NANOSYSTEMS: PHYSICS, CHEMISTRY, MATHEMATICS, 2014, 5 (4), P. 590–596

# SYNTHESIS AND STUDY OF NANOSIZED BIOMATERIALS BASED ON HYDROXYAPATITE

E. A. Bogdanova, N. A. Sabirzyanov

Institute of Solid State Chemistry, Ural Branch of the Russian Academy of Science, Ekaterinburg, Russia

chemi4@rambler.ru, sabirzyanov@ihim.uran.ru

**PACS 82.70.Kj**

Directed synthesis of fluorine- and silicon-containing apatites was carried out. The limiting degrees of substitution of silicate groups for phosphate groups were proposed. The physico-chemical properties of the produced materials were investigated.

**Keywords:** biomaterials, fluorine-substituted hydroxyapatite, silicon-substituted hydroxyapatite.

*Received: 16 June 2014*
*Revised: 30 June 2014*

## 1. Introduction

Recent progress in the field of biomaterials intended to replace and reconstruct bone tissue is due to the application of hydroxyapatite (HAp) as bone implants. Numerous HAp-based biocomposite materials containing antioxidants, antibiotics, regenerating and anti-inflammatory agents have been designed and implemented in clinical practice.

However, in spite of attractive biological properties Hap, materials based thereon have some drawbacks, such as low bioresorption rate *in vivo*, poor stimulating effect on the growth of new bone tissues, low crack resistance and small fatigue durability in the physiological environment.

Chemical modification of HAp allows one to vary the characteristics of the materials over a wide range. The application of modified HAp opens up the opportunities for the preparation of artificial bone substances for implants and a large variety of drugs for curing different lesions of bone, soft and mucous tissues of the individual.

A promising method of modification is the introduction of fluorine or silicon into the primary structure with the formation of fluorine- or silicon-substituted HAp. The introduction of fluorine increases the resistance to biodegradation [1-3] and improves the adsorption of proteins [4] and adhesion of the coating to the metal substrate [5, 6]. Besides, fluorine is necessary for the growth and strength of bones and is a natural stimulator of bone tissue, tooth enamel and dentin formation. The application of fluorine-substituted HAp as a component of pharmaceutical compositions is thought to improve their stability in the reactive environment of the human body and to enhance the therapeutic effect.

Silicon-substituted HAp is of particular interest because of requirements to the implant to grow actively together with the bone tissue forming an intermediate area consisting of biogenic nanocrystalline HAp [7, 8]. Silanol (Si–O–H) bonds on its surface promote the formation of a new phosphate layer accelerating the implant adnation with the bone (osseointegration) [7-9]. Moreover, silicon is also necessary for formation of all body tissues including the basic substances of bone and cartilage tissues, since its physiological role is generally connected with synthesis of glycosaminoglycans and collagen.

To obtain new composite HAp-based materials combining biocompatibility with enhanced strength properties, the authors synthesized silicon-substituted HAp with different degrees of dispersion and a predetermined content of substituting anion while examining the physical and chemical properties of the obtained material.

## 2. Methods and materials

The monophasic samples were produced by the standard method of chemical condensation using additional silicon- and fluorine-containing reagents:

$$10\mathrm{Ca(OH)_2} + 6\mathrm{H_3PO_4} \rightarrow \mathrm{Ca_{10}(PO_4)_6(OH)_2} + 18\mathrm{H_2O}$$

$$\mathrm{Ca_{10}(PO_4)_6(OH)_2} + x\mathrm{F^-} \rightarrow \mathrm{Ca_{10}(PO_4)_6(OH)_{2-x}F_x} + x\mathrm{OH^-}$$

$$(x = 1;\ 1.5;\ 2)$$

$$10\mathrm{Ca(OH)_2} + (6\text{-}x)\mathrm{H_3PO_4} + x\mathrm{Si(OR)_4} \rightarrow$$

$$\mathrm{Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}} + 4x\mathrm{ROH} + (18\text{-}3x)\mathrm{H_2O}$$

