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SYNTHESIS AND PROPERTIES  
OF INORGANIC COMPOUNDS

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## Modifying Brushite-Containing Phosphate Cements by Complexing Additives

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**Abstract**—The use of citric acid, sodium pyrophosphate, and sodium tri- and hexametaphosphates is studied as additives intended to retard the curing of brushite cements for convenience of their osteoplastic use. The effects of these additives on the curing rates, microstructures, and mechanical properties of these cements are studied.

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The concept of materials used for osteoplastic purposes has been altered seriously during the last two decades. Within the frame of what is referred to as a regeneration approach, the accent is made on the substitution of a native growing tissue for an implant, while the material is intended to play the role of a source of the elements that are necessary for building up the bone, where the implant performs a support function only initially [1, 2]. In currently use there are two major groups of calcium-containing compact biomaterials, namely: (a) highly resorbable, but low-strength phosphate–biopolymer composites and calcium phosphate cements (CPCs); and (b) highly strong, but poorly resorbable phosphate ceramics. Recently, the first group of materials (in particular phosphate cements) has received a noticeable preference, for the reason that the low-temperature technologies thereof provide their efficient filling in with bioactive elements (special proteins or stem cells) and thereby additionally enhances their osteosynthetic activity [3].

Depending on the products to be formed in cement stone, the calcium phosphate cements (CPCs) are classified into apatite CPCs (the major product being hydroxylapatite (HAP)  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) and brushite CPCs (the major product being brushite  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ). Cement stone is formed due to the formation and growth of produced crystals and their mutual overlapping. The unique feature of curable CPCs consists in that the forces binding crystals of the target product are very weak, so that individual crystals can easily be flaked from the cement stone mass, especially after the material is partially degraded in a living body.

A property of paramount importance for the practical use of CPCs is the rheology of the cement mass,

which is determined by the solid-to-liquid (S/L) phase ratio in the mass. In this context, apatite cements are “thick,” although their solidification (curing) times are relatively long; extensive research has been targeted to reduce these times. The solubilities of apatite cements in aqueous solutions are close to those of bone tissues. This means that apatite CPCs are virtually insoluble in neutral solutions, but their solubilities strongly increase as pH is reduced. Solvability is an important characteristic of resorbable bone implants, for the reason that they can be subject to controlled degradation by special cells (osteoclasts). Unlike apatite cements, brushite cements are fluid, and therefore they can be injected through a syringe into a closed bone defect, for example for filling in a defect cavity. As opposed to apatite CPCs, brushite CPCs are cured very rapidly. Acidic pH values that appear when a brushite CPC is brought in contact with a solution, are responsible for its high resorption rates in a body compared to an apatite CPC. The high bioresorption rates of brushite cements render them very attractive in terms of the regenerative approach to healing bone defects [4], but their practical use is hampered by high rates of chemical reactions [5]. In this context, a search for efficient retardant additives is one of the major lines of research for brushite CPCs [6–8].

Here, as the additives such that influence the formation, growth, and dissolution of brushite crystal, we will consider the following biocompatible compounds: citric acid, sodium pyrophosphate, sodium trimetaphosphate, and sodium hexametaphosphate. As regards the first two of these additives, the available literature is scarce [6], and the last two are considered here for the first time.

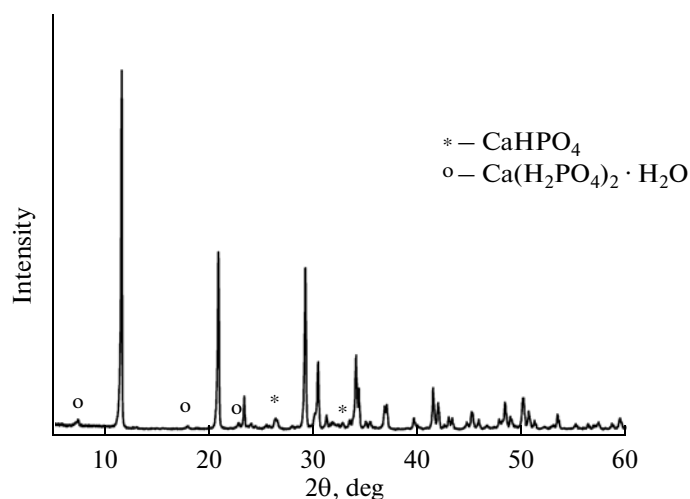
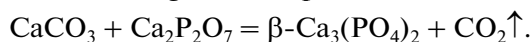


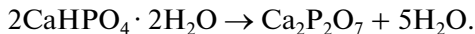
Fig. 1. X-ray powder diffraction pattern for brushite cement.

