Study of the effects of heat-treatment of hydroxyapatite synthesized in gelatin matrix

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Abstract. In the study, the isothermal thermogravimetric analysis (TGA) of hydroxyapatite synthesized in gelatin matrix (HAG) has been performed. 3 wt.% HAG samples were synthesized from the solution simulating the human extracellular fluid (SBF). X-ray diffraction and IR spectroscopy were used to determine the composition. During the experiment, increase in the calcination temperature up to 200°C-800°C was found to cause weight loss. The study of phase composition revealed that heat treatment does not affect the phase composition of the solid phase, which is composed of hydroxylapatite (HA). The prepared HAG (3 wt.% gelatin) samples are shown to have low thermal stability; the degradation of the samples occurs at 400° C.

1. Introduction

The global bioceramics demand on the market currently reaches ~40 billion euros per year, the estimated annual growth rate is of 7-12% and the required amount of the material to meet the demand is estimated at tens of tons [1-3]. The number of patients in need of surgery to restore the integrity of the bone is increasing every year: in the US, the annual number of patients exceeds more than 1 million people (of which over 300 thousand people need hip and knee prosthetics, the same holds true for dental implants).

Materials based on hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ are analogs of the mineral component of bone, and they are considered the most promising biocomposites to be used as substitutes for bone defect repair due to their high bioactivity [4–9].

It is known that the main components of bone are type I collagen ($\sim 20\%$), a mineral phase (\sim 60%), water (~ 9%) and non-collagenous proteins (~ 3%), the rest being polysaccharides and lipids [10, 11]. The organic part of bone is composed of collagen fibers and proteins, such as osteocalcin, osteonectin and fibronectin [12]. Gelatin is a natural polymer formed through hydrolysis of collagen. In contrast to the latter, it is more stable and exhibits less antigenicity, thereby can be effectively used as a calcium phosphate based organic matrix biomaterial [13]. Gelatin contains biologically active functional amino acid groups, and it is an advanced material for bone regeneration, including its combination with hydroxyapatite [14]. Since gelatin is a denatured form of collagen and it contains significant amounts of biologically functional amino acid groups, the investigation of properties of hydroxyapatite synthesized in the gelatin matrix (HAG) under near-physiological conditions is a challenging and relevant physicochemical problem.

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2. Materials and Methods

The SBF was used as a model solution of extracellular fluid. A solid phase was prepared by precipitation from aqueous solutions; the starting components of the system were $CaCl_2$, $MgCl_2$, K_2HPO_4 , $NaHCO_3$, Na_2SO_4 , NaCl [15] solutions and 3 wt.% gelatin (figure 1).



Figure 1. Scheme for HAG synthesis from SBF.

Thermal effects were studied by isothermal thermogravimetric analysis. Hydroxyapatite samples synthesized in the presence of gelatin were placed in dry ceramic crucibles. Then the samples were placed in a muffle furnace and kept for 2 h. The calcination temperature was varied from 200 to 800°C by increment of 200°C. After that, the crucibles were allowed to cool to room temperature, and then weighed on an analytical balance.

The solid precipitates were analyzed by FT-IR spectroscopy and X-ray diffraction (XRD) – qualitative and quantitative phase analysis, measurement of the crystallite sizes (coherent scattering regions – CSR). The IR spectra were recorded using a FT-801 spectrometer (the samples were prepared in the form of KBr pallets). The XRD of the powder samples was performed with the Bruker D8 Advance X-ray diffractometer, and the diffraction patterns were identified using the software package EVA (developed by Bruker AXS).

3. **Results and Discussion**

The XRD results showed that the samples synthesized in SBF under varying concentration of gelatin were single-phase and represented hydroxyapatite. The diffraction patterns indicated HA reflexes at 25.8° (002), 31.7° (211), 32.2° (112) and 32.9° (300) (figure 2).





Figure 2. Diffraction pattern of the hydroxyapatite powder crystallized in the presence of 3% gelatin solution.



4000 4500 5000

The lattice parameters of the prepared solids are increased (Table. 1) as compared to those of the unmodified hydroxyapatite that can be attributed to possible HA sorption in the gelatin matrix.

Sample	Initial gelatin	Lattice constants, Å	
	concentration, %	a	С
HA	-	9.414	6.865
Gelatin	3	9.432	6.881

Table 1. Lattice parameters.