$$(\mathrm{R} = \mathrm{Et};\ x = 0.5;\ 1)$$

The phase composition of the synthesized samples was studied by X-ray phase analysis (Shimadzu XRD 700 $\mathrm{Cu}_{k\alpha}$-radiation) and infrared spectroscopy (Spectrum One; Nicolet 6700, FT-IR). The morphological peculiarities of the obtained specimens were examined using scanning electron microscopy (JEOL JSM 6390 LA with an energy-dispersion analyzer). The thermal stability was studied by differential thermal analysis and thermogravimetry with a TG-DTA-92 device (Setaram). The lattice constants were calculated on the basis of powder patterns using the Celref software.

## 3. Results and discussion

Examination of the microstructure of the samples by scanning electron microscopy showed that the synthesis products are poorly crystallized and consist of nanosized particles integrated in agglomerates (Fig. 1), which can be destroyed by grinding the dried product in ball mills.

The X-ray data reveal that the powder patterns of the obtained compounds are identical to those of HAp. Since different minerals known as apatites give similar powder patterns with minor variations, and the considerable width of the scattering peaks caused by small sizes of crystals reduces the accuracy of comparative analysis of powder patterns, we used infrared spectroscopy method to confirm the presence of fluorine in Hap and identify the chemical bonds of the phosphate and silicate groups emerging with the introduction of silicon into the HAp structure.

The IR spectra of the fluorine-substituted (Fig. 2.1) and silicon-substituted HAp (Fig. 2.2) samples with different substituting ion content indicate the presence of valence and deformation oscillations of the main groups and bonds typical of these compounds: absorption bands of P–O groups at 1053, 1021 and 950 $\mathrm{cm}^{-1}$ (asymmetric $\nu_3$ and symmetric $\nu_1$ valence oscillations of bonds in phosphate tetrahedron $\mathrm{PO_4^{3-}}$), doublets at 601 and 569 $\mathrm{cm}^{-1}$(asymmetric deformation $\nu_4$ oscillations of P–O bonds) and valence oscillations of O–H bonds at 3570 $\mathrm{cm}^{-1}$, deformation oscillations at 1646 $\mathrm{cm}^{-1}$ and valence oscillations of water absorbed during synthesis at 3435 $\mathrm{cm}^{-1}$.

The substitution of fluorine for hydroxyl groups reduces the resolution of the oscillation bands of O–H bonds at 3570 $\mathrm{cm}^{-1}$ owing to partial loss of O–H groups, which indirectly confirms the expected mechanism of substitution.

