

Mesoporous iron-doped hydroxyapatite synthesized in the presence of acetone

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Synthesis of materials

The following reagents were used to synthesize the powders: acetone ($\text{C}_3\text{H}_6\text{O}$, analytical grade, Component-Reaktiv Ltd., Moscow, Russia), ammonium hydrophosphate ($(\text{NH}_4)_2\text{HPO}_4$, analytical grade, Himcity Ltd., Moscow, Russia), calcium hydroxide ($\text{Ca}(\text{OH})_2$, analytical grade, Labtech Ltd., Moscow, Russia), and iron chloride (FeCl_3 , analytical grade, Himcity Ltd., Moscow, Russia)

For the synthesis, solutions and suspensions were prepared based on mixtures of distilled water and acetone, mixed in a 1:1 ratio. The ammonium hydrophosphate solution was added dropwise to the mixture of calcium hydroxide and iron chloride under constant stirring under a glass overhead stirrer. After the reaction was complete, the precipitate was stirred for another hour. The pH value of the reaction mixture was maintained at a level of 11–12 due to alkalinity of $\text{Ca}(\text{OH})_2$. The powders were ripened in mother solutions at 37 °C for 21 days. The resulting powders were washed out in distilled water, filtered and dried at 60 °C for 24 hours.

To synthesize the 1FeHA sample, 36.5 g of calcium hydroxide was dissolved in a mixture of 200 ml of water and 200 ml of acetone. This mixture was placed under an overhead stirrer and stirred constantly. 0.9 g of FeCl_3 was dissolved in 10 ml of water and added to the $\text{Ca}(\text{OH})_2$ suspension. A solution of ammonium hydrophosphate was obtained by dissolving 39.5 g of the reagent in a mixture of 100 ml of water and 100 ml of acetone. This solution was then added dropwise to the iron chloride and calcium hydroxide mixture using a separatory funnel. The resulting product was 50 g of iron-substituted hydroxyapatite.

Materials and Methods

For the determination of the chemical composition of the obtained materials, powders were heat-treated at 900 °C for water removal, dissolved in $\text{HCl} - \text{HNO}_3$ mixture, and analyzed by atomic emission spectrometry with inductively coupled plasma by NCS TESTING TECHNOLOGY CO. Wavelengths of 393.366 nm for calcium, 214.914 nm for phosphorous, and 238.204 nm for iron were chosen for quantification.

The phase composition of the powders was characterized by the X-ray diffraction (XRD) method (Shimadzu XRD-6000, $\text{CuK}\alpha$ radiation) with identification according to ICDD and PCPDFWIN databases. Quantitative phase analysis and lattice parameter estimation based on the Rietveld method, as well as a calculation of crystallite size (D) according to the Scherrer equation, were performed applied the PHAN% software.^{S1}

Fourier-transform infrared spectroscopy (FTIR) absorption spectra of the synthesized powders were recorded in the range from 4000 to 400 cm^{-1} using the KBr method on a Thermo Nicolet Avatar 330 FTIR spectrometer.

The particle investigations were conducted by transmission electron microscopy (TEM) with X-ray energy dispersive spectroscopy (XEDS) in the mapping mode to establish the distribution of the elements in powders (JEOL JEM 2100 with Oxford Inca, carbon sputtered specimens, accelerating voltage of 200 kV, 1000 mm of the diffraction length, 25.1).

Zeta potential was measured on the standard suspensions of powders particles in distilled water, which were prepared at a concentration of 5 µg/10 ml using Photocor Compact – Z equipment (Tallin, Estonia). The suspensions were subjected to dispersion in an ultrasonic bath for 30 minutes to remove agglomerates and uniform distribution of particles in water. Then 500 µl of the suspension was placed in a measuring cuvette and the total volume was adjusted to 2 ml with distilled water.

The SSA according to Brunauer, Emmett, and Teller (BET), pore volume and pore size distributions were determined using the BJH analyzer model (Barrett, Joyner, and Halenda) of the materials were determined by low-temperature nitrogen adsorption measurements (Micromeritics TriStar analyzer).

