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Doped Calcium Carbonate-Phosphate-Based Biomaterial for Active Osteogenesis

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1. Introduction

The problems of modern medicine and biotechnology involve not only creation of implants replacing bone tissues and organs, but also synthesis of biologically active materials promoting the fullest restoration of tissues and maintenance of necessary functions of an organism. It is well known that calcium is one of the elements important for a living organism, for its cations control the transportation of inorganic ions and organic substances through cell membranes in the metabolic process involving the delivery and removal of reaction products from a cell. Interacting with regulatory proteins, calcium participates in nerve impulse transmission to muscles. Calcium is necessary for blood coagulation and participation in the synthesis of hormones, neuromediators and other controlling substances (1). Calcium is a building material for the bone tissue, its inorganic part. The solid residual of the bone tissue contains 70 % of calcium hydroxide phosphate (calcium hydroxyapatite) $Ca_{10}(PO_4)_6(OH)_2$ and 30 % of an organic component, namely, collagen fiber. The bone tissue should be characterized as an organic matrix impregnated by amorphous $Ca_3(PO_4)_2$ and crystals of calcium hydroxide phosphate synthesized in bone tissue osteoblast cells (2).
Ions Na^+, K^+, Mg^{2+}, Fe^{2+}, Cl^- and CO_3^{2-} are contained in the structure of calcium hydroxide phosphate of the bone tissue besides Ca^{2+} and PO_4^{3-}. The content of anions CO_3^{2-} in calcium hydroxide phosphate of the bone material can make up to 8 wt. %, and they substitute hydroxyl or phosphate groups. Therefore, in view of the carbonate groups introduced into the structure of calcium hydroxide phosphate, its probable formula will be as follows (3 – 5): $Ca_{10}(PO_4)_6(CO_3)_x(OH)_{2-x}$.
Actually, the crystal structure, as well as the structure of chemical bonds, of calcium hydroxide phosphate is much more complex because of vacancies in the crystal structure of both anion and cation nature. The vacancies can be filled with bivalent cations of trace elements received by a living organism and with anions SiO_{2x}^{2-}, SO_4^{2-} and Cl^-, F^-. The crystal structure of calcium hydroxyapatite is considered in (4, 5) where there is a simplified form of an elementary cell. However, practically in all scientific works accessible for viewing it is

not mentioned that the structure of chemical bonds in calcium hydroxyapatites and apatites of the kind is more complex than their empirical formula and that it is not completely representative. Taking into consideration that phosphoric acids and their salts have basically polymeric structure with the formation of inorganic polymers due to hydrogen bonds and oxygen bridges, one can assume that calcium hydroxide phosphates are also characterized by the formation of inorganic polymers.

It is well known that in an organism there is a complex system of storage and release of calcium, which involves the hormone of the parathyroid gland, calcitonin and vitamin D_3. If an organism is unable to assimilate calcium because of age-related and hormonal changes, the lack of calcium begins to be filled with the dissolution of calcium hydroxide phosphate of the bone tissue. As a result, the bone tissue becomes less strong. Besides, deposition of phosphate salts in the cartilaginous connective tissue and on vessel walls is observed. A prominent feature of the growth of bones, teeth and other structures is the accumulation of calcium. On the other hand, the accumulation of calcium in atypical sites leads to the formation of stones, osteoarthritis, cataracts and arterial abnormalities (1). The entrance of calcium into an organism can proceed in the form of easily assimilated phosphates, which are also necessary for the synthesis of adenosine triphosphoric acid accumulating energy and participating in active transportation of ions through cell membranes. As after 55 the majority mankind suffers from various diseases of joints, lower strength of the bone tissue, osteochondrosis, osteoporosis and frequent fractures, it is necessary to create a material based on inorganic calcium phosphates easily assimilated by a living organism, and not only through the gastrointestinal tract. It is well known that, when calcium phosphate (hydroxyapatite) is introduced into the bone tissue, as a result of slow resorption in an organism and involving in metabolism, osteogenesis improves, but calcium phosphates fail to get into an organism through the skin. The solution to this problem is biomaterial developed on the basis of nanocrystalline doped microelements of calcium carbonate phosphates with a rapid impact on the process of osteogenesis and with the ability to penetrate into the organism through the skin, i.e., through the membranes of living cells (6 -8).

Calcium phosphates are studied all over the world. Methods of synthesizing calcium hydroxide phosphates are known. They consist in the following: precipitation from salts of calcium (or hydroxide, or oxide, or carbonate) with addition of *o*-phosphoric acid or mono- or double-substituted phosphate salts with the subsequent hydrolysis in the solution, under hydrothermal conditions, or as a result of pyrolysis (9 – 23). Methods for synthesizing calcium hydroxide phosphates are most exhaustively discussed in (4). It is hardly possible to adduce all the references. The issues concerning methods of production of calcium phosphates, their structure and properties are most fully elucidated in (14).

These are problem of a resorption of calcium hydroxyapatite and osteogenesis in vivo organisms important (24 - 27). However, the patent and scientific literature does not offer any preparations based on inorganic calcium phosphates influencing the metabolism of calcium in a living organism through the skin.

The aim of this work is to synthesize calcium carbonate-phosphates doped with cations, which are easily assimilated by a living organism, including through the skin. It presents a study of their crystal phases, chemical composition and particle size analysis, as well as their biological activity in the processes of osteogenesis.

2. Materials and methods

For synthesizing samples of doped calcium carbonate-phosphate, calcium carbonate of three crystal structures was used. They are calcite (rhombohedral), vaterite (hexagonal) and aragonite (orthorhombic). Precipitation of calcium carbonate-phosphate was performed by *o*-phosphoric acid (2 mol/l), which was added dropwise into a calcium carbonate suspension in an ammonium chloride solution (2 mol/l) at 45 to 55°C. The size of the pH environment varied between 5.2 and 6.5 depending on the molar ratio Ca/P (1.55 to 1.67). Doping cations were added during calcium carbonate precipitation: Fe^{2+} and Mg^{2+} 0.0004–0.06; Zn^{2+} 0.0015–0.002; K^{+} 0.001– 0.01; SiO_2 0.0002– 0.006; and Mn^{2+} 0.00002 – 0.001 mol %. The choice of the calcium-phosphorus - cations-doped molar ratio was caused by the known concentrations of these elements in the bone tissue (1). The precipitate of synthesized calcium carbonate-phosphates was separated by filtering, washed by water and dried at temperatures not higher than 75°C.

