Synthesis and biochemical characteristics of bioactive calcium-phosphate materials obtained from alcohol solutions

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Abstract. Sol-gel method synthesized 2 series of powders with different TiO₂ content in the system. The percentage of elements in terms of the oxide system SiO₂–P₂O₅–CaO–TiO₂ is: composition 1: 52–14–24–10, composition 2: 52–17–29–2 wt.%, respectively. Solutions of composition 1 are stable up to 11 days. Solutions of composition 2 are stable up to 2 days. The physicochemical properties of calcium phosphate materials were studied using the methods of IR spectroscopy, thermal analysis and elemental analysis. The biochemical activity of the tablets according to the Kokubo method showed a significant absorption of Ca²⁺ and Mg²⁺ ions within 7 days.

1. Introduction
Creation of new bioactive calcium-phosphate materials for regeneration of damaged bone tissue structure is one of the most important tasks of modern surgery, orthopedics, dentistry and traumatology [1-3]. At violation of bone tissue integrity it is important to find and provide optimal conditions for regenerative processes, which is recognized as one of the promising and priority directions of scientific research in the XXI century. The presence of Ca and P is a prerequisite for the biomaterial, since they are the main components of bone tissue. The introduction of various additives into the biomaterial composition leads to the improvement of its properties. One of these is titanium.

Calcium-phosphate materials are bioactive substances that are intended to bind to biological systems in order to improve the effectiveness of treatment, formation or replacement of any organ tissue in the performance of certain functions of the body. Biological activity refers to the effect or influence that materials have on cells by controlling or activating them to a specific reaction and behaviour. [1-4]

Titanium dioxide is considered harmless in contact with human tissue, but it should not exceed 10 wt% [5-6]. The main purpose for such material in natural conditions is to combine osteogenic activity, in reconstruction of bone tissue with the possibility of gradual resorption [7-10].

A promising method for obtaining calcium-phosphate materials is sol-gel technology, as there is a possibility of obtaining materials with different properties: uniform at the molecular level distribution of components in the initial solution, the possibility of obtaining uniform materials of complex composition on the basis of solid solutions.

The purpose of this paper is to study the effect of TiO₂ additive on the biochemical characteristics of bioactive calcium-phosphate materials obtained from alcohol solutions based on tetrabutoxytitanium,
tetraethoxylene, orthophosphoric acid and calcium nitrate with different content of components in the system.

2. Experimental

To study the physical and chemical properties of calcium-phosphate materials, samples of powders for the SiO$_2$-P$_2$O$_5$-CaO-TiO$_2$ system of two compositions with the percentage of elements in conversion to the oxide system 52–14–24–10, mas % (cipher "Ti_10") and 52–17–29–2 mas % (cipher "Ti_2") were obtained. Calcium nitrate (Russia), orthophosphoric acid (Russia), tetrobutoxytitanium (Acros, USA), tetroetetoxyselan (Russia), and butanol-1 (Russia), have been used to prepare solutions. The kinematic viscosity in Ostwald's capillary viscometer was used to determine the "life" time of the solution after the expiry time of the aliquot liquid. The Ostwald viscometer is a U-shaped glass tube, in one knee of which the capillary is soldered. To study the surface morphology of bioactive calcium-phosphate materials obtained at different annealing temperatures was used HITACHI TM-3000, equipped with an attachment for energy dispersion microanalysis Quantax-70. Images of synthesized disperse materials with magnification from 500 to 3000 times in vacuum were obtained on microscope. The Agilent Cary 630 FTIR IR spectrometer was used to obtain infrared spectra. Surface characteristics are important when studying the properties of biomaterials, because the adhesion of proteins, cells and bioreabsorability of materials when implanted in the body depends on them. Acid-base properties of solid body surface, which are formed in the course of its synthesis and reflect the peculiarities of its structure and reactivity, are studied by pH-metry method. The method is based on the change of pH value of the water suspension in time. Measurements were taken at multi-test device with ESC mark electrode - 10601/7. Error of pH determination is 0.05.