Electron paramagnetic resonance (EPR) spectra were obtained in the X-band with an operating frequency of 9.6 GHz on a Bruker Elexsys E580 spectrometer. In steady state, the second modulation frequency was 100 kHz, the amplitude was 1 Hz, and the output power was 200 µW. In pulse mode, a Hahn sequence was used with pulses of 16 and 32 ns and an interval of 250 ns. A flow helium cryostat was used for low-temperature experiments.

Mössbauer studies of a series of HA samples with iron impurities at calcium positions were carried out in transmission geometry on a WissEl spectrometer operating in constant acceleration mode. A ⁵⁷Co(Rh) source with an activity of ~ 40 mCi was used. The spectrometer's velocity scale was calibrated using the spectrum of thin metallic iron foil. The spectra at low temperature were obtained in a CFICEV (Ice Oxford) flow cryostat. The experimental spectra were processed using the SpectrRelax program. The central shifts are given relative to the center of gravity of the metallic iron spectrum at room temperature.

Table S1 Chemical composition of as-synthesized powders with the theoretical formula $\text{Ca}_{(10-x)}\text{Fe}_{2x/3}(\text{PO}_4)_6(\text{OH})_2$.

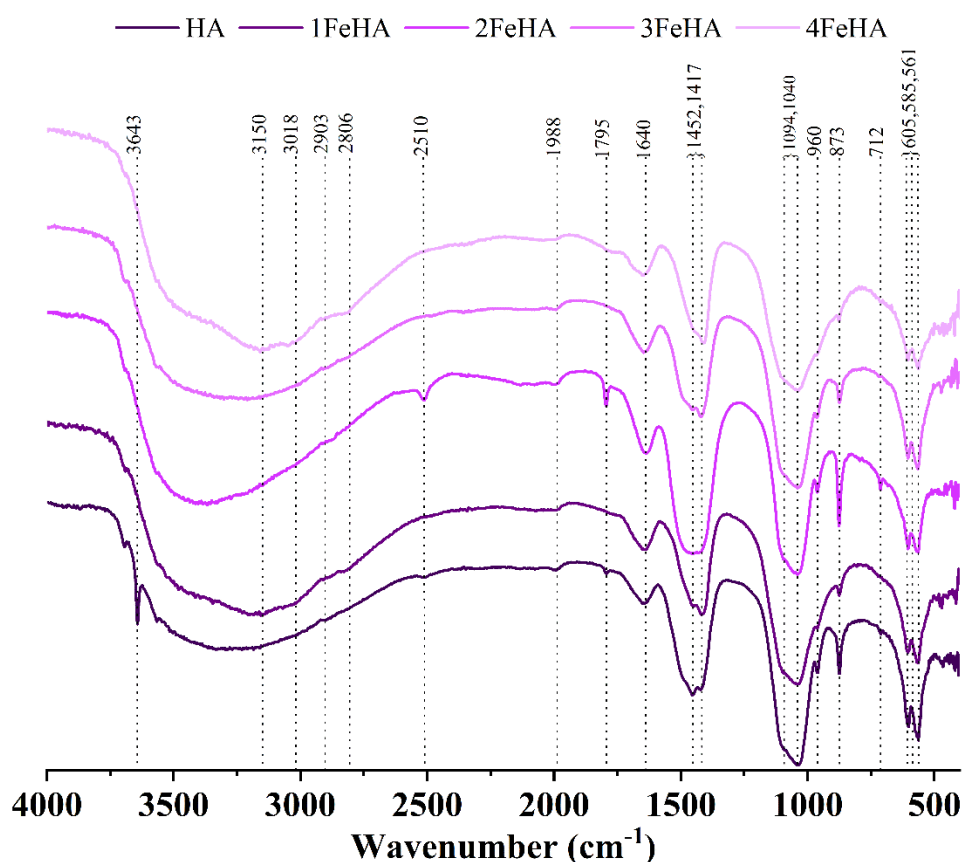
Sample	Value of x (theor.)	Ca- substitution by Fe (mol%)	Concentration (mol%)						(Ca+Fe)/P	
			Ca		P		Fe			
			Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.
HA	0	0	22.73	22.76	13.64	11.59	0	0	1.67	1.96
1FeHA	0.005	1	22.52	22.12	13.65	12.50	0.15	0.27	1.66	1.79
2FeHA	0.05	5	21.67	21.79	13.69	12.29	0.76	0.85	1.64	1.84
3FeHA	0.1	10	20.61	20.39	13.74	12.62	1.53	1.68	1.61	1.74
4FeHA	0.2	20	18.46	17.13	13.85	11.93	3.08	4.70	1.56	1.83