The samples thus obtained were characterized by X-ray diffraction (XRD) (DRON-2 diffractometer, Cu*K*α radiation; STADI-P diffractometer, software for diffraction peak identification using JCPDS–ICDD PDF2 data); IR spectroscopy (Shimadzu JR-475 spectrophotometer, KBr disk method) and differential thermal analysis (DTG) (MOM thermoanalytical system) at a heating rate of 10 to 11deg/min within the range of temperatures from 20 to 1000°C, with a weight of 500 mg. The particle size analysis of the samples was performed by gravitational centrifugal sedimentation with the use of the SA-CP2 analyzer produced by Shimadzu, Japan (dispersion medium viscosity 0.0093 P, density 1.0 g/cm^3).

The chemical composition (Ca, P, Fe, Mg, Zn, Mn, K, Si) was determined by standard techniques of complexometric method (28) and X-ray fluorescent analyses with the use of the EDX-900HS energy dispersion spectrometer (Shimadzu, Japan). The mechanical strength of the bone and dental tissues was studied with the application of the method of stress determination in the bone tissue transverse section (29, 30). The calculation was made by the formula $P=a\ F/S$, where P is mechanical strength (shearing stress), MPa; S is the cross-sectional area of the specimen to produce the stress, mm^2; F is the load applied to cut the bone and dental tissues, kg-wt. The relative error of the method was 2.5 %. *Figure* 1 is presented apparatus for research transverse mechanical strength of the bone and dental tissues.

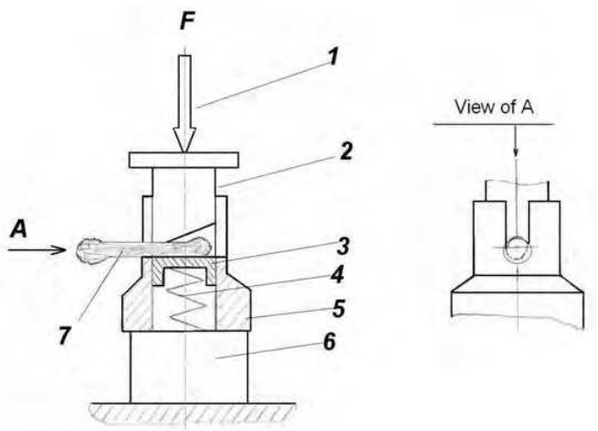


Fig. 1. Instrument for research transverse mechanical strength of the bone and dental tissues: 1-screw press; 2- poise; 3- pillar; 4- spring; 5 – spigot-matrix; 6 – force sensor; 7- sample of bone and dental tissue

3. Results and discussion

3.1 The synthesis of doped calcium carbonate- phosphate

The mechanism of obtaining this biomaterial is quite difficult, and this process can be considered oscillating reactions of calcium in a living organism. Reaction of the synthesis of doped calcium carbonate phosphates, which were described in (6-8), include several initial compounds as a calcium carbonate of three polymorphic crystal forms (calcite, aragonite, and vaterite), ortho–phosphoric acid, ammonium chloride, ammonium hydroxide, and microelements of the living organism (K^+, Mg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}, SiO_2). The formation of complex of $M_{8-x}M_x(OH)_2[(CO_3)_{x-2}\cdot H_2O]$ were described in (7, 8).

For example, in the medium of ammonium hydroxide, three polymorphic forms of $CaCO_3$ can form ammonium met stable Hydroxycarbonates complexes on the following scheme:

$$CaCO_3 + NH_4OH \rightleftarrows NH_4CaCO_3OH \quad (1)$$

Or in general terms:

$$A \rightleftarrows X \quad (2)$$

The formation of three types of crystal structures of calcium carbonate (in the medium of ammonium hydroxide and ammonium chloride) is typical for the reaction (1): calcite, vaterite, and aragonite, which was proven by the data of XRD (*Figure 2)*. SEM micrographs of synthetic calcium carbonate: calcite, vaterite, and aragonite shown in *Figure* 3.

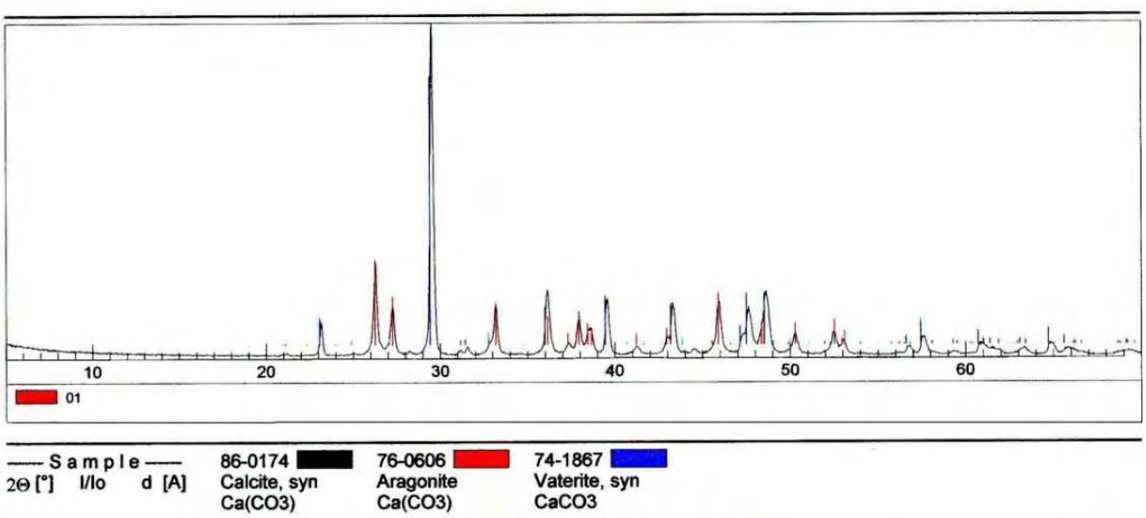
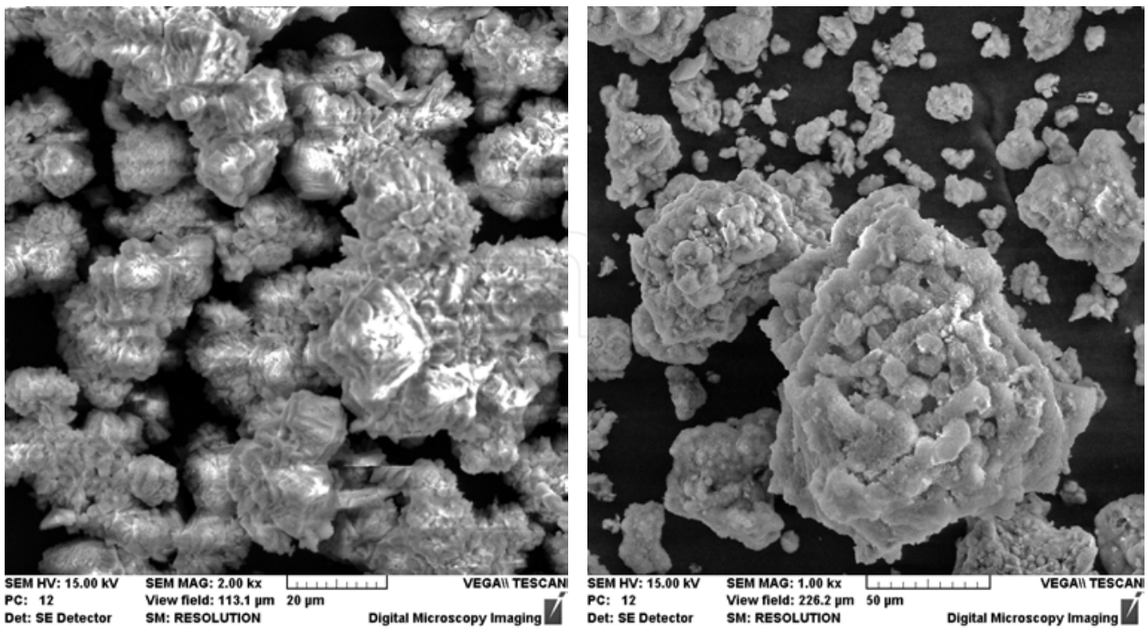


