

Influence of synthesis conditions on the crystallinity of hydroxyapatite obtained by chemical deposition

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Abstract. The hydroxyapatite synthesis on a variety of substrates under various conditions was studied. It was shown that the increase in the temperature of the reaction medium increases the amount of nanocrystalline phase with an average crystallite size of 25 nm. Studies revealed that in addition to the pure hydroxyapatite, β -Ca₃(PO₄)₂ along with calcium carbonates and carbonate-substituted hydroxyapatites were formed. A significant increase in phase crystallinity during the heating of reactants up to the reaction temperature was shown.

1. Introduction

Calcium phosphates are widely used as an artificial material to treat or replacement the bone since they reduce reactions of inflammation and rejection of the implant, and are involved in the processes of calcium metabolism and osteogenesis [1,2,3]. Besides, hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂, HA) and tricalcium phosphate (Ca₃(PO₄)₂) are successfully used as the components of surface coatings of metal implants [2,3,4].

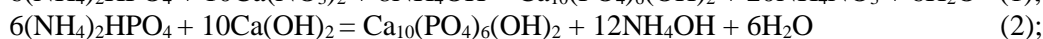
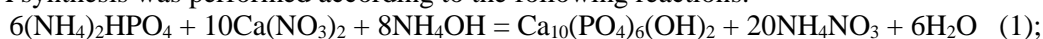
As a bone component, HA generates a crystalline acicular structure and forms a scaffold [5,6]. Therefore, depending on the functionality the implants must include HA in a designated form to provide bone restoration or integration [3, 5].

The crystalline HA is usually obtained by the methods of liquid or solid-phase synthesis. The latter is carried out at high a temperature that leads to a reduction in defect structure of crystal lattice, particle growth and a decrease of the specific surface of a powder. This makes the powder not suitable for bulk implants obtaining [7,8]. In addition, the products of solid-phase synthesis usually have chemical and mineralogical inhomogeneity, along with chemical inertness. The liquid-phase synthesis on the contrary, provides product with an inherent chemical homogeneity. The dispersity of a product is determined by the concentration of precursors; fine-crystalline product is produced from precursors with lower concentration [11]. Furthermore, there is a possibility to control the phase composition and the crystallinity of the product. [9, 10].

The aim of this study is to determine the liquid phase synthesis conditions to obtain fine-crystalline calcium phosphates.

2. Materials and research techniques

HA synthesis was performed according to the following reactions:



The precursors with a high purity were used. Solutions were prepared by degassed boiling of distillate with a concentration ratio $[Ca^{2+}]/[PO_4^{3-}] = 0.5M/0.3M$ and $pH = 8.73$. The value of pH was controlled by electronic pH-analyzer Mettler Toledo S700 with a gel electrolyte.

Details of synthesis conditions are shown in Table 1. Samples 1-1, 1-2, 1-3, 1-4, 1-5 and 1-6 were obtained by reaction (1), samples 2-1, 2-2, 2-3, 2-4, 2-5, 2-6 were obtained by the reaction (2). A part of the reaction products were soaked in the mother liquor (1-3, 2-3, 2-5, 2-6) for fixed time. All products were separated of the mother liquor, then washed in the distillate water at least 4 times until neutral pH was reached, then were centrifuged, dried and dehydrated at 105 °C, except the sample 1-6.

The synthesis temperature has been defined by calculation of Gibbs free energy and provided shift the equilibrium toward the reaction product. After drying, the samples were subjected to XRD studies performed on a DRON-3M diffractometer ($Cu_{K\alpha}$ - radiation, wavelength 1.54056 Å) with the following scanning parameters: accelerating voltage 90 kV, beam current of 30 mA, scan step of 0.03 ° in the 10-70 ° 2 θ range, preset time of 1s. The degree of the crystallinity of samples was calculated using the software packages POWDERCELL 2.4 and Crystallographica Search-Match. As a reference, HAP 2007 ICDD №01-074-0565 was used, it had X-ray reflexes obtained under similar scanning conditions [12]. Morphology of the powders was studied by scanning electron microscopy (SEM) JEOL JSM 6000 with its attachment that was used for elemental analysis. Infrared (IR) spectra of the powders were recorded on a Nicolet 5700 device.

Table 1. Synthesis conditions for HA obtained.