### EXPERIMENTAL

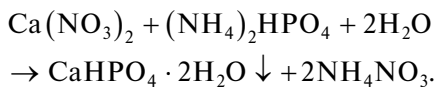
Tricalcium phosphate ( $\beta$ -TCP) was prepared at  $950^\circ\text{C}$  for 6 h using the solid-phase reaction



Calcium pyrophosphate was prepared from a brushite powder which was decomposed via rapid insertion into a furnace maintained at  $600^\circ\text{C}$  for 15 min by the reaction

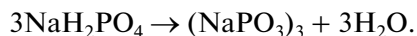


Brushite was prepared by rapidly combining 0.5 M solutions of calcium nitrate and ammonium hydrogenphosphate (both salts were of chemically pure grade):



The resultant precipitate was washed three times by decantation and then filtered on a Buchner funnel and left to dry at room temperature for 24 h.

Sodium trimetaphosphate was prepared by dehydrating sodium dihydrogenphosphate (chemically pure grade) at a temperature of  $600^\circ\text{C}$  for 2 h and then slowly cooling the product to room temperature:



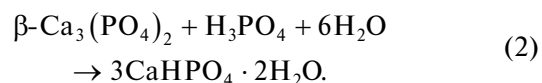
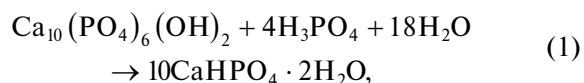
Sodium pyrophosphate was prepared from sodium hydrogenphosphate (chemically pure grade) via annealing at  $600^\circ\text{C}$  for 2 h:



The sodium hexametaphosphate used was a commercially available chemical (Sigma-Aldrich, p.a.), which was X-ray amorphous and apparently contained polyphosphates  $(\text{NaPO}_3)_n$  ( $n \geq 3$ ).

Brushite cements were prepared as a result of the acid–base reaction of powdery HAP (Riedel-de-Han,

puriss.; reaction (1)) and the as synthesized powdery  $\beta$ -TCP (reaction (2)) with orthophosphoric acid:



The solid-to-liquid (S/L) phase ratio (in g/mL) was varied from 0.6 to 0.8 for the HAP cements and from 0.8 to 0.9 for the  $\beta$ -TCP cements. Both additive-free cements and cements with a retardant (which was citric acid from Sigma-Aldrich, p.a.; sodium trimetaphosphate; or sodium hexametaphosphate) were prepared. The retardant additives were predissolved in phosphoric acid (which served as the grouting fluid). Additive concentrations were varied from 0.04 to 0.08 mol/L for sodium tri- and hexametaphosphates and from 0.2 to 0.6 mol/L for citric acid. Phosphoric acid was poured in portions to the HAP (or  $\beta$ -TCP), while the paste was vigorously stirred to remove nascent lumps. The total stirring time was 20–40 s; then, the cement mass was poured into a Teflon die and formed into columns 10 mm in diameter and 20 high to be used in subsequent mechanical tests.

The brushite formation kinetics was studied with and without retardants in the HAP + phosphoric acid system. The ratio S/L was 0.7 (g/mL). The modifier used was citric acid (0.6 mol/L) or sodium trimetaphosphate (0.08 mol/L). After the cement paste was kneaded, samples were taken to measure X-ray powder diffraction patterns. In order to stop the reaction, a sample was transferred to a beaker filled with acetone and vigorously stirred.

The mechanical strength values of brushite cements were used to manufacture brushite/HAP and brushite/ $\beta$ -TCP composites. The composites were as

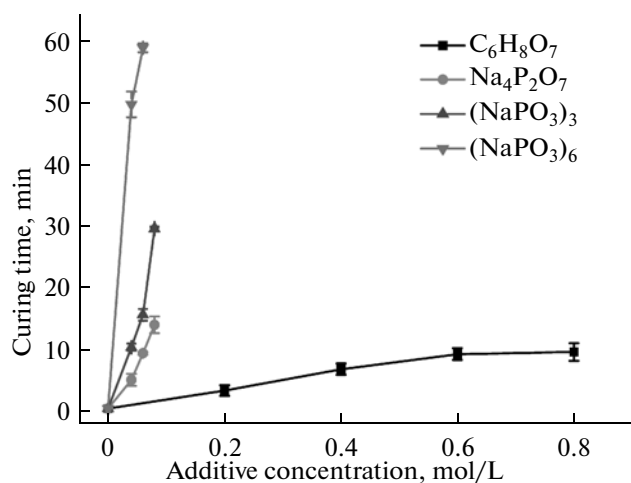


Fig. 2. Curing time of brushite cements versus retardant amount (HAP with phosphoric acid; S/L = 0.8 (g/mL)).