The analysis of the IR-spectra (figure 3) identified the bands that determine the HA structure: 1040–1080 (v₃), 960, 840 (v₂), 602, 574 (v4) and 473 (v₂) cm⁻¹ corresponding to the phosphate group vibrations, a broad band at 3440–3570 cm⁻¹ related to deformation vibrations of the OH⁻-groups and valent vibrations of the adsorbed water. Carbonate ions are observed to localize in the phosphate ion lattice position with the corresponding absorption bands of the C–O deformation vibrations of the CO_3^{2-} ion bonds at 875 cm⁻¹ and valent vibrations at 1415–1481 cm⁻¹ in the IR-spectra [16]. A corresponding band of the deformation vibrations of the C=C bond in the region of 1658 cm⁻¹.

The study of the prepared hydroxyapatite samples synthesized in the presence of gelatin showed continuous weight loss (figure 4).



Figure 4. Diagram of TGA for 3 wt.% HAG samples.

The TGA diagram demonstrates the highest weight loss at 200°C and 400°C that can be attributed to adsorption and removal of the chemically bound water from the HAG samples. An increased weight loss at 400°C can be caused by burn out of the organic phase (gelatin) in the HA samples. At 600°C, the weight decreases insignificantly, while at 800°C, the weight loss increases by 2 times (compared to that at 600°C), which indicates the removal of the carbonate groups from the HA structure. These data is consistent with the results obtained by FT-IR spectroscopy (figure 5).



Figure 5. Results of FT-IR spectroscopy for 3% HAG samples subjected to heat treatment at 200, 400, 600 and 800°C.

Figure 6. X-ray diffraction patterns for 3% HAG after heat treatment.

The X-ray diffraction patterns (figure 6) of the HAG samples subjected to heat treatment show peak splitting at 400°C. In our opinion, this is caused by degradation of the gelatin components (amino acids), and thereby release of the carbonate component that has been partially released in the form of carbon dioxide, and partially entered the calcium phosphate structure. At 600°C and 800°C, the intensity of peak splitting did not increase and remained at the same level. This is most likely due to

the fact that no orbitals are left vacant to bind carbonate ions in calcium phosphate as compared to that in case of unsubstituted hydroxyapatite.

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The results of FT-IR spectroscopy showed that the spectrogram of the samples not subjected to heat treatment (figure 3) is identical to that of the samples heated at 200°C (figure 5). This suggests that no significant change in the HAG structure occurs at 200°C. During the analysis, several overlapping spectra were observed: in the region from 2700 cm⁻¹ to 3800 cm⁻¹, which, apart from the - OH water vibrations, include vibrations of the amino groups, and methyl and methylene groups. In addition, $-H_2$ groups overlap the carboxyl groups in the region of 1400 cm⁻¹.

As can be seen in Fig 4, at 400°C, the peak significantly decreases in the region of 3500 cm⁻¹ that indicates removal of water from the structure and degradation of amino acids, and methyl and methylene groups. The shape of the carboxyl group doublet in the region of 1415–1466 cm⁻¹ changed as well that can be confirmed by the removal of the $-CH_2$ group from the prepared sample.

When the temperature rises to 600°C, the intensity of the C=C band (1658 cm⁻¹) is not observed, and the intensity of the C-O band (1420–1466 cm⁻¹) decreases, which indicates the start of the carbonate component destruction. In addition, the O-H band (3569 cm⁻¹) reduces, which is also indicative of the start of the hydroxyapatite destruction.

At 800°C (figure 6), the spectrogram shows further reduction of the O-H band (3569 cm⁻¹) and C-O band (1420–1466 cm⁻¹).

At 200°C, a corresponding deformatio band of the C=C bond is observed in the region of 1658 cm⁻¹. At temperature increase up to 400°C, the peak intensity falla, and at temperature up to 600°C, it completely disappears, which is consistent with the data in [17].

4. Conclusion

The isothermal thermogravimetric analysis revealed four stages of thermal decomposition of composite materials: 1) removal of water and easily volatile impurities (t= $25^{\circ}C-280^{\circ}C$); 2) gelatin pyrolysis (t= $280^{\circ}C-400^{\circ}C$); 3) calcium pyrophosphate phase formation (t= $400^{\circ}C-600^{\circ}C$); 4) removal of carbonate ions from the HA structure and its decomposition (t > $600^{\circ}C$).

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