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Biocomposites based on selenium-substituted hydroxyapatite

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Abstract. When creating biocompatible calcium phosphate materials with good bioresorbability, calcium hydroxyapatite (HA) is used, which has a similar structure to bone mineral and has good biocompatibility, bioactivity and high resistance to proteins, chemotherapeutic drugs and antigens. The synthesis of HA/SeO_3 was carried out and it was shown that selenite ions can be included in the structure of HA, which is the predominant phase, the crystallites have a lamellar morphology and the same size, which indicates that the replacement of HA with SeO_3^{2-} ions does not lead to a change in its structure. The solubility of the modified samples in 0.9% NaCl and acetate buffer increases compared to pure HA, which confirms the inclusion of selenite ions in the HA structure. The synthesis of HA/SeO_3/chitosan composites was carried out and it was found that the predominant phase is HA, and the morphology of SeO_3-HA/chitosan aggregates are complex bulk particles of irregular shape. Dissolution of samples in 0.9% NaCl/acetate buffer of the HA/SeO_3 composition occurs less intensively than for samples of the HA/SeO_3/chitosan composition, which allows the former to be used in the case of passive resorption, and HA/SeO_3/chitosan composites for active resorption.

Keywords: hydroxyapatite, chitosan, selenium, composite, morphology

1. Introduction

Nowadays, of particular interest in medicine is the possibility of reconstructing bone tissue using non-toxic materials, which over time are resorbed in the body and replaced by new bone tissue. Such materials consist of an inorganic and an organic phase. Calcium phosphates (CP) are widely used as inorganic materials, since they consist of Ca^{2+} and PO_4^{3-} ions necessary for the construction of bone tissue [1–4]. To create biocompatible calcium phosphate materials with good bioresorbability, calcium hydroxyapatite (HA) is used, which has a similar structure to bone mineral and has good biocompatibility, bioactivity and high resistance to proteins, chemotherapeutic drugs and antigens [5–8]. Research has shown that hydroxyapatite modified by ion doping also has some special biological properties.

The element selenium has antitumor, antibacterial and anti-aging effects, and can also enhance human immunity. Selenium intake plays an important role in bone formation [3, 8]. Therefore, it is very important to obtain a multifunctional new bone repair material with good osteoconductivity, as well as antitumor, antibacterial and other effects by incorporating selenium into hydroxyapatite [9].

Selenium-enriched HA is most often prepared by wet deposition methods using SeO_3^{2-} ions as a source of selenium. The use of natural resources such as cuttlefish bone to produce composites is a promising direction because they contain, in addition to calcium, many useful ions found in human bones [10, 11]. The purpose of the work is to synthesize and study the physicochemical properties of HA/SeO_3/chitosan composites.

2. Materials and methods

The synthesis of composites is carried out by the “wet method” at room temperature. The synthesis is based on the reaction:

$$(10\text{-}x)\ Ca(NO_3)_2 + x\ Na_2SeO_3 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10-x}(PO_4)_6(SeO_3)_x(OH)_{2-x} + 20NH_4NO_3 + xNa_3PO_4 + 6H_2O$$

The synthesis is carried out at a constant concentration of Ca^{2+} (0.068 mol/L). The concentrations of HPO_4^{2-} and SeO_3^{2-} ions vary so that $n1 = SeO_3^{2-} / (HPO_4^{2-} + SeO_3^{2-})$ varies from

1.5; 3.0; 5.0; 7.5; 10 g/L, and $n_2 = Ca^{2+}/ (HPO_4^{2-} + SeO_3^{2-}) = 1.67$. Add 25% aqueous NH_4OH solution to pH 10.5. The precipitate is filtered 2 days after the solution is completely added.

Next, the precipitate is dried in an oven at a temperature of $T = 80$ °C to completely remove unbound water. The dried sediment is transferred to a container and weighed on an analytical balance.

To obtain chitosan gel, we used low molecular weight chitosan obtained from the shells of Kamchatka crabs without any impurities. A 2% solution of chitosan was prepared in 0.5% acetic acid for 24 hours. The pH of the resulting gel was kept at least 7.0. The resulting gel forms a thin, moderately soluble film on the skin or polymer substrate within 10 minutes. Powdered material (HA/SeO_3 (3.0, 7.5, 10 g/L)) was introduced into the resulting gel in an amount of 10, 30 wt.% and subjected to intensive stirring. The resulting foam was placed in a crucible and dried at $T = 22–25$ and 200 °C.

