doi: 10.56761/EFRE2024.N1-O-048803

Results of formation of a mixture of calcium phosphates

O.A. Golovanova[*]*, A.A. Tsyganova*

Omsk State University F.M. Dostoevsky, Omsk, Russia
[*]*golovanoa2000@mail.ru*

Abstract. A theoretical calculation was made of the possibility and conditions for the formation of mineral phases. It has been established that under these conditions the formation of a mixture of calcium phosphates is possible, with hydroxyapatite being the most stable phase. Based on the calculated data, a synthesis was carried out, as a result of which it was established that the resulting sediments were represented by the phases of hydroxyapatite, octacalcium phosphate and brushite. It was found that the synthesized mixture of calcium phosphates can be used to create dense ceramics. Thus, the range of use of synthesized materials is expanding. It has been proven that in an SBF solution a calcium phosphate layer is formed on the surface of the samples, which indicates the bioactivity of the samples. The prospect for further development of the topic is the use of ceramics as materials for replacing bone defects or a biologically active layer on the surface of implants.
Keywords: calcium phosphates, surface, ceramic, bioactivity.

1. Introduction

Calcium phosphates are chemical compounds of interest in many interdisciplinary fields of science, including geology, chemistry, biology and medicine. According to the literature, the first attempts to establish their chemical composition were made by Berzelius in the mid-19th century [1]. About 70 years later, the existence of different crystalline phases of calcium phosphates was hypothesized [2–4]. In mammals, calcium phosphates are the main inorganic component of both normal (bones, teeth, antlers) and pathological (dental and kidney stones, atherosclerotic deposits, etc.) hard tissues. With the exception of certain parts of the inner ear, all hard tissues of the human body are composed of calcium phosphates in the form of finely crystalline, non-stoichiometric, Na-, Mg- and carbonate-containing hydroxyapatite (often called biological apatite) [1, 5, 6].

It is known that the structural and morphological characteristics of the material are the most important indicators affecting the quality of osteoregenerative processes during the restoration of bone defects. Such indicators usually include: micro- and macroporosity of the material, shape and size of pores, their volume fraction in relation to bone matter, specific surface area. The ideal material for implantation should be as close as possible to human bone in terms of the above characteristics [7].

Thus, the goal of the work is to carry out a theoretical calculation of the possibility and conditions for the formation of a mixture of calcium phosphates and, based on the calculation results, to carry out the synthesis of materials based on a mixture of calcium phosphates.

1.1 Calculation of thermodynamic functions to assess the possibility and conditions for the formation of calcium phosphates

To build the model, a solution was taken with the initial concentrations of sediment-forming ions Ca^{2+} and PO_4^{3-} 50.0 mM and 37.5 mM, respectively, the concentration of the addition of Mg^{2+} ions was 12.5 mM and pH = 6.0–7.0 [8, 9]. Electrostatic interaction was taken into account using the ionic strength of the solution. The main provisions of the thermodynamic model are presented in [10, 11]. A comparison of IS (supersaturation) and Ks/allowed us to establish that in the studied pH region the formation of the following compounds is thermodynamically probable: $CaHPO_4 \cdot 2H_2O$, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, β-$Ca_3(PO_4)_2$, α-$Ca_3(PO_4)_2$, $Ca_{10}(PO_4)_6(OH)_2$ (HA). According to the calculation results, hydroxyapatite (HA) has the highest degree of supersaturation.

In descending order of IS values, the resulting calcium phosphates can be arranged in the following series: $Ca_{10}(PO_4)_6(OH)_2$>$Ca_8H_2(PO_4)_6\cdot 5H_2O$>β-$Ca_3(PO_4)_2$>α-$Ca_3(PO_4)_2$>$CaHPO_4\cdot 2H_2O$. The dependence of supersaturation indices on pH is presented in Fig. 1.

2. Materials and methods

Based on theoretical calculations, an experiment was carried out.

