# In-Vitro Investigation of Magnetron-Sputtered Coatings Based on Silicon-Substituted Hydroxyapatite

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**Abstract**—Silicon-containing calcium phosphate (Si-CaP) coatings on titanium and austenite steel substrates have been prepared by method of high-frequency magnetron sputtering. The powder of silicon-containing hydroxyapatite $Ca_{10}(PO_4)_{6-x}(SiO_4)_x(HO)_{2-x}$ (Si-HA), where $x = 0.5$ obtained using a mechanochemical technique, was used as a target material. The obtained coatings were X-ray amorphous; the elemental composition of the coatings depended on the composition of the target to be sputtered. The coatings were heated in air for 3 hours to the temperature 700°C with the aim of changing their structure. The bioactivity of the coatings was studied using in-vitro tests. The solution of the simulated body fluid (SBF) oversaturated with respect to HA was used as a model medium. The phase elemental composition and morphology of the deposited and annealed Si-CaP coatings before and after submersion into the solution were controlled using the methods of X-ray phase analysis (XPA), energy-dispersive X-ray analysis (EDXA), and scanning electron microscopy (SEM). According to the XFA and IR-spectroscopy data, heat treatment in the air yields the formation of an apatite-like phase in the coating. Thermostating of "metal + coating" specimens in the solution of an artificial intertissue fluid revealed that all obtained coatings were biologically active, and a calcium phosphate layer was formed on the coating surface during mineralization. The annealed coatings show a higher chemical stability under physiological conditions as compared to amorphous coatings.

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## INTRODUCTION

Isomorphic types of apatite remain the most widely-used biocompatible material both in the form of a stoichiometric pure hydroxyapatite (HA) and its various substitutions [1]. The HA lattice makes it possible to accomplish a large variety of substitutions in two cation positions and in two positions of the anion sublattice [2]. The authors of [3] synthesized apatite with different substitutions (potassium, zinc, copper, barium, magnesium) in the cation sublattice using a mechanochemical method. Having investigated the functional reply of the marrow cells to the synthesized material extracts, it was demonstrated that depending on the addition of microelements they can exert a multidirected regulatory effect on the marrow and the bone tissue.

The HA with phosphate partially substituted for the $SiO_4^{4-}$-group has recently aroused particular interest [4]. It was established that silicon belongs to a number of vitally important elements for higher animals and humans [5]. The participation of silicon in the organization and vital activity of connective and bone tissues has been revealed. Biologically active silicates in the form of organosilicon compounds are not toxic as macroparticles and can form strong bonds in bulk on interface surfaces. By the example of glass ceramics, it is known that silicon in the bulk bulk material and on its surface intensifies the joining of an implant with a bone (osteointegration) [6]. Coatings based on silicon-substituted hydroxyapatite (Si-Ha) are the subject of current research. The possibility of using silicon-substituted apatite as a coating for medical implants has been poorly studied, though all investigations have been performed abroad. The main technological methods for production of biocompatible coatings are plasma sputtering [7, 8], laser ablation [9], methods based on the crystallization of coatings from different solutions [10, 11], and high-frequency (HF) magnetron sputtering [12–14]. In some methods the parent material undergoes changes during deposition and the coating obtained represents itself a new multiphase system. It is important for the applied method to preserve the chemical composition of the parent material when the coating is deposited on the implant. HF-magnetron sputtering [15] meets this requirement. This study implements a new approach of synthesizing a Si-HA powder and using it as a target for HF-magnetron sputtering, which allows coatings with uniform compositions to be obtained.

Bioactivity is a complex characteristic of materials compatible with an organism that takes into account (in addition to the effect on the biological processes of growing and the differentiation of cells) such parame-

Comparison of the ion composition of human blood plasma and SBF (mmol/l)

| @Ион \ @Среда | $Na^+$ | $K^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $Cl^{2-}$ | $HPO_4^{2-}$ | $HCO_3^-$ | $SO_4^{2--}$ |
|---|---|---|---|---|---|---|---|---|
| SBF | 142.0 | 5.0 | 1.5 | 2.5 | 147.8 | 4.2 | 1.0 | 0.5 |
| Plasma | 142.0 | 5.0 | 1.5 | 2.5 | 103 | 27.0 | 1.0 | 0.5 |

ters as the rate of the material dissolving in a subacid medium created by certain groups of cells and the rate of HA deposition from intertissue fluid of the organism on the material surface [16].

