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Peculiarities of influence of anionic modification on formation of Na^+, Mg^{2+}, Zn^{2+}-containing calcium phosphates

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The paper presents a study of the effect of adding carbonate or borate anions to the initial solution of the Ca^{2+}-Mg^{2+}-Zn^{2+}-Na^+-PO_4^{3-} system on the formation of calcium phosphates containing Na^+, Mg^{2+}, Zn^{2+}. In the Ca^{2+}-Mg^{2+}-Zn^{2+}-Na^+-PO_4^{3-} system, the biphasic calcium phosphates (mixture of phases based on $Ca_{10}(PO_4)_6(OH)_2$ and β-$Ca_3(PO_4)_2$) have been obtained. It was established that an increase in the amount of Zn^{2+} in the initial solution leads to an increase in the content of phase β-$Ca_3(PO_4)_2$ from 25wt% to 75wt% in biphasic calcium phosphates and its particles size from 35 to 71 nm. *In vitro* tests of the modified calcium phosphates with different anionic composition in model solution at 37°C showed the highest activity: an increase in pH more than 35% after first 48 hours and for 14 days of the study for calcium carbonate apatite phosphate containing Na^+ (0,5wt%), Mg^{2+} (0.5wt%) and Zn^{2+} (1.7wt%). At the same time, borate-containing phosphate with a similar content of trace elements showed activity only during first 48 hours with a pH change of 20%. Obtained results showed the possibility of obtaining apatite-related modified calcium phosphate with different dissolution rates by changing the nature of anionic dopant when designing materials for orthopedics.

Key words: zinc; magnesium; sodium; hydroxyapatite; tricalcium phosphate.

Особливості впливу аніонного модифікування на формування Na^+, Mg^{2+}, Zn^{2+}-вмісних кальцій фосфатів. *Н.Ю. Струтинська, Є.О. Комащенко, О.В. Лівіцька, М.С. Слободяник*

У представленій роботі наведено результати дослідження впливу додавання карбонат- чи борат-аніонів до вихідного розчину систем Ca^{2+}-Mg^{2+}-Zn^{2+}-Na^+-PO_4^{3-} на формування Na^+, Mg^{2+}, Zn^{2+}-вмісних кальцій фосфатів. У випадку системи Ca^{2+}-Mg^{2+}-Zn^{2+}-Na^+-PO_4^{3-} отримані біфазні кальцій фосфати (суміш фаз на основі $Ca_{10}(PO_4)_6(OH)_2$ та β-$Ca_3(PO_4)_2$). Встановлено, що збільшення вмісту Zn^{2+} у вихідному розчині призводить до зростання вмісту фази на основі β-$Ca_3(PO_4)_2$ з 25мас% до 75мас% в біфазних кальцій фосфатах та розміру наночастинок з 35 до 71 нм. Тестування *in vitro* синтезованих модифікованих кальцій фосфатів з різним аніонним складом в модельованому розчині з pH =7.45 при 37°C показало, що найбільшу активність, зі збільшенням значення pH більше, ніж на 35% впродовж перших 48 годин і протягом наступних 14 днів встановлено для карбонат-вмісного гідроксиапатиту, що містив катіони Na^+ (0,5мас%), Mg^{2+} (0,5мас%) та Zn^{2+} (1,7мас%). В той час, як борат-вмісні фосфати з таким самим вмістом мікроелементів продемонстрували активність лише впродовж перших 48 годин зі зміною pH лише на 20%. Отримані результати свідчать про можливість одержання кальцій фосфатів апатитового типу з різною швидкістю розчинення залежно від природи аніонного замісника при розробці матеріалів для ортопедії.