Sample	Type of mixing	Heating	Reaction temperature, °C	ΔG , kJ	Curing in the mother liquor		The calcination temperature, °C
					soaking, days	Temperature, °C	
1-1	dripping	resistance	60	-1134	-	-	-
1-2	draining	resistance	60	-1134	-	-	-
1-3	dripping	microwave	60	-1134	-	-	-
1-4	draining	resistance	100	-3381	-	-	-
1-5	draining	resistance	100	-3381	1	25	-
1-6	draining	resistance	60	-1134	-	-	400
2-1	draining	resistance	100	-824	-	-	-
2-2	draining	resistance	100	-824	-	-	800
2-3	draining	resistance	100	-824	1	37	-
2-4	draining	resistance	100	-824	1	37	800
2-5	draining	resistance	100	-824	14	37	-
2-6	draining	resistance	100	-824	14	37	800

3. Results and discussion

3.1. XRD analysis

The X-ray diffraction patterns of the reaction (1) products and reference sample are shown in Figure 1. All of the samples are presented of HA.

The average size of crystallites is determined using by characteristic HAP reflex (002) according to the Scherrer formula (data are presented in Table 2):

$$L = k\lambda / \beta \cos \theta$$

where L means the average crystallite size, k – constant close to unity, λ – X-ray wavelength ($Cu_{K\alpha} = 1.54056$ Å), β – broadening of the X-ray reflex $\beta = (B^2 - b^2)^{1/2}$, where B – full-width at half-maximum (FWHM) of the (002) diffraction line for the sample, b – FWHM of the diffraction line of the reference sample, θ – Bragg's diffraction angle for (002) line [11]. As one can see from the data above, the highest concentration of HA in a product, namely 95%, was reached by drip mixing along

with synthesis at 60° C (sample 1-1); the least concentration of 52% (sample 1-2) was obtained by pouring together precursors and maintaining the temperature of 60° C; respectively sizes crystals were 40 and 35 nm.

Table 2. The average size of the crystallites of synthesis products

Sample	1-1	1-2	1-3	1-4	1-5	1-6	2-1	2-2	2-3	2-4	2-5	2-6	ICDD 2007 01- 074- 0565
Crystallite size, nm	~40	~35	~37	~35	~30	~24	~37	~40	~35	~40	~20	~27	~14
HA content wt. %	95	52	58	68	70	70	68	90	70	90	70	90	99

Complete pouring together of heated precursor and reacting at 100° C resulted in a formation of fine-grained product with a crystallite size of 24 nm (1-6); wherein the HA content of 70% was achieved when the additional heat treatment of the precipitate at 400° C was performed. Maturing precipitate in the mother liquor for one day at room temperature does not give the same effect as heating for the sample 1-5 [13]. The use of microwave heating was not conducive to the increase of the yield of HA or the decrease of the crystallite size. Obtained data are explained by the Onsager law, namely by tensor direction of the reaction toward the higher temperatures, and by the fact that product (precipitation) is fast extracted from the reaction zone.