The studies [17—19] used a technique for evaluating the bioactivity of the materials not requiring much time and not as expensive as in vivo testing. When performing relatively simple in vitro experiments simulating real processes in the human body, this technique makes it possible to evaluate the bioactivity of the materials at early stages. For example, using a simulated body fluid (SBF) as a model medium, the powders based on calcium phosphate were arranged according to the degree of their bioactivity. The maximum "activity" in the solution of an artificial intertissue fluid was demonstrated by Si-HA.

This work studies the behavior of coatings based on Si-HA in an SBF-solution identical to blood plasma in its mineral composition and ion concentration produced using the of HF-magnetron sputtering method.

## EXPERIMENTAL

The target for HF-magnetron sputtering (220 mm in diameter and 10 mm in thickness) was prepared based on ceramic technology: compacting of the powder at a pressure of 70 MPa followed by annealing of the obtained blanks at the temperature 1100°C for 1 h in air. The $(Ca_{10}(PO_4)_{6-x}(SiO_4)_x(HO)_{2-x}$ (Si-HA) powder, where $x = 0.5$ for target preparation, was obtained by mechanochemical methods [20]. Commercially pure titanium Bt1-0, KBr (for investigating molecular bonds in the coating using the infrared (IR) spectroscopy method) and austenite steel 12X18H10T were used as substrates for sputtering.

The coatings were sputtered using the modified 08PKhO-100T-005 industrial setup with a magnetron source (5.28 MHz). The parameters for the coating formation were as follows: the working pressure was 0.1 Pa (the limiting pressure in the vacuum chamber was $10^{-4}$ Pa), the distance between the target and the substrate was 40 mm, the sputtering time was 3 hours, the working gas was argon, and the power of the HF generator was 290 W. The structure of the coatings was varied by heating in air for 3 h up to a temperature of 700°C.

The surface morphology and the elemental composition of silicon-containing calcium phosphate (Si-CaP) coatings were investigated using the ESEM Quanta 400 FEG scanning electron microscope manufactured by FEI with an imbedded EDX-analyzer (EDS analysis system Genesis 4000, S-UTW-Si(Li)detector) operating under high vacuum conditions ($10^{-5}$ Pa). In addition, using energy-dispersive X-ray analysis (EDXA), a qualitative analysis of the element distribution in the coating was performed as a result of the surface scanning of the specimen. Prior to the analysis, the specimens were coated with gold and palladium. The phase composition of the coating was determined using X-ray phase analysis (XFA) on a Siemens Diffraktometer D500 (Cu$K_\alpha$ radiation with $\lambda = 1.5406$ Å at 40 kV and 20 mA). The X-ray photographs were interpreted using the International Center for Diffraction Data (ICDD) database: the number of the card for synthetic HA was 09-432; for commercially pure titanium, 44-1294. The chemical bonds of phosphate ($PO_4$) and substituting ($SiO_4$) groups were revealed using IR spectroscopy. The optical absorption spectra were recorded by the Thermo Nicolet 5700 facility in the range 400—2000 cm$^{-1}$.

The bioactivity of the coatings was studied using in vitro tests. The SBF solution, oversaturated with respect to HA, which represented an artificial nonorganic part of human blood plasma (Table 1), was used as a model medium. The SBF solution was prepared in accordance with [21] by dissolving NaCl, KCl, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 6H_2O$, $Na_2SO_4 \cdot 10H_2O$, $NaHCO_3$, $K_2HPO_4 \cdot 3H_2O$ in distilled water. The value of the buffer pH was reduced to 7.4 (±0.1) by addition of sodium hydroxide solution or HCl.