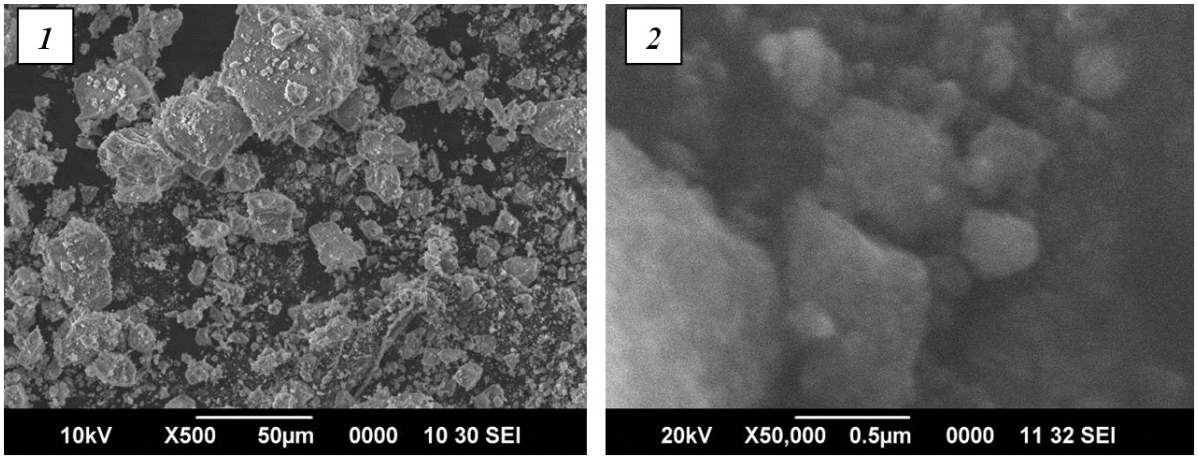


FIG. 1. Micrographs of crystalline fluorine-substituted HAp of the composition $Ca_{10}(PO_4)_6F_2$, magnification: ×500 ***(1)***, ×50000 ***(2)***

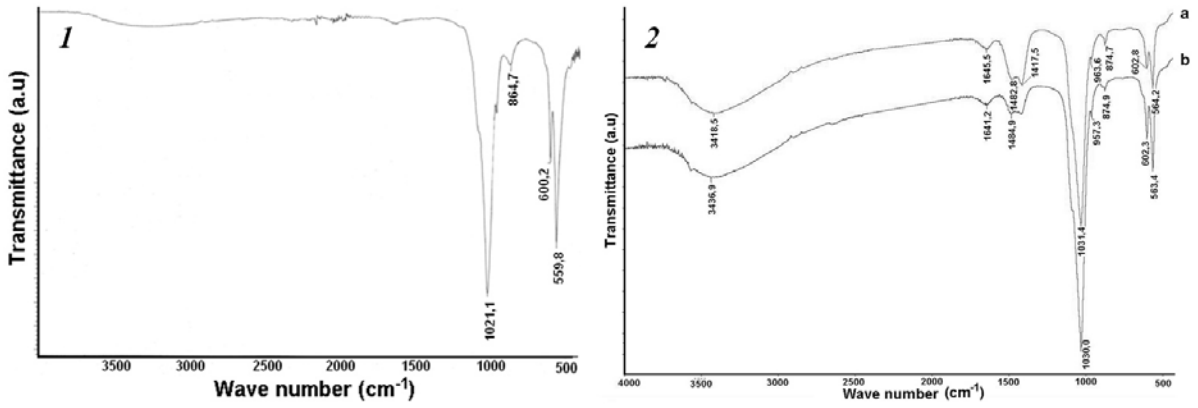


FIG. 2. IR spectra of $Ca_{10}(PO_4)_6(OH)_{2-x}F_x$ for x = 1; 1.5; 2 ***(1)***, $Ca_{10}(PO_4)_5(SiO_4)(OH)$ ***(2a)*** and $Ca_{10}(PO_4)_{5.5}(SiO_4)_{0.5}(OH)_{1.5}$ ***(2b)***

The absence of hydroxyl vibration oscillations at 630 $cm^{-1}$ near the low-frequency band typical of P–O oscillations (601 $cm^{-1}$) is due to the reduction of the number of $OH^-$ ions in the compound to compensate the extra charge emerging with the introduction of silicate groups into the HAp lattice. The absorption bands typical of oscillations of Si–O bonds are also present: absorption band with a frequency of 500 – 520 $cm^{-1}$, typical of oscillations of the silicate group bonds in the apatite structure, is due to the deformation oscillations of Si–O bonds; the valence oscillations bands of these bonds at 945 $cm^{-1}$ overlap with the intense absorption bands of the phosphate group in the 900 – 1200 $cm^{-1}$ range. The intensity of the $PO_4^{3-}$ bands for the silicon-substituted HAp samples decreases with an increase in the substitution degree. The presence of oscillations of the $CO_3^{2-}$ group at 1420 – 1480 $cm^{-1}$ in the IR spectra is explained by a considerable affinity of the silicon-substituted HAp to the carbonate-anion, which, being located in the phosphate ion position [10], compensates for the silicate anion charge. The substitution degree of $CO_3^{2-}$ increases with the content of silicon in the sample, which is confirmed by an increase in the band intensity of this group on the IR spectra.