For investigation of physical and chemical properties of powders the methods of infrared spectroscopy, thermal analysis, RFA and elemental analysis were used [11-13]. Thermogravimetric analysis was carried out on the thermal analyzer NETSCH Jupiter STA 449 F1 in the temperature range of 25 - 1000 °C with a heating rate of 30 °/min in the atmosphere, the control of released products of decomposition in the gas phase was carried out with a mass spectrometer NETSCH QMS 403 D Aëolos. The kinetic parameters of the samples were determined using the Metzger-Horowitz approximation method [14]. Bioactive properties of the obtained powder were considered by Kokubo method [15]: tablets made by pressing with 5 mm diameter were studied in SBF solution at 37 °C during 7 days with daily updating of the solution, which by its mineral composition and ion concentration is identical to human plasma. Ca$^{2+}$ and Mg$^{2+}$ ions were measured using the trilonometric titration method [15].

3. Results and discussion

The increase in viscosity of solutions in time can be due to the processes of hydrolysis and polycondensation, which are accompanied, as well as due to electrostatic interaction between dissolved ions and solvent molecules. The viscosity of freshly prepared solutions was in the range of 1.01 - 1.03 mm$^2$/s. It was found that on the eleventh day of maturation the solution for the first composition reached the value of 1.6 mm$^2$/s. After 12 days of ripening the solution becomes unsuitable for obtaining materials. In the second solution the formation of solid phase is much faster, as the processes of hydrolysis and polycondensation due to the presence of water in a larger volume of added orthophosphoric acid accelerates the processes of hydrolysis and polycondensation, so the solution is suitable for obtaining materials on the second day.

Thermal analysis data (Fig. 1) showed that the first stage in the temperature range up to 300 °C is the removal of physically and chemically bound water. Further in the temperature range of 300-500 °C is the combustion of alcohol and products of thermal-oxidative degradation of ethoxygroups and the mass of the sample changes slightly. At the last stage, at temperatures above 600°C, crystallization and polymorphic transformations of oxides in the system take place. Kinetic parameters of processes are calculated by Metzger-Horowitz method. Relatively low activation energy values from 33 to 57 kJ/mol indicate the removal of physically adsorbed water and solvent. Such a low Ea value confirms the destruction of intermolecular bonds (Van der Waals (Ea ~ 10-20 kJ/mol [14]) and/or hydrogen bonds (Ea < 40 kJ/mol [14]) with hydrate water molecules. Activation energy of subsequent stages is from 149
to 224 kJ/mol, which is typical for the destruction of chemical bonds in compounds. The loss of mass was 11 g.

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IR spectroscopy data confirm the presence of adsorbed water (1638-1640 cm\(^{-1}\)) and organic solvent (3431-3438 cm\(^{-1}\)) in formed samples (60 °C). Chemical bonds characterized by valence oscillations of P=O, -PO\(_4^3-\) groups (933-943 and 1146 cm\(^{-1}\)), chains of siloxanes \(\delta\) (Si-O-Si) (1039-1042 cm\(^{-1}\)) and valence oscillations of bonds of calcium with oxygen (413-430 cm\(^{-1}\)) are fixed.

Fluctuations in the bond between titanium and oxygen at 601 cm\(^{-1}\) are fixed only at 800 °C (Fig. 2). The nature of the IR spectra illustrates the process of successive structuring of the material as a result of heat treatment at temperatures between 60 and 800 °C.

Based on the results of X-ray it was found that samples at 600 °C are amorphous, so it was difficult to determine the phase composition of products using this method. With RFA it was found that the formation of crystalline structures occurs at 800 °C.