The investigations were carried out in stationary conditions simulating a zone of aseptic inflammation inevitably occurring after implantation of any item: an acid medium, the absence of a directed fluid motion (tissue edema). The annealed specimens were placed in the SBF-solution at 37°C for 1—7 days; unannealed specimens, for 1—28 days. At some specified time intervals the specimen was taken out in order to analyze the morphology and the elemental composition of the coating; the concentration of calcium in the solution was determined using atomic-absorption spectroscopy (AAS, an AA-spectrometer of the M-series with a GF 95 graphite furnace).

## DISCUSSION OF RESULTS

According to the SEM images (Fig. 1), the surface of the Si-CaP coating formed using HF-magnetron sputtering is homogeneous and dense and does not contain visible flaws, cracks, or chips over the entire
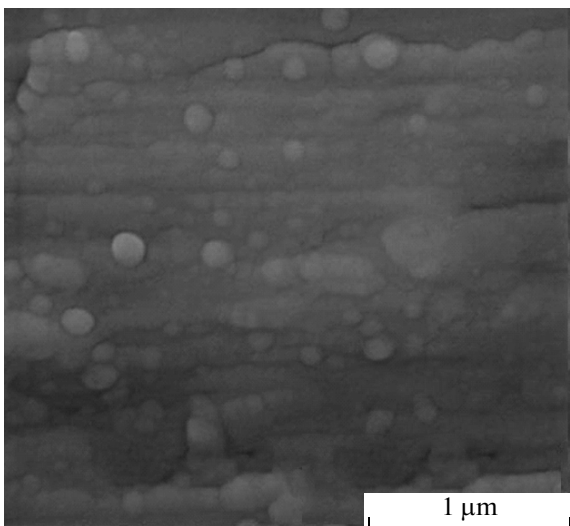
**Fig. 1.** SEM image of the coating surface formed by HF-magnetron sputtering.

area. The key elements of the surface profile are the islands shaped as "domelike" grains. The structure of the coating and the dynamics of their growth are discussed in [22, 23]. The maps of the element distribution ("mapping") along the surface of the Si-CaP coatings formed on a titanium substrate, obtained using EDXA, showed that all the elements present in the coating, namely calcium, phosphorous, silicon, and oxygen, are uniformly distributed. In [24], the Si-CaP coatings were formed using two separate targets for sputtering, made of silicon and stoichiometric HA. However, the XPA studies of the coating revealed Si and CaO phases in addition to the HA phase. According to the data of [8], Si-CaP coatings obtained using HF-magnetron sputtering of the target produced by simple agitation of Si and HA have a nonuniform dis-

tribution of the elements in the coating. In our case, the nature of the element distribution over the specimen surface demonstrates the advantage of this approach to the formation of a coating using the target made of synthetic Si-HA.

The results of the XPA of the formed unannealed coatings showed the absence of crystalline phases corresponding to calcium phosphates (Fig. 2a). Only the presence of diffraction maxima of the substrate material (Ti) was revealed. Thus, the coatings deposited by the modes used are X-ray amorphous, which corresponds fully to the data of [17, 24]. The amorphous structure of the obtained coatings was confirmed by the results of the IR-spectroscopy. Earlier [25] we showed that distinct from the parent powder, the target material represented a mixture of two phases, namely HA stabilized with silicon, and tribasic calcium phosphate (TCP); in this case, the phase composition of Si-HA does not differ from that of pure HA [26]. It suggests that the coating can also contain Si-HA and TCP.

