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Microemulsion Method for Producing Hydroxyapatite

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Abstract—Hydroxyapatite nanoparticles were obtained in the course of reaction in microemulsion system stabilized by surfactant (sodium bis-2-ethylhexylsulfosuccinate). By the methods of X-ray phase analysis, IR spectroscopy, elemental analysis, and electron microscopy the presence of crystals in the samples of hydroxyapatite as the main phase, and detected the formation of additional calcium phosphate compounds was demonstrated.

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Calcium Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is the main inorganic component of the animal and human teeth and bones and is used in reconstructive surgery, dentistry, and is recommended as a component of toothpastes and gels, cosmetics, and hygiene means and food additives. Current active development of hydroxyapatite materials is directed to optimizing mechanical characteristics, improving biocompatibility, increasing bioactivity, etc. All properties of this promising material depend crucially on their microstructure. The control over porosity, size distribution, and morphology of individual grains or crystallites is extremely important for obtaining a material with desired properties. However, obtaining nanosized hydroxyapatite is rather difficult: Aggregates are often formed with clusters larger than 100 nm.

Therefore, among the many different ways of synthesis of hydroxyapatite, a specific one is the poorly investigated emulsion method, since it allows to reduce the particle size, to limit their growth, and to provide virtually nonhydrated disperse phase. Previously this method was used to produce semiconductors, catalysts, magnetic particles, and drugs [1].

The principle of the method consists in dissolving one reagent in water microdroplets of the "water in oil" microemulsion, and the second in the microdroplets of another "water in oil" microemulsion. Then the two microemulsions are mixed. Owing to the very small size, the droplets are involved in Brownian movement. They constantly suffer collisions and form dimers and other aggregates. These aggregates have short lifetime and undergo rapid decay into the drops with the initial size. As a result of continuously occurring processes of coalescence and spontaneous dispersion, the content of microdroplets is evenly distributed over all the drops, and reaction proceeds in the drops. Eventually, the reaction product is precipitated.

Microemulsion of "water in oil" type is prepared by using sodium bis-2-ethylhexylsulfosuccinate (commercial brand AOT) as this surfactant is stable in a wide range of compositions and consist of monodisperse droplets. The size of the droplets calculated from geometric considerations, is proportional to the molar ratio W = [water]/[surfactant]. This was confirmed experimentally by the light scattering methods [2].

The size of droplets in the first approximation can be calculated by the equation:

$$r = 0.18W + 1.5,$$

where r is hydrodynamic radius, nm. Thus, the droplet size can be varied by setting the value of W. It is assumed that isolated water drops control the growth of particles. In fact, the system is somewhat more complicated, because growth of the primary droplets induces secondary processes that affect the final size of the particles. Such processes include Ostwald riprning

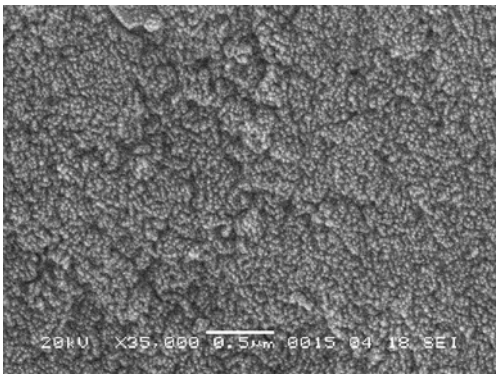

Fig. 1. Photomicrograph of the surface of the sample I. Tag 0.5 μm.

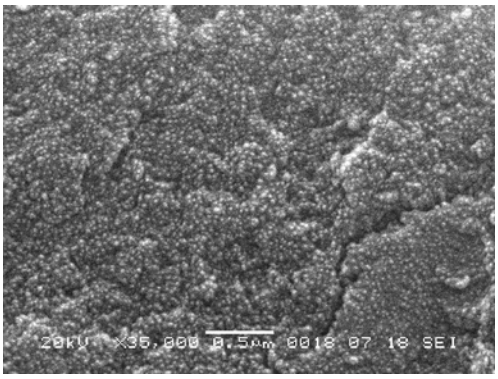

Fig. 2. Photomicrograph of the surface of the sample II. Tag 0.5 μm.

and flocculation. It was found in the study of similar processes [3] that the rate of nucleation and growth in the microemulsion medium is limited by the rate of interdrop exchange. For the microemulsions based on AOT at $W = 5$ the duration of formation of the nuclei is ~ 1 μs.

It should be noted that the particle size is usually much smaller than the size of droplets containing the particles. For example, in [2] the found value of the radius of microemulsion droplets is approximately tenfold larger than the radius of the obtained CdS particles.