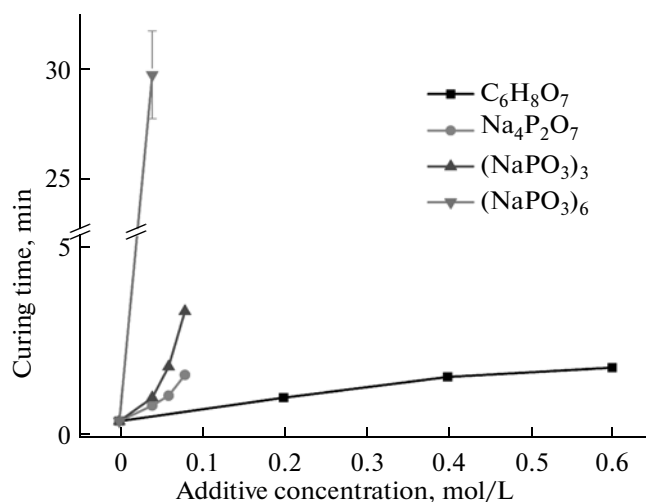


Fig. 3. Curing time of brushite cements versus retardant amount ( $\beta$ -TCP with phosphoric acid; S/L = 0.8 (g/mL)).

follows: (a) 100 wt % brushite + 0 wt % HAP( $\beta$ -TCP), (b) 80 wt % brushite + 20 wt % HAP( $\beta$ -TCP), and (c) 60 wt % brushite + 40 wt % HAP( $\beta$ -TCP). The solid/liquid phase ratio was 0.8 (g/mL); the retardant additive used was citric acid (0.4 mol/L).

The curing time of the cement paste was determined by the Wick method. The paste was prepared from 1.6 g HAP ( $\beta$ -TCP) and 2 mL phosphoric acid of an appropriate concentration (with or without additives). The paste was vigorously stirred for 40 s and then left to cure. The period of time when Wick's needle (6 g in weight) stopped immersing into the paste was recorded. Three to six measurements were carried out in order to evaluate the convergence of the results.

An X-ray diffraction experiment was carried out on a Rigaku D/Max-2500 diffractometer equipped with a rotating anode. Reflection (Bragg–Brentano) geometry with CuK $\alpha$  radiation was used.

Microstructures were examined using scanning electron microscopy (SEM) on a LEO SUPRA 50VP (Carl Zeiss) electron microscope equipped with an autoemission source with accelerating voltages of up to 10 kV in secondary electrons (SE2 and InLens detectors). In some cases, samples were sputtered with gold (on a Fine Coat (JEOL) setup).

The  $\zeta$  potential of HAP particles upon addition of modifiers was measured on a Zeta-analyzer (Nano ZS, Malvern Instruments). Samples for measurements were prepared by suspending HAP in distilled water (by 20-s ultrasonication).

For carrying out mechanical tests which were intended to determine strength properties, cylinder-shaped samples were prepared with the ratio diameter : height = 1 : 2 (diameter: 10 mm; height: 20 mm). These samples were subjected to uniaxial compression (along the axis of the cylinder) at a straining rate of

1 mm/min. The tests were carried out on a P-05 universal testing machine equipped with a Spider multi-channel measurement system.

## RESULTS AND DISCUSSION

The cements obtained are not single phase as probed by X-ray powder diffraction (Fig. 1) In addition to brushite, they can contain minor phases: monetite (CaHPO<sub>4</sub>) results from heating the cement paste [9]; and calcium dihydrogenphosphate (monocalcium phosphate monohydrate, MCPM, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> · H<sub>2</sub>O) appears there where phosphoric acid is in local excess. The heating of the cement paste, which results in monetite formation and which is an adverse factor when cements are used in vivo, may be explained as arising from the heat effects of the appropriate acid–base reactions. In this context, it is of paramount importance to retard heat evolution during curing of brushite cements.

Figures 2 and 3 show the curing time depending on the modifier and modifier concentration for brushite cements in the HAP + phosphoric acid system and  $\beta$ -TCP + phosphoric acid system, respectively. As one can see from these plots, our proposed retardants (sodium tri- and hexametaphosphates) are more efficient than pyrophosphate, because far longer curing times are attainable with them at the same concentration.

The effect of these additives may be explained by taking in account the following two circumstances: (1) the adsorption of an anion on the surface of the HAP (or TCP) and the hindered access of a proton from solution to the HAP (TCP) surface (proton adsorption on the apatite surface is the initial act of HAP (TCP) dissolution in an acid medium); and (2) the formation of strongly bonded complexes of calcium cations with

**Table 1.**  $\zeta$  potentials of a polydisperse HAP suspension in the presence of various additives

Suspension	HAP	HAP + (C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> ) (0.6 M)	HAP + (Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> ) (0.08 M)	HAP + (NaPO <sub>3</sub> ) <sub>3</sub> (0.08 M)	HAP + (NaPO <sub>3</sub> ) <sub>6</sub> (0.08 M)
$\zeta$ Potential average, mV	-9.30	-1.15	-30.9	-28.5	-29.8
$\zeta$ Potential scatter, mV	6.92	13.6	36	41	14.7

**Table 2.** Stability constants of calcium complexes with various ligands (L)  
Ca<sup>2+</sup> + L  $\leftrightarrow$  (CaL) log K<sub>1</sub>

L	HPO <sub>4</sub> <sup>2-</sup>	Ci <sup>3-</sup>	HCi <sup>2-</sup>	H <sub>2</sub> Ci <sup>-</sup>	P <sub>2</sub> O <sub>7</sub> <sup>4-</sup>	HP <sub>2</sub> O <sub>7</sub> <sup>3-</sup>	P <sub>3</sub> O <sub>9</sub> <sup>3-</sup>	P <sub>4</sub> O <sub>12</sub> <sup>4-</sup>
log K <sub>1</sub>	2.98–3.01 <sup>a</sup>	4.62	3.05	1.15	5.6	3.6	3.45	5.42

<sup>a</sup> According to [10], where Ci stands for the citrate anion.

ionized additive species [10], which inhibits the occurrence of the reaction  $\text{Ca}^{2+} + \text{HPO}_4^{2-} \rightarrow \text{CaHPO}_4$  and thereby brushite nucleation.