To change the structure of the coatings, the specimens were heated in air up to a temperature of 700°C. Figure 2 shows the X-ray photographs and the IR-spectra of the annealed coatings. The X-ray photographs of the coating after heating reveal reflexes corresponding to HA and Ti. The reflexes of other calcium phosphates (tribasic calcium phosphate, tetracalcium phosphate, etc.), crystalline CaO, and silicon-containing crystalline compounds are absent on the X-ray photographs. The IR-spectra of annealed coatings (Fig. 2b) revealed the absorption bands attributed to oscillations of the P-O bonds in the range of deformation vibrations (at 603 and 560 cm$^{-1}$), and stretching vibrations of these bonds at 1057 and 1028 cm$^{-1}$. The absorption band of the coatings at 945 cm$^{-1}$ (marked with an arrow in Fig. 2b) is related to the stretching vibrations of the Si-O bonds in the apatite
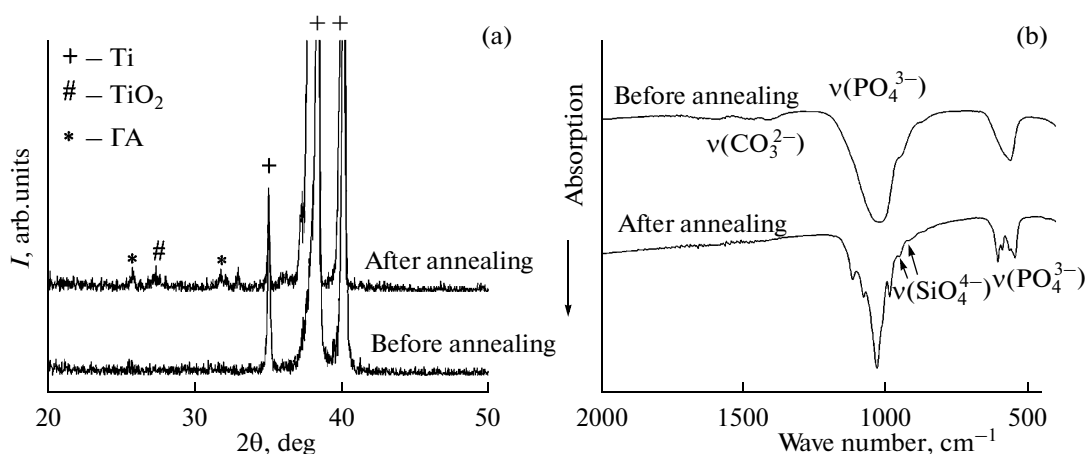


**Fig. 2.** X-ray photographs (a) and IR spectra (b) of Si–CaP coatings before and after annealing. Substrate material: (a) titanium BT1-0, (b) KBr.
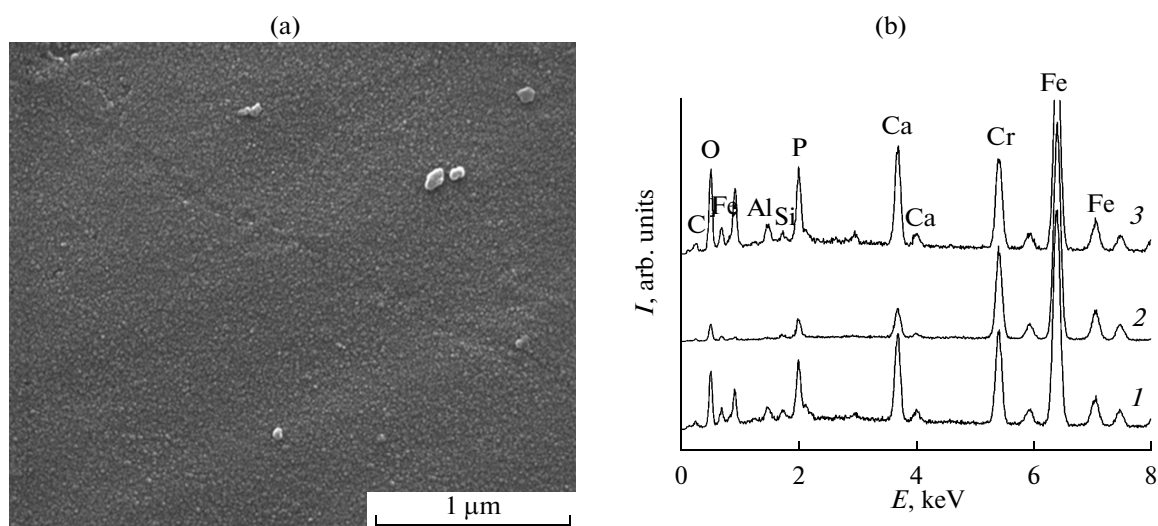
(a)