The phases of the following composition for Ti_10 are formed: Ca\(_5\)(PO\(_4\))\(_2\),\(_{8.5}\)(SiO\(_4\))\(_{0.15}\)O - calcium silicate, Ca\(_{10}\)(PO\(_4\))\(_{5.5}\)(HPO\(_4\))\(_{0.15}\)(SiO\(_4\))\(_{0.33}\)(OH)\(_{1.66}\)O\(_{0.19}\) - hydroxyapatite, CaSiO\(_3\), anatase, Ca\(_3\)(PO\(_4\))\(_2\); for Ti_2: Ca\(_{10}\)(PO\(_4\))\(_{5.22}\)(HPO\(_4\))\(_{0.15}\)(SiO\(_4\))\(_{0.33}\)(OH)\(_{1.66}\)O\(_{0.19}\) - hydroxyapatite, CaSiO\(_3\).

According to the data of thermal analysis, infrared spectroscopy, X-ray conditions of material formation are established, temperature processing is necessary at 800 °C.
Microphotographs in Fig. 3 show that the surface structure of powders is porous and granular, which is favorable for practical application [12].

![Microphotographs in Fig. 3](image)

**Figure 3.** Microphotographs and micro-X-ray analysis of the powder surface of the system for the SiO$_2$–P$_2$O$_5$–CaO–TiO$_2$ system at 800 °C, where:

$a$ – Ti$_{10}$ composition; $b$ – Ti$_{2}$ composition

Changes in the pH of the suspension of samples dried at 800 °C are due to the dissolution of calcium ions, because they are still in the composition of dissolved nitrate. On the surface of the samples there are various acid and main centers, such as Si$^+$ (Lewis acid center), –OH group (Brensted main centers), Si–O–Si (Lewis main centers). The kinetic curves of changes in acidity of the suspension of the samples are shown in Fig. 4.

![Kinetic curves in Fig. 4](image)

**Figure 4.** Kinetic curves of changes in the acidity of the suspension of samples dried at 800 °C

During the first 30 sec the hydroxylhydrate cover is desorbed from the air, and therefore the pH of the suspension sharply increases. After 8 minutes the pH value stabilizes in the interval from 8 to 10. Such stabilization indicates that the specimen surface represents the base. Since no Si–OH bonds have been identified in the samples calcined at 800 °C according to IR spectroscopy, the surface represents the main Lewis center. While in the solution, the samples of the main centers interact with protons of
water molecules. Less rigidly bound hydroxo groups of water pass into the solution from the material surface, so the basicity of the medium increases sharply. The scheme of such interaction is shown in Fig 5. After the interaction of the surface with the aqueous solution surface becomes the basis of Brennsted.

![Figure 5. Scheme of the mechanism of interaction of the main centers with protons of water molecules](image)

The study of bioactive properties of the obtained samples (Fig. 6) showed that on the first day there is a rapid deposition of ions on the material surface.

![Figure 6. Time dependency of Ca$^{2+}$ and Mg$^{2+}$ ion concentration for Ti_10 and Ti_2](image)

This is due to the fact that there is a rapid exchange of ions of alkali and alkaline earth metals due to the formation of hydroxyl groups on the surface of the material [16]. Then Ca$^{2+}$, PO$_4^{3-}$ ions migrate, forming a calcium-phosphate layer on the surface of the material due to the accumulation of ions and the speed slows down over time [17]. It has been established that the samples under study have high biological activity. The content of titanium oxide in the system does not affect the biological properties of materials and both compositions are suitable for further study of biological activity.

4. Conclusions
The sol-gel method produced solutions based on tetrabutoxytitanium, tetraethoxysilane, orthophosphoric acid and calcium nitrate with different content of components in the system. It was found that solution Ti_10 is stable for 12 days and suitable for obtaining materials up to 11 days. Solution Ti_2 is stable for two days. According to the data of thermal analysis and IR-spectroscopy the conditions of material formation are established. Up to 300 °C, physically and chemically bound water is removed, at 300-500 °C - oxidation of alcohol and products of thermal degradation of ethoxygroups, at 500-800 °C transition of amorphous modifications of compounds to crystalline.

