Full Articles

Sorption and cocrystallization binding of Zr^{IV} ions with hydroxyapatite as a promising carrier of medical radionuclide ^{89}Zr

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A significant difference in behavior of Zr^{IV} ions during their cocrystallization and sorption binding to hydroxyapatite (HAP) is demonstrated. When the concentration of doping ions exceeds 10^{-4} mol L^{-1}, a chemical reaction with the formation of amorphous zirconium phosphate and complete dissolution of the sorbent occurs in the system rather than sorption. According to the X-ray diffraction and transmission electron microscopy (TEM) data, a similar reaction is also possible at lower concentrations of zirconium ions. The effect of doping ions on the morphology and structure of HAP is significantly lower for the cocrystallization introduction of Zr. In addition, according to high-resolution TEM data, doping ions can uniformly be distributed over the carrier surface or volume. Therefore, this method of binding method can be recommended for the preparation of a target HAP-Zr complex.

Key words: ^{89}Zr, hydroxyapatite, sorption, cocrystallization, morphology, sorption isotherm.

Positron emission tomography (PET) is a popular and actively developed method of nuclear diagnostics due to the insertion of new radionuclides and diverse methods for their delivery to the functioning site. At present, isotope ^{89}Zr is one of attractive diagnostic radionuclides owing to its nuclear physical characteristics.[1] Unlike other popular PET radionuclides (^{68}Ga, ^{64}Cu, ^{86}Y), ^{89}Zr possesses a longer half-life period ($T_{1/2}$ = 78.41 h), which makes it possible to use this isotope for diagnostics of comparatively slow processes in the human organism. The developed radiopharmaceuticals (RPh) based on ^{89}Zr form a "classical" group in which the radionuclide is chelated by diverse complexes and then binds to antibodies[2] and an "alternative" group where various nanoparticles act as carriers of ^{89}Zr. High-density lipoprotein,[3] liposomal nanoparticles,[4] gold nanoparticles,[5] and others are studied as similar nanocarriers. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) has a number of advantages due to biocompatibility and bioactivity and the ability to be completely assimilated by the human organism (bioresorbability). These properties make it possible to consider HAP as a promising carrier of various medical radionuclides (^{177}Lu, ^{223}Ra, ^{69m}Zn, ^{225}Ac, and others).[6—9]

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Serious attention is given to the preparation of HAP nanocomposites with zirconium for the use in stomatology and manufacturing bone implantants. High-temperature syntheses of these nanocomposites make it possible to enhance the mechanical strength of the units.[10] The sorption and cocrystallization methods of zirconium introduction into HAP are used to a lower extent. The sorption of ^{89}Zr without carrier or with it (taken in a minimum concentration) was shown[11,12] to proceed predominantly *via* the ion-exchange mechanism (*i.e.*, in HAP calcium is replaced by zirconium). In this case, the structure and morphology of the sorbent remain unchanged.

The purpose of this work is the cocrystallization and sorption introduction into HAP of zirconium ions in a wide range of concentrations for the evaluation of possibilities of HAP as a carrier of medical radionuclide ^{89}Zr. The sorption kinetics and the influence of introduction of the multicharge Zr^{IV} cation on specific features of the structure and morphology of the formed product are considered.

Experimental

Used sorbents. The HAP-based sorbents of two types were used in experiments on sorption binding of Zr^{IV} ions: HAP in the form of an aqueous suspension (HAP_0) synthesized using a known procedure[13] and a solid powder of HAP_0 subjected to high-temperature treatment[14] (HAP_T).

Synthesis of HAP_0 as an aqueous suspension was described earlier.[15] The solid phase content in the samples determined by the gravimetric method was 8.3±0.8 wt.%. The estimated surface area with allowance for nanoparticle agglomeration was 300 $m^2\ g^{-1}$.

Synthesis of HAP_T was described earlier.[14,16] The powder was prepared by the calcination of the HAP_0 sample in a MIMP furnace (Russia) at 1100 °C for 4 h. The specific surface area determined by the method of thermal nitrogen desorption[14,16] was 42±2 $m^2\ g^{-1}$.