(b)



**Fig. 3.** SEM images of the surface (a) and EDXA spectra (b) of unannealed Si−CaP coatings. Substrate material: 12X18H10T steel: (a) after 7-day soaking, (b) before soaking (*1*), after 1-day (*2*) and 7-day (*3*) soaking in the SBF-solution

structure. In addition to the absorption bands corresponding to HA, the bands of the P-O groups (542, 585, and 984 cm$^{-1}$) associated with TCP [27, 28] were not fixed by the XPA. The obtained results gave all grounds to assume that the coating contains Si-HA and silicon-containing X-ray amorphous phases.

The behavior of the Si-CaP coatings produced using the HF-magnetron method, subsequently annealed in the SBF solution (dissolving−precipitation), was controlled with the XPA, EDXA, and SEM methods. The results of the investigation of the phase and elemental composition, and morphology of the coating before and after soaking in the SBF solution are shown in Figs. 3−5.

The microphotographs (Fig. 3a) of the analyzed unannealed Si-CaP coatings after a seven-day-long soaking in the artificial intertissue fluid solution demonstrated "tiny sources" of crystallization uniformly distributed over the entire surface.

The elemental analysis (Fig. 3b) revealed Ca and P in the coatings; the intensity of their maxima decreases after the first day of soaking in the artificial biological medium. However, in seven days the intensity of these maxima substantially increased exceeding the values obtained for the coatings prior to soaking in the SBF-solution. Analogous nanosized structures on the surface of annealed Si-CaP specimens were observed as early as after 1-day-long soaking in SBF. It follows from Fig. 4b that the structures increased in size by the 7th day of the experiment. The analysis of the surface layer composition (Fig. 4d) in annealed specimens pointed to a regular decrease in the concentration of Ca and P in the coating after the first day of soaking in the solution; then the concentration and, consequently, the layer thickness increased.

The observed increase in the maxima on the EDXA-spectra is related to variations in the thickness of the calcium phosphate layer on the substrate surface. It suggests that the observed "tiny sources" of crystallization (Figs. 3a and 4b) are the nuclei of the calcium phosphate crystals. The growth of the nuclei due to the absorption of $Ca^{2+}$ and $PO_4^{3-}$ ions from the oversaturated salt solution yields the formation of an openwork structure formed from lamellar crystals (Fig. 4c). According to the XPA data (Fig. 5), these crystals are crystals of HA. The crystal growth in the direction perpendicular to the coating surface is attributed to the formation of diffusion flows following the "dissolving−precipitation" mechanism [18]. After 28 days of soaking in the SBF solution, the boundaries between individual crystals cannot be seen, i.e., their conglomeration takes place [29]. The formation of such a layer qualitatively points to the biological activity of the Si-CaP coatings. As in the case of biologically active glasses, the high surface activity of Si-HA can be attributed to silanol (Si-O-H) groups which are formed on the surface of the material and actively participate in the process of interface mineralization [30]. The surface intensively exchanges calcium and phosphorous ions with the solution; in this case, the silanol groups strongly bind the calcium ions facilitating the formation of an amorphous calcium phosphate layer on the surface. Subsequent lamination and crystallization of the layer yield the formation of an openwork structure consisting of tiny lamellar HA crystals (according to XPA data, Fig. 5d).