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 ($\lambda = 690$ 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.

Selenite ions were determined by the photometric method. The method is based on the catalytic action of Se(IV) in the reaction of methylene blue with sulfide ions. Spectrophotometer B-1100, $\lambda = 618$. The error of determination is within 2–4%.

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%.

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

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

3. Results and its discussion

The study of solid phases by X-ray phase analysis showed that the main mineral component is hydroxyapatite. The main intense lines of hydroxyapatite according to the 2θ school are angles 23.0; 25.9; 31.9; 39.8; 49.5 and 53.3 (Fig. 1). Also, in the diffraction patterns at a concentration of selenite ions of 7.5 g/L, angles of 12.14; 17.82; 31.75, which characterizes the presence $CaSeO_3*H_2O$. Using the Debye-Scherrery formula, the average crystallite sizes were calculated from the diffraction patterns of the samples HA/SeO_3. It is clear from the data that the crystallites have the same size, this indicates that the replacement of HA with SeO_3^{2-}- ions does not lead to distortion of its structure (1.5 g/L – 0.53 Å; 3.0 g/L – 0.54 Å ; 5.0 g/L – 0.54 Å; 10.0 g/L – 0.55 Å).

Using Fourier transform infrared spectroscopy, it was found that the synthesized SeO_3^{2-}/HA samples have the entire set of bands characteristic of calcium phosphates and selenium ions. In the composition of SeO_3^{2-}/HA, the IR spectra show absorption bands of the O-P-O bond in PO_4^{3-} and HPO_4^{2-}, where the absorption maxima are 1000, 1034 cm^{-1} – stretching vibrations, 563 cm^{-1}, 604 cm^{-1} – bending vibrations (Fig. 2).

Vibrations of the C-O bond in CO_3^{2-} are observed at 872–874 cm^{-1} (carbonate-substituted hydroxyapatite is B-type). Vibrations characteristic of the Se-O bond are observed in the region of 820–890 cm^{-1}. The intensity of the absorption bands increased with increasing SeO_3^{2-} concentration;

these bands are related to symmetric and asymmetric vibrations of the Se-O bond. This is proven by the inclusion of selenium ions in the HA structure.

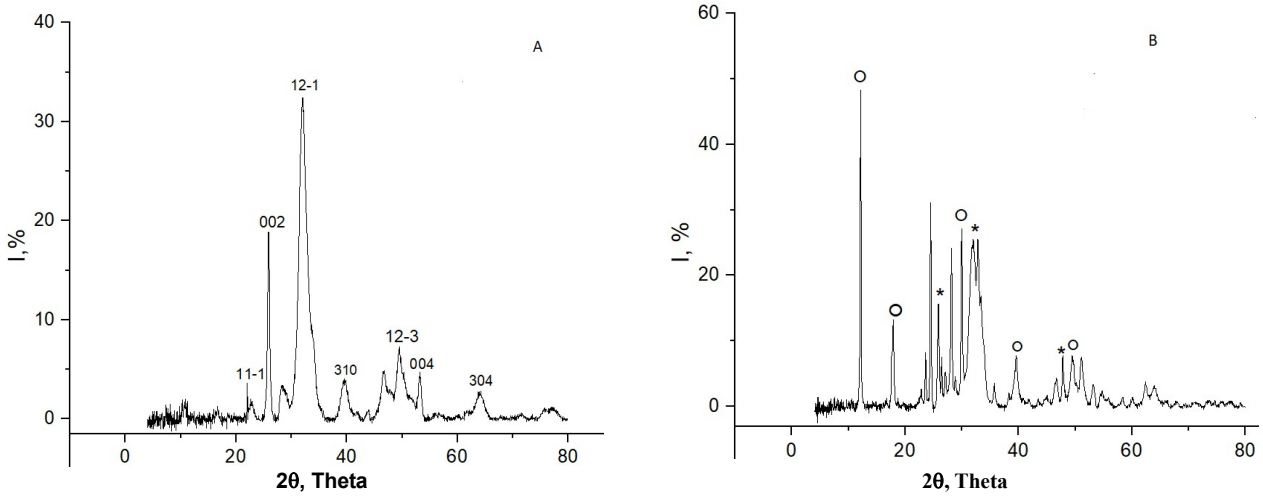


Fig. 1. X-ray diffraction patterns of HA/SeO_3 samples with different concentrations of selenite ions (A – 1.5; B – 7.5 g/l, *HA, ° – $CaSeO_3$*H_2O).