Fig. 2. XRD patterns of calcium carbonate: calcite (53 wt.%), vaterite (6 wt.%) and aragonite (41 wt.%)

Under the action of *ortho*-phosphate acid in the presence of magnesium cations and silicon dioxide, carbonate is replaced in the phosphate acid with the formation of $CaHPO_4$ (brushite) or $Ca_8H_2(PO_4)_6\cdot$ according to the reaction:

$$NH_4CaCO_3OH + H_3PO_4 = NH_4OH + CaHPO_4 \quad (3)$$

$$10NH_4CaCO_3OH + 6H_3PO_4 = Ca_8H_2(PO_4)_6 + 2CaCO_3 + 8CO_2 + 10NH_4OH + 8H_2O \quad (4)$$



a) b)

Fig. 3. Scannig electron micrographs of calcium carbonate (a): calcite, vaterite, and aragonite; of doped Fe 0.004; Mg 0.007; Zn 0.002; Mn 0.00002 mol.% calcium carbonate-phosphate (b)

Or in general terms:

$$B + X \rightarrow Y + C \tag{5}$$

In the environment of ammonium chloride with the addition of *o*-phosphoric acid, with the pH environment from 5.2 to 6.5, calcium phosphate chloride may form from precipitated calcium carbonate, for example, by the following equation (for convenience of writing equations by integers in a formula, while XRD analysis the number of atoms shows fractional):

$$5CaCO_3 + 2H_3PO_4 + 2NH_4Cl + 2\,NH_4OH = 2Ca_5(PO_4)_2(OH)_2Cl_2 + 5CO_2 + \\ + 5H_2O + 4NH_3 \tag{6}$$

$$Ca_8H_2(PO_4)_6 + 2CaCO_3 + NH_4Cl + NH_3 \rightleftarrows Ca_{10}(PO_4)_6OHCl + 2NH_4HCO_3 \tag{7,8}$$

Or in general terms:

$$C \rightleftarrows R$$

According to the law of mass action speed of responce (7, 8) characterize by the equation:

$$\frac{dx_4}{dt} = k_4[NH_4^+][Cl^-][NH_3];$$

$$\frac{dx_5}{dt} = k_5[NH_4^+]^2[HCO_3^-]^2$$

$$3CaHPO_4 + 2\,CaCO_3 + 2\,NH_4Cl + 2\,NH_3 + 3\,H_2O + CO_2 \rightleftarrows$$
$$\rightleftarrows\ Ca_5(PO_4)_2(OH)_2Cl_2 + 3\,NH_4HCO_3 + NH_4H_2PO_4 \qquad (9,10)$$

Or in general terms:

$$C \rightleftarrows R \qquad (11)$$

Reaction rate (9, 10) characterize by the equation:

$$\frac{dx_6}{dt} = k_6\left[NH_4^+\right]^2\left[Cl^-\right]^2\left[NH_3\right]^2\left[CO_2\right]$$

$$\frac{dx_7}{dt} = k_7[NH_4^+]^4[HCO_3^-]^3[H_2PO_4^-].$$

In addition is transfomation cycle with response:

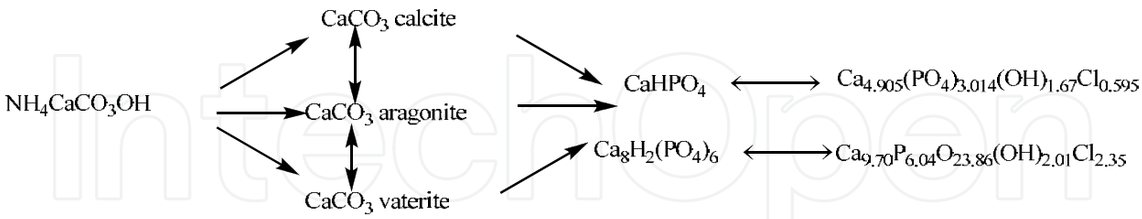
$$CaHPO_4 \rightleftarrows CaCO_3$$

Or in general terms:

$$C \rightleftarrows A \qquad (12)$$

Doping of calcium carbonate-phosphate with Mg^{2+} - cations leads to the formation of the following phases in them: octacalcium phosphate hydrogen, brushite, besides, the phases of calcite and aragonite partially remain there. Simultaneous doping with Fe^{2+} and Mg^{2+} cations causes the formation of the same phases as in case of introduction of Fe^{2+} cations alone, however, the calcium hydrogen phosphate $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ and calcium phosphate chloride hydroxide $Ca_{9.70}P_{6.04}O_{23.86}(OH)_{2.01}Cl_{2.35}$. The insertion of cations Fe^{2+}, Mg^2 leads to the basic crystal phase of octacalcium phosphate hydrogen $Ca_8H_2(PO_4)_6\ 5H_2O$.