The microphotographs and the results of the EDXA obtained for the coatings formed in the results of their soaking in SBF and pointing to the precipitation of a calcium phosphate layer from the solution to the coating surface are in good agreement with the
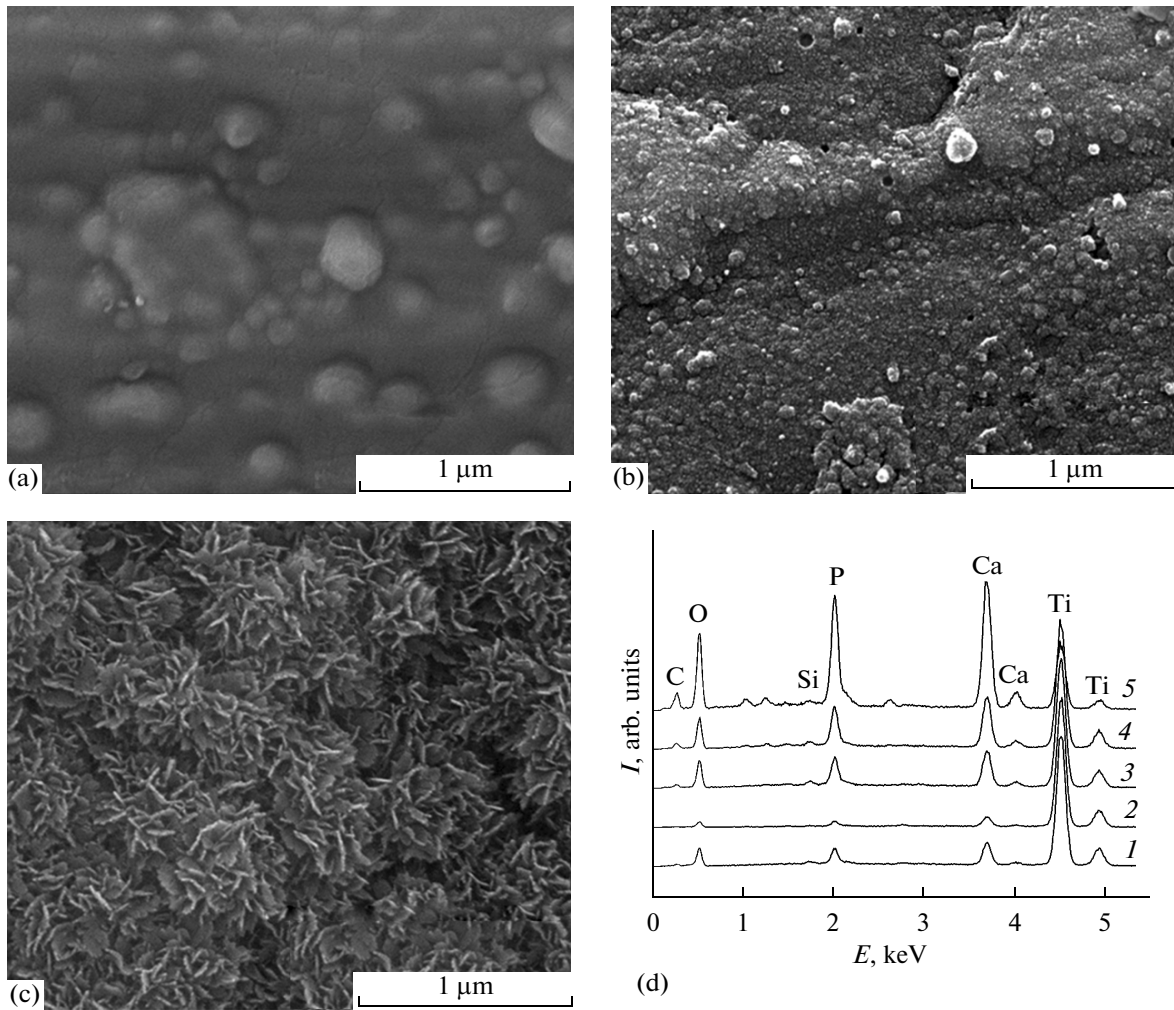
**Fig. 4.** SEM images of the surface (a–c) and EDXA-spectra (d) of annealed Si-CaP coatings before and after soaking in SEF-solution. Substrate material: titanium: (a) before soaking, (b) after 7-day soaking, (c) after 28-day soaking, before (*1*) and after 1-day (*2*), 7-day (*3*), 14-day (*4*), and 28-day soaking in the SBF-solution.
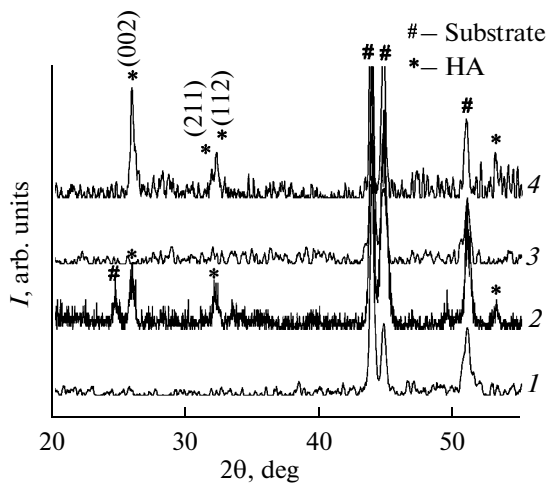