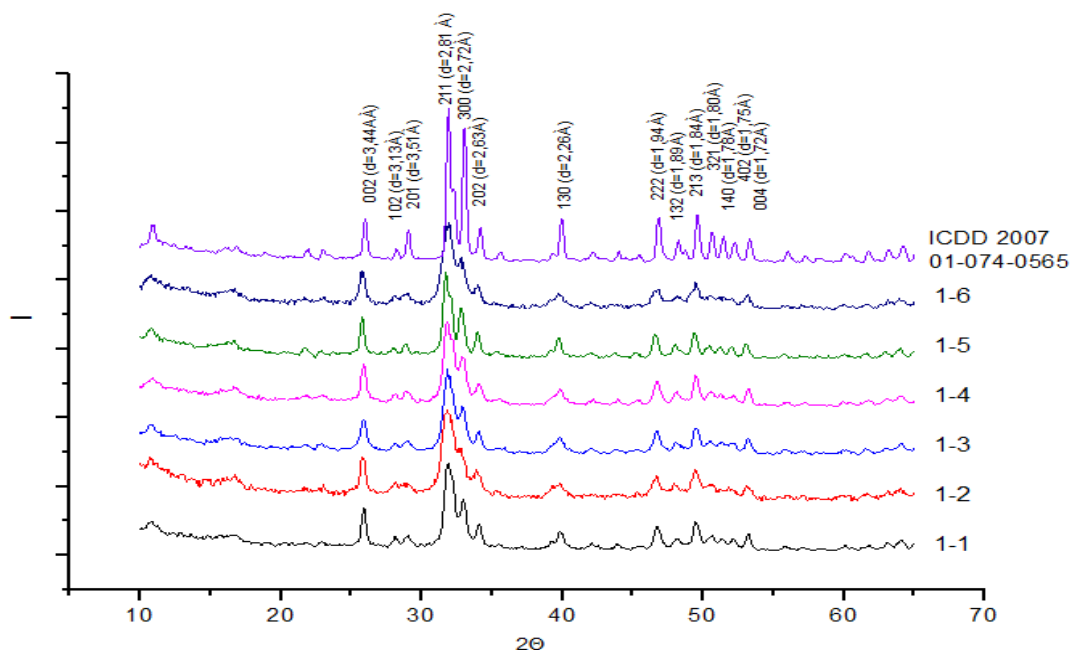


Figure 1. X-ray diffraction patterns of the reaction (1) products and of reference sample.

As shown in Figure 2, the powders obtained by the reaction (2) consist of HA mainly. The average crystallite size in the samples 2-1, 2-3, 2-5, obtained at 37° C is respectively 37 nm, 35 nm, and 20 nm. At that 2-3, 2-5 were kept for 1 day and two weeks respectively in the mother liquor. All these X-ray diffraction patterns indicated comparatively low intensity of the reflexes. However, after the calcination at 800 ° C, the crystalline phase content of the HA increased up to 90%.

Thus, the increase of the degree of product crystallinity is observed when preheated precursors are mixed at 100° C and precipitation is kept in the mother liquor for more than a day. Rapid removal of the product from the solution entails to an increase of the portion of the fine-crystalline phase.

Comparison of the thermodynamic conditions of the (1) and (2) reactions demonstrates that the decrease in Gibbs free energy effects positively on increase of the portion in the product of HA with the smallest crystallite size (1-4, 1-5, 1-6, 2-5).

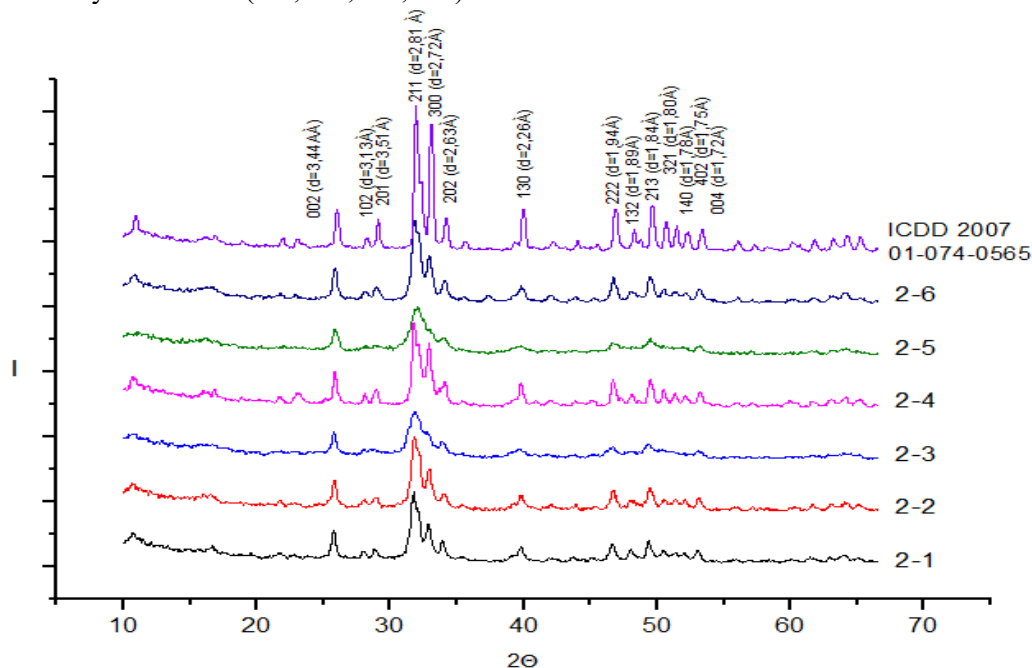


Figure 2. X-ray diffraction patterns of the reaction (2) products and of reference sample.

