Influence of heating time and microwave radiation power on the microstructure and phase composition of calcium-phosphorus compounds during formation

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Abstract. This work is devoted to experimental methods for producing calcium-phosphorus compounds, including calcium hydroxyapatite, using microwave radiation. Two types of synthesis were used: template method and chemical precipitation. During synthesis, the heating time and power were changed. The obtained samples were studied using scanning electron microscopy, X-ray diffraction analysis, and the BET (Brunauer–Emmett–Teller) sorption method.

1. Introduction
Calcium hydroxyapatite ((Ca₁₀(PO₄)₆(OH)₂, HAp) is an inorganic material that is the main component of human bone tissue. Natural HAp makes up about 50% of the total bone mass and almost 96% of the human enamel.

Important features of hydroxyapatite are deviations from the ideal stoichiometric ratio Ca/P=1.67 and variable composition. Therefore, the chemical-physical and chemical-biological properties of hydroxyapatite are able to vary depending on the method of preparation. Accordingly, a relevant task is to study methods for obtaining and modifying HAp with different properties for certain applications in different fields of science and medicine.

The main methods for obtaining hydroxyapatite powders include chemical precipitation from solutions, hydrothermal synthesis, sol-gel method, ultrasonic synthesis, microemulsions method, solid-phase methods, and template synthesis [1]. The aim of the work was to study the features of template synthesis and chemical precipitation of calcium hydroxyapatite using a microwave oven.

2. Experiment
In this study two methods of obtaining HAp using microwave radiation with the same precursors were selected: template synthesis [2] and chemical precipitation method [3].
In the template synthesis, according to [2], calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·4H$_2$O) and orthophosphoric acid (H$_3$PO$_4$) were used as precursors of calcium and phosphorus. The eggshell membrane (ESM) was separated from the eggshell by dipping the broken eggshell in a 2M solution of hydrochloric acid HCl. Then the ESM was purified with distilled water, then it was impregnated with phosphate and calcium ions, submerged in a 0.6 M solution of phosphoric acid for 24 hours. Then solution of 1M of the calcium precursor was prepared by dissolving the required amount of calcium nitrate hexahydrate in distilled water. It was added while continuously stirring on a magnetic stirrer at a speed of 1000 rpm. ESM was kept in solution for 24 hours, and then dried at room temperature for 24 hours. ESM loaded with precursors was calcined in a microwave oven, varying the power modes.

In the synthesis by chemical precipitation of hydroxyapatite powders, 0.5 M of calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·4H$_2$O) and 0.3 M of orthophosphoric acid (H$_3$PO$_4$) were used. Distilled water was used as a solvent. The solutions were mixed on a magnetic stirrer for 10 minutes at a speed of 500 rpm with the addition of ammonia water to achieve pH=10. Then the solution was placed for heating in a microwave oven at a power of 700 W.

SEM images were obtained using a scanning electron microscope TESCAN S9000G (accelerating voltage 5 kV, beam current 30 pA). Before examining the samples, a thin layer of carbon was sprayed on their surface.

The phase composition of the synthesized samples was studied using a Rigaku SmartLab (CuKα) diffractometric complex. The diffraction patterns were recorded in the quasi-parallel beam mode in the range of angles 2θ = 10÷80° with a step Δ(2θ) = 0.02°.

The specific surface area was measured using the SorbiMS equipment (CJSC “META”, Novosibirsk, Russia) based on the analysis of high-purity nitrogen adsorption isotherms [4, 5].

3. Results and discussion

Table 1 shows the modes of microwave influence on the membrane loaded with precursors during the template synthesis of hydroxyapatite (series M$_01$–04).

**Table 1. M-series (membranes)**

| Sample number | The power of microwaves, W | Time, minutes |
|---------------|-----------------------------|---------------|
| M$_01$        | without affecting           |               |
| M$_02$        | 140                         | 5             |
| M$_03$        | 616                         | 5             |
| M$_04$        | 700                         | 5             |

When synthesizing materials by chemical precipitation, the obtained powder was divided into two parts and placed in a muffle furnace for 1.5 (table 2, P$_01$) and 3 (table 2, P$_02$) hours.

**Table 2. P-series (powders)**

| Sample number | Impact in the muffle furnace |
|---------------|------------------------------|
| P$_01$        | 500 °C, 90 minutes           |
| P$_02$        | 500 °C, 180 minutes          |
| P$_02$ (resynthesis) | 500 °C, 180 minutes |

The sample P$_02$ (resynthesis) was synthesized under conditions similar to the preparation of sample P$_02$ in large volumes to verify the reproducibility of the results.