The next stage in the presence of such doping microelements as Fe^{2+}, Mg^2, Zn^{2+} K^+, Si^{4+}, Mn^{2+} is the formation of calcium phosphate chloride hydroxide according to the following scheme:



The simplest classic example of the existence of autooscillations in the system of chemical reactions is the trimolecular model ("brusselator") offered by I.R. Prigozhine and R. Lefebre (31). The main purpose for the study of this model was to determine the qualitative types of behavior, which are compatible with the fundamental laws of chemical and biological kinetics. In this context, the brusselator plays the role of a basic model, like a harmonic oscillator in physics. A classic brusselator model describes the hypothetical scheme of chemical reactions:

$$A \rightarrow X$$

$$B+X \rightarrow Y+C$$

$$2X+Y \rightarrow 3X$$

$$X \rightarrow R$$

$$A+B \rightarrow R+C. \quad (13)$$

The key is the stage of transformation of two *X* molecules and one *Y* molecule into *X* (the so-called trimolecular reaction). Such a reaction is possible in processes with the participation of ferments with two catalytic centers. The nonlinearity of this reaction, coupled with processes of diffusion of the substance, well as the formation spatial structures in an initially homogeneous system of morphogenesis. Although the trimolecular stage in chemical kinetics is not as common as in biomolecular processes, expressions for the speed of some chemical reactions in some definite cases can be called cubic - type. Such equations are called "reaction diffusion"equations. The whole system has an oscillating character and can be presented as a brusselator of the simplest implementation of cubic nonlinearity by the following chemical reaction:

$$2X + Y \rightarrow 3X \quad (14)$$

If the final products *C* and *R* are immediately removed from the reaction, then the scheme of the reactions (in the case of a point system) can be given by the following system of equations:

$$\frac{dx}{dt} = A + X^2Y - (B+1)X$$

$$\frac{dy}{dt} = BX - X^2Y.$$

Inserting doping cations Mg^{2+} and K^{+} leads to the synthesis of the basic phase of calcium phosphate hydrogen $Ca_8H_2(PO_4)_6$ as the additional phase of calcium phosphate chloride hydroxide $Ca_{9.70}P_{6.04}O_{23.86}(OH)_{2.01}Cl_{2.35}$ (up to 7 wt %) and calcium carbonate phosphate and potassium hydrate phosphate hydrogen $Ca_8H_2(PO_4)_6{\cdot}H_2O$-$KHCO_3$-$H_2O$ (up to 6 wt %, *Table 1*). The regularities of the concentration change of hydroxychlorapatite, chloride, and magnesium in the products of reaction in the synthesis of calcium carbonate phosphate for the reaction with visible cations Mg^{2+}, Fe^{2+}, Zn^{2+}, and Mn^{2+} are shown in *Figure 4.*

The kinetic curves of concentration changes in the synthesis of doped calcium carbonate-phosphate are similar to the kinetics of concentration changes and the phase picture of the fructose-6-phosphate and fructose - diphosphate system.

Therefore, the oscillating dynamics of the brusselator model and modeling with waves, which are proposed for the fructose-6-phosphate and fructose-diphosphate system in (31, 32). For comparison, see model of intracellular calcium oscillations, as described in (33-37), *Figure 5.*

Oscillating character synthesis of the doped calcium carbonate-phosphate reply in the filtering and washing process doped calcium carbonate-phosphate precipitation what shown in *Figure 6.*

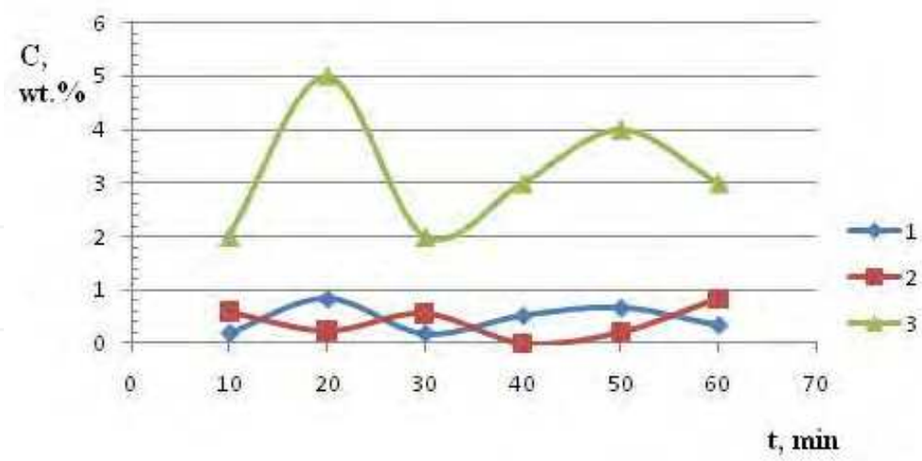


Fig. 4. The kinetics of concentration dependencies of the chloride-ions (1), Mg^{2+} (2) and calcium chloride-hydroxide phosphate (3) precipitation on a time reaction

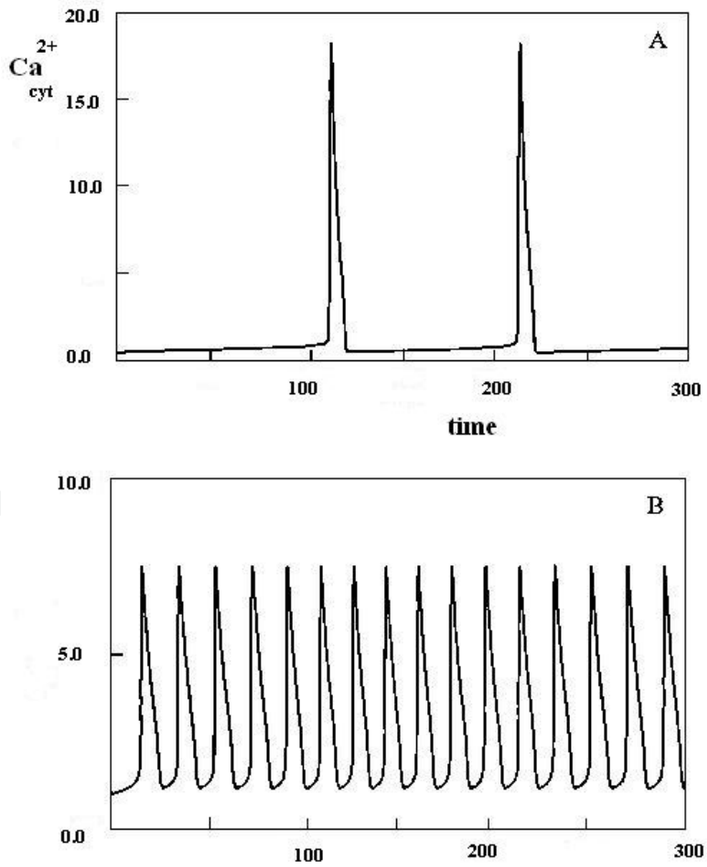


Fig. 5. Model fluctuations in intracellular calcium. Kinetics of Ca concentration in different settings (33)