3.2. Analysis of IR spectra

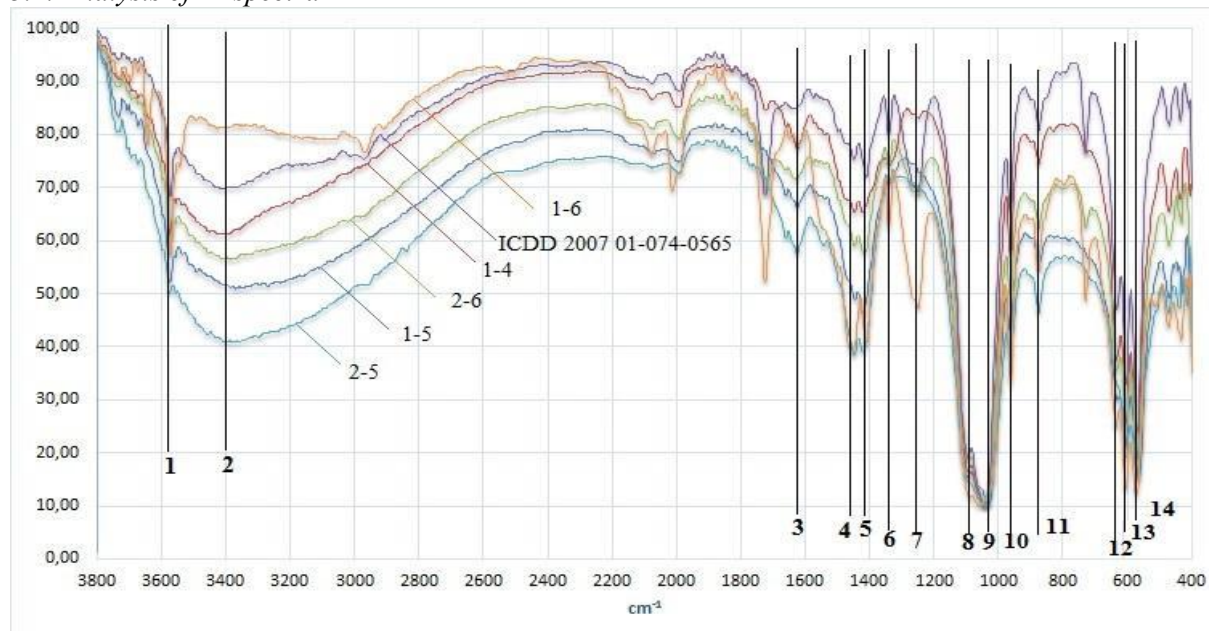


Figure 3. IR spectrum of the sample.

The IR spectra of the samples (Figure 3, Table 3) include the bands corresponding to the vibrations of the phosphate groups in the range of wave numbers 955-962, 520-660 and 1020-1090 cm^{-1} [15]. A broad band of deformation vibrations OH^- -groups at 3440-3570 cm^{-1} corresponds to the water adsorbed. The low intensity of the band at 3570 cm^{-1} and 630 cm^{-1} responsible for the stretching and

deformation vibrations of OH⁻-groups in HA obtained without maturing the precipitate in the mother liquor (sample 1-6) indicates a low content of HA phase in a product. Vibrations corresponding to the bands in the ranges 870-875 cm⁻¹ and 1300-1500 cm⁻¹ are associated to the C=O groups in the carbonates. The presence of carbonate groups in all samples was caused by adsorption of carbon dioxide from the air during the synthesis. Besides, in the spectra of the calcined samples (1-6, 2-6), the intensity of the respective peaks in the ranges of 1300-1500 cm⁻¹, 3440-3570 cm⁻¹ and 1630-1650 cm⁻¹ was significantly reduced due to the desorption of molecules of water and carbon gas from the powder surface and decomposition of the carbonate-HAP [14]. The obtained data demonstrates the presence of the carbonate groups in the products of reactions.

Table 3. Infrared vibrational modes of synthesized hydroxyapatite, standard and published data.

№	Characteristic frequencies	Vibrational frequency, ν cm ⁻¹						
		1-4	1-5	1-6	2-5	2-6	reference	literature [15,16]
1	OH structural	3570	3571	3573	3570	3572	3572	3572
2	OH deformation	3400–3570	3400–3570	–	3400–3570	3400–3570	3400–3570	3420–3570
3	CO ₃ structural	1632	1634	1635	1631	1634	1632	1630–1650
4	CO ₃ group ν^3	1453	1456	1453	1454	1454	1459	1450–1460
5	CO ₃ group ν^3	1414	1416	1413	1420	1420	1420	1429
6	groups of adsorbed	1100–	1100–	–	1100–	–	–	1100–1300
7	H ₂ O	1300	1300		1300			
8	PO ₄ bending ν^3	1022	1040	1032	1030	1044	1040	1050
9	PO ₄ bending ν^3	1090	1089	1087	1086	1088	1089	1090
10	PO ₄ stretching ν^1	963	962	960	963	961	965	961
11	CO ₃ group ν^3	879	873	870	880	881	879	879
12	OH structural	627	630	630	650	631	631	635
13	PO ₄ bending ν^4	602	602	598	610	601	601	607
14	PO ₄ bending ν^4	573	573	573	564	574	574	571