Figures 1–4 show X-ray diffractograms obtained during the study of the samples M$_01$-04. The numbers 01-070-0359 and 00-009-0077 are the card numbers in the ICCD (The International Center for Diffraction Data) database. We used this database for phase identification.
Since the samples obtained by the template synthesis are films with an uneven surface, the crystalline phases were identified based mainly on the position of the Bragg’s peaks, and not on the ratio of their intensity. From the diffraction patterns it is seen that the crystalline phase in the samples M_01-M_03 is a mixture of brushite and monetite. The presence of high brushite peak (020) on the diffraction patterns for these samples indicates the predominance of this phase. In the sample M_04, the Bragg’s peaks corresponding to the brushite phase are practically absent; the X-ray diffraction pattern can be identified as the diffraction pattern of monetite.

Figures 5-6 show the results of the study of the phase composition of powders obtained by chemical precipitation from solutions.
The X-ray diffraction pattern of sample P_01 is not shown, since we assumed the presence of peaks on it, similar to the diffraction pattern of sample P_02 according to [3].

Figures 5 and 6 show that the P_02 sample contains peaks corresponding to four phases: calcium hydroxyapatite (mainly), calcite, calcium hydrochloride, and calcium oxide (in very small amounts).

Using the Scherrer method, the average crystallite sizes in the samples were estimated. According to Scherrer’s formula, the average crystallite size $d$:

$$d = \frac{0.9 \cdot \lambda}{\beta \cos(\theta)}$$

where $\lambda = 0.154$ nm is the X-ray wavelength; $\beta$ is the width of the Bragg’s peak at half maximum; $\theta$ is the angle of incidence of X-ray radiation.

To estimate the average crystallite size, we used the most intense Bragg’s peaks. Tables 3 and 4 show the obtained $d$ values determined for various crystalline phases.

**Table 3. $d$ values for M-series**

| Sample number | Brushite, nm | Monetite, nm |
|---------------|--------------|--------------|
| M_01          | 46±3         | 44±8         |
| M_02          | 46±2         | 30±3         |
| M_03          | 40±4         | 24±5         |
| M_04          | -            | 26±3         |

**Table 4. $d$ values for P-Series**

| Sample number | Calcium Hydroxide, nm | Calcium Oxide, nm | Hydroxylapatite, nm | Calcite, nm |
|---------------|-----------------------|-------------------|---------------------|-------------|
| P_02          | 18±2                  | 33±4              | 33±2                | 33±2        |
| P_02 (resynthesis) | 30±8                  | 21±7              | 30±4                | 22±4        |

It is known that broadening of Bragg’s peaks can also be affected by mechanical stresses in the structure. In this case, the peak broadening depending on the angle would change according to the function $tg(\theta)$. In the present work, we assumed that the broadening of the Bragg’s peaks is caused not by mechanical stresses, but by the size effect.

Figures 7-10 show SEM images of the samples obtained during template synthesis after exposure to microwave radiation of different power. The arrows in the figures show calcium-phosphorus phases.

**Figure 7.** SEM images of the sample M_01, scanning field: a) 50x50 µm; b) 28.1x28.1 µm
Figure 8. SEM images of the sample M_02, scanning field: a) 30.8x30.8 µm; b) 20.3x20.3 µm

Figure 9. SEM images of the sample M_03, scanning field: a) 50x50 µm; b) 10x10 µm

Figure 10. SEM images of the sample M_04, scanning field: a) 100x100 µm; b) 50x50 µm

Figures 11-12 show SEM images of structures obtained as a result of hydrochemical deposition (P_01 and P_02).
In figures 7-10 it can be seen that there are large spherical particles attached to the plexus of the egg membrane – this is part of the membrane itself according to [2]. However, there are spherical particles much smaller than membrane particles with a diameter of 60-340 nm, which are calcium-phosphate phases. There are also crystal structures that are either the remains of the initial reagents, or, as the results of X-ray analysis show, the monetite phase.

Figures 11-12 show that hydrochemical synthesis and subsequent heat treatment resulted in formation of nanorods with a diameter of 70-100 nm and a length of 100 to 500 nm. Nanorods are combined into agglomerates, the average size of which is units of micrometers.

Such sizes of nanorods agglomerates are consistent with the results of measuring the specific surface area of powders obtained by hydrochemical deposition. Studies of the specific surface area of powders, depending on the heat treatment conditions, showed a slight decrease in the specific surface area after two hours of annealing (from 10 to 7 m²/g), which may indicate a slight increase in the particle size as a result of sintering.

4. Conclusions

It was found that the microstructure and phase composition of the formed materials are significantly influenced by microwave modes when obtaining calcium-phosphate compounds using the template method. In the samples obtained by this method the phases of monetite and brushite (or a mixture of them) were revealed mainly. In the case when the power of microwave radiation was 700 W (with equal values of the exposure time), the X-ray diffraction pattern can be identified as a diffraction pattern of monetite only. It is also possible that when using this synthesis method, hydroxyapatite inclusions were present in an amorphous state.
In the samples obtained by chemical precipitation, hydroxyapatite makes up most of the crystalline phase. Thus, hydroxyapatite crystals were not found in template samples, but were found in the samples obtained by the chemical method. This method is comparatively simple and allows one to get calcium hydroxyapatite in powder form in large quantities.

References
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