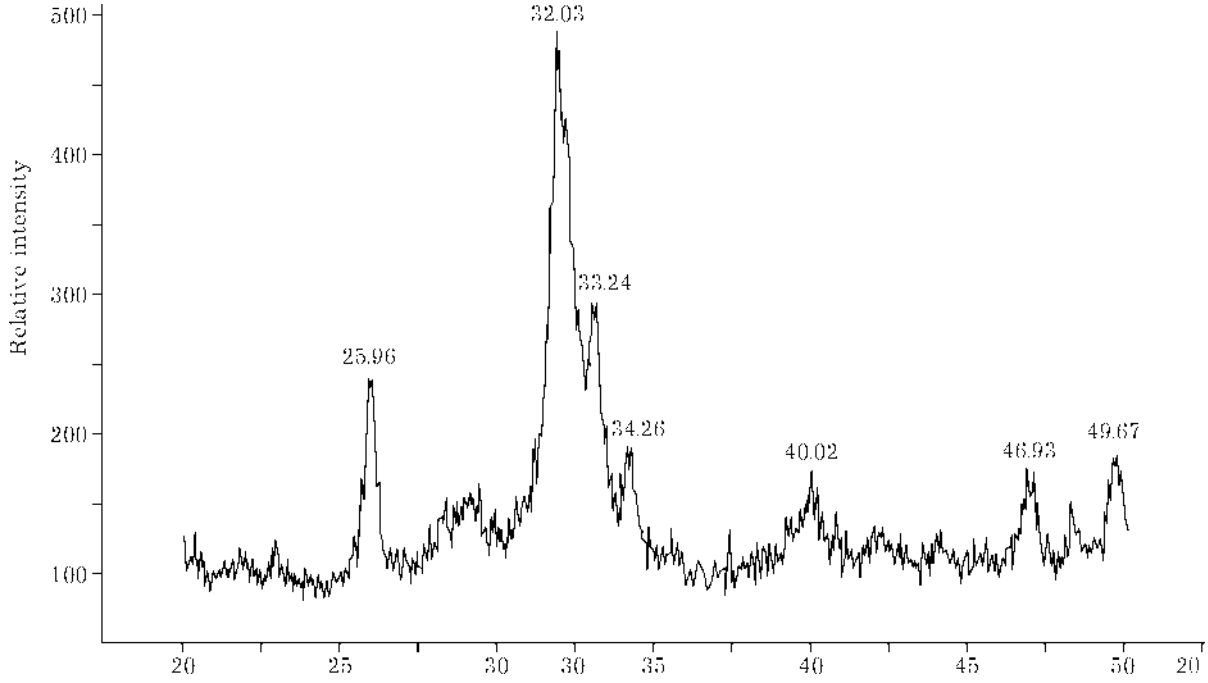


Fig. 7. Diffractogram of hydroxylapatite $Ca_9Mg(PO_4)_6(OH)_2$ (sample No. 8) synthesized mechanochemically and having calcium partially replaced by magnesium after annealing at 500 °C for 2 h.

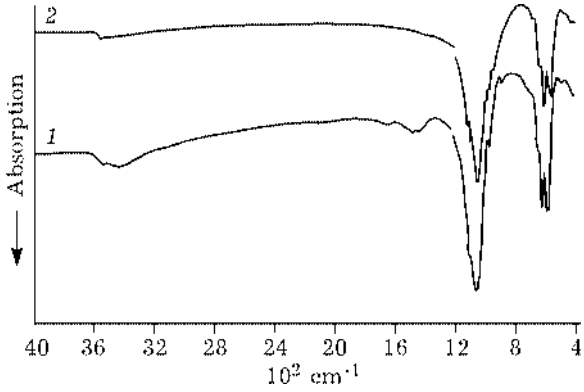


Fig. 8. IR spectra of sample No. 8 after annealing at 500 (*1*) and 900 °C (*2*) for 2 h.

structure, and its loss as a result of annealing at 900 °C led to a transformation of some part of the latter into β-tricalcium phosphate. In the IR spectrum of this sample, the bands of the O–H bond vibrations of water are absent, while the P–O vibration bands typical for the β form of trisubstituted calcium phosphate appear in the range 900–1100 cm$^{-1}$ [25] (Fig. 8, spectrum *2*, sample No. 8).

Mechanochemical synthesis of apatite with barium partially substituted for calcium is only feasible if one calcium ion per unit cell is replaced (see Table 1, sample No. 7). An attempt to have two or more calcium ions replaced by barium led to an amorphous phase (see Table 1, sample No. 9). This is explained by the large difference in lattice parameters: $a = 0.94176$ nm, $c = 0.6881$ nm for $Ca_{10}(PO_4)_6(OH)_2$; $a = 1.0177$ nm, $c = 0.7731$ nm for $Ba_{10}(PO_4)_6(OH)_2$ [25].