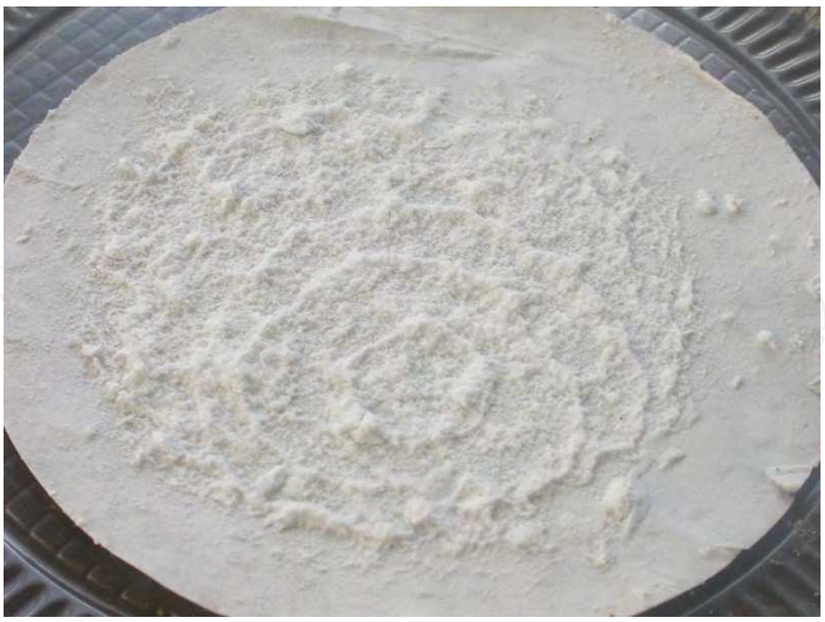


Fig. 6. Oscillating character in the filtering and washing process of the doped calcium carbonate-phosphate precipitation

The results of X-ray diffraction and chemical analysis confirm this. Typical X-ray diffraction pattern of doped calcium carbonate-phosphate samples are presented in *Figure* 7. SEM-micrographs of synthetic doped calcium carbonate-phosphate shown in *Figure 3.*

The composition is also confirmed by the chemical analysis data and the obtained IR-spectra of calcium carbonate-phosphate samples. *Figure* 8 presents typical IR-spectra of calcium carbonate-phosphate samples doped with iron, magnesium, zinc, manganese where there are bands of absorption of valence vibration δ_v of the PO_4^{3+} group 525, 560, 600 cm^{-1}, bands of absorption of symmetric vibrations ν_1 865-870 and 960-980 cm^{-1} and asymmetric vibrations ν_3 1040 - 1050 and 1100 -1130cm^{-1}, and also bands of absorption of the deformation vibration ν_3 of the CO_3^{2-} group 1400 cm^{-1}. The band of absorption of 1630 -1650 cm^{-1} corresponds to the deformation vibrations of OH^- water groups. The band of absorption 3150, 3480 cm^{-1} corresponds to the valence vibration of water and characterizes the presence crystallization water. The values for the triplet of the valence vibration of the phosphate group δ_v are close to those presented in (9 – 11). A comparison between the IR-spectra of the calcium carbonate-phosphate samples and brushite $CaHPO_4 \cdot 2H_2O$ formed from calcium oxide revealed a difference. It has been found that brushite is characterized by bands of absorption of valence vibration δ_v of group PO_4^{3+} 530, 575, 600 cm^{-1}, bands of absorption of symmetric vibrations ν_1 790, 870 and 985 cm^{-1} and asymmetric vibrations ν_3 1060, 1135 and 1210 cm^{-1}, as well as bands of absorption of deformation vibration of the OH^- group 1645 cm^{-1}.

DTG-analysis establishes that the calcium hydroxyapatite crystallization temperature is 840°C, which proves to be true judging by the endothermic effect on the thermograph. At temperatures 130, 190 and 240°C, endothermic effects are caused by the dehydration of crystallization waters and the removal of hydrogen ions. The general loss of the weight of the samples dried up at a temperature of 75°C makes 17.5 to 18.5 wt. %, and this agrees well with the chemical and phase analysis *(Figure* 9*).*