The energy dispersive X-ray analysis – EDAX confirmed that the composition of the synthesized samples corresponds to pre-assigned stoichiometry (Fig. 3). Analysis performed through 20 points in different parts of the samples gave an insignificant scatter of results.

No fluorine-free points were found in the fluorine-substituted and silicon-free points in the silicon-substituted HAp samples confirming the formation of solid solutions in these systems.

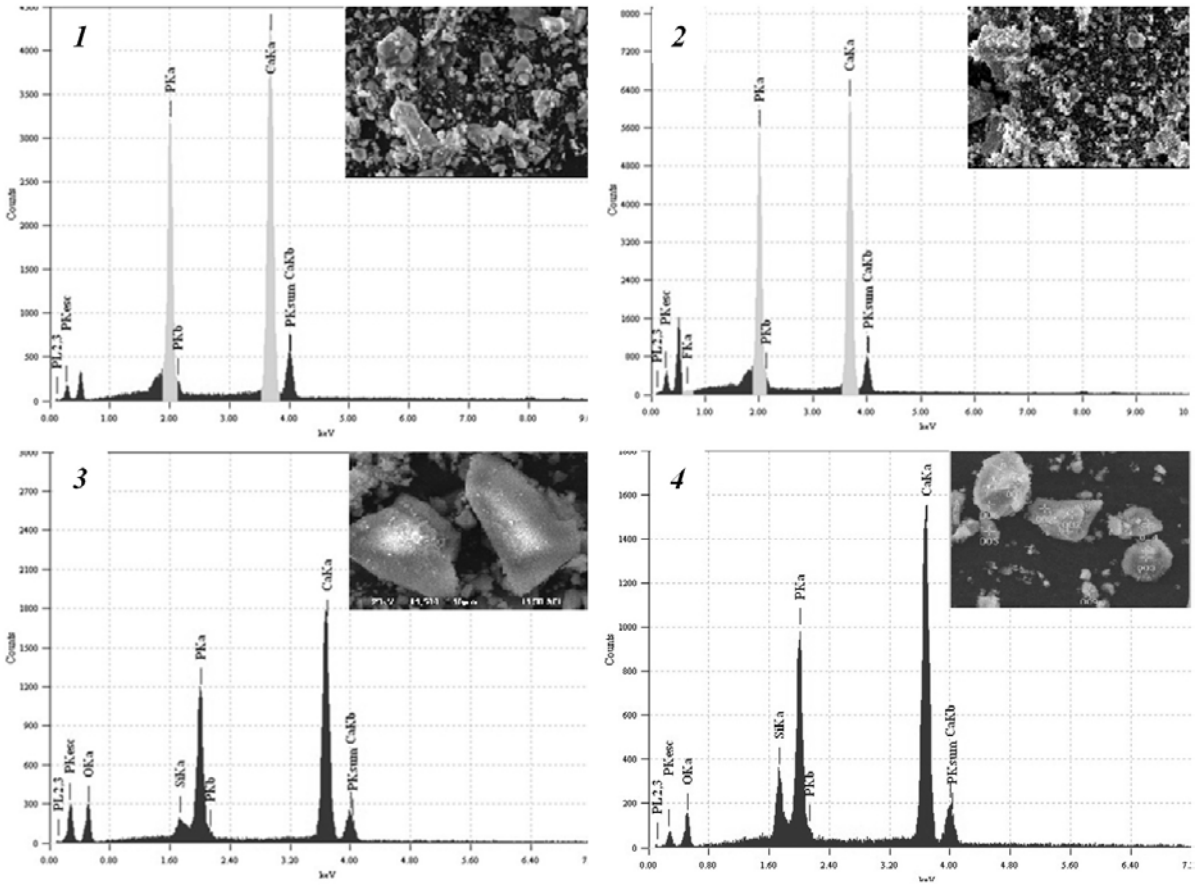


FIG. 3. EDAX-spectra of HAp ***(1)*** and anion-substituted HAp with the formula $Ca_{10}(PO_4)_6F_2$ ***(2)***, $Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}$ for x = 0.5 ***(3)*** and x = 1 ***(4)***

Thus, the assumption that fluorine or silicon can be embedded into the HAp lattice is confirmed by the experimentally established facts: the absence of phases of other calcium phosphates or anion-containing compounds in the powder patterns; the change in the spectral pattern in the characteristic fields as well as by the EDAX data.