It was found that the formation of crystalline structures occurs at 800 °C. The phases of the following composition for Ti_10 are formed: calcium silicate, hydroxyapatite, CaSiO$_3$, anatase, Ca$_3$(PO$_4$)$_2$; for Ti_2: hydroxyapatite, CaSiO$_3$. 


At research of behaviour of materials in artificial conditions (in solution SBF on method Kokubo) it is established, that investigated samples possess high biological activity. The content of titanium oxide in the system does not affect the biological properties of materials and both compositions are suitable for further study of biological activity.

Acknowledgments
This study was supported by the Tomsk State University competitiveness improvement programme.

References
[1] Barinov S M 2010 Calcium phosphate-based ceramic and composite materials for medicine Russ. Chem. Rev. 79 13
[2] Bagherpour I, Naghib S M, Yaghtin A H 2018 Synthesis and characterisation of nanostructured hardystonite coating on stainless steel for biomedical application IET nanobiotechnology 12 895
[3] Li X, Wang M, Deng Y, Xiao Y, Zhang X 2017 Fabrication and Properties of Ca-P Bioceramic Spherical Granules with Interconnected Porous Structure ACS Biomaterials Science and Engineering 3 1557
[4] Bjornoy S H, Bassett D C, Ucar S, Andreassen J-P, Sikorski P 2016 A correlative spatiotemporal microscale study of calcium phosphate formation and transformation within an alginate hydrogel matrix Acta Biomaterialia 44 254
[5] Li H, Chang J 2013 Stimulation of proangiogenesis by calcium silicate bioactive ceramic Acta Biomaterialia 9 5379
[6] Oliveira W F, Arruda I R S, Silva G M M, Machado G, Coelho L C, Correia M T S 2017 Functionalization of titanium dioxide nanotubes with biomolecules for biomedical Applications Mater. Sci. Eng. C. 81 597
[7] Kalantari E, Naghib S M, Naimi-Jamal M, Mozafari M 2017 Green solvent-based sol–gel synthesis of monticellite nanoparticles: a rapid and efficient approach J. Sol-Gel Sci. Technol., 84 87
[8] Kukueva E V, Putlyaev V I, Tikhonov A A and Safronova T V 2017 Octacalcium phosphate as a precursor for the fabrication of composite bioceramics Inorg. Mater. 53,2 212
[9] Xiaoyan P. 2004 Phase transformation of nanocrystalline anatase powders induced by mechanical activation J. Am. Ceram. Soc. American Ceramics Society 6 1164
[10] Liu G 2012 A red anatase TiO₂ photocatalyst for solar energy conversion Energy Environ. Sci. 11 9603
[11] Ochiai T, Photochem J 2012 Photoelectrochemical properties of TiO₂ photocatalyst and its applications for environmental purification Photochem. Rev. 4 247
[12] Henderson M A 2011 A surface science perspective on TiO₂ photocatalysis Surf. Sci. Rep. 6 185
[13] Yin Z F 2013 Recent progress in biomedical applications of titanium dioxide Phys. Chem. Chem. Phys 14 4844
[14] Joost U 2015 Photocatalytic antibacterial activity of nano-TiO₂ (anatase)-based thin films: Effects on Escherichia coli cells and fatty acids Photobiol. B Biol. 142 178
[15] Yamaguchi S, Nath S, Matsushita T, Kokubo T 2014 Controlled release of strontium ions from a bioactive Ti metal with a Ca-enriched
[16] Kaur M, Singh K. 2019 Review on titanium and titanium based alloys as biomaterials for orthopaedic applications Mater. Sci. Eng. 844
[17] Bellucci D, Salvatori R, Anesi A, Chiariini L., Cannillo V. 2019 SBF assays, direct and indirect cell culture tests to evaluate the biological performance of bioglasses and bioglass-based composite Mater. Sci. Eng. 757