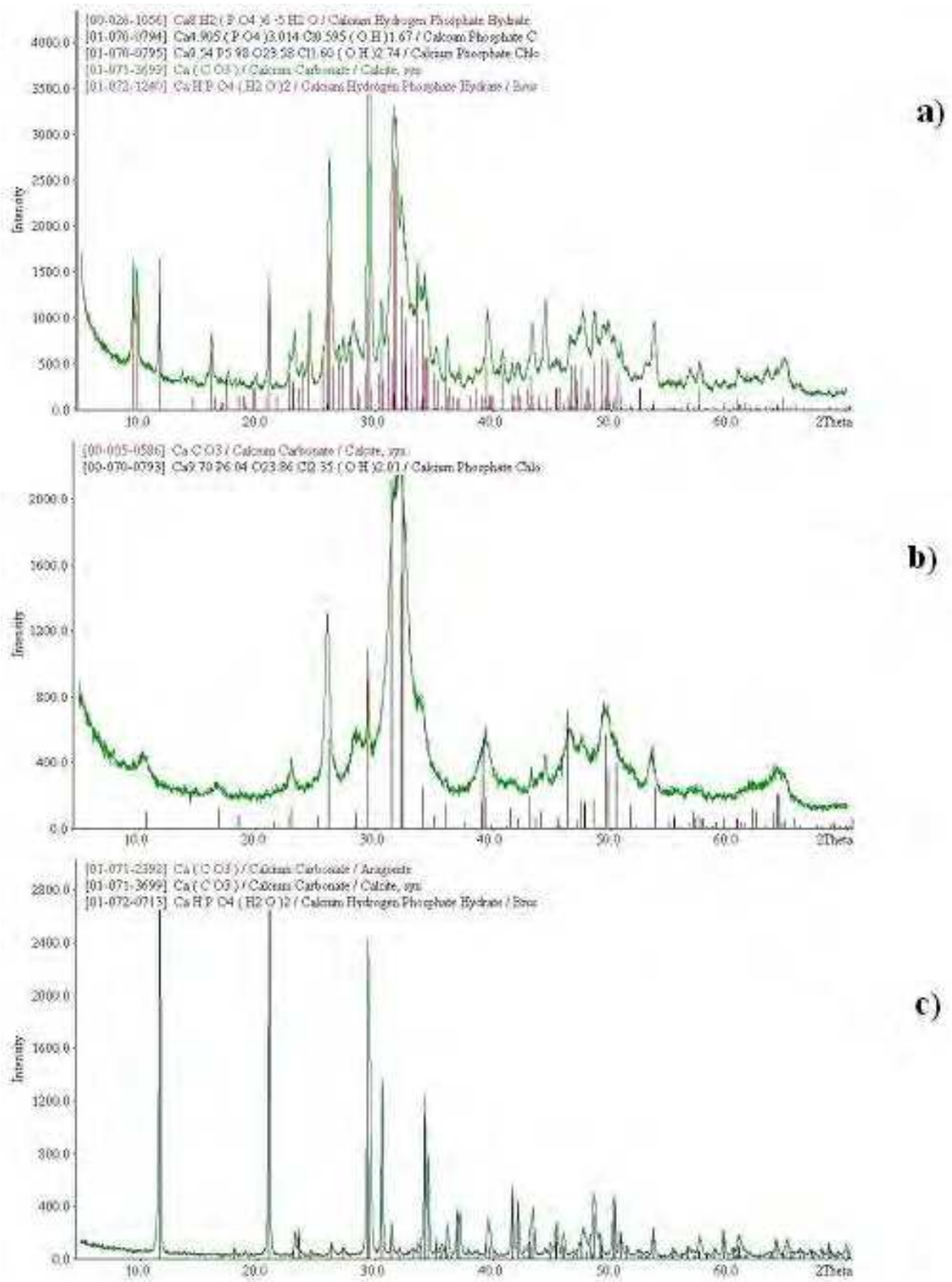


Fig. 7. XRD patterns of (a) iron, magnesium, zinc doped; (b) iron, magnesium, zinc, manganese doped; (c) iron, magnesium, silica doped calcium carbonate phosphate

Found, mol. %						Solid phase concentration, wt.%
Fe	Mg	Zn	K	Mn	SiO_2	
0.001	0.02	-	-	-	-	$Ca_8H_2(PO_4)_6*5H_2O$ 76% $Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 18%
0.001	0.005	0.002	-	0.001	-	$CaHPO_4*2H_2O$ 72% $Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 13%
0.001	0.004	0.002				$CaHPO_4*2H_2O$ 72% $Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 13%
0.002	0.06	0.002				$CaHPO_4*2H_2O$ 70% $Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 18%
0.002	0.01	0.002				$CaHPO_4*2H_2O$ 70% $Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 18%
0.003	0.02	-	0.001	-	-	$CaHPO_4*2H_2O$ 80%
-	0.06	-	0.001	-	-	$Ca_8H_2(PO_4)_6*5H_2O$ 85% $Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 7 % $Ca_8H_2(PO_4)_6*H_2O$-$KHCO_3$-H_2O 6%
-	0.003	-	-	-	0.002	$CaHPO_4*2H_2O$ 60% $Ca_8H_2(PO_4)_6*5H_2O$ 16%
-	0.01	-	0.001	-	-	$CaHPO_4*2H_2O$ 80%
0.0004	0.035	0.002	-	-	-	$Ca_8H_2(PO_4)_6*5H_2O$ 86% $Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 2% $CaHPO_4*2H_2O$ 6% $Ca_{4.905}(PO_4)_{3.014}Cl_{0.595}(OH)_{1.67}$ 1%
-	0.02	-	-	-	-	$CaHPO_4*2H_2O$ 10% $Ca_8H_2(PO_4)_6*5H_2O$ 50%
0.0004	0.02				0.0006	$CaHPO_4*2H_2O$ 84%
0.004	0.007	0.002	-	0.00002	-	$Ca_{9.70}P_{6.04}O_{23.86}Cl_{2.35}(OH)_{2.01}$ 75% $CaCO_3$ 25%

Table 1. The phase and chemical composition of calcium carbonate-phosphate samples

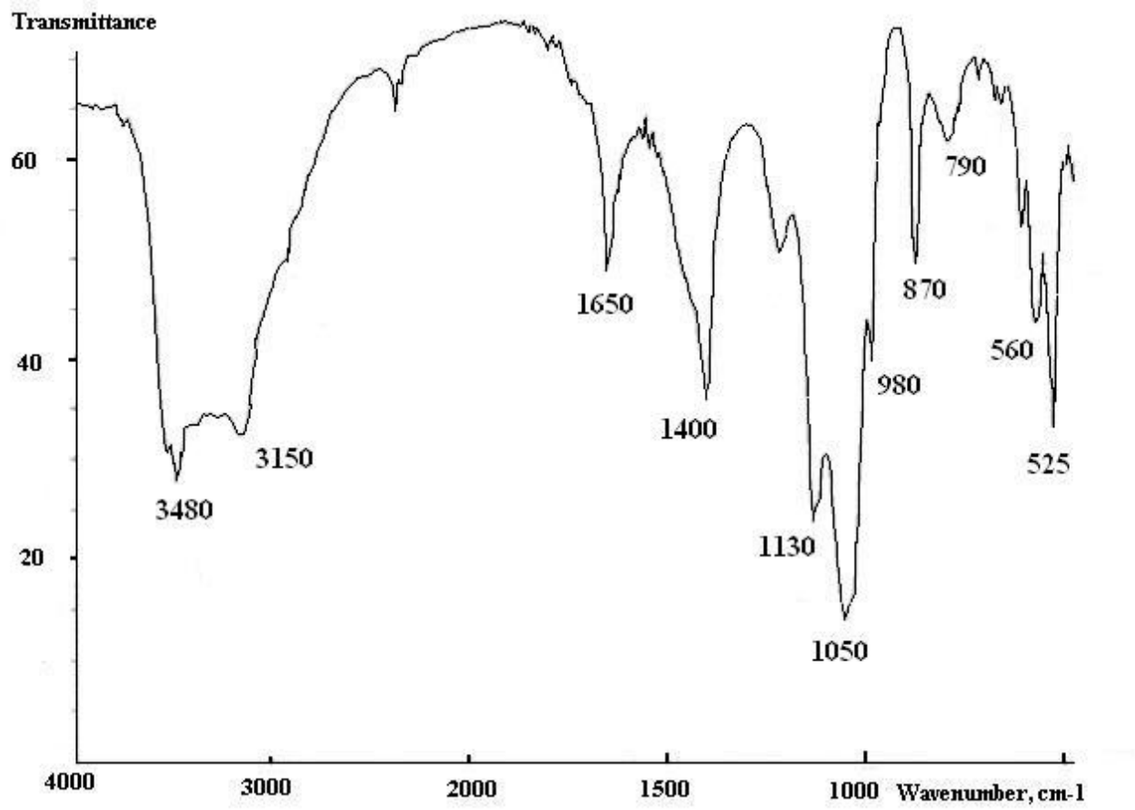


Fig. 8. Typical IR-spectra of calcium carbonate-phosphate samples doped with iron, magnesium, zinc, manganese.

Thus, doping cations of Fe^{2+}, Mg^{2}, Zn^{2+} K^{+}, Mn^{2+} and Si^{4+} have an effect on the phase equilibrium in the system $CaCO_3$ – H_3PO_4 – NH_4Cl, as well as on the end products of the process of sedimentation and their properties. It is calcium phosphate chloride and calcium hydrogen phosphate that are general crystal phases for iron- and magnesium-doped samples. The particle-size analysis has shown that the composition of calcium carbonate-phosphate samples is polydisperse.