Cocrystallization introduction (coprecipitation) of Zr^{IV} ions into formed nanocrystals of HAP was carried out using a published procedure.[15,16] Zirconium was introduced into the reaction mixture (before the onset of phosphoric acid supply to the reactor) as a solution of zirconium oxochloride, which was prepared beforehand by the dissolution of a precise weighed sample of $ZrOCl_2 \cdot 8H_2O$ (reagent grade) in H_2O (5 mL) on stirring. The concentration of the salt was chosen in such a way that the molar ratio Zr/Ca would be approximately 1/10 (11 mol.%). The reaction course was monitored using an Elit 3305 pH meter (Great Britain). After the end of the reaction (pH 6—7), a portion of a suspension of HAP-Zr was sampled for the gravimetric determination of the solid phase content and subsequent morphological analysis. A mother liquor was separated after centrifugation from the rest part of the suspension to determine the contents of zirconium, calcium, and phosphate ions. The solid phase was dried at 80 °C to a constant weight, after which a portion of the powder was subjected to high-temperature treatment (see earlier), and the phase composition of all samples (HAP-Zr and HAP-Zr_T) was determined.

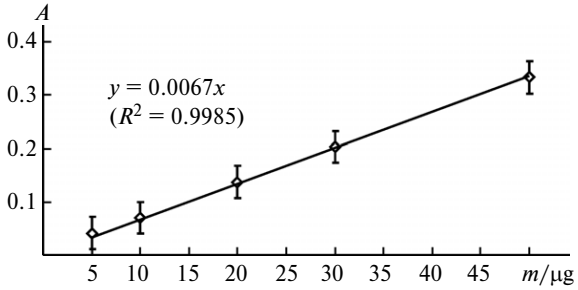

Fig. 1. Calibration plot for the determination of the zirconium content in the solution; m is the weight of zirconium in the sample, and A is absorbance.

Chemical analysis of solutions to the content of target ions (Zr^{4+}, Ca^{2+}, PO_4^{3-}) was performed by spectrophotometry (Shimadzu UV-1280, Japan).

Content of zirconium ions was determined by a previously described method[17] using the xylenol orange reagent with which zirconium ions in 0.2 M H_2SO_4 give a colored complex in a molar ratio of 1 : 1. The absorption maxima of the complex lie at λ = 535—540 nm, and the absorption maximum of the reagent corresponds to λ = 440 nm. The calibration plot was preliminarily constructed (Fig. 1), and the molar absorption coefficient of the complex was determined (ε = 0.0285 L mol^{-1} cm^{-1}).

Content of calcium ions was determined on a special Calcium-Olvex set (Russia) with *o*-cresolphthalein complexone with which calcium ions in an alkaline medium form a colored complex. The analysis was carried out at the wavelength λ = 570 nm.

Content of phosphate ions was determined using a special FN-Olvex set (Olvex Diagnostikum, Russia) at λ = 340 nm.

Sorption of zirconium on HAP_0 and HAP_T. In order to study the sorption of zirconium ions on HAP_0 and HAP_T, solutions of zirconium oxochloride with concentrations of $1.1 \cdot 10^{-4}$ mol L^{-1}, 0.14 mol L^{-1} (in this case, a monolayer can be formed on the sorbent surface) and 0.81 mol L^{-1} (saturated solution) were prepared.

In experiments with the HAP_0 sorbent, a suspension of HAP_0 (1 mL) and a solution of zirconium oxochloride with a necessary concentration (4 mL) were placed in each of eight 10-mL plastic bottles (SARSTEDT, Germany). The samples were stirred on a Multi Bio RS-24 rotor (Latvia), and at certain intervals the samples were centrifuged (5 min at 3000 *g*,) on an MLW T.51.1 centrifuge (German Democratic Republic), after which the mother liquor (1 mL) was sampled to determine the contents of zirconium and calcium ions.

When a HAP_T powder was used as the sorbent, HAP_T (0.088 g, amount of the solid phase of HAP in 1 mL of the suspension from the first series of experiments) was placed in eight 10-mL plastic bottles, and a solution (4 mL) with a chosen concentration of zirconium ions was added. Time counting and stirring were started at the moment of addition of a solution of zirconium oxochloride. After necessary time passed, the suspension was centrifuged for 3 min at 3000 *g*, the mother solution was separated from the precipitate, and the content of zirconium and calcium ions was measured as described above. Experiments were carried out room temperature (22±3 °C).