The synthesis is carried out by precipitation from an aqueous solution at a temperature of 313 K. The precipitate is obtained by pouring solutions of calcium chloride ($CaCl_2\cdot 2H_2O$) and disodium sodium phosphate ($Na_2HPO_4\cdot 3H_2O$), with a previously adjusted pH value = 6.5 ± 0.05. The OCP deposition process is carried out in the presence of magnesium ions. After settling the heterogeneous system for 48 hours.

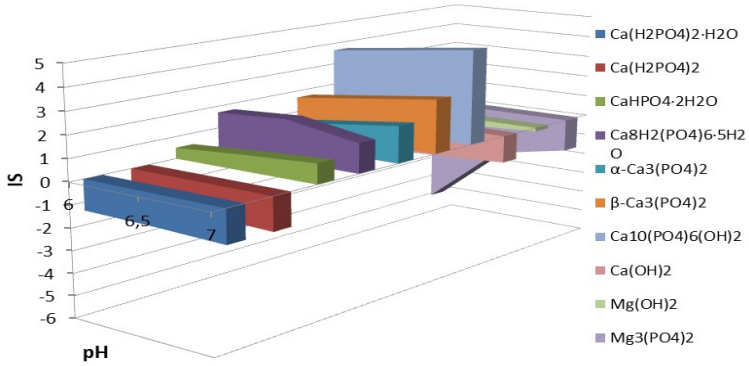

Fig. 1. Graphical dependence of IS of poorly soluble compounds on pH.

Obtaining ceramics. We introduce a plasticizer (paraffin, to get rid of cracks) dissolved in CCl_4 in a ratio of 10% by weight of the powder into the powder, mix the resulting paste and dry it for an hour. Then we sift the paste (sieve size 400 microns), press (P = 200–600 MPa) and anneal at T = 900, 1000, 1100 °C for 3 hours at a heating rate of 5 °C/min (samples are cooled along with the oven). In order to remove the plasticizer, we hold it at 250 °C for 2 hours.

Determination of the concentration of calcium ions during the study of the solubility of synthetic calcium phosphates is carried out by direct potentiometry using an ion-selective electrode.

Orthophosphate ions in the presence of excess molybdate in an acidic environment form phosphomolybdic heteropolyacid (PMH). Using a KFK-2 device, using a red light filter (λ = 690 nm) and cuvettes with a layer thickness of 2 cm. The determination is repeated three times and a calibration graph is constructed using the average optical densities: D = f {$C(PO_4^{3-})$}, the regression equation is calculated .

The phase composition of the obtained samples was studied using X-ray diffraction. (D8 Advance diffractometer, Bruker Company). In order to obtain additional information about the composition of the synthesized samples, the method of infrared spectroscopy was used.IR spectra were obtained on an FSM 2202 spectrophotometer, Infraspec, Russia. The detection limit was 5%.

Measurement of the specific surface of samples using the BET method (SBET-N_2) using this technique was carried out on a Gemini 2380 adsorption device for the adsorption of standard nitrogen gas at 77.4 K at one point of the nitrogen adsorption isotherm in a helium flow (at a relative nitrogen vapor pressure P/P0 = 0.075) for 3 hours. Specific surface measurement range from 0.5 to 999 m^2/g. The limit of permissible relative error in measuring the specific surface area in the multiple measurement mode is no more than 5%.

The sediments obtained during the syntheses are examined by optical microscopy using a binocular microscope of the XSP-104 series. The morphology of the powders was studied using a

MIRA 3 LMH scanning electron microscope (SEM) and a JEOL JSM-6610LV scanning electron microscope (SEM) in low-vacuum mode, the accelerating voltage of the electron beam was 20 keV.

To study the stability of calcium phosphate samples synthesized under various conditions, they are dissolved in a solution of hydrochloric acid, NaCl 0.9%, and acetate buffer.

Study of the bioactivity of materials, i.e. the ability to form a calcium phosphate layer on the surface in model solutions of biological fluids was carried out according to the method of [9]. Tablets produced by pressing weighing 0.2 g and 13 mm in diameter were placed in an SBF solution, the mineral composition of which was identical to human blood plasma.