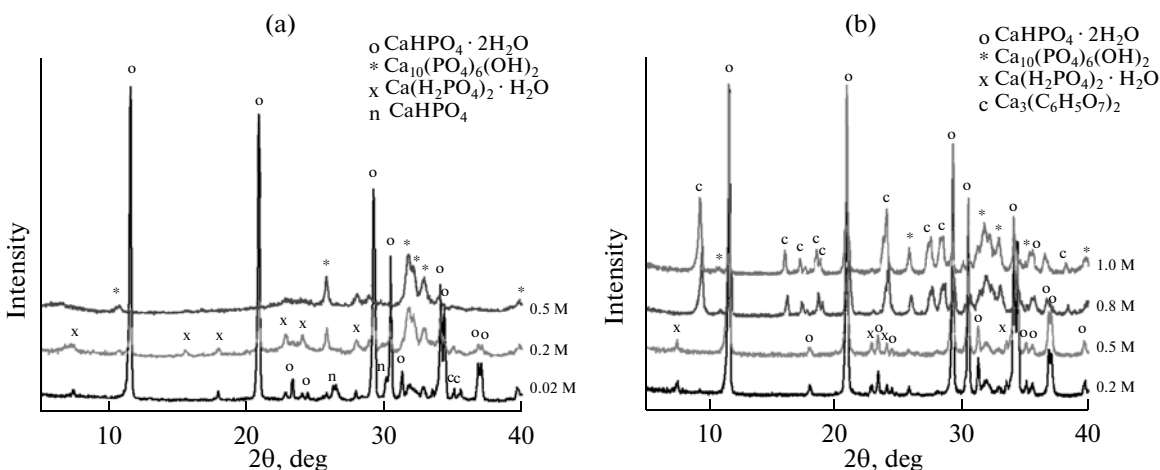
In this study, the adsorption properties of an additive were ascertained by the effect caused on the  $\zeta$  potential of a suspension of HAP particles (Table 1). A comparison of  $\zeta$  potential values for the systems containing additives and for a net HAP suspension shows that, when polyphosphate additives are used, the potential experiences a distinct shift toward negative values, which this may be related to the adsorption of the multicharged polyphosphate anion on the HAP surface, whereas for citric acid, the potential shifts toward positive values (from -9.3 to 1.15 mV, Table 1) and this is likely to arise from the adsorption of positively charged species (i.e., protons).

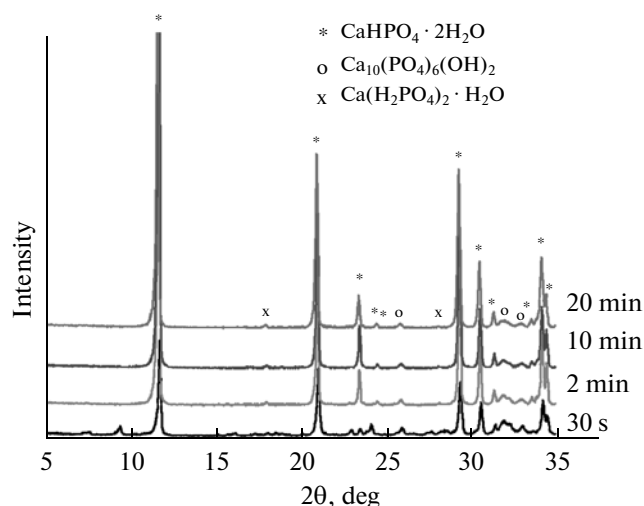
Table 2 displays the stability constants of various complex ions with reference to the stability constant of the CaHPO<sub>4</sub><sup>0</sup> ion pair. Obviously, the stability constant values decrease as pH is reduced because of a reduction in the negative charge of the ligand and a reduc-

tion in the Coulombic term of the calcium–ligand bond energy. Comparing the constants for unprotonated species allows the additives to be arranged in the order of increasing calcium binding efficiencies (thereby, in the order of increasing their effects on the solubility of HAP) as follows: citric acid < trimetaphosphate < pyrophosphate ~ tetrametaphosphate < hexametaphosphate; this order suggests that the hexametaphosphate has a greater complexing ability than tetrametaphosphate.