Sorption isotherm. The sorption isotherm of zirconium ions was obtained using HAP_T samples as the sorbents. The calculated amount of water, a precise volume of a solution of zirco-

Table 1. Scheme of the preparation of mixtures for the construction of the sorption isotherm of zirconium ions on HAP

m(HAP)/g	C_1[a]·10^3/mol L^{-1}	Volume/mL (C_2[b]/mol L^{-1})	
		Solution of Zr^{4+}	H_2O
0.088	0.1	5.0 ($1.1 \cdot 10^{-4}$)	0
	1	1.0 ($8.1 \cdot 10^{-3}$)	7.00
	8	0.1 (0.81)	9.90
	41	0.1 (0.81)	9.50
	81	1.0 (0.81)	9.00
	810	5.0 (0.81)	0
0.089	4	0.05 (0.81)	9.95
	16	0.2 (0.81)	9.80
	162	0.1 (0.81)	8.00

[a] Specified concentration of a solution of Zr^{4+}.
[b] Concentration of a solution of Zr^{4+} used in the indicated volume for the preparation of the mixture.

nium (of a required concentration), and a precise weighed sample of the HAP_T powder were placed in nine 10-mL plastic tubes, and time counting was started. The scheme of sample preparation for this experiment is presented in Table 1. Stirring on a rotor was performed for 30 min, then the phases were separated by centrifugation for 3 min (3000 *g*), the mother solution was separated from the precipitate, and the concentration of zirconium ions in the mother liquors was measured. Experiments were carried out at room temperature (22±3 °C).

Effect of the concentration and pH of a zirconium solution on the structure and morphology of the sorbent. Three experiments were carried out to determine the effect of the sorption interaction on the morphology and structure of the sorbent. In each experiment, multiply increased volumes of zirconium solutions with three concentrations used (40 mL each) were added to a suspension of HAP_0 (10 mL). The obtained mixtures (designated as Zr_1, Zr_2, and Zr_3 in order of decreasing concentrations of zirconium ions) were stirred for 1 h with pH-metric control. After the end of stirring, samples were taken from the mixtures to study by transmission electron microscopy (TEM). The remained mixture was centrifuged (as indicated above), the mother liquor was separated, and the precipitate was washed with distilled water and dried in a drying box at 70 °C to a constant weight for X-ray diffraction analysis (XRD).

Phase composition of samples obtained in all experiments was determined by XRD. XRD patterns were obtained on a DRON-3 automated X-ray diffractometer in a Bragg—Brentano mode with a graphite monochromator on a diffracted beam controlled by the EXPRESS computer program. The measurements were performed on a detector with the Co-Kα anode (radiation wavelength 0.179021 nm, step-to-step scan mode in the range 2θ = 10—80° with an increment of 0.1—0.05°). The exposure time per point was 3—5 s.

Morphology of nanoparticle samples was studied using high-resolution transmission electron microscopy (HR-TEM) on a Jeol JEM-2100 F microscope (Japan) with the possibility of local energy dispersive analysis. The samples were prepared by the deposition of a droplet of an aqueous suspension of the studied crystals, which was diluted with distilled water in a ratio of 40 : 1, onto a special copper lattice with the formvar film. Then the samples were dried in air and stored in special pencil-cases.

Results and Discussion

As shown by preliminary experiments on the sorption of zirconium ions on HAP_0 from a solution with a concentration of 0.14 mol L^{-1}, zirconium ions are instantly removed from the solution on contact with the sorbent. At the same time, when high concentrations (0.81 mol L^{-1}) are used, a suspension of HAP_0 is nearly completely dissolved to form a foreign solid phase. A solution of zirconium oxochloride with a solution of 0.81 mol L^{-1} has too low pH value, which results in the dissolution of a HAP suspension. A solution with a concentration of 0.14 mol L^{-1} has pH 2—3, which also results in a substantial damage of the sorbent. Only at low concentrations ($1.1 \cdot 10^{-4}$ mol L^{-1}) the pH of the solution turned out to be in an appropriate range of 3.5—6.0. Thus, it is impossible to use HAP_0 in the form of an aqueous suspension to study the sorption of zirconium ions at relatively high concentrations.

Sorption kinetics of zirconium ions on HAP_T from solutions with concentrations of 0.14 and $1.1 \cdot 10^{-4}$ mol L^{-1} is presented in Fig. 2. The degree of sorption characterizes the fraction of zirconium that passed to the solid phase to the introduced amount of zirconium. As expected, zirconium ions pass to the solid phase very rapidly, especially at low concentrations. According to the chemical analysis results, this process is accompanied by an intensive escape of calcium ions to the solution. From the formal point of view, the kinetic curves can be described by the Lagergren model of the pseudo-second order.[18] However, more or less reliable data on the rate constants were obtained only for sorption from a zirconium solution with the concentration 0.14 mol L^{-1}: k = 4.90 (g HAP) (mg Zr^{IV})$^{-1}$ min^{-1}, Γ_e = 0.68 (g Zr^{IV}) (g HAP)$^{-1}$, and R^2 = 0.98.