When determining porosity, a sample of mass m0 is placed in a graduated cylinder containing a known volume of ethanol and held for 30 minutes. The sample is then removed, weighed and the remaining volume of alcohol is measured.

To calculate the geometric and relative density of the samples after pressing and firing, their mass and linear dimensions were measured.

The method for determining bending strength is carried out on an Instron installation using the three-point bending method with a rigid loading system at strain rates from 0.02 to 20 mm/min.

3. Results and its discussion

Using X-ray diffraction, it was established that as a result of synthesis, a mixture of calcium phosphates (octacalcium phosphate, brushite and HA phases) is formed, the main intense lines of which correspond to angles 2Θ (Fig. 2): octacalcium phosphate – 11.4, 22.8; HA – 25.9, 29.6, 31.8; brushite – 20.4, 47.3, 35.2, with crystallite sizes determined by the Selyakov-Scherrer method, presented in Table 1.

The IR spectrum of the resulting sample contains bands characteristic of phosphate groups (Fig. 3): asymmetric stretching vibrations at 1024 and 1154 cm^{-1}, characteristic of O–P–O bonds, as well as peaks at 530, 574 cm^{-1}, corresponding to P vibrations = O in PO_4^{3-}. This indicates that, under these conditions, octacalcium phosphate and brushite should be considered metastable phases with respect to HA. Consequently, the crystallization processes of these calcium phosphates will be characterized by lower ΔG values at a lower level of system supersaturation (SI).

Using the BET method, it was found that the specific surface area of the powder material is 23 m^2/g, which, in comparison with most ceramic materials based on PC, which have a specific surface area of no more than 1 m^2/g, indicates a high potential for practical application, since it has now been established that that increasing the specific surface area of the implantation material has a positive effect on the kinetics of bone formation and, therefore, improves the bioactivity of the material.

From the results of optical microscopy (Fig. 4) it is clear that the resulting crystals have a “rosette” morphology consisting of plate-like crystals characteristic of octacalcium phosphate, crystals of a monoclinic structure characteristic of brushite and crystals of a hexagonal system characteristic of HA (unit cell formula $Ca_5(PO_4)_3(OH)$). $PO_4)_3(OH)$).

At the next stage of the work, a study was carried out on the possibility of using a mixture of calcium phosphates as a basis for producing dense ceramics. As a result, dense ceramic samples were obtained using uniaxial pressing and sintering at temperatures of 900, 1000 and 1100 °C for 3 hours (Fig. 5).

When determining the physical and mechanical properties of the samples, it was established (Table 2) that with increasing temperature the density of the resulting ceramics increases, which is due to a decrease in the number of large pores, which opens up the possibility of using these materials as implants that bear significant mechanical loads during implantation (for example, replacement cortical bone of the jaw).

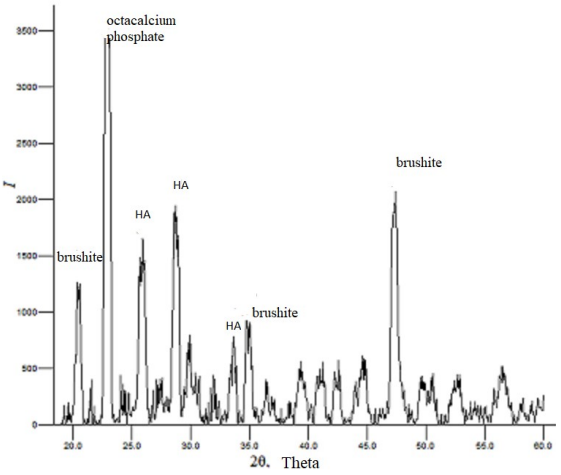

Fig. 2. Diffraction pattern of a mixture of calcium phosphates.