Table S2 Unit cell parameters and D of HA и FeHA.

Sample	a (nm)	c (nm)	c/a	V (nm ³)	D (nm)
Hydroxyapatite ICDD №09-0432	0.9418	0.6884	0.7309	0.5288	—
HA	0.9429	0.6889	0.7306	0.5304	13.4
1FeHA	0.9416	0.6882	0.7309	0.5284	12.3
2FeHA	0.9434	0.6881	0.7294	0.5303	8.0
3FeHA	0.9452	0.6881	0.7280	0.5324	6.5
4FeHA	0.9418	0.6888	0.7314	0.5291	5.9

As the Fe^{3+} content increased, the intensity of the peaks corresponding to the vibrations of hydroxyl groups in HA at 3643 and 633 cm^{-1} decreased, and the regions belonging to adsorbed water in the range 2900–3400 and 1640 cm^{-1} turned into a shoulder. At 873 cm^{-1} , peaks associated with HPO_4 vibrations were observed, decreasing in intensity (Figure S1).

Additional bands at 712 cm^{-1} and 2510 cm^{-1} for 3FeHA were observed for $\text{OCO}^{\text{S}2}$ and $\text{C=O}^{\text{S}3}$ respectively due to tiny CaCO_3 formation.^{S2} It is worth noting the absence of bands indicating Fe–O bonds characteristic of iron oxides, as the region of their appearance is in the wave number range below 400 cm^{-1} .^{S4}