Sorption isotherm (Fig. 3) cannot be described in terms of the traditional Langmuir and Freundlich models. However, the isotherm shape and regularities of the be-

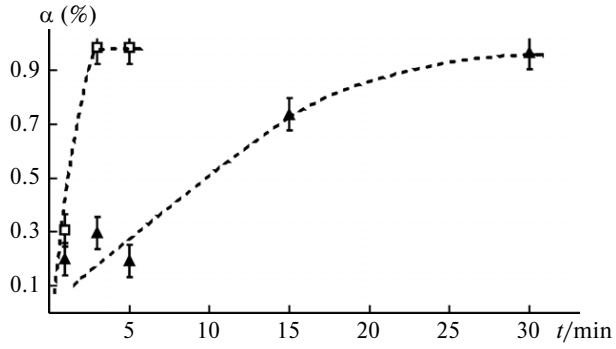

Fig. 2. Kinetics of Zr^{4+} sorption on HAP_T at the concentration of zirconium solutions $1.1 \cdot 10^{-4}$ (white squares) and 0.14 mol L^{-1} (black triangles); α is the degree of sorption.

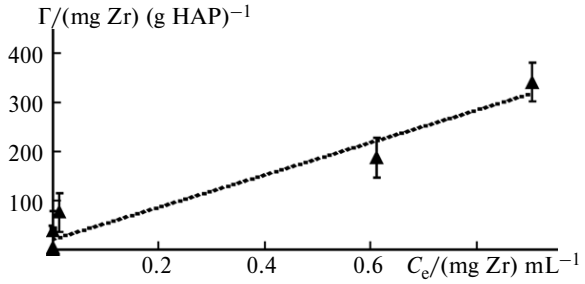

Fig. 3. Sorption "isotherms" of zirconium ions on HAP_T; C_e is the equilibrium concentration of Zr.

havior of Zr^{IV} resemble the sorption behavior of Bi^{3+} ions on contact with HAP of different types[16] where a chemical reaction between the components of the system was observed rather than sorption. Probably, the situation in the case of zirconium ions is similar, and the chemical reaction of zirconium oxochloride and HAP occurs instead of sorption. The reaction is accompanied by the dissolution of HAP and formation of zirconium phosphate. The XRD data of the Zr_{1-3} samples confirm the advanced assumption (Fig. 4). An amorphous phase is formed at high concentrations of zirconium, and only at the lowest concentration the XRD pattern of the sample (Zr_3) is close to that of HAP. The composition of the amorphous phases of the Zr_{1-2} samples cannot be determined from diffraction patterns, but they represent, most likely, either amorphous zirconium phosphate, or its hydroxo form. The HR-TEM results for the Zr_{1-3} samples confirmed this assumption. Figure 5 shows that Zr_1 and Zr_2 samples are in the amorphous phase indeed. In addition, the data of local energy dispersive analysis show that the Zr_1 sample contains no calcium (*i.e.*, this is amorphous zirconium phosphate). In the case of the Zr_2 sample, the peaks of calcium are observed, but the phase remains amorphous (possibly consisting of a mixture of amorphous calcium and zirconium phosphates or their mixed phosphate). Only the Zr_3 sample corresponds to HAP by morphology and qualitative composition.

No similar substantial changes in HAP are observed for the cocrystallization introduction (coprecipitation method) of zirconium. The synthesis gave a suspension of the HAP-Zr product with the solid phase content 5.4±0.4%. The chemical analysis of the mother liquor after the synthesis showed a higher content of calcium ions over the mother liquor of the HAP_0 suspension: HAP-Zr, $(7.9\pm0.2)\cdot10^{-3}$ mol; HAP_0, $(9.8\pm0.2)\cdot10^{-5}$ mol. Phosphate and zirconium ions were nearly absent. These data and gravimetric analysis results of the obtained thermal phase after the thermal treatment provide the following empirical formula: $Ca_{9.2}Zr_{0.4}(PO_4)_6(OH)_2\cdot$ $\cdot 0.76\ Zr(OH)_4\cdot 0.7\ H_2O$.