As per IR spectra, maturing products in the mother liquor entail the increase the degree of its crystallinity as well as the increase of the content of carbonate groups, presumably as a part of the calcium carbonate and carbonate-containing HA. It should be noted that the inclusion of the carbonate anion in the structure of the HA in a small amount did not change the phase composition and was not observed in the X-ray diffraction patterns [17]. No differences in IR spectra of samples 2-5 and 2-6 were observed, thus, the calcination does not result in the increase of the content of crystal phase in a product.

3.3. Scanning electron microscopy

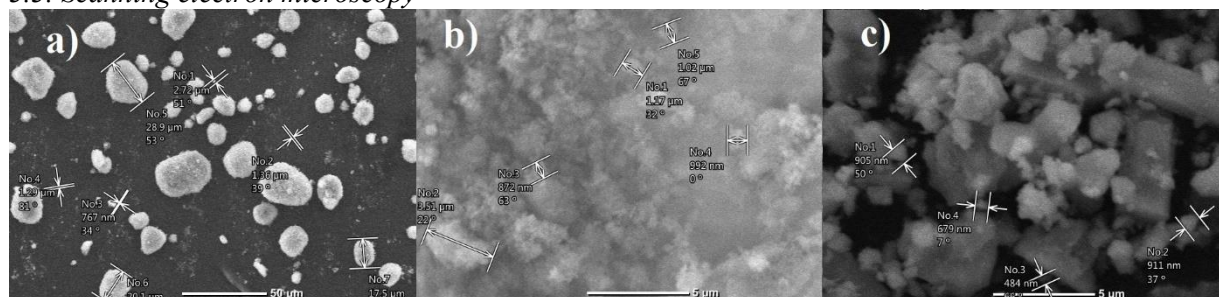


Figure 4. SEM images of the samples a) – 1-4; b) – 1-5; c) – 2-5.

All products represent as powder systems which include the aggregates of 10-100 μ sized that were formed due to the surface activity (Figure 4a). The aggregates and precipitates themselves have a loose structure (Figure 4b) in which the crystalline phase is distributed statistically. The only exception was the sample 2-5 (Figure 4c), which had been kept in the mother liquor for 2 weeks. The sample 2-5 structure includes predominantly amorphous particles of irregular shape ranging in the size from 100 up to 500 nm, and larger particles (greater than 500 nm) have denser structure. Apparently, the evolution of the precipitate went the same way as for the other samples. During the first day, the precipitate had a loose structure, wherein the partial crystallization occurred. While keeping precipitation in the solution for 2 weeks, the accumulation of a crystalline product and formation of a lamellar crystal with a regular faceting took place.

The synthesis process has no significant effect on the Ca/P ratio, as shown by elemental analysis (Table 4). Maturing precipitation in the mother liquor bring the Ca/P ratio close to the stoichiometric value.

Table 4. Elemental Composition (atomic %) of the synthesized samples.

Index	Ca	P	O	C	Ca/P
1-4	16.02	10.16	39.93	10.81	1.61
1-5	19.98	12.09	35.52	17.74	1.65
2-5	21.01	12.55	30.11	15.04	1.67
Standard	14.01	8.01	40.8	20.87	1.75

4. Conclusion

It was found that the products synthesized in reactions (1) and (2) primarily consist of hydroxyapatite. The conditions for obtaining a nanocrystalline product (20-27 nm) with a HA fraction of 70 to 90 wt.% were determined. Draining precursors at a temperature close to the boiling point provides active convection, uniform distribution of the reagents in the bulk that accelerates the synthesis. It is preferable to keep the precipitation in the mother liquor more than a day and up to two weeks to increase the content of nanocrystalline phase. Further calcination the product is not reasonable because of the low energy efficiency of the process.

It is shown that the initial state and the method of the heating precursor influence insignificantly on the crystallinity, Ca/P ratio and the content of the nanocrystalline phase.

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