1. Introduction

Calcium phosphates (hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, β-$Ca_3(PO_4)_2$ and their biphasic mixtures) are considered as bone replacement or repair materials due to their biocompatibility, osteoconductivity and bioactivity. Their flexible framework allows for doping with various ions, that expands the functionalization of calcium phosphates and the impact on the properties of the resulting materials [1-4]. Depending on the type of structure, calcium phosphates can be used for various purposes due to their different properties. Thus, hydroxyapatite is a more stable phase, and the use of biphasic mixtures improves osteoinductivity and affects solubility [5-6]. The partial substitution of Ca^{2+} ions for Mg^{2+} and Zn^{2+} in the cation lattice promotes not only osteogenesis and increased biological activity, but also stabilization of the β-$Ca_3(PO_4)_2$ phase in synthetic materials; the effect of zinc is more significant [7-8]. Zn-containing materials have antimicrobial properties and stimulate the formation of a new bone. The presence of zinc is necessary for metabolic processes and DNA replication, and its deficiency can increase the healing time of bone tissue [9-10]. Doping with Na^+ ions allows the composition to be brought closer to the inorganic component of natural bone and increases osteoconductivity, and they also participate in cell adhesions and bone metabolism [11-12]. The widespread partial substitution in the anion sublattice (PO_4^{3-} ions for CO_3^{2-}) makes the material similar to bone apatite and allows influencing the rate of osteoclast resorption, and accordingly can be used to regulate the rate of bioabsorption [13-14]. The study of the introduction of boron into the structure of calcium phosphates is possible as in the case of carbonate anion according to the principle of partial substitution of PO_4^{3-} groups for BO_3^{3-} or OH^- for linear BO_2^-. This is an actual issue that needs further study. Boron, as a trace element, promotes faster healing of wounds, plays an important role in the metabolism of bone tissue and has certain antibacterial properties, and its presence in bones is significant [15-16].

The main goal of present work was to investigate the influence of anionic sublattice modification on peculiarities of phase formation of calcium phosphates in the presence of a complex of trace elements (Na^+, Mg^{2+}, Zn^{2+}). The *in vitro* activity of modified calcium phosphates was assessed depending on their anionic and phase composition. These data are important for choice of synthetic materials for bone restoration.

2. Experimental

2.1 Preparation of samples

Calcium phosphates containing Na^+, Mg^{2+} Zn^{2+} (0.5-1.7 wt%) have been synthesized in aqueous solutions at molar ratio Ca^{2+}:Mg^{2+}:Zn^{2+}:Na^+:PO_4^{3-}:An^- = (10-x-y/2) : 0.25 : x : 0.25 : (6-y) : y, (x = 0.1, 0.25, 0.5; y = 0 or 0.5) (An – CO_3^{2-}, BO_3^{3-}) and temperature 25 °C with further heating of powders to 600 °C (2 hours). The following compounds were used as initial components: $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $NaNO_3$, $(NH_4)_2HPO_4$, NH_4HCO_3 and H_3BO_3 (analytical grade). The resulting mixture of nitrates in the required molar ratios was dissolved in 25 ml of deionized water, and then 25 ml of solution containing phosphate or phosphate-carbonate/borate anions was added with constant stirring at room temperature. Then 3 ml of $NH_3{*}H_2O$ was added. Then water was evaporated and powders after grinding were annealed at 600 °C in a muffle furnace SNOL-7.2/1100 (TermoPro-601 temperature controller).

2.2 Methods of characterization

The phase composition of phosphates was studied using X-ray powder diffraction. XRD patterns were recorded using a Shimadzu XRD-6000 diffractometer (2θ in the range 5-60° with rate 1.2°/min). The apatite-type crystalline phase was identified from the ICDD (International Center for Diffraction Data) database reference for $Ca_{10}(PO_4)_6(OH)_2$ (PDF2 #01-089-6495). The crystallite sizes for modified calcium phosphates were calculated using the Debye-Scherrer equation.

Fourier transform infrared spectroscopy was used to detect the presence of different anions in synthesized phases. A PerkinElmer Spectrum BX spectrometer was used (frequency range 400-4000 cm^{-1}). The samples were pressed into KBr pellets.

The *in vitro* activity of synthesized calcium phosphates modified with a complex of trace elements (sodium, zinc and magnesium) and different anions (carbonate or borate) was investigated by their soaking in the phosphate-buffered saline (PBS) at pH = 7.45 and temperature of 37 °C. The pH values of the PBS solution with the sample were measured every 24 hours using a pH meter (OHAUS Started 2100).