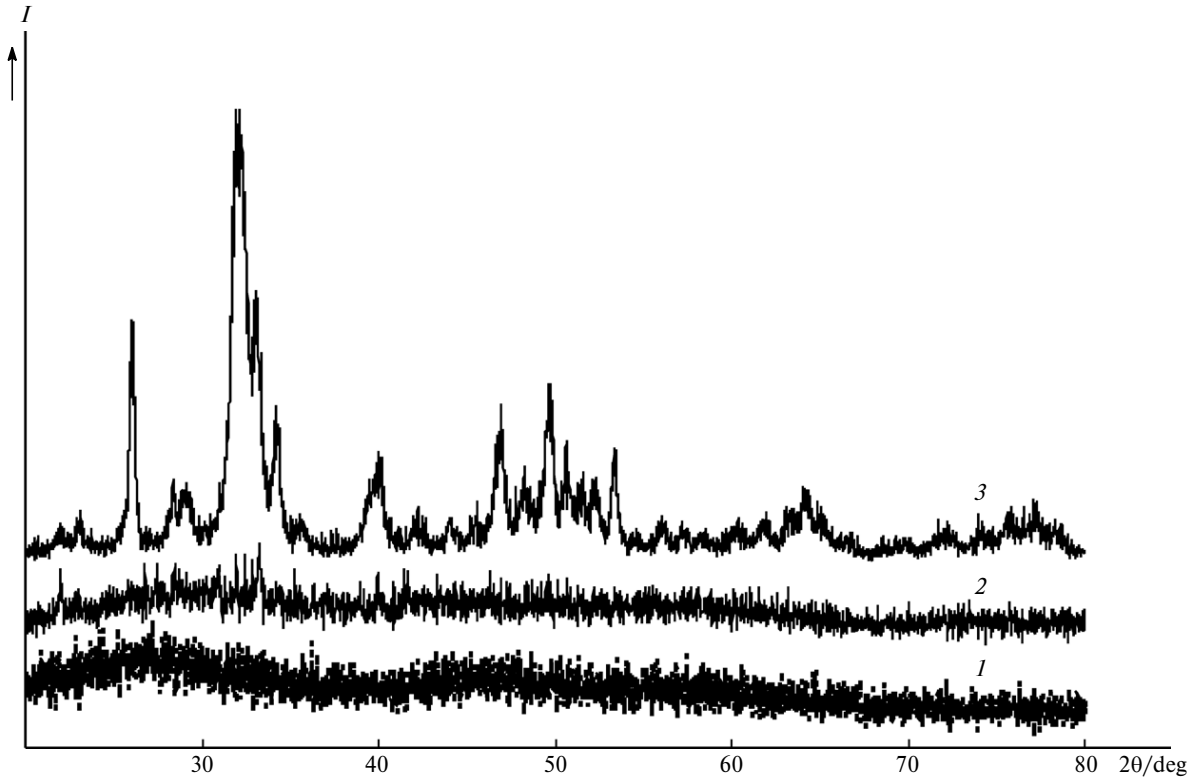

Fig. 4. XRD data for the solid phase samples after contact with HAP_0 for 1 h with solutions of Zr^{4+} of various concentrations: 0.81 (*1*), 0.14 (*2*), and $1.1\cdot10^{-4}$ mol L^{-1} (*3*).