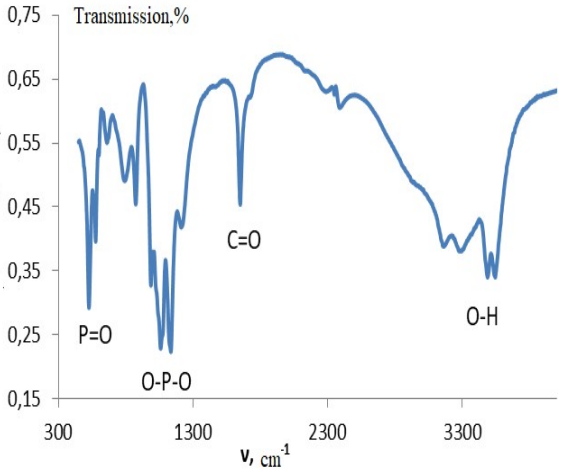

Fig. 3. IR spectrum of a mixture of calcium phosphates.

Table 1. Sizes of crystallites of the resulting phases.

Phase	Octacalcium phosphate	Brushite	Hydroxyapatite
Sizes of crystallites, nm	2.96	2.45	2.15

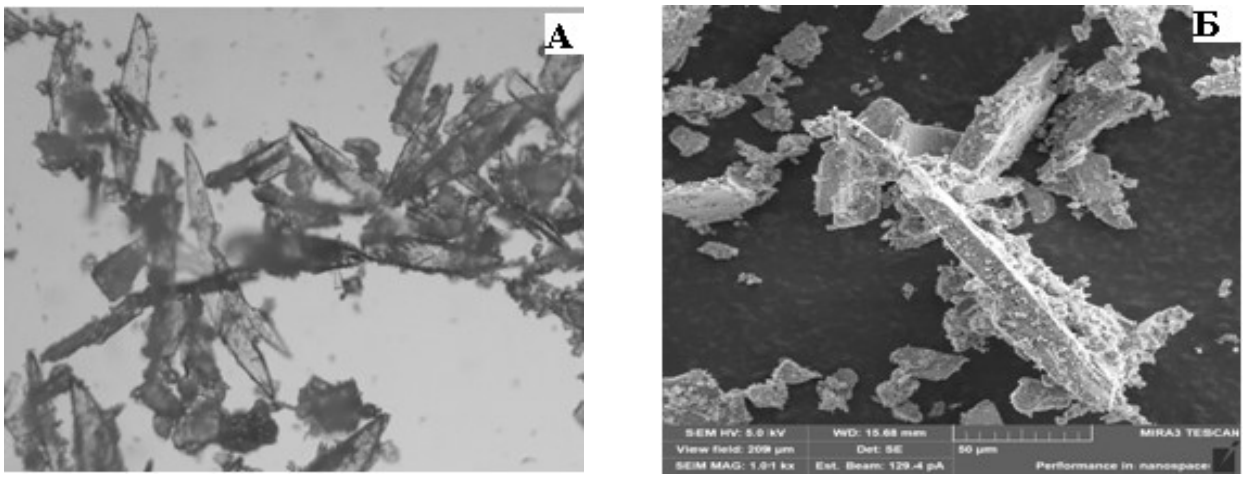

Fig. 4. Type of particles of a mixture of synthesized calcium phosphates: a) magnified ×120, b) a photograph taken on an electron microscope.

Table 2. Physical and mechanical properties of the obtained samples.

Processing temperature, ˚C	Density, %	Specific surface area, m^2/g	Porosity , %
900	76	5.5	6.8
1000	81	4.4	4.5
1100	88	3.8	1.9

It has been shown that the sample obtained at a sintering temperature of 1100 °C has the greatest strength, which is due to the initial dense packing of the sample and the high sintering temperature (Fig. 6).

At the next stage of the study, the samples were dissolved in a 0.1 M HCl solution, an acetate buffer solution, and a 0.9% NaCl solution. From the data obtained, it follows that the resorbability of the resulting ceramics decreases with increasing processing temperature, which is associated with a decrease in the specific surface area of the samples, while the highest initial dissolution rate is observed in the acidic medium of a 0.1 N hydrochloric acid solution and decreases as the pH of the dissolution medium approaches neutral values (Fig. 7).