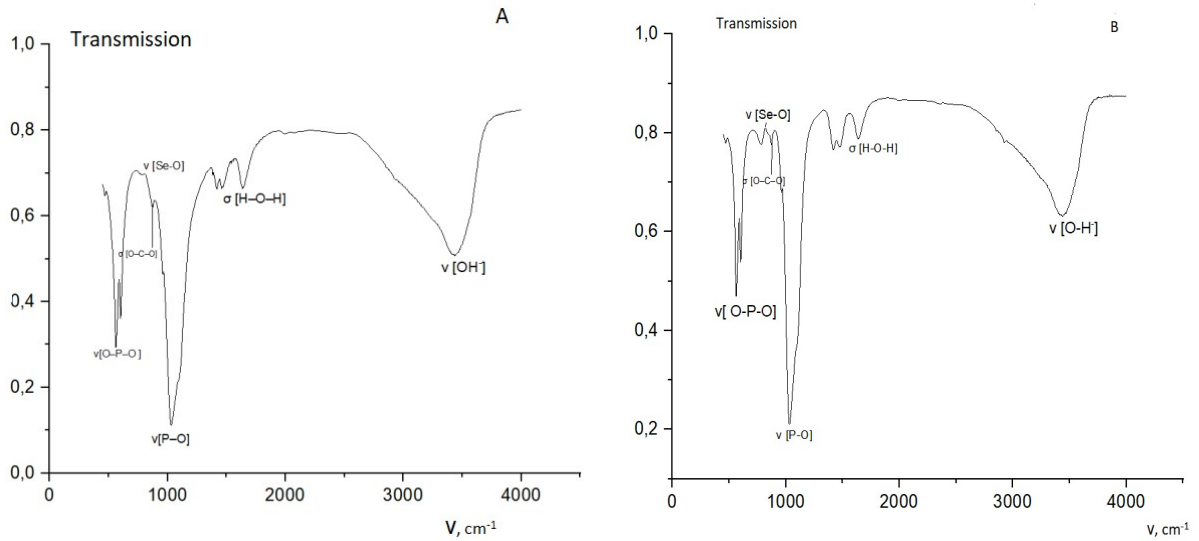


Fig. 2. IR spectra of synthesized HA-SeO_3, with different concentrations of SeO_3^{2-} ions, g/l (A – 1.5; B – 7.5).

Chemical analysis of the supernatant showed the presence of selenium ions in the resulting solid phases. The Ca/P coefficient was calculated, which for biogenic hydroxyapatite is 1.67. With an increase in the concentration of selenite ions in the solution during synthesis, the Ca/P coefficient increases and amounts to 2.07, which confirms the possibility of including the selenite ion in the HA structure at the position of phosphate ions.

The study of solid phases by X-ray phase analysis in the system $Ca(NO_3)_2$-Na_2SeO_3-$(NH_4)_2HPO_4$-NH_4OH-H_2O – chitosan showed that the main mineral component is hydroxyapatite.

Using Fourier transform infrared spectroscopy, it was found that the synthesized SeO_3^{2-} HA/chitosan samples have the entire set of bands characteristic of calcium phosphates and selenium ions. In the composition of SeO_3^{2-}/HA/chitosan, absorption bands of the O-P-O bond in PO_4^{3-} and HPO_4^{2-} appear in the IR spectra, where the absorption maxima are 1000, 1034 cm^{-1} – stretching vibrations, 563 cm^{-1}, 604 cm^{-1} – bending vibrations (Fig. 3). Vibrations of the C-O bond in $CO_3^{2-}$are observed at 872–874 cm^{-1} (carbonate-substituted hydroxylapatite is B-type).

Vibrations characteristic of the Se-O bond are observed in the region of 820–890 cm^{-1}. The intensity of the absorption bands increased with increasing SeO_3^{2-} concentration; these bands are related to symmetric and asymmetric vibrations of the Se-O bond. A wide absorption band in the far region of the spectrum at 3237, 2955 cm^{-1} relates to stretching and bending vibrations of –OH and –NH_2 groups. These groups are involved in the formation of intra- and intermolecular bonds in the chitosan molecule. For chitosan in the composition of the composite, absorption bands are visible with a frequency of 3737 cm^{-1}, related to vibrations of the O-H bond, and at a frequency of 1436 cm^{-1}, the bending vibration of the amino group –NH_2.