Summing up our results, we may claim that the different behaviors of citric acid and the polyphosphate additives may be related to the stronger adsorption of the polyphosphates on HAP particles and their stronger binding to calcium cations to enhance calcium cation solubilization and retard brushite crystal nucleation.

For the  $\beta$ -TCP + phosphoric acid system (reaction (2)), there is a similar relationship between the curing times, on the one hand, and the modifier and its concentration, on the other (Fig. 3). Noteworthy, in this case the cement paste is cured far more rapidly.

**Fig. 4.** X-ray powder diffraction patterns for brushite cement samples produced using sodium hexametaphosphate (a) or citric acid (b) as a retardant additive.



**Fig. 5.** X-ray powder diffraction patterns for brushite produced without additives.

This is associated with the lower solubility of HAP compared to  $\beta$ -TCP.

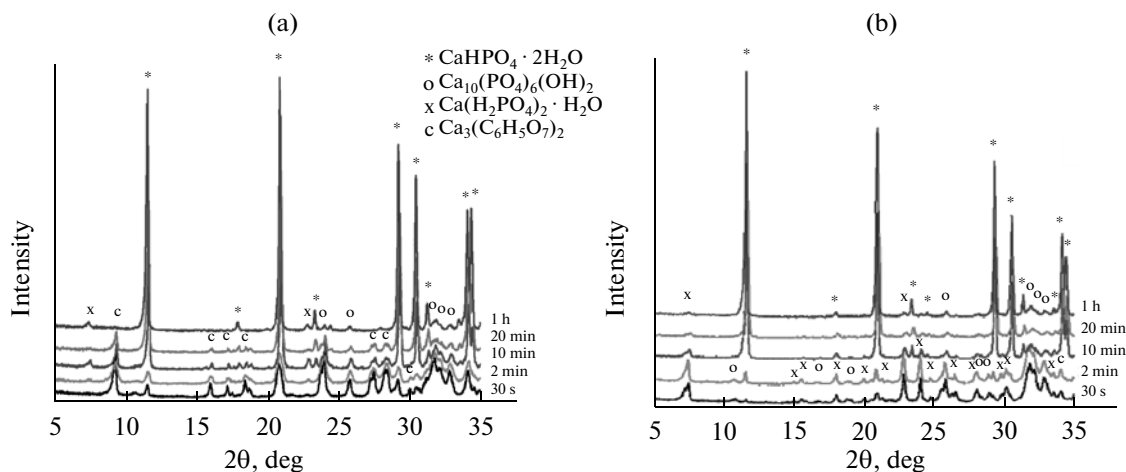
The curing time data were used to select the working concentrations of the additives to be used in further experiments, which were as follows: 0.04–0.08 mol/L for sodium tri- and hexametaphosphates and 0.2–0.6 mol/L for citric acid. Greater amounts of an additive are inappropriate for some reasons. First, the curing times of cements increase so that they become much longer than the time required for surgical intervention. Second, high modifier concentrations retard brushite formation to an extent such that the reaction occurs incompletely upon mixing equimolar amounts of HAP ( $\beta$ -TCP) with phosphoric acid (Fig. 4a). When the retardant is citric acid, not only do its high concentrations result in an incomplete proceeding of

the reaction (HAP remains in the system), but they also result in the appearance of a calcium citrate impurity (Fig. 4b).

The kinetics of brushite formation with retardant additives (citric acid and sodium trimetaphosphate) was studied in the reaction of HAP with phosphoric acid. The reference was a brushite sample obtained by the same reaction but without additives. X-ray powder diffraction pattern (Fig. 5) show that the sample is inhomogeneous: in addition to brushite and HAP, the sample contains an MCPM minor phase in an amount that virtually does not change over time. The reaction stops completely in 2–10 min.

When brushite cement is manufactured in the presence of citric acid, some considerable differences are observed in the X-ray diffraction patterns of samples (Fig. 6a). First, brushite formation is retarded. Second, an intermediate (calcium citrate) appears; its fraction decreases with time, and the released calcium subsequently participates in brushite formation. Third, the relative intensities of the (0 2 0) reflection ( $2\theta = 11.65^\circ$ ) and the (1 2  $-1$ ) reflection ( $2\theta = 21^\circ$ ) in X-ray diffraction patterns change compared to those for the brushite prepared without citric acid; this change may be assigned to the particle morphology changing from isometric to platy as a result of the preferred adsorption of the modifier on the basal (010) faces of brushite crystals (Fig. 10).

When sodium trimetaphosphate is used, the brushite formation reaction is retarded to a greater extent (Fig. 6b). Once the cement paste is kneaded, the nascent brushite fraction is as low as 10%. The most acidic of the calcium phosphates, namely MCPM, is first formed to subsequently convert in acidic solution to the lower soluble brushite, as follows:



**Fig. 6.** X-ray powder diffraction patterns for brushite produced in the presence of 0.4 M citric acid (a) and 0.06 M sodium trimetaphosphate (b) as a retardant additive.