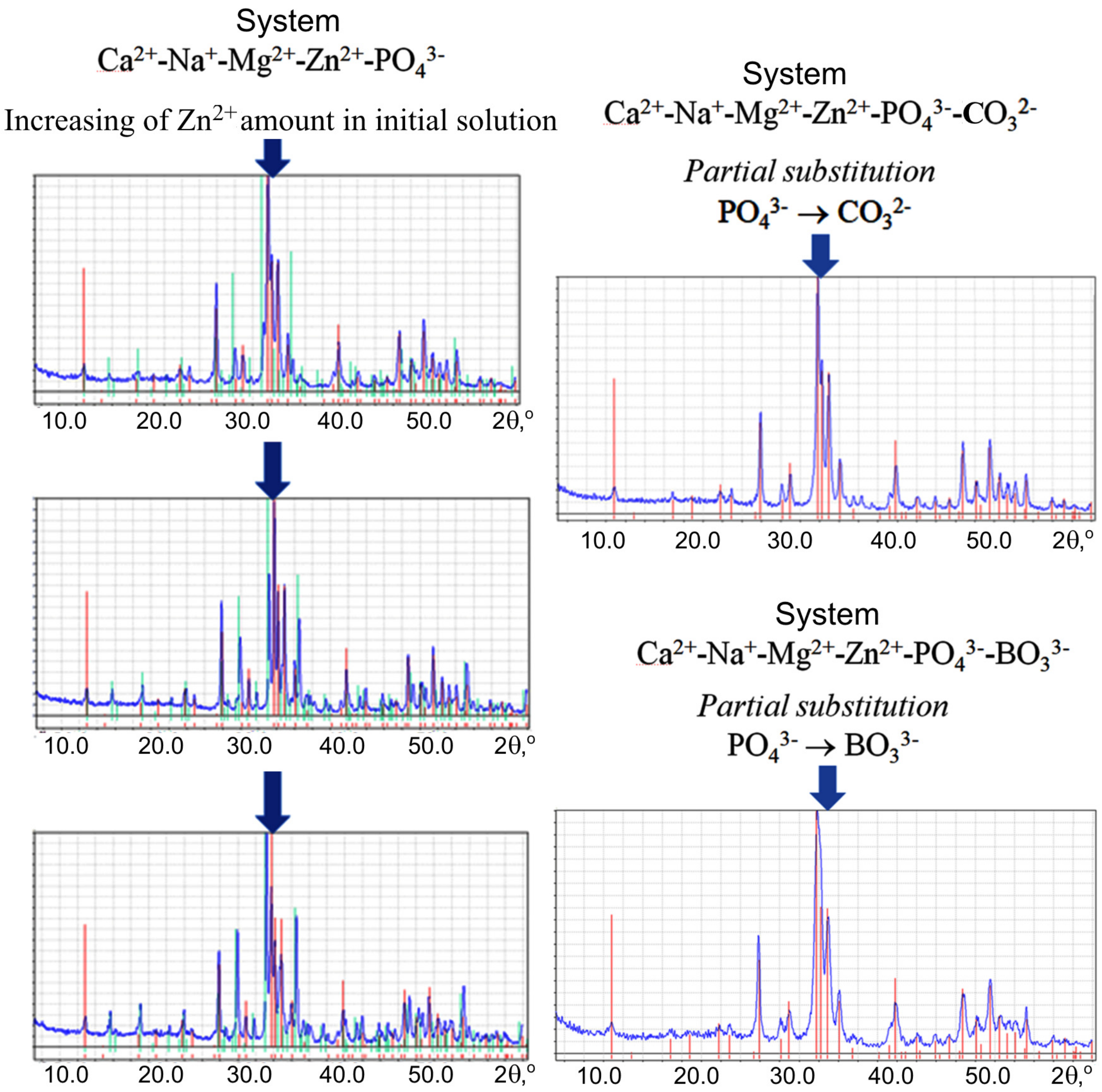

Fig. 1. XRD patterns of obtained calcium phosphates at molar ratios: $Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-}$= (9.625-$x$) : 0.25 : 0.25 : x : 6, for x = 0.1, 0.25 and 0.5; $Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-} : CO_3^{2-}$= 9.125 : 0.25 : 0.25 : 0.25 : 5.5 : 0.5, and $Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-} : BO_3^{3-}$= 9.375 : 0.25 : 0.25 : 0.25 : 5.5 : 0.5, and heat-treated at 600°C for 2 hours.