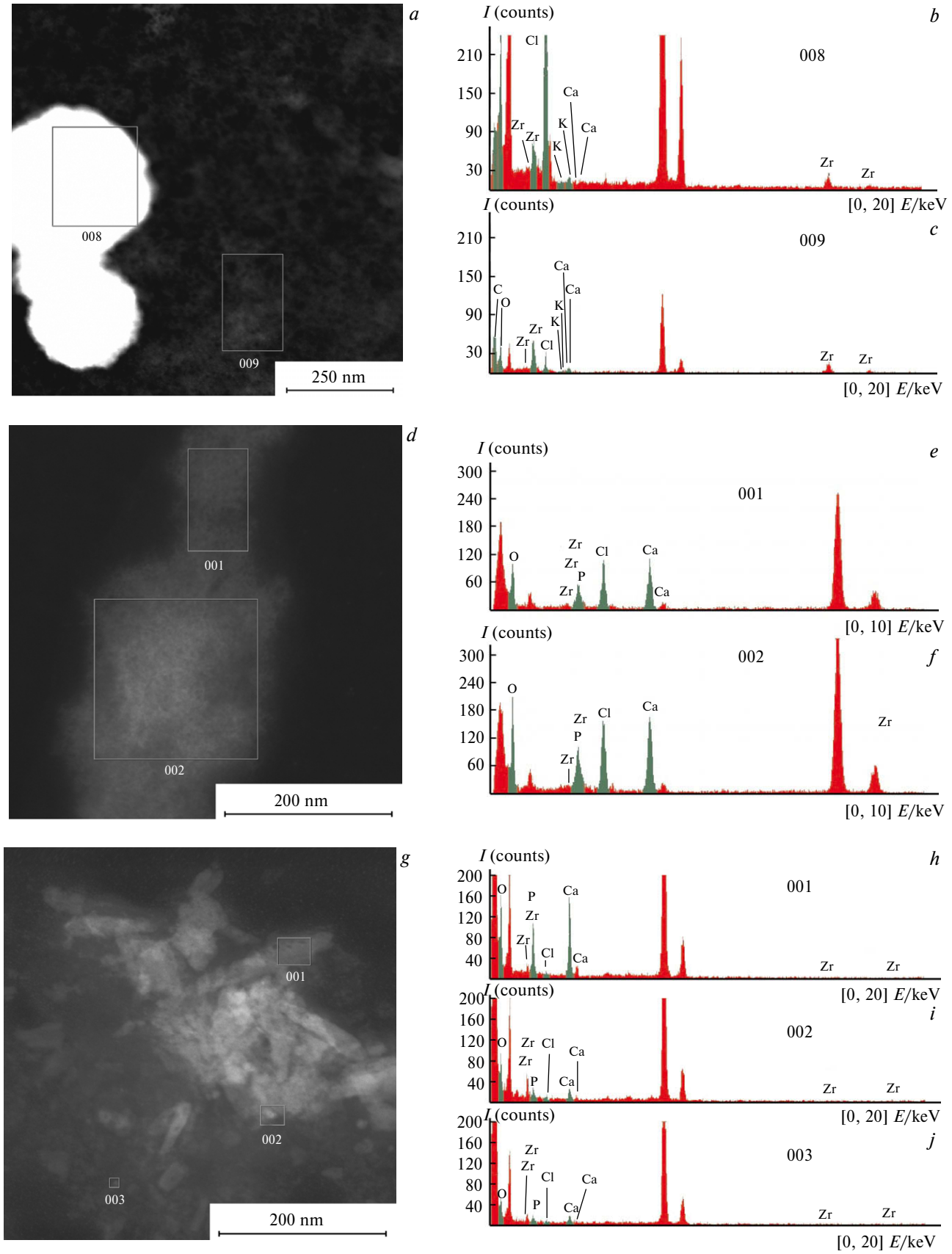

Fig. 5. HR-TEM results and content of elements (*I*) in the solid phase samples after contact with HAP_0 for 1 h with solutions of Zr^{4+} of various concentrations: 0.81 (*a*—*c*), 0.14 (*d*—*f*), and $1.1 \cdot 10^{-4}$ mol L^{-1} (*g*—*j*) (peaks without designations refer to Cu).

Only the main lines of HAP were detected by XRD (Fig. 6, *a*) on the diffraction patterns, and no reflections of foreign phases were revealed. In addition, no substantial shift of the main lines toward the lines of pure HAP was observed. This indicates that a minor amount of zirconium, which probably replaces calcium in the HAP structure, exerts a weak effect on the structure and morphology of the sample. The diffraction patterns showed no formation of zirconium hydroxide. Therefore, either this phase exists in a very low amount (and, hence, its reflections are disguised by the reflections from HAP), or the phase is uniformly distributed as nanoclusters over the surface of HAP particles. The latter is confirmed by the HR-TEM data by mapping the elements across the sample surface (Fig. 7). The reflections corresponding to the ZrO_2 phase formed upon the decomposition of zirconium hydroxide are more pronounced after the thermal treatment as indicated previously[19,20] (Fig. 6, *b*).

Thus, at the concentrations of zirconium ions in the solution higher than 10^{-4} mol L^{-1} no sorption was observed because of the chemical reaction that occurs with the dissolution of HAP formation of the amorphous phase