Fig. 5. Photograph of ceramic material obtained at a sintering temperature of 900 °C, magnified ×10.

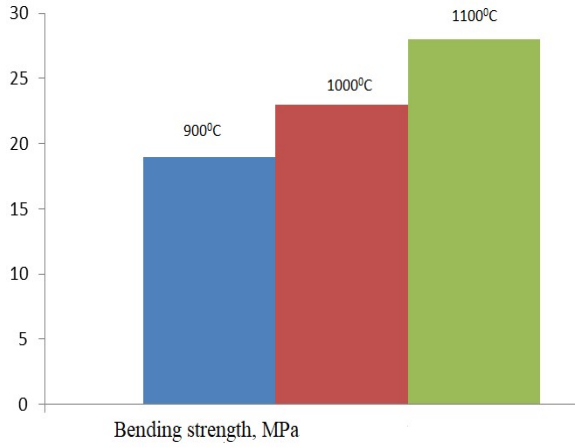

Fig. 6. Dependence of bending strength on the firing temperature of samples.

To assess the bioactivity of the synthesized materials, tablets made from them by pressing were placed in an SBF solution, which is identical in mineral composition to human blood plasma, and Tris-buffered physiological solution. The tablets were kept in solutions at 37 °C, the rate of dissolution/formation of a calcium phosphate layer on the surface was assessed by the increase/decrease in the concentration of calcium ions on days 1, 4, 7, 14, 21 and 28 of the experiment.

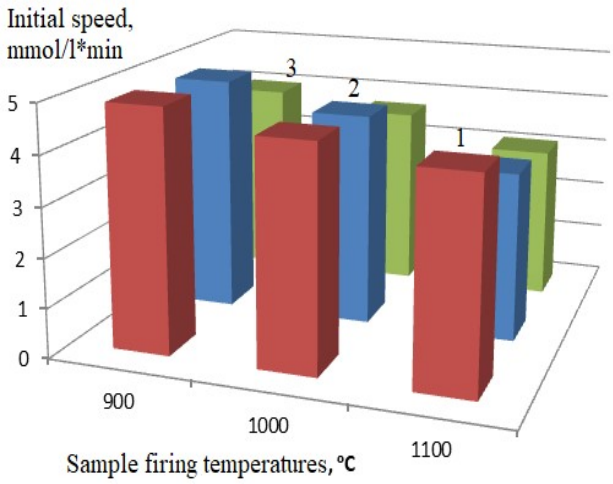

Fig. 7. Initial dissolution rate of ceramic materials in various media (1 – 0.01 mol hydrochloric acid; 2 – acetate buffer solution; 3 – 0.9% sodium chloride solution).

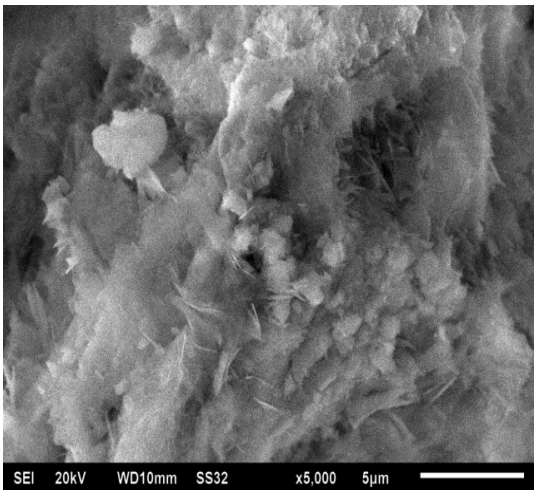

Fig. 8. Photo of the surface of samples after 28 days in SBF: mixture of calcium phosphates.