3. Results and discussion

The features of modification of calcium phosphates with microelements (Na^{+}, Mg^{2+}, Zn^{2+}) were studied with different amounts of zinc cations in aqueous solutions at molar ratios of $Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-}$= (9.625-$x$) : 0.25 : 0.25 : x : 6, x = 0.1, 0.25 and 0.5 as well as a fixed amount of Zn^{2+} (x =0.25) with the addition of carbonate or borate anion at a molar ratio of $PO_4^{3-} : An^{-}$ = 11. According to XRD data, the presence of an additional type of anions in the initial solution changed the phase type of the obtained phosphates (Fig. 1). In the case of the Ca^{2+} - Na^{+} - Mg^{2+} - Zn^{2+} - PO_4^{3-} system with different amounts of zinc cations, two-phase calcium phosphates were obtained (a mixture of phases based on $Ca_{10}(PO_4)_6(OH)_2$ – hexagonal system, space group $P6_3/m$ and β-$Ca_3(PO_4)_2$ – trigonal system, space group R-$3c$) (Fig. 1). It was found that an increase in the amount of Zn^{2+} in the initial solution (value of x from 0.1 to 0.5) leads to an increase in the content of the phase based on β-$Ca_3(PO_4)_2$ from 25 wt% to 75 wt% (Fig. 1). At the same time, the addition of carbonate or borate anions to initial solutions (at a molar ratio of $PO_4^{3-} : An^{-}$ = 11) at a fixed molar ratio of cations ($Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+}$ = 9.375 : 0.25 : 0.25 : 0.25) resulted in the formation of single-phase modified calcium phosphates of the apatite type (Fig. 1). In this case the introduction of a carbonate or borate anion into the apatite-type structure plays) resulted

Table 1. Calculated particles size for calcium phosphates obtained at different molar ratios of components in initial solutions

Molar ratios	Phase type	Particle size, nm
System Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-}		
9.525 : 0.25 : 0.25 : 0.1 : 6	$Ca_{10}(PO_4)_6(OH)_2$	24
	β-$Ca_3(PO_4)_2$ (25wt%)	35
9.375 : 0.25 : 0.25 : 0.25 : 6	$Ca_{10}(PO_4)_6(OH)_2$	38
	β-$Ca_3(PO_4)_2$ (42wt%)	55
9.125 : 0.25 : 0.25 : 0.5 : 6	$Ca_{10}(PO_4)_6(OH)_2$	49
	β-$Ca_3(PO_4)_2$ (75wt%)	71
System Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-}: CO_3^{2-}		
9.125 : 0.25 : 0.25 : 0.25 : 5.5 : 0.5	$Ca_{10}(PO_4)_6(OH)_2$	26
System Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-}: BO_3^{2-}		
9.375 : 0.25 : 0.25 : 0.25 : 5.5 : 0.5	$Ca_{10}(PO_4)_6(OH)_2$	18

in the formation of single-phase modified calcium phosphates of the apatite type (Fig. 1). In this case the introduction of a carbonate or borate anion into the apatite-type structure plays an important role in its stabilization and reducing the influence of Zn^{2+} for formation of a phase based on β-$Ca_3(PO_4)_2$.

It was found that the composition of the initial solution affects the size of the synthesized particles of apatite-containing calcium phosphates; calculations using the Debye-Scherrer formula revealed the formation of particles with a size of 26 nm or 18 nm for carbonated or borate-containing hydroxyapatites, respectively. For the apatite-type phase in two-phase calcium phosphates obtained in the system Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-}= (9.625-x) : 0.25 : 0.25 : x : 6 the size of particles increased from 24 nm to 49 nm with an increase in the amount of zinc in the initial solution (x from 0.1 to 0.5). At the same time, for the phase based on β-$Ca_3(PO_4)_2$, the particle size is somewhat larger and also increases from 35 to 71 nm with an increase in the amount of zinc in the solution (x from 0.1 to 0.5) (Table 1). The obtained results indicate that anionic modification of apatite-type calcium phosphates has virtually no effect on particle size, whereas zinc cations promote the formation of larger particles.