The basic fraction of particles ranges from 5 to 20 microns for samples doped with cations simultaneously. In addition the ultradispersed fraction with the size of particles up to 10 nm in amounts of 1.5 % is observed, and this allows the material to be especially active in all the samples. It is noted that material dispersiveness enables the material to get through the skin of an organism. The characteristic curves of the particle-size analysis of the samples are adduced in *Figure 10*.

3.2 Studying biological activity

The influence of the doped calcium carbonate-phosphate on the bone and dental tissues of a living organism was investigated experimentally in white rats. A 1 % water suspension of calcium carbonate-phosphates was introduced inside animals through an enteric tube in amounts of 5 ml within 40 days, 30 mg per 1 kg of live weight (there were five groups of 10 animals, namely, I – placebo, II – within 10 days, III – within 20 days, IV – within 30 days, V

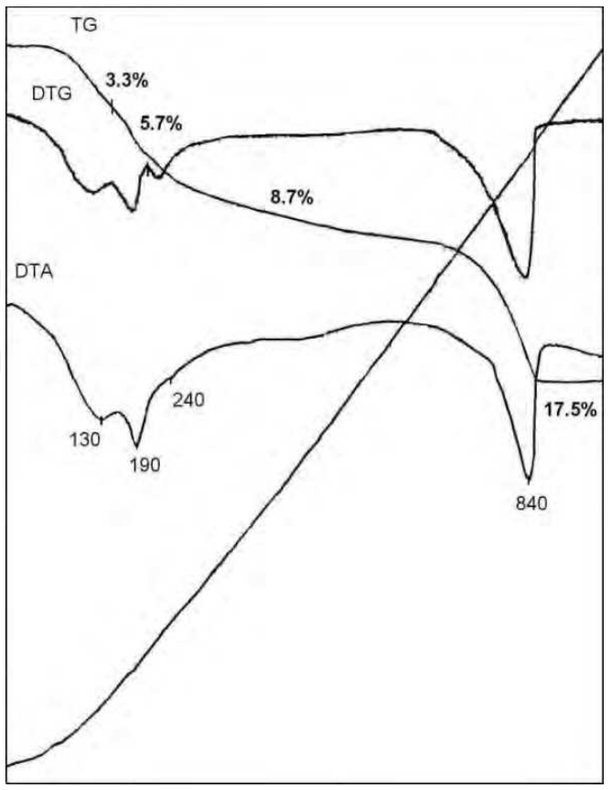


Fig. 9. A characteristic DTA-curve of calcium carbonate-phosphate samples doped with cations of iron and magnesium

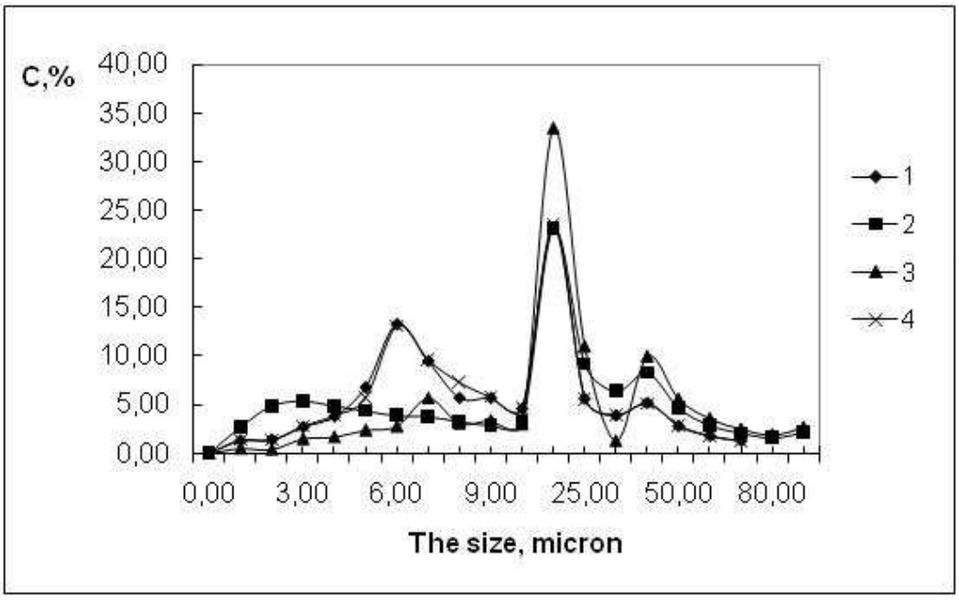
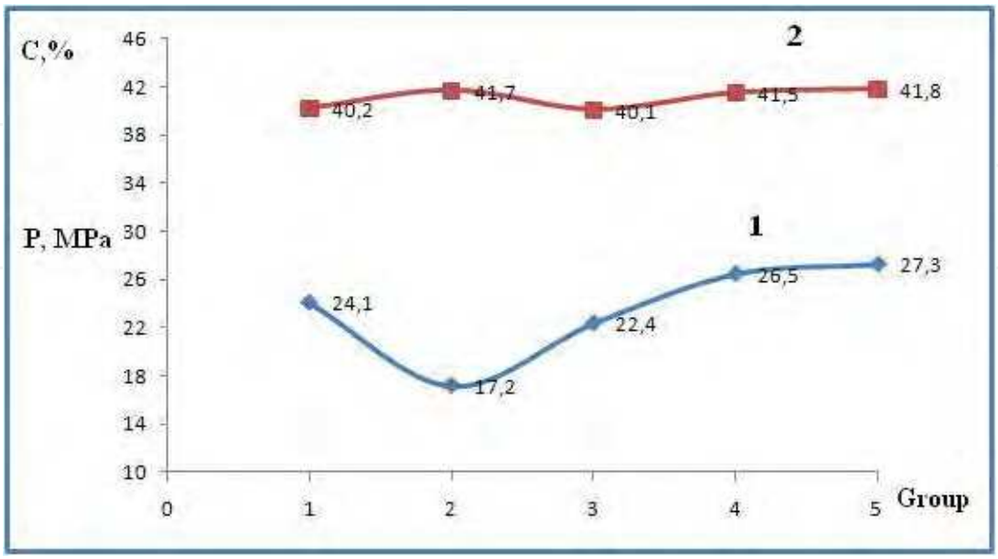


Fig. 10. Differential particle size distributions of calcium carbonate phosphates prepared from them: (*1*) doped with 0.02 mol % Fe, (*2*) doped with 0.02 mol % Mg, (*3*) doped with 0.02 mol % Fe and 0.02 mol% Mg, (*4*) undoped

– within 40 days). The results are illustrated in *Table* 2 showing the transverse mechanical strength (shear stress) of the bone tissue (femur) and the dental enamel as a function of the duration suspension introduction into animals. As a result, it has been found that there is a 13% increase in the mechanical strength (shear stress) of the bone tissue and a 7 % increase in the strength of the dental tissue (enamel), and this enables us to make an assumption of strengthened osteogenesis in a living organism.