**Fig. 5.** X-ray photographs of amorphous (a, c) and annealed (b, d) Si-CaP coatings on steel before and after soaking in the SBF. Soaking duration: 7 days for amorphous coatings and 28 days for annealed coatings.

data on the calcium concentration in the solution. The results of the investigation of the time dependence of the calcium concentration in the solution are shown in Fig. 6. The determination of the cumulative concentration of calcium in the SBF solution for all types of coatings shows that the time dependence of the calcium concentration is similar. Two regions can be clearly distinguished on the graphic chart: at the initial stage, an increase in the number of $Ca^{2+}$ ions in the solution is observed; then this section gives place to a nonlinear section where the slope angle changes its sign. A substantial difference in the dissolving rates in annealed and unannealed specimens at the initial stage (4–7 days) should be noted. Amorphous unannealed coatings are characterized by a higher initial resorption rate as compared to the annealed coatings. A qualitative coincidence of the dependence character is related to one and the same process, namely "dissolving–precipitation" [18].
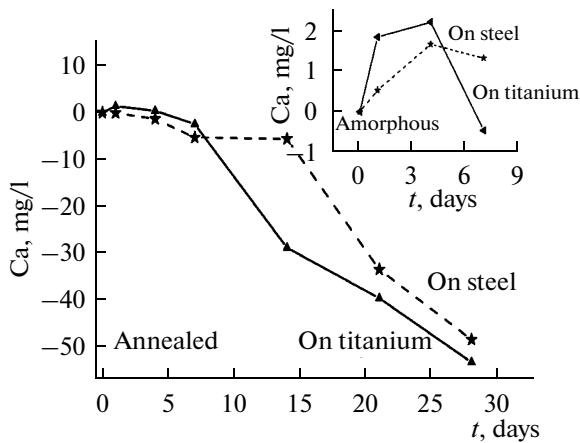
**Fig. 6.** Cummulative concentration of calcium in SBF-solution. Final values are determined by the difference between the calcium concentration in the reference specimen (SBF prior to submersion of "composite+substrate" composite) and calcium concentration after the specimen had been taken out for each time instant. Duration of the experiment is 7 days for unannealed coatings and 28 days for annealed coatings.

## CONCLUSIONS

The coatings based on silicon-substituted hydroxyapatite were produced using the HF-magnetron sputtering method. The coatings formed were X-ray amorphous and their elemental composition depended on the composition of the target for sputtering. The distribution of the elements over the coating thickness was uniform. Heat treatment in air yields formation of an apatite-like phase in the coating.

Thermostating of "metal+coating" specimens in the solution of an artificial intertissue fluid showed that all obtained specimens demonstrated bioactive properties: a calcium phosphate layer was formed on the surface by the mineralization process. Annealed crystalline coatings demonstrate a high chemical stability in vitro as compared to amorphous coatings.

## ACKNOWLEDGMENTS

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