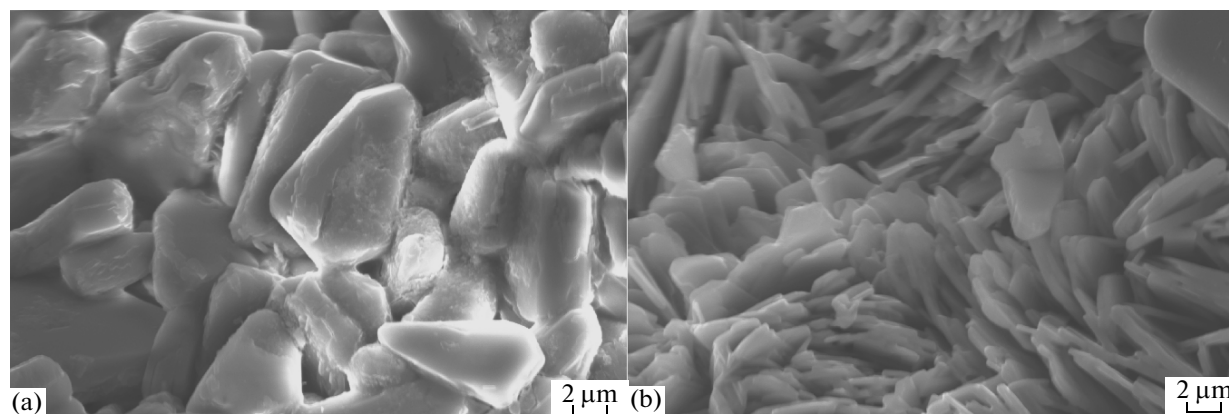
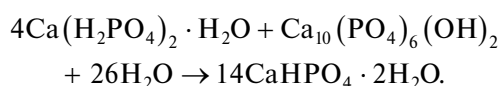


Fig. 7. Micrographs of brushite cement samples produced from (a) HAP and (b)  $\beta$ -TCP.



When HAP (or  $\beta$ -TCP) is the starting reagent, the differences between the strengths of samples are in direct relationship to the differences between the micromorphologies of the resulting cements (Fig. 7). For  $\beta$ -TCP, the higher degrees of supersaturation, which are due to the higher solubility of  $\beta$ -TCP ( $-\log \text{SP} = 28.9$ ) compared to that of HAP ( $-\log \text{SP} = 116.8$ ), result in the appearance of a lot of thin platy crystallites having sizes of  $\sim 5 \mu\text{m}$ .

The microstructure element that is responsible for the strength of a cement sample is phase contact, namely a crystal concretion, which has an intercrystallite boundary with the specific surface energy  $\gamma_{\text{pc}}$ . Upon disintegration, two crystals in the phase contact are displaced with respect to each other to perform a work against autohesion forces:  $A = P_{\text{pc}}l' = (2\gamma - \gamma_{\text{pc}})S_{\text{pc}}$ , where  $P_{\text{pc}}$  is autohesion forces in the phase contact,  $l'$  is the displacement pathway,  $\gamma_{\text{pc}}$  is the (specific) energy of the intercrystallite boundary,  $\gamma$  is the surface energy of a face that is parallel to the boundary, and  $S_{\text{pc}}$  is the contact surface area.

The simplest model of the phase contact consists of two platy crystals pressed together and rotated by a  $\theta$  angle relative to the axis that is normal to or lies in the plane of the boundary. Then,  $l' = lf(\theta)$ , where  $l$  is the characteristic size of a brushite crystal and  $f(\theta)$  is some function of the misalignment of crystals in the contact; the surface energy of the face and the energy of the boundary are also functions of the misalignment angle, so that the autohesion is also a function of the angle  $P_{\text{pc}}(\theta) = (2\gamma(\theta) - \gamma_{\text{pc}}(\theta))S_{\text{pc}}/(lf(\theta))$ .

The macroscopic compressive strength is derived from  $\sigma_{\text{c}} = (P_{\text{pc}}N)/S$ , where  $N$  is the number of contacts in the cross section of the sample and  $S$  is the cross-sectional area. Evidently, the density of contacts in the plane  $N/S$  is proportional to the number of brushite crystals per unit volume, i.e., factually to the density of the sample. Thus, the strength is determined by the

number and properites of phase contacts, namely, by the crystal size  $l$ , which characterizes a possible contact area, and energies  $\gamma_{\text{pc}}(\theta)$  and  $\gamma(\theta)$ .

The use of TCP instead of HAP and the use of modifiers in the cement paste virtually do not reduce the brushite crystal size, but change the crystal morphology and apparently increase the density of phase contacts (see the stack of platelets in Fig. 7).