**Figure S1** Full-range FTIR spectra of HA и FeHA samples.

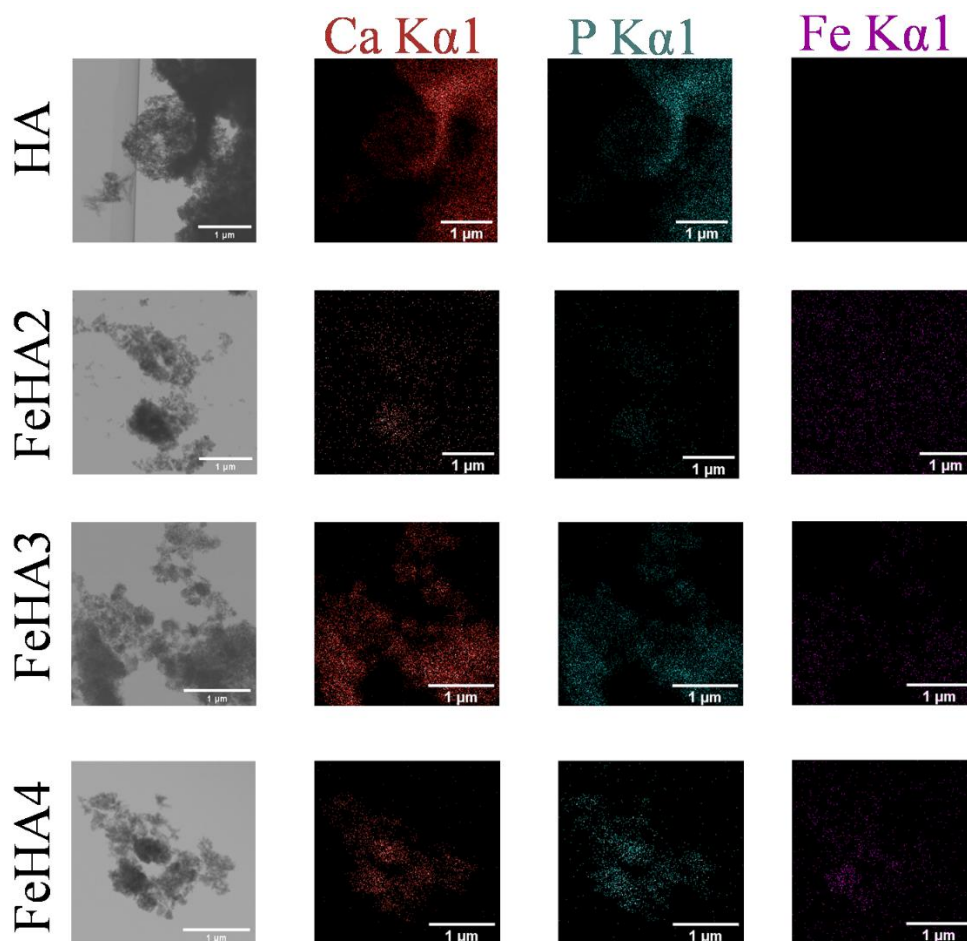


Figure S2 EDX analysis of samples.

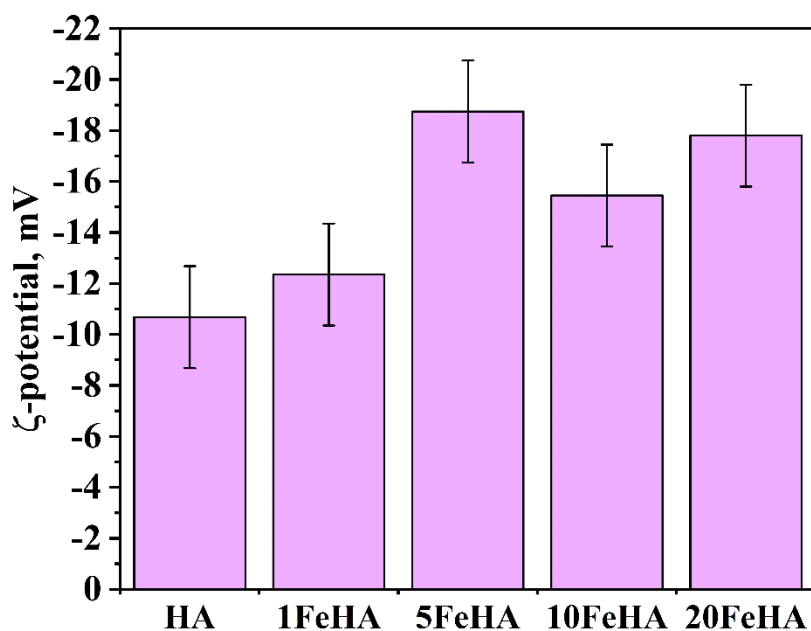


Figure S3 ζ-Potential measurements results.

Low temperatures were necessary due to the extremely short spin-phonon relaxation times T_1 arising from the interaction of Fe^{3+} with the vibrational modes of the local environment. The inset shows the modulation signals of the electron spin echo due to the anisotropic ultra-fine interaction of the electron spin of Fe^{3+} ions with the structural nuclei of hydrogen and the presence of forbidden transitions. The

resonance lines in the frequency domain are located at 14 MHz, which, at a certain gyromagnetic ratio and a fixed magnetic field of 340 mT, correspond to ^1H $I = 1$ nuclei with a natural prevalence of 99%. This fact indicates the localization of Fe^{3+} ions in the HA structure.

The Mössbauer spectra of the 4FeHA sample are presented below, with the spectra accumulated at two different temperatures: room temperature (a) and 80 K (b). It is evident that when the temperature is reduced to 80 K, there is an absence of qualitative alterations in the spectrum. The changes affected only the ultra-fine parameters of the components. It is noteworthy that the spectra do not exhibit any magnetically ordered components (sextets), including those associated with iron oxides such as hematite and magnetite.