The introduction of fluorine or silicate ions into the initial HAp structure should change the lattice constants. The lattice constants of the synthesized samples of anion-substituted HAp were calculated on the basis of the powder patterns using the Celref software. The calculated results reveal (Tabl. 1) that the lattice constants of the synthesized samples of fluorine- and silicon-substituted HAp different from the stoichiometric HAp, which indirectly confirms anionic substitution. In the literature, there are different values of the lattice constants of silicon-substituted Hap obtained by different methods. For example, in [11] the lattice constants of silicon-substituted HAp were found to increase with the silicon content. At the same time, reduction of the lattice constants for silicon-substituted HAp was reported in [12]. Analysis of the obtained data correlates with the data [7] showing that the introduction of silicon into the HAp lattice decreases the parameter $a$ and increases the parameter $c$. The introduction of fluorine into the initial HAp structure also decreases the lattice constants.

Table 1. The lattice constants of HAp and anion-substituted forms

| Compound | a, Å | c, Å | Volume of crystal unit, Å$^3$ |
|---|---|---|---|
| $Ca_{10}(PO_4)_6(OH)_2$ | 9.418 | 6.884 | 528.80 |
| $Ca_{10}(PO_4)_{5.5}(SiO_4)_{0.5}(OH)_{1.5}$ | 9.389+0.027 | 6.893 ± 0.002 | 526.21 ± 2.28 |
| $Ca_{10}(PO_4)_5(SiO_4)(OH)$ | 9.246+0.017 | 6.885 ± 0.001 | 509.69 ± 0.92 |
| $Ca_{10}(PO_4)_6(OH)F$ | 9.375± 0.015 | 6.887± 0.001 | 524.19± 0.85 |
| $Ca_{10}(PO_4)_6(OH)_{0.5}F_{1.5}$ | 9.376± 0.030 | 6.883± 0.001 | 524.09± 1.68 |
| $Ca_{10}(PO_4)_6F_2$ | 9.383± 0.033 | 6.881± 0.001 | 524.70± 1.88 |

The results of thermogravimetric analysis (Fig. 4) reveal the following stages of thermal decomposition accompanied by weight loss of the samples: the loss of bound water and some amount of carbonate ions in the 200 – 600°C interval and considerable loss of carbonate ions in the interval from 600 to 1000°.

The $Ca_{10}(PO_4)_5(SiO_4)(OH)$ sample loses weight (16.8 wt.%) more readily than the other samples since it contains, along with bound water, carbonate ions, to which silicon-substituted HAp shows considerable affinity. For silicon-substituted HAp $Ca_{10}(PO_4)_{5.5}(SiO_4)_{0.5}(OH)_{1.5}$, the weight loss is 9.95 wt.%, which is explained by a lower carbonate ion content in this sample.

The fluorine-substituted forms have greater thermal stability increasing with the degree of substitution. The weight loss of fluorine-substituted Hap was: $Ca_{10}(PO_4)_6(OH)F$ – 12.3 wt.%; $Ca_{10}(PO_4)_6(OH)_{0.5}F_{1.5}$ – 10.2 wt.%; $Ca_{10}(PO_4)_6F_2$ – 9.7 wt.%.