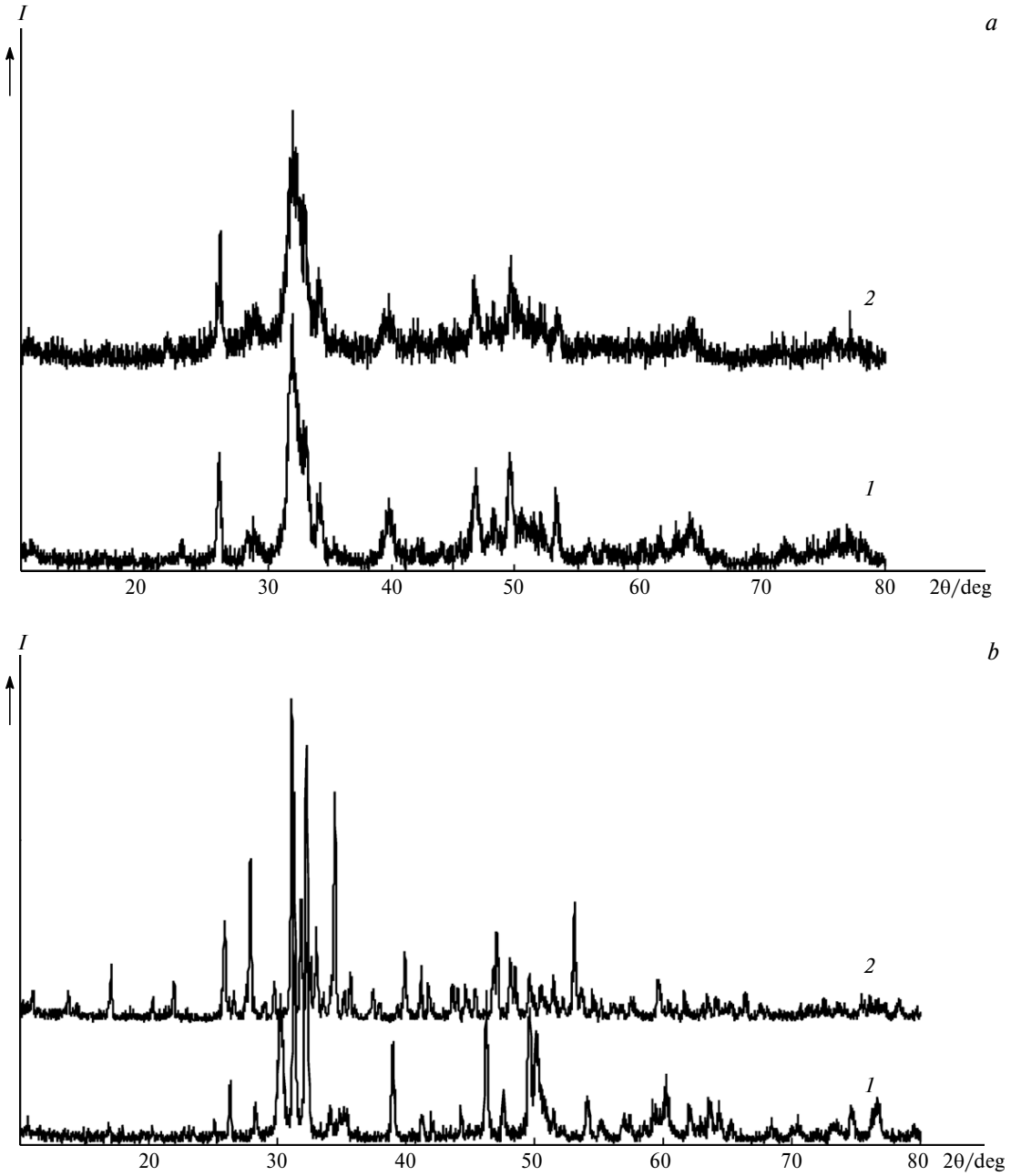

Fig. 6. XRD patterns of the HAP_0 (*1*) and HAP-Zr (*2*) samples after the synthesis (*a*) and high-temperature treatment (*b*).