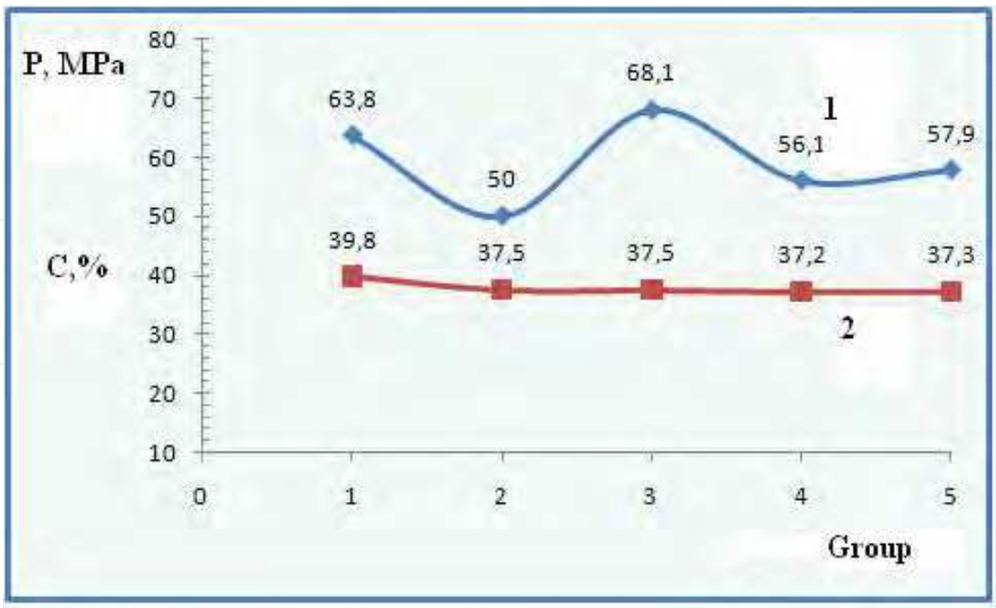
Groups on 10 animals	Shear strength of the bone tissue, MPa	Standard deviation, S^2	Content of Ca of the bone tissue, wt. %	Standard deviation, S^2	Shear strength of dental enamel, MPa	Standard deviation S^2	Content of Fe, wt. %	Standard deviation, S^2	Content of Mg, wt. %	Standard deviation, S^2
I	24.1	0.23	40.25	0.14	63.8	1.95	0.78	0.07	0.06	0.01
II	17.2	0.98	41.72	0.45	50.0	1.20	0.40	0.09	0.24	0.04
III	23.0	0.15	40.06	0.69	68.1	1.83	0.63	0.03	0.26	0.01
IV	26.5	0.46	41.53	0.12	56.1	1.75	0.92	0.05	0.16	0.01
V	27.3	0.59	41.78	0.10	57.9	1.90	0.70	0.05	0.22	0.03

Table 2. Processed experimental data on the effect of doped calcium carbonate-phosphate on the mechanical strength of the bone tissue and the concentration of iron and magnesium in dental enamel



a)

Fig. 11. (Continued)



b)

Fig. 11. Impact doped calcium carbonate-phosphate an bone tissue strength (curve 1, fig. a) and bone tissue calcium concentration (curve 2, fig. a) and impact an tooth tissue strength (curve 1, fig. 11b) and tooth tissue calcium concentration (curve 2, fig. 11 b)

It is necessary to note that the first 10 days see a decrease in mechanical strength for the bone and dental tissues, and this is attributed to the insufficient number of receptors generated to assimilate calcium and phosphorus at the first stage. The results obtained have been processed by means of methods of mathematical statistics and the sampling has been verified for the normal distribution of the results. The comparison of the results with known ones (29) demonstrates a good agreement.

The content of calcium in the bone tissue, iron and magnesium in the dental enamel of animals as dependent on the introduction of calcium phosphates doped with magnesium and iron is presented in *Table 2* and in *Figure 11.* The changes in the mechanical strength of dental enamel and the content of iron and magnesium prove the activity of calcium phosphates and their influence on osteogenesis.

4. Conclusion

Thus, synthesized and investigated doped calcium carbonate-phosphate doped with cations represent a complex phase composition and constitute a biologically active material. The introduction of cations Fe^{2+}, Mg^{2+}, Zn^{2+}, K^{+}, Mn^{2+} and Si^{4+} changes the phase equilibrium in the $CaCO_3$ – NH_4Cl – H_3PO_4 system and leads to the formation of calcium phosphate chloride hydroxide, octacalcium hydrogen phosphate, brushite as the most active components participating in osteogenesis and the strengthening of the bone and dental tissues. By virtue of the kinetic data of the reaction of the interaction between orthophosphate acid and calcium hydroxycarbonate complexes in the synthesis process of nanocrystalline doped calcium carbonate phosphate, this system can be submitted as being chemically oscillating, i.e., oscillating in time. To describe this oscillating system, one can use

a brusselator of the simplest cubic nonlinear realization. The kinetic curves of concentrated changes in the synthesis of nanocrystalline doped calcium carbonate phosphates are similar to the kinetics of concentration changes and phase pattern of the fructose-6-phosphate and fructose di-phosphate systems, respectively. The final reaction product output is determined as a result of oscillations. Doping cations have an impact on the formation of biologically active phosphate compounds. Doped calcium carbonate-phosphate are promising biocompatible materials designed to strengthen the bone and dental tissues and to replenish calcium in a living organism.

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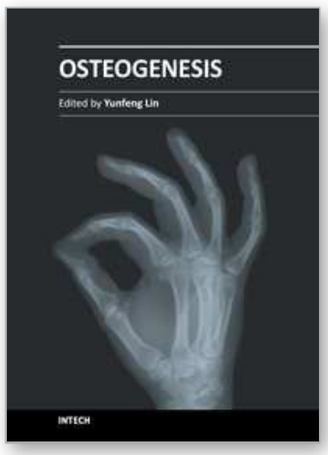

Osteogenesis

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This book provides an in-depth overview of current knowledge about Osteogenesis, including molecular mechanisms, transcriptional regulators, scaffolds, cell biology, mechanical stimuli, vascularization and osteogenesis related diseases. Hopefully, the publication of this book will help researchers in this field to decide where to focus their future efforts, and provide an overview for surgeons and clinicians who wish to be directed in the developments related to this fascinating subject.

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