Analysis of the kinetic curves shows that adsorption of Ca^{2+} ions from the SBF solution occurs on the surface of the ceramic material throughout the entire time the material is kept in the solution. The most likely mechanism for the formation of a calcium phosphate layer on the surface of materials is the mechanism proposed in [221]. Hydroxyl groups on the surface of the tablets, due to a partial negative charge, attract calcium ions from the solution, which, when adsorbed, give the surface a partial positive charge. Next, phosphate ions join, resulting in the formation of a film of poorly soluble calcium phosphates on the surface. Thus, gradual filling of the surface leads to a decrease in the rate of adsorption of calcium ions on the surface. The SEM results indicate the formation of calcium phosphate layers on the surface of the tablets (Fig. 8).

4. Conclusion

The thermodynamic functions of the formation of mineral phases were calculated at concentrations of sediment-forming ions Ca^{2+} and PO_4^{3-} 50.0 mM and 37.5 mM, the addition of Mg^{2+} ions 12.5 mM, and pH = 6.5. It has been established that under these conditions the formation of a mixture of calcium phosphates is possible, with HA being the most stable. Based on the calculated data, a synthesis was carried out, as a result of which it was established that the resulting precipitates were represented by the phases of HA, octacalcium phosphate and brushite. It was established that in the SBF solution a calcium phosphate layer is formed on the surface of the samples, which indicates the bioactivity of the samples.

Acknowledgement

The work was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation (topic No. 075-03-2023).

5. References

[1] V.S. Komlev, S.M. Barinov, I.I. Bozo, et al., Bioceramics composed of octacalcium phosphate demonstrate enhanced biological behavior, *ACS Appl. Mater. Interfaces*, vol. **6**(19), 16610, 2014; doi: 10.1021/am502583

[2] C. Combes, S. Cazalbou, C. Rey, Apatite biominerals, *Minerals,* vol. **6**, 34, 2016; doi.org/10.3390/min6020034

[3] H. Morgan, R.M. Wilson, J.C.Elliott, et al., Preparation and characterisation of monoclinic hydroxyapatite and its precipitated carbonate apatite intermediate, *Biomaterials*, vol. **21**, 617, 2000; doi: 10.1016/S0142-9612(99)00225-2

[4] M. Mathew, S. Takagi, Structures of biological minerals in dental research, *J. Res Nat Inst Stand and Techn*, vol. **106**. № 6, 1035, 2001; doi: 10.6028/jres.106.054

[5] E. Noam, N. Metoki, Calcium Phosphate Bioceramics: A Review of Their History, Structure, Properties, Coating Technologies and Biomedical Application, *Materials*, vol. **10**, № 4, 334, 2017; DOI: 10.3390/ma10040334

[6] V. Tyagi, A.H. Harris, N.J. Giori, Survival of hydroxyapatite-coated versus non-hydroxyapatite-coated total hip arthroplasty implants in a veteran population, *J. Arthroplast*, vol. **37**, 1143, 2022; doi: 10.1016/j.arth.2022.02.067

[7] O. A. Golovanova, A. A. Tsyganova, E. S. Chikanova, Targeted synthesis of octacalcium phosphate and a study of its properties, *Glass physics and chemistry,* vol. **42**, № 6, 615, 2016; doi: 10.1134/S1087659616060043

[8] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity?, *Biomaterials*, № 27, 2907, 2006; doi: 10.1016/j.biomaterials.2006.01.017

[9] A.A. Tsyganova, O.A. Golovanova, Role of Mg^{2+}, Sr^{2+}, and F^- ions in octacalcium phosphate crystallization, *Inorganic materials,* vol. **53**, № 12, 1292, 2017; doi: 10.1134/S0020168517120184

[10] O.A. Golovanova, Thermodynamic modeling of poorly soluble compounds formation in biological fluid, *J. of thermal analysis and calorimetry*, vol. **133**, № 2, 1219, 2018; doi: 10.1007/s10973-018-7369-6.

[11] A. Solodyankina, A. Nikolaev, O. Frank-Kamenetskaya, O. Golovanova, Synthesis and characterization of nanocrystalline apatites from solution modeling human blood, *J. of Molecular Structure*, vol. **1119**, 484, 2016; doi: 10.1016/j.molstruc.2016.04.080