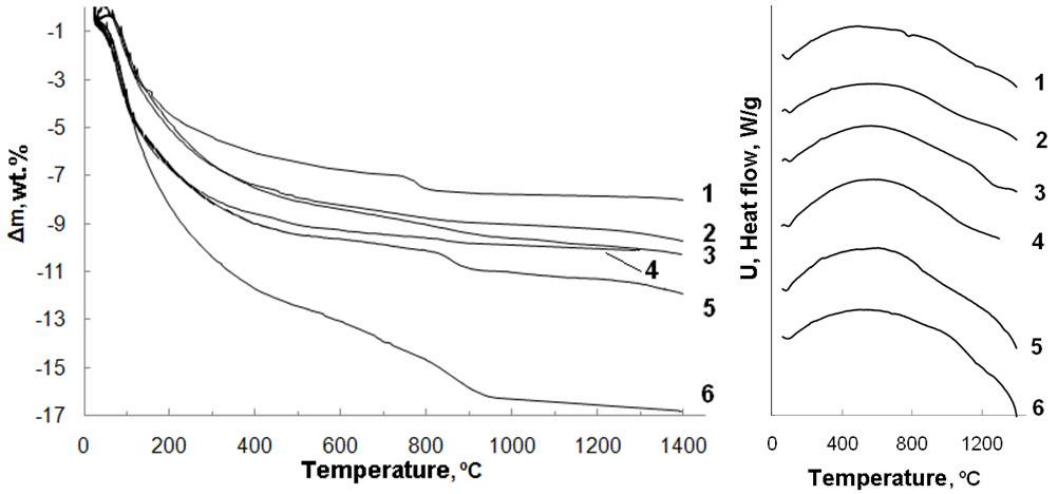


Fig. 4. DTG and DTA curves for: ***1*** – $Ca_{10}(PO_4)_6(OH)_2$, ***2*** – $Ca_{10}(PO_4)_6F_2$, ***3*** – $Ca_{10}(PO_4)_{5.5}(SiO_4)_{0.5}(OH)_{1.5}$, ***4*** – $Ca_{10}(PO_4)_6(OH)_{0.5}F_{1.5}$, ***5*** – $Ca_{10}(PO_4)_6(OH)F$, ***6*** – $Ca_{10}(PO_4)_5(SiO_4)(OH)$ samples

Pure HAp loses 8 wt.%, but this sample is unstable. The endothermic effect at 800°C on the DTA curves (Fig. 5) is attributed to partial decomposition of HAp and to the formation of tricalcium phosphate, which is typical of HAp obtained by precipitation. The occurrence of the tricalcium phosphate impurity phase was confirmed by X-ray, while the powder patterns of anion-substituted HAp samples with varying degrees of substitution annealed at 800 and 1000°C are monophasic and correspond to those of HAp. EDX

confirmed that composition of the annealed samples corresponds to the initial compositions with predetermined degrees of substitution.