The FTIR spectra in the frequency range of 400-4000cm^{-1} for synthesized samples are represented in Fig. 2. The main characteristic bands of phosphate (PO_4^{3-}) were detected in the ranges of 540-650 см$^{-1}$ (v_4) and 970-1185 см$^{-1}$ (v_1 and v_3). The typical band of hydroxyl (OH^-) group is at 3582 cm^{-1} while modes of sorbed water were observed at 3200–3600 and 1580-1700 cm^{-1}. The FTIR spectra confirmed the formation of B-type carbonated hydroxyapatite which is indicated by the typical CO_3^{2-} bands at 873–885 and 1405–1460 cm^{-1} [17]. The formation of B-type hydroxyapatite was reported to show lower crystallinity of the powder and enhances the solubility of the apatite structure [18]. Characteristic bands of the BO_3^{3-} group were detected at 1302-1216 cm^{-1} and 784 cm^{-1} and were attributed to the antisymmetric stretching v_3 and the symmetric bending v_2 modes (Fig. 2, curve 2). The absence of a pair of weak peaks in range of 2005-1920 cm^{-1} of the antisymmetric stretching mode v_3 of ^{10}B-O and ^{11}B-O bonds of the linear BO_2^- groups confirmed the implementation of only partial substitution of $PO_4^{3-}\rightarrow BO_3^{3-}$, but not $OH^-\rightarrow BO_2^-$.

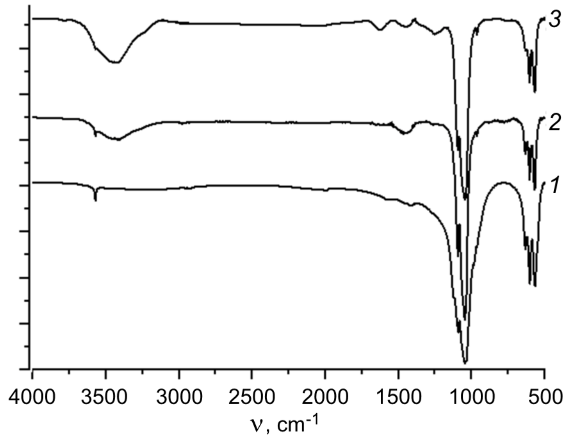

Fig. 2. FTIR spectra of modified calcium phosphates prepared at molar ratios: Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-} : An^-= (10-y/2) : 0.25 : 0.25 : 0.25 : (6-y) : y, (y = 0 (curve 1) or 0.5 (curves 2-3) An^- - CO_3^{2-} (curve 2) and BO_3^{3-} (curve 3), heat-treated at 600°C for 2 hours.

Results of *in vitro* activity testing of synthesized biphasic calcium phosphate with different weight ratio of component and car-