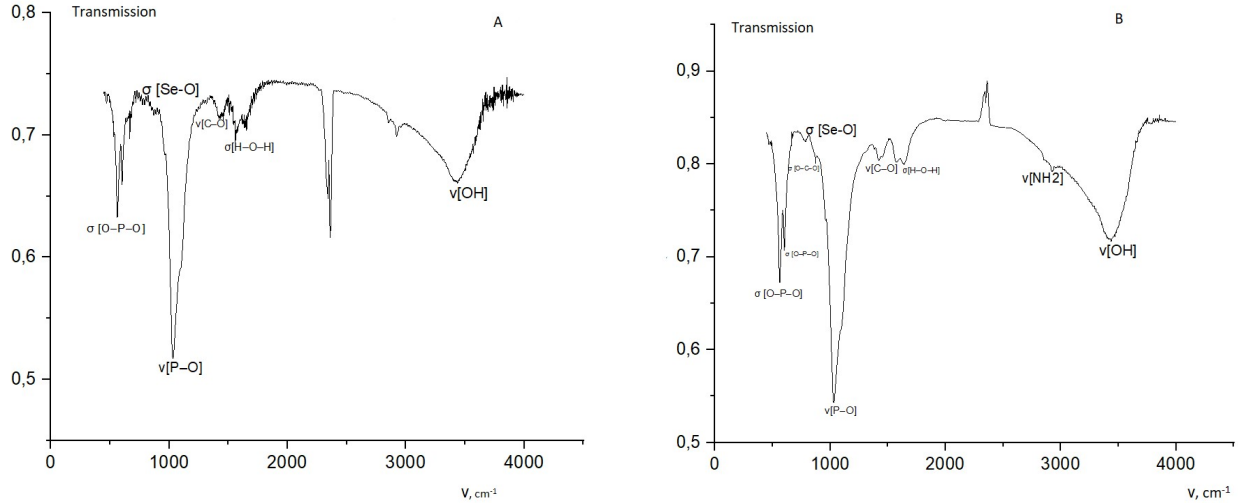
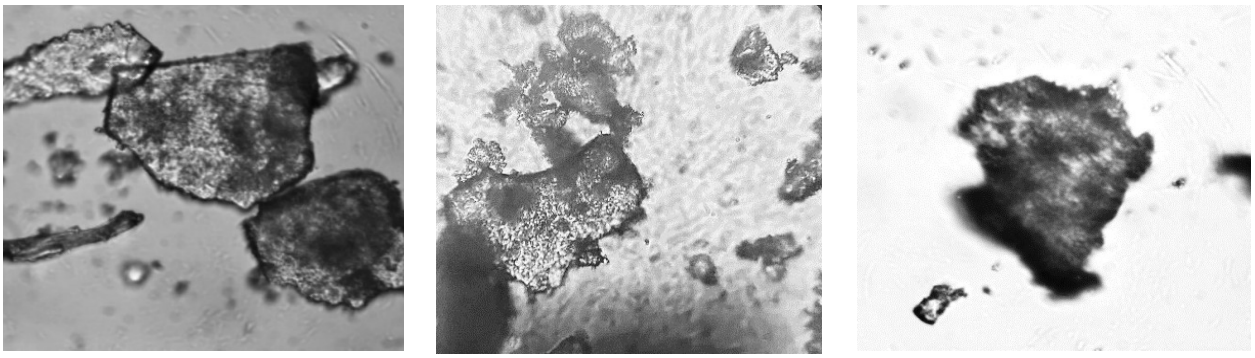


Fig. 3. IR spectra of HA/SeO_3/chitosan samples: A) HA/SeO_3(3.0 g/L)/chitosan, 10%; B) HA/SeO_3(3.0 g/L)/chitosan, 30%.

The morphology of solid phase particles was determined using optical microscopy. From Figure 4 shows that the surface of SeO_3-HA powder particles has a lamellar morphology, typical of calcium orthophosphate and often observed in biogenic and natural apatites. The addition of chitosan leads to a change in the morphology of the particles, forming complex voluminous particles of irregular shape.