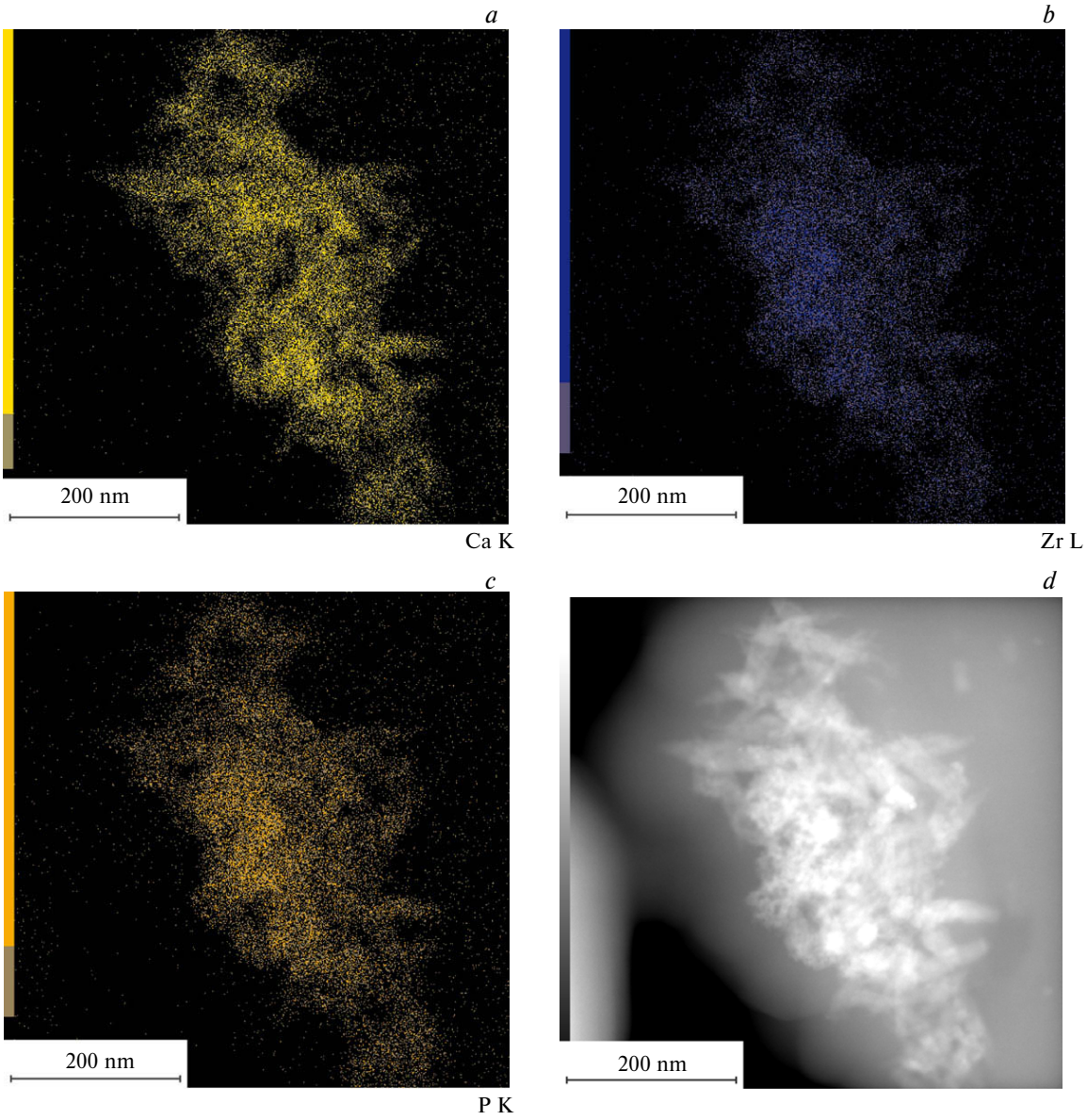

Fig. 7. HR-TEM results for the HAP-Zr sample: mapping of the region of the sample surface with respect to various elements: calcium (*a*), zirconium (*b*), and phosphorus (*c*) and initial processed image of the sample obtained by the coprecipitation method (*d*); K and L are lines of characteristic X-ray radiation of the elements.

of zirconium phosphate. At a concentration of 10^{-4} mol L^{-1}, in spite of zirconium sorption, the chemical reaction cannot be excluded and irreversible chemisorption should be considered. The question about the route *via* which the reaction proceeds (in the liquid phase through the dissolution of the sorbents or directly on the sorbent surface (topochemical reaction)) remains unanswered. However, it should be mentioned that a transparent mother liquor separated from the solid phase (after sorption on HAP_T) transformed into a non-transparent gel upon storage for 1—2 days. The study XRD and HR-TEM studies of this gel showed that the substance was analogous in properties to the Zr_2 sample, *i.e.*, is a mixture of amorphous calcium and zirconium phosphates. This fact indicates in favor of the variant of reaction occurrence *via* dissolution.

Therefore, when HAP is applied as a carrier of medical zirconium radionuclide (^{89}Zr), it is desirable to use the radionuclide without a "radiochemical carrier" (solution of the same compound but with the stable isotope) in the sorption method of introduction. The cocrystallization binding method can be used if the radionuclide with the carrier must be introduced in a significant concentration.

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