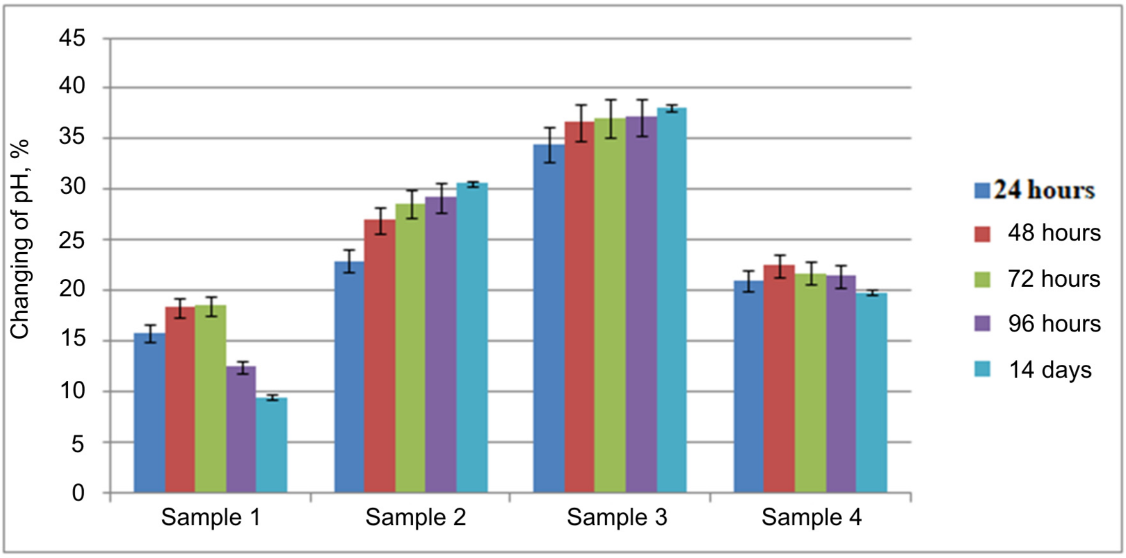

Fig. 3. The changing of pH values of model solutions in presence of obtained modified calcium phosphates prepared at molar ratios: Ca^{2+} : Na^{+} : Mg^{2+} : Zn^{2+} : PO_4^{3-} : An^{-}= (10-x-y/2) : 0.25 : 0.25 : *x* : (6-y) : y, (y = 0: *x* = 0.1 (Sample 1) or 0.5 (Sample 2); An^{-} - CO_3^{2-} (*x* = 0.25 – Sample 3) and BO_3^{3-} (*x* = 0.25 – Sample 4) after 24, 48, 72, 96 hours and 14 days at temperature 37 °C.

bonate- or borate-containing hydroxyapatite are presented in Figure 4: a change in the pH value of the model solution is observed during the first 96 hours (at the 24-hour stage) of the study and after 14 days. It was established that a biphasic calcium phosphate containing 75 wt% of phases based on $Ca_{10}(PO_4)_6(OH)_2$ and (25wt%) of β-$Ca_3(PO_4)_2$ (0,5wt% of Na^+, 0.5wt% of Mg^{2+}, 0.7wt% of Zn^{2+}) has the least effect on the pH value (only 18%) during the first 72 hours. At the same time, an increase in the amount of the phase based on β-$Ca_3(PO_4)_2$ to 75wt% led to an increase in the activity of the sample even after 14 days of exposure to the solution (Fig. 3, sample 2). The most active in increasing pH by more than 35% after the first 48 hours was carbonated apatite-related calcium phosphate (0.5 wt% of Na^+, 0.5 wt% of Mg^{2+}, 1.7 wt% of Zn^{2+}) (Fig. 3, sample 3). The main feature of this sample is the increased pH value even after 14 days, which indicates its ability to partially dissolve throughout the entire study period. Unlike carbonated hydroxyapatite, the borate-containing calcium phosphate with the same content of other dopants (0.5wt% of Na^+, 0.5 wt% of Mg^{2+}, 1.7 wt% of Zn^{2+}) is active only during the first 48 hours with a pH increase of 20%.The obtained results indicate the importance of the amount of phase based on β-$Ca_3(PO_4)_2$ in biphasic calcium phosphate and the presence of carbonate anions in apatite-related calcium phosphate for regulating the activity of synthetic calcium phosphates when they are used for bone tissue restoration.

4. Conclusions

It has been established that anionic modification of the apatite structure with a carbonate or borate group has an important effect on the formation of single-phase calcium phosphates containing Na^+(0.5wt%), Mg^{2+}(0.5wt%) and Zn^{2+} (1.7wt%). For the Ca^{2+}-Na^+-Mg^{2+}-Zn^{2+}-PO_4^{3-} system, the possibility of increasing the amount of the phase based on β-$Ca_3(PO_4)_2$ to 75wt% in the composition of biphasic calcium phosphates by increasing the amount of Zn^{2+} in the initial solution has been established. An *in vitro* study of synthesized calcium phosphates in a model solution at 37°C showed the potential of using the carbonated apatite-related calcium phosphate containing Na^+(0.5 wt%), Mg^{2+}(0.5wt%) and Zn^{2+} (1.7 wt%) as an active additive during the first 14 days of synthetic materials for bone tissue restoration.

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