The kinetics of apatite formation and product stability were found to be affected by the starting components and by water liberated by reaction between the latter as a result of mechanical activation of reaction mixtures of samples No. 10 and 11 aimed at synthesizing barium hydroxylapatite. Reaction of sample No. 10 with barium hydroxide liberated a large amount of water. Apatite formed in 5 min after activation had started and did not change within 10 min of treatment. When barium oxide was used (reaction of sample No. 11), apatite was also synthesized within 5 min after mechanical activation had started, but after 10 min of treatment, trisubstituted barium phosphate formed. Therefore, one can suggest that increased time of treatment plays the same role as sample annealing.

The substituted apatites synthesized mechanochemically were analyzed for biocompatibility.

### *Biocompatibility test for synthesized apatites*

The functional methods for determining cell activity (in particular, testing how they stick to plastic or glass) are highly sensitive to the nature of the material being tested [5]. Therefore, it was interesting to examine the functional response of marrow cells to extracts of mechanochemically synthesized stoichiometric calcium-deficient hydroxylapatite [$(Ca/P)_{at} < 1.67$] with various atoms substituted into the cation sublattice.

In our studies, the sticking of marrow cells to plastic was investigated in BPB containing no $Ca^{2+}$ and $Mg^{2+}$ salts [31] in the presence of a small amount of embryonal calf serum. This model revealed that the soluble (extractable) fractions of synthesized apatites are bioactive.

Testing the extracts obtained by dissolving synthetic apatites with different Ca/P ratios and microelement additions led to ambiguous results (Table 2). The extracts of nonstoichiometric apatites with $(Ca/P)_{at} < 1.67$ at the concentrations used unambiguously suppressed the ability of the cells to stick to plastic compared to biological hydroxylapatite (titers 1 : 8 and 1 : 4) and solvent (titer 1 : 4); the suppression was statistically meaningful ($P_U < 0.05$) and dose-dependent. This agrees with literature data on cytotoxicity of amorphous calcium phosphates [32, 33]. According to [34], accelerated ceramic solution in the organism stimulates the osteoinducing activity but decreases biocompatibility of the biomaterial. Osteoinduction caused by apatites with a calcium to phosphorus ratio between 1.50 and 1.67 may be due to mineralization of the collagen matrix [30].

The increased calcium to phosphorus ratio in the samples led to increased adhesive activity of marrow cells, which was especially pronounced at high extract concentrations (see Table 2). The extracts of stoichiometric apatite with $(Ca/P)_{at} = 1.67$ and $(Ca/P)_{at} = 1.8$ were comparable to the extracts of biological hydroxylapatite in their effect on cell adhesion. Moreover, in the 1 : 8 titer, the extracts of sample No. 4 (see Table 1), where $(Ca/P)_{at} = 1.8$, even increased cell survivability in the culture compared to the biological analog (Table 3).

TABLE 2

Cellularity of myelokaryocytes in a liquid culture after 1 h of cultivation with seven-day extracts of hydroxylapatites synthesized mechanochemically and biological hydroxylapatite (average data)

| Sample No. | Extract of the apatite sample | Number of sticking marrow cells, %, in the titer of extracts | |
|---|---|---|---|
| | | 1 : 8 | 1 : 4 |
| 1 | $Ca_9HPO_4(PO_4)_5(OH)$; $(Ca/P)_{at} = 1.5$ | 32.94* | 0.59#* |
| 2 | $Ca_{9.6}(PO_4)_6(OH)_{1.2}$; $(Ca/P)_{at} = 1.6$ | 52.94 | 56.47 |
| 3 | $Ca_{10}(PO_4)_6(OH)_2$; $(Ca/P)_{at} = 1.67$ | 63.53 | 40.00 |
| 4 | $Ca_{10.8}(PO_4)_6(OH)_{3.6}$; $(Ca/P)_{at} = 1.8$ | 64.71 | 49.41 |
| 5 | $Ca_{10}\ K_{0.04}(PO_4)_{6.04}(OH)_2$ | 77.65# | 64.71 |
| 6 | $Ca_{10}(Cu,Zn)_{0.08}(PO_4)_6(OH)_{2.16}$ | 80.00#* | 72.94 |
| 7 | $Ca_9Ba(PO_4)_6(OH)_2$ | 48.24 | 17.65#* |
| 8 | $Ca_9Mg(PO_4)_6(-OH)_2$ | 42.35 | 43.53 |
| | Toxicity control for samples: biological hydroxylapatite | 49.41 | 63.53 |
| | isotonic solution of NaCl, 7 days in a thermostat (solvent) | 58.82 | 51.76 |
| | Toxicity control for solvent: fresh isotonic solution of NaCl | 49.41 | 49.41 |

#, * Statistically significant differences ($P_U < 0.5$) according to Wilcoxon–Mann–Whitney's test for polymer articles with respect to biological hydroxylapatite and solvent, respectively.