Hydroxyapatite nanoarticles are formed as a result of the following reaction:

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca10(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O.$$

The main feature of this process consists in the contact between the two water-soluble salts at a short-term merging of the droplets. This is a very fast process with a second-order rate constant equal to 10^6–10^7 l mol^{-1} s^{-1}, and its value to some extent depends on the size of the droplets. Through a series of sequential processes stable nanoparticles of hydroxyapatite are formed. It is particularly important to obtain the hydroxyapatite particles of extremely small diameter, since this defines the properties of the materials. In accordance with the selected ratio W = [water]/[surfactant] the expected size of particles is less than 5 nm.

The X-ray patterns of these samples are diffuse and do not allow to analyze the phase composition. Preparation of micrographs is also difficult. Therefore further the samples were heated to 250°C and held at that temperature for 24 h (sample I) or 48 h (sample II). According to [4], in this temperature range there is a gradual enlargement of the particles without significant change in the phase composition. Figs. 1 and 2 depict the micrographs of samples I and II.

Earlier [5] by the methods of high-resolution transmission electron microscopy (HRTEM) and electron micro-diffraction (nano-diffraction) it has been shown that at moderate pH and temperature the deposited hydroxyapatite has crystalline structure regardless of the particles size. It was assumed in the early studies of the formation mechanism of hydroxyapatite that in the initial stage of the reaction amorphous phase is precipitated consisting of clusters that form the basis for the future crystal. The amorphous state of the material was substantiated by the lack of clear peaks on the X-ray photograph. However, further electron diffraction studies showed that the broadening of diffraction reflections is due to the small size of crystals. Furthermore, there is weakening and change in the relative intensity of the reflections at the scattering on very small particles. Thus, only a combination of research methods provides the correct interpretation of experimental data on the phase composition and morphology of the particles. In [5] only the use of electron probe with diameter 3.5–10 nm made it possible to identify the crystal structure of the nanoparticles of hydroxyapatite.

Our data confirmed the limitations of the method of X-ray diffraction in determining phase composition in the case of small objects.

A typical infrared spectrum of the samples (Fig. 3) is characterized by the bands of OH group vibrations (ν = 3606, 3380 cm^{-1}), stretching and bending vibrations of phosphate and carbonate groups. The difference in the IR spectra of the nanocrystalline

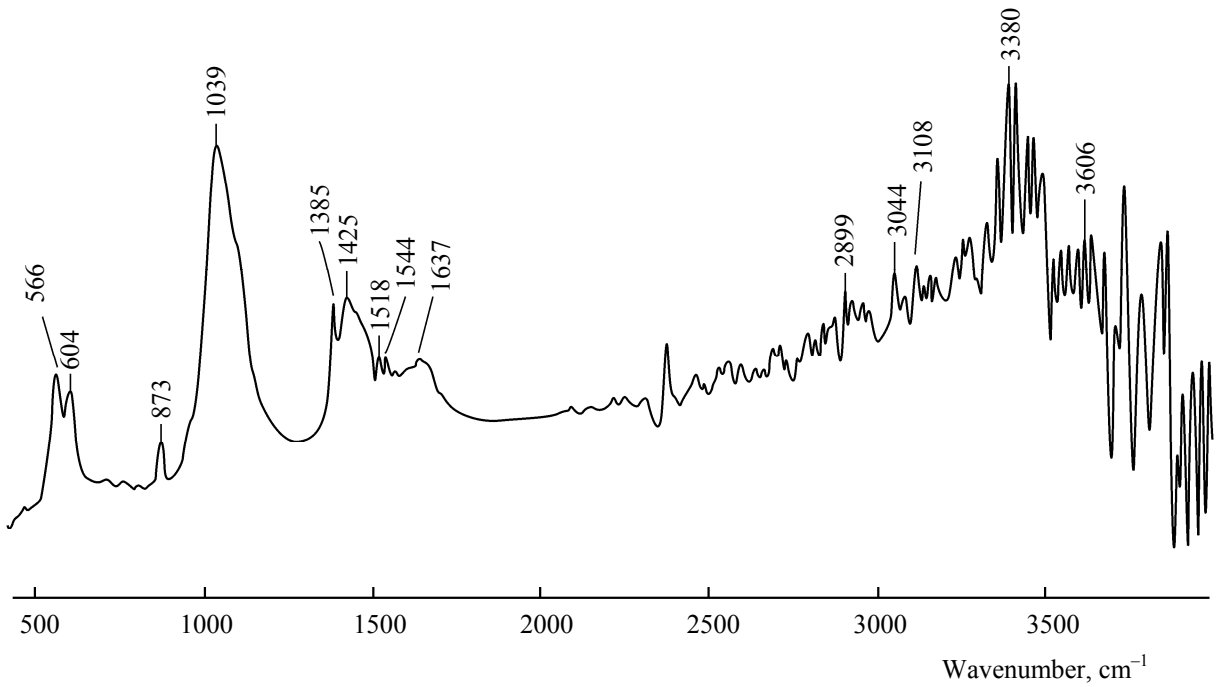

Fig. 3. IR absorption spectrum of hydroxyapatite.