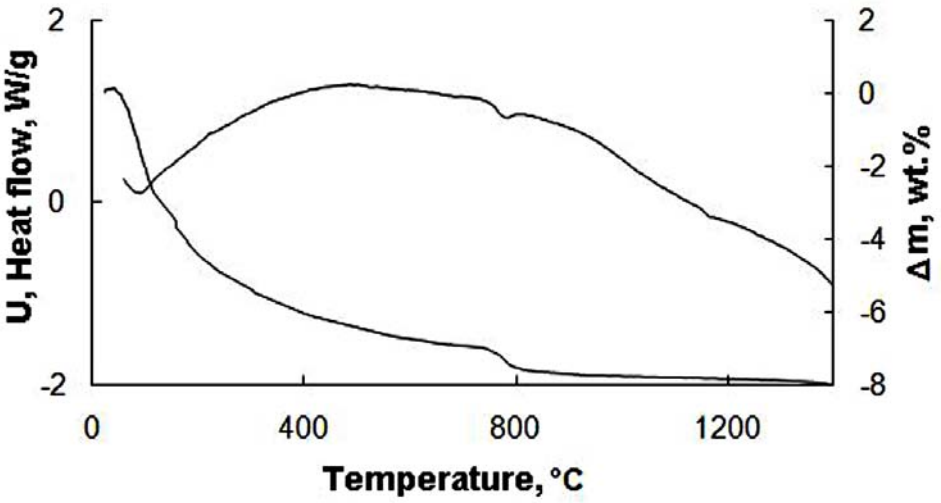


FIG. 5. DTA curves of pure Hap

Thus, the study of the influence of anionic substitution on the thermal stability of HAp showed that in comparison with pure HAp the anion-substituted forms have greater thermal stability. The most thermally stable sample is $Ca_{10}(PO_4)_6F_2$. The thermal stability of fluorine-substituted HAp can be explained by the difference in the energies of chemical bonds of $F^-$and $OH^-$ ions with calcium ions in Ca-channels and by structural stabilization.

Inasmuch as one of the crucial requirements to modern materials for biomedical applications is high bioactivity allowing not only for biological processes of cell growth and differentiation but also for the rate of material dissolution, we have studied the behavior of the synthesized samples in a sub acid medium (acetate buffer solution with pH = 4). It was established that in comparison with pure HAp, silicon-substituted HAp has a higher solubility, increasing with the degree of substitution of silicate ions for phosphate ions (Fig. 6).

The solubility study shows that the introduction of fluorine into the structure, be it complete or partial replacement of $OH^-$ groups, exerts a stabilizing effect and promotes production of a material with improved strength properties, because it increases the resistance to biodegradation and to the action of acids.

## 4. Conclusions

Thus, a method for changing the physicochemical properties of HAp by partial anionic substitution has been proposed. Directed synthesis of silicon-containing apatites was carried out, which is a complex physicochemical problem. The limiting degrees of substitution of silicate groups for phosphate groups were proposed.

The thermal stability study showed that the introduction of fluorine or silicon stabilizes the apatite structure. Examination of the behavior of the produced materials in the model solution revealed a greater resorbing ability and consequently, greater bioactivity for the silicon-modified samples in comparison with pure HAp. The application of these materials for medical purposes is very promising.

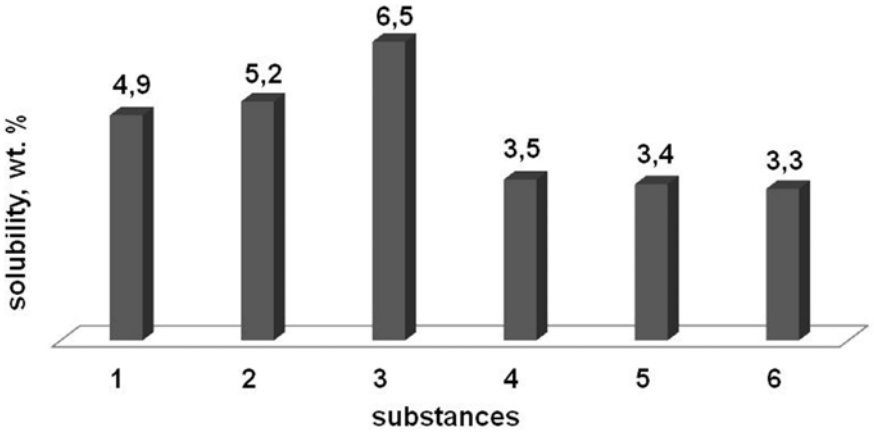


FIG. 6. The solubility for: ***1*** – $Ca_{10}(PO_4)_6(OH)_2$, ***2*** – $Ca_{10}(PO_4)_{5.5}(SiO_4)_{0.5}(OH)_{1.5}$, ***3*** – $Ca_{10}(PO_4)_5(SiO_4)(OH)$, ***4*** – $Ca_{10}(PO_4)_6(OH)F$, ***5*** – $Ca_{10}(PO_4)_6(OH)_{0.5}F_{1.5}$, ***6*** – $Ca_{10}(PO_4)_6F_2$ samples

## Acknowledgments

This work was supported by the Presidium of the Ural Branch of RAS (program No. 12-P-3-1003).

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