A B C

Fig. 4. Type of particles of SeO_3/HA composites with different contents of selenite ions, g/L: A – 7.5; B – SeO_3/HA/chitosan, 30%; C – HA/SeO_3 (10.0 g/ L)/chitosan, 10%, (magnification 10x40).

The dissolution patterns of HA-SeO_3 composites were carried out for 2 hours in 0.9% NaCl to study the biodegradability of the samples. The solubility of samples containing from 1.5 to 3.0 g/L increases compared to pure HA, which indicates the inclusion of selenite ions in the HA structure. At high SeO_3^{2-} concentrations, the solubility of the samples decreases, since selenite ions are

adsorbed on the surface of the sample and the insoluble compound $CaSeO_3$ is formed (Solubility product = $4.7 \cdot 10^{-6}$), Table 1.

The patterns of dissolution of HA-SeO3 composites in acetate buffer (pH = 5.5) showed that the initial dissolution rate for samples increases with riseing content of selenite ions in samples with a concentration of 1.5; 3.0; 5.0 g/L, which confirms the likelihood of selenite ions entering the HA structure. In samples containing selenite ions of 7.5 and 10.0 g/L, the dissolution rate decreases due to the adsorption of selenite ions on the surface of the samples.

Table 1. Initial dissolution rates.

C selenite ions in solution, g/ L	Speed, min^{-1}, in 0.9% NaCl	Speed, min^{-1}, in acetate buffer
0	0.011	0.011
1.5	0.015	0.040
3.0	0.014	0.061
5.0	0.003	0.079
7.5	0.004	0.020
10.0	0.013	0.043

The dissolution patterns of HA-SeO_3/chitosan composites were carried out in 0.9% NaCl to study the biodegradability of the samples. It has been established that with an increase in the content of selenite ions, the concentration of calcium ions in the solution increases. Based on the obtained experimental dependences pCa = $f(t)$, the initial values of the rates of release of calcium ions in solution were calculated (Table 2).

Table 2. Value of initial dissolution rates.

HA/SeO_3/ chitosan		Speed, min^{-1}, in 0.9% NaCl	Speed, min^{-1}, in acetate buffer
C selenite ions in solution, g/L	Solid phase content, %		
3.0	10	0.006	0.042
7.5		0.033	0.017
10.0		0.050	0.012
3.0	30	0.001	0.049
7.5		0.025	0.009
10.0		0.048	0.015

From the data obtained it is clear that the initial dissolution rate for the samples increases with increasing content of selenite ions in the samples. The increase in speed is associated with greater solubility of calcium selenite (Solubility product = $4.7 \cdot 10^{-6}$). For samples containing 30% solid phase, the same dependences are observed as for samples with 10% solid phase. The patterns of dissolution of HA-SeO_3 composites were carried out in acetate buffer (pH = 5.5). It was found that the dissolution rate for samples decreases with increasing content of selenite ions in the samples, which is associated with the formation of chitosanium ion $C_6H_9NH_3^+$, which binds to PO_4^{3-} ions and imparts viscosity of the solution.

4. Conclusion

The synthesis of HA/SeO_3 was carried out: the study of the solid phase by X-ray diffraction and IR spectroscopy showed that selenite ions can be included in the structure of HA, which is the predominant phase; particles have a lamellar morphology, and the same size, this indicates that the replacement of HA with SeO_3^{2-} ions does not lead to a change in its structure; the solubility of the modified samples in 0.9% NaCl and acetate buffer increases compared to pure HA, which confirms the inclusion of selenite ions in the HA structure.

The synthesis of HA/SeO_3/chitosan composites was carried out: the study of the composites by X-ray diffraction and IR spectroscopy showed that the predominant phase is HA.

When comparing the synthesized samples: the crystallite sizes in the HA/SeO_3 samples are close to those in the HA/SeO_3/chitosan composites; the addition of chitosan leads to a change in the morphology of the particles, forming complex bulk particles of irregular shape; dissolution of samples in 0.9% NaCl/acetate buffer of the HA/SeO_3 composition occurs less intensively than for samples of the HA/SeO_3/chitosan composition, which allows the former to be used in the case of passive resorption, and HA/SeO_3/chitosan composites for active resorption.

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