hydroxyapatite and its finely crystalline counterpart is the most pronounced in the region of a triplet in the spectrum of fine crystalline hydroxyapatite (approximately 630, 600, 570 cm^{-1}), that is absent in the spectrum of nanocrystalline counterpart (in our case, 604 and 566 cm^{-1}) [6].

In the spectrum the bands at 1425 and 873 cm^{-1} are present corresponding to vibrations of carbonate groups and the bands of water stretching vibrations. A peak at 1039 cm^{-1}, which does not form clearly defined triplet, confirms the formation carbonato-hydroxyapatite [7]. Thus, all the studied hydroxyapatite samples contain some amount of CO_3^{2-} groups.

Elemental analysis showed that at the decrease in particle size a problem appears of the presence of additional calcium phosphate phases in the samples. The impurity content decreases with time. Perhaps one reason is the local variation of pH in the droplets of the microemulsion, as it is known that at lower pH increases a probability of formation of various calcium phosphates, with a molar ratio of Ca:P varied from 0.5 to 2.0 [5]. Deviation of this ratio from the ideal value of 1.67 to larger or smaller side is dependent also on the order of mixing the reagents. Adding calcium precursor to phosphate leads basically to the formation of the calcium-deficient phase (Ca:P ≈ 1.5), while adding phosphate emulsion to the calcium containing emulsion, on the contrary, results in enrichment with calcium (Ca:P = 2.0). Also interesting is the fact that tetracalcium phosphate $Ca_4(PO_4)_2O$, which is structurally similar to hydroxyapatite, is formed in non-aqueous media or at low pressure of steam, typically at the temperatures above 1300°C. In our case, all samples obtained by adding phosphorus precursor to calcium and aged less than 5 days contained a certain amount of this phase.

At the increase in the surfactant concentration formed an elastic material that included nanosized particles of hydroxyapatite. A layer of such material applied in a gel-like state on the surface of titanium fixed well owing to adhesive properties of sodium ethylhexylsulfosuccinate. As the sodium ethylhexylsulfosuccinate is used in cosmetics and is considered to be a non-toxic surfactant, this method can be offered for coating. In this case, microemulsions based on toluene are more convenient than based on octane.

Thus, emulsion synthesis method allows the preparation of nanoparticles of hydroxyapatite, but requires additional time for “maturing” the crystals. While in the aquatic environment the hydroxyapatite precipitate can be obtained in a few hours after dropwise adding phosphorous precursor to calcium one [8], the emulsion synthesis under our conditions requires more than 8 days. Improvement of the method

probably consists in use of another methods of mixing the reagents, separation and drying the formed crystals.

EXPERIMENTAL

Electron microscopy was carried out on a JSM-6380 LV instrument (Japan), elemental analysis was performed using an "Energy-250" setup.

The IR spectra were recorded in the range of 4000–400 cm^{-1} on IR spectrometers Infralyum FT-02 and Specord-IR-75, the samples were pressed into pellets with KBr. The diffraction patterns were obtained on DRON-3M and DRON-7 devices, radiation CuK_α, scanning rate 2 deg min^{-1}.

As surfactant for emulsifying sodium bis-2-ethylhexylsulfosuccinate, 96% (Acros) was used, as the nonpolar (oil) phase served octane ("chemically pure") or toluene ("chemically pure"), as the polar phase was used distilled water.

The first emulsion was prepared from organic solvent and aqueous 0.1 M calcium nitrate solution (Schering-Kahlbaum, Germany), brought to pH = 8 with aqueous ammonia. The value for W = [water]/[surfactant] was varied from 10 to 20. The temperature was maintained constant (20°C).

The second emulsion was prepared from organic solvent and aqueous 0.06 M solution of ammonium hydrophosphate (Merck, Germany), with the same solvent, stabilizer, W ratio and the temperature as for preparing the first emulsion.

Then 50 ml of second emulsion was added dropwise to 50 ml of the first one. Under such conditions, the Ca:P ratio corresponded exactly to the stoichiometriy (1.67).

The solution was stirred with a magnetic stirrer for 24 h. The precipitates formed were dried in air or in a temperature-controlled unit at 250°C.

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