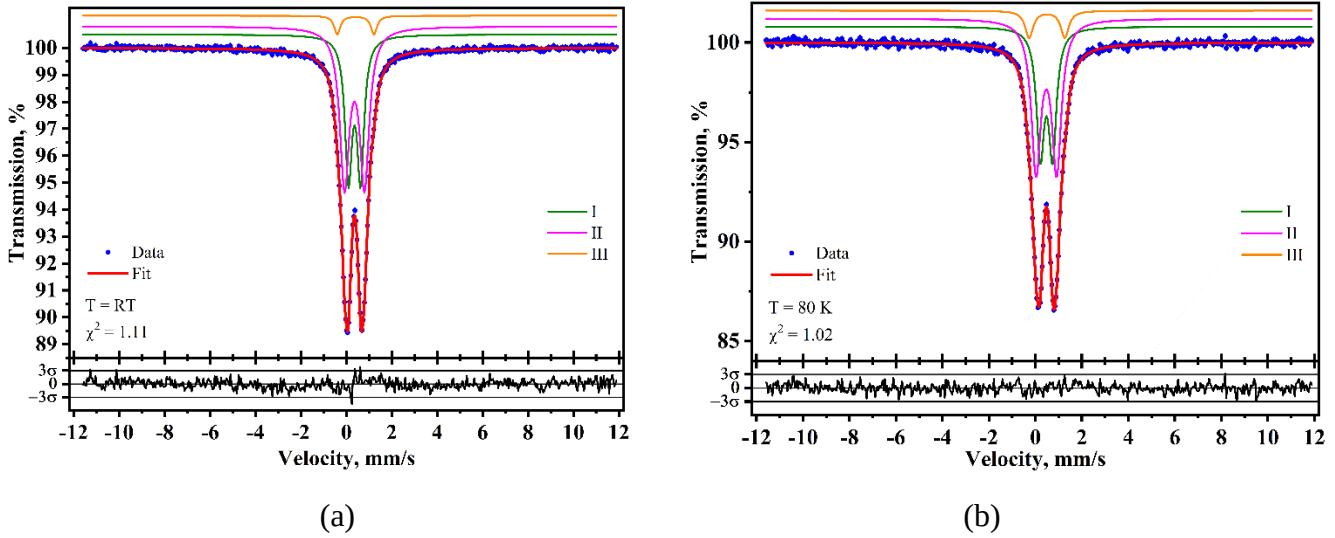


Figure S4 Mössbauer spectra of 4FeHA obtained at room temperature (a) and 80 K (b).

It is notable that the central shifts of the spectrum components exhibit an increase as the temperature decreases. These alterations are associated with the second-order Doppler effect, and the experimental dependencies are well described by the ratio obtained within the framework of the Debye model of solids.

$$CS(T) = \delta_0 - \frac{9k_B\Theta_D}{2cM} \left[\frac{1}{8} + \left(\frac{T}{\Theta_D} \right)^4 \int_0^{\frac{\Theta_D}{T}} \frac{x^3}{e^x - 1} dx \right],$$

where the first term, δ_0 is the isomeric shift determined by the electron density on the nucleus and is considered to be independent of temperature, and the second term is due to the second-order Doppler effect. In the following formula, T denotes the temperature, Θ_D is the Debye temperature, k_B Boltzmann's constant, M is the atomic mass, and c is the speed of light.

The quadrupole splittings of the two doublets demonstrate minimal sensitivity to temperature variations. This is an indication of trivalent iron ions, attributable to their electronic configuration. It has been determined that Fe^{3+} has five electrons in its outer shell, with these being distributed evenly among five $3d$ orbitals. It is important to note that the half-filled $3d$ shell is spherically symmetric and therefore does not create an electric field gradient on the nucleus. Consequently, the phenomenon of quadrupole splitting is predominantly governed by the lattice contribution, which exhibits a negligible temperature dependence.

References

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