The density of contacts  $P_{\text{pc}}(\theta) = (2\gamma(\theta) - \gamma_{\text{pc}}(\theta)) \times S_{\text{pc}}/(lf(\theta))$ , which is responsible for the macroscopic properties of a cement sample, for equal  $S_{\text{pc}}/l$  increases as  $\gamma(\theta)$  increases or  $\gamma_{\text{pc}}(\theta)$  decreases. We may demonstrate that  $\gamma$  is minimal for basal faces ((001) and  $(-101)$ ) and sloping faces ((120),  $(-111)$ , and  $(1-10)$ ). For this reason, while paradoxically, the strongest contact appears provided that brushite samples grow together by their sides rather than flatwise. However, the flatwise phase contact of brushite crystals is most

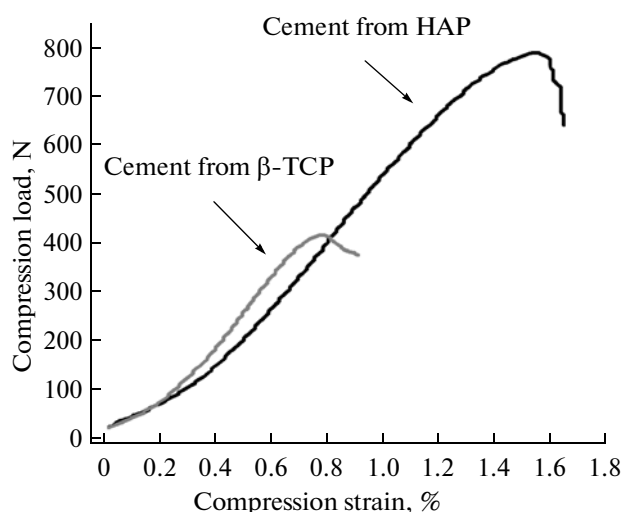
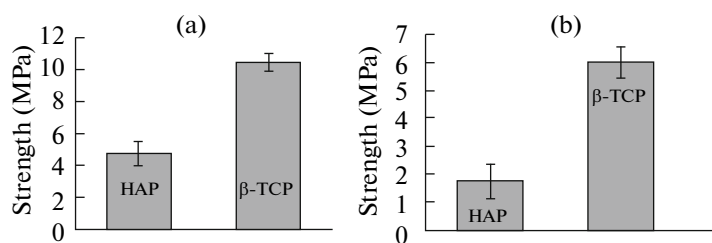
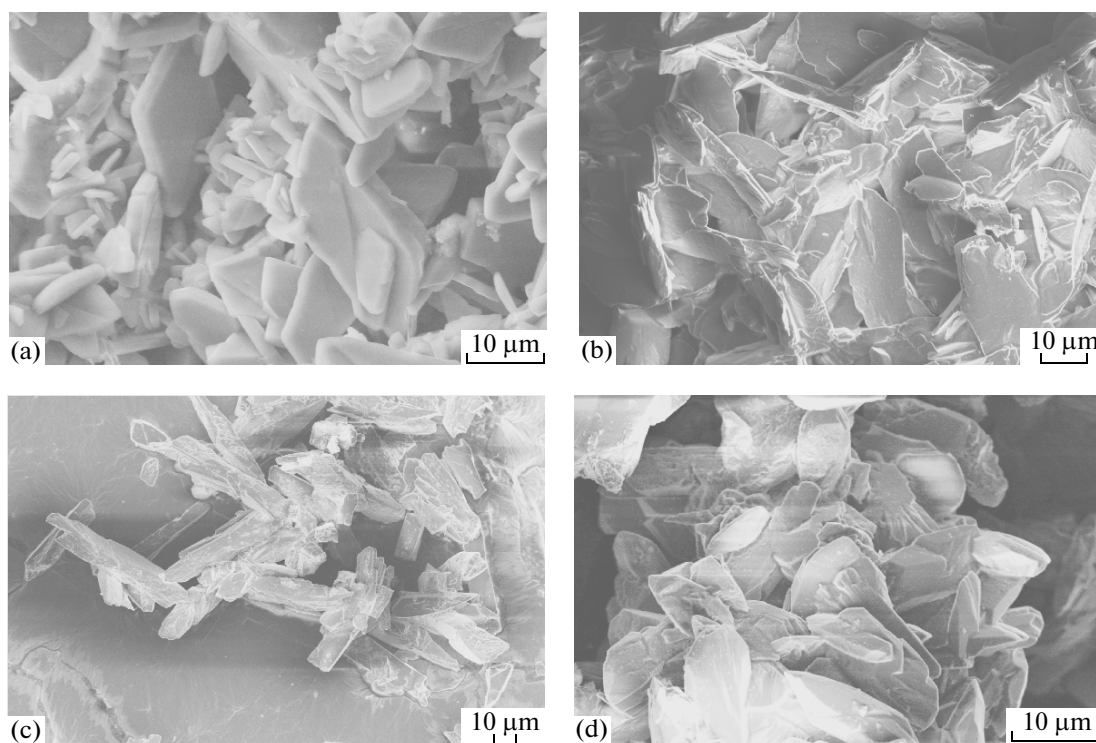


Fig. 8. Loading curves for samples produced from HAP and  $\beta$ -TCP (retardant: 0.4 M citric acid; S/L = 0.8 (g/mL)).