On the other hand, the biological activity of mechanochemically synthesized hydroxylapatite may be changed by introducing various micro- and macroelements in the apatite lattice. Thus adhesion of myelokaryocytes in the culture was adversely affected by the soluble fraction of apatite powders with barium substituted for one calcium ion (see Table 2, sample No. 7), which was especially conspicuous for the 1 : 4 titer. At the same time, introduction of a potassium atom in the apatite structure can stimulate cell adhesion (see Table 2, sam-

TABLE 3

Viability of myelokaryocytes in a liquid culture after 1 h of cultivation with seven-day extracts of synthetic and biological hydroxylapatite powders (average data)

| Powder extract, $n = 3$ | Number of viable marrow cells, %, in the titer of extracts | |
|---|---|---|
| | 1 : 8 | 1 : 4 |
| $Ca_9HPO_4(PO_4)_5(OH) + 14H_2O$; $(Ca/P)_{at} = 1.5$ | 93.06 | 90.27 |
| $Ca_{9.6}(PO_4)_6(OH)_{1.2} + 15H_2O$; $(Ca/P)_{at} = 1.6$ | 93.37 | 96.83 |
| $Ca_{10}(PO_4)_6(OH)_2 + 15H_2O$; $(Ca/P)_{at} = 1.67$ | 90.18 | 96.64 |
| $Ca_{10.8}(PO_4)_6(OH)_{3.6} + 15H_2O$; $(Ca/P)_{at} = 1.8$ | 98.00# | 95.29 |
| $Ca_{10}K_{0.04}(PO_4)_{6.04}(OH)_2 + 15H_2O$ | 93.46 | 93.58 |
| $Ca_{10}(Cu,Zn)_{0.08}(PO_4)_6(OH)_{2.16}$ | 87.87 | 96.53 |
| $Ca_9Ba(PO_4)_6(OH)_2 + 23H_2O$ | 91.26 | 94.11 |
| $Ca_9Mg(PO_4)_6(OH)_2 + 14H_2O$ | 93.98# | 94.30 |
| Biological hydroxylapatite | 86.26 | 97.13 |
| Isotonic solution of NaCl (solvent) | 88.87 | 86.00 |

*Note.* $n$ is the number of holes bearing the powder extract.

#Statistically significant differences ($P_U < 0.5$) according to Wilcoxon–Mann–Whitney's test $U$ with respect to biological hydroxylapatite.

ple No. 5, titer 1 : 8). Hydroxylapatite suspensions with calcium partially replaced by magnesium slightly increased the viability of marrow cells (see Table 3).

Apatite extracts with 0.04 % copper and zinc ions in the structure (1 : 4 titer) showed the maximal stimulating effect on cell adhesion. Differences in the optical activity of the culture reached statistical values relative to the values of the solvent and biological hydroxylapatite (see Table 2).

Copper and zinc, which are present as microelements in the organism, modulate various cell and tissue functions via the dependent metalloenzymes [35]. At the same time, they obviously possess bactericidal properties [36]. Today we still need an effective and inexpensive antimicrobial material which is capable of

1) liberating an antimicrobial agent for prolonged periods of time and at a constant therapeutically active level; and

2) which is applicable to a wide range of devices and materials;

3) possesses acceptable shelf time; and

4) exhibits low toxicity for mammals [37].

Therefore, synthetic apatites with copper and zinc atoms incorporated in the lattice may prove useful as materials for bulky articles and as metal coatings possessing both regulatory and antimicrobial activities. This new class of articles will prove helpful in solving problems at an interface bone/implantate and hence will possess improved biocompatibility.

The extracts of all samples showed satisfactory characteristics of toxicity in the cell culture *in vitro* (see Table 3). Thus apatite samples synthesized mechanochemically did not inflict any detrimental damage on cells. Moreover, they possessed bioactivity and selective regulatory activity with respect to bone marrow and bone tissue depending on microelement additions. Thus it is known that copper, zinc, and magnesium hinder the loss of bone mass [39].

## CONCLUSIONS

1. Hydroxylapatites with different calcium to phosphorus ratios and with superstoichiometric potassium, copper, and zinc cations introduced in their structure have been synthesized mechanochemically.

2. Hydroxylapatite with 1/10 of all calcium ions replaced by magnesium in the unit cell has been synthesized, the number of substituted calcium ions being ten times higher than that obtained during synthesis from solution and by the thermal method. The structure of apatite is stabilized by structurally bound water; when the latter is lost, apatite is partially transformed into trisubstituted phosphates.

3. Biocompatibility analysis of the samples indicated that apatites synthesized mechanochemically are bioactive. They dissolve in biological liquids, producing multidirectional effect on the functional activity of marrow cells depending on their composition.

4. All of the tested synthetic calcium phosphates possess satisfactory cytotoxicity. However, calcium-deficient apatite, in particular, the sample with $(Ca/P)_{at} < 1.67$ and with calcium partially replaced by barium suppresses the adhesive activity of myelokaryocytes *in vitro*.

5. On the contrary, cell adhesion is stimulated by hydroxylapatites with copper and zinc atoms introduced in their structure. These metals have an antimicrobial activity, which is important for preventing post-implantation infection.

6. Apatites synthesized mechanochemically and possessing adjustable bioactivity are promising materials for manufacturing articles of new generation, as well as coatings, for orthopedy and traumatology.

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