**Fig. 9.** Strength of cements produced from HAP and  $\beta$ -TCP using 0.4 M citric acid (a) and 0.06 M sodium trimetaphosphate (b) at S/L = 0.8 (g/mL).



**Fig. 10.** Micrographs of brushite cement samples produced from HAP: (a) without additives and (b–d) in the presence of (b) 0.4 M citric acid, (c) 0.06 M brushite, and (d) 0.06 M sodium hexametaphosphate.

frequent: despite its lower strength, it provides a greater contact area and a greater number of contacts per unit volume, which ultimately gives a gain in the macroscopic strength of the sample.

Figure 8 shows representative loading curves. The destruction occurs on an elastic segment within a strain range of 1–2%, which is quite great for a continuous brittle body and provides evidence of a compaction of the sample due to a continuous destruction of phase contacts between brushite platelets and gliding of the platelets relative to each other.

Noteworthy, a nearly twofold gain in strength is observed for the cements modified with either citric acid or sodium trimetaphosphate (Fig. 9). Mechanical tests for samples modified with sodium hexametaphosphate have not been carried out because these samples experienced nonuniform shrinkage upon drying.

When citric acid serves as a retardant additive, brushite particles are enlarged and flattened (Fig. 10). However, morphological alterations are not as strong as expected from the results of  $\zeta$  potential measurements (Table 1). Brushite platelets are grown together with each other to yield lateral phase contacts, thereby providing a greater strength of the cement. In the presence of sodium trimetaphosphate, the shapes of brushite crystallite more resemble rods (with lengths of up to 50  $\mu$ m), which provides an adverse variant of crystallites growing together and a decrease in strength (Fig. 11).

Having regard to the above values of the strengths of compact brushite cement samples, we chose conditions so as to obtain cements that would be capable of withstanding maximal loads; these conditions were as follows: the modifying additive: 0.4 M citric acid; and

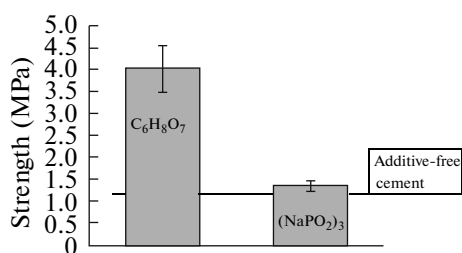


Fig. 11. Strength of brushite cements brushite cements produced from HAP using retardant additives.

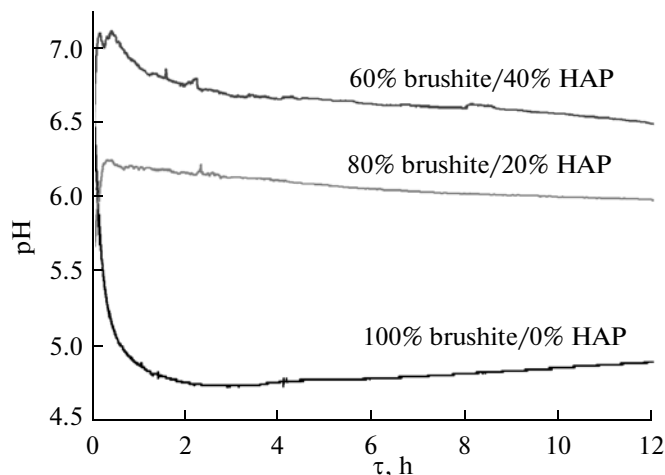


Fig. 12. Behavior of cements in aqueous solutions.

S/L = 0.8 (g/mL). Composites consisting of brushite (the better soluble phase) and a less soluble (resistive) component, which was either HAP or  $\beta$ -TCP, were manufactured under the same conditions. The intention to design these composites is motivated not only by the desire to retard implant resorption rates but also by the need to reduce the “acidity” of the cement (cf. the variation dynamics of aqueous pH in contact with brushite CPCs and composites in Fig. 12). The too low pH values, which are just intrinsic to brushite CPCs, can induce the necrosis of surrounding tissue.

In summary, this study proposes new metaphosphate additives to calcium phosphate cements that provide curing times longer than 10 min (up to 60 min). These additives bring about an increase in size of the  $CaHPO_4 \cdot 2H_2O$  crystals formed upon cement curing.

Of the modifying additives tested, citric acid has the strongest effect on the strength characteristics of cements (it provides a fourfold increase in compressive strength).

The effects of the modifiers on the micromorphology and strength of brushite cements are due to the adsorption of a multicharged anion on the calcium phosphate surface and the formation of strongly bonded complexes of calcium with an ionized modifier species, which inhibits brushite nucleation.

HAP/brushite composites consolidated by brushite cement have been manufactured. The curing times of the composites have been found to lengthen and pH levels in solutions brought in contact with these materials have been found to increase from 4.75 (for pure brushite cement) to 6.0 (for HAP/brushite = 20/80 (wt/wt) composite).

#### ACKNOWLEDGMENTS

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