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Thermal effects of carbonated hydroxyapatite modified by glycine and albumin

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Abstract. In this work calcium phosphate powders were obtained by precipitation method from simulated solutions of synovial fluid containing glycine and albumin. X-ray diffraction and IR spectroscopy determined that all samples are single-phase and are presented by carbonate containing hydroxyapatite (CHA). The thermograms of solid phases of CHA were obtained and analyzed; five stages of transformation in the temperature range of 25-1000°C were marked. It is shown that in this temperature range dehydration, decarboxylation and thermal degradation of amino acid and protein connected to the surface of solid phase occur. The tendency of temperature lowering of the decomposition of powders synthesized from a medium containing organic substances was determined. Results demonstrate a direct dependence between the concentration of the amino acid in a model solution and its content in the solid phase.

1. Introduction

Among a wide range of modern biomaterials used for the restoration of bone defects, composites, based on carbonated hydroxyapatite (CHA) and organic components such as collagen and non-collagenous proteins, polysaccharides, polymers, etc., are in wide demand [1-5]. Crystal-chemical similarity of CHA with inorganic basis of bone mineral provides a high biocompatibility of such materials used in implantation; namely, it induces biochemical reactions similar to those of bone remodeling [6, 7]. The use of organic compounds in the quality of dopants approximates the conditions of the synthesis of materials to bone mineralization *in vivo*, taking place on a collagen matrix and with biological fluids [1-3, 8]. Therefore, it is possible to obtain CHA similar in crystallinity and morphological characteristics to the native bone that also improves the osteointegration of the implant with the surrounding living tissues. Despite a large number of works on the preparation of biocomposites [1-8], the influence of organic materials on the structure and properties of CHA, the mineral component of bone tissue, are not adequately covered. Thus, the role of amino acids of the polypeptide chain of collagen, composing the crystalline segments -Gly-X-Y-, (Gly – glycine, proline, etc.) and amorphous regions (lysine, histidine and others) are not fully described [9, 10]. The impact of organic components of biofluids (albumins, globulins, lipids, carbohydrates, etc.) on the characteristics of the solid phase is not fully considered [11]. Therefore, the present work is devoted to the preparation of CHA powders from model solutions of human synovial fluid containing glycine and albumin, study of their composition and thermal effects.

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2. Material and methods

CHA was produced by "wet synthesis" from the model solution approximated by electrolyte composition to the synovial fluid of an average healthy person at values pH = 7.40±0.05 and ionic strength - 0.174±0.001 [8]. This CHA was used as a control sample. Two series of model experiments were carried out. In the first series of syntheses, in the initial solution containing anions (HPO_4^{2-}, HCO_3^-, Cl^-, SO_4^{2-}) glycine was added (CH_2NH_2COOH, α- amino acetic acid of CP brand, the range of concentrations from 0.04 to 0.24 mol/l), the second – albumin (bovine serum albumin (BSA) of A brand; the range of concentration from 5 to 10 g/l). When selecting the concentrations of glycine and albumin, we were guided by their content in the collagen of the bone tissue and biological fluids (synovia, blood plasma), respectively [12, 13]. The solution containing the anions (HPO_4^{2-}, HCO_3^-, Cl^-, SO_4^{2-}) and the organic substance was spilled to the solution with calcium and magnesium cations at a rate of 5 ml/min. Further, the acidity of the model system was adjusted by adding a 10% solution of HCl until the value of pH = 7.40±0.05 was reached. Crystallization of the solid phase was carried out at room temperature (20-22 °C) for 7 days. At the end of this period, the precipitate was separated from the solution by filtration, washed three times with water, dried at 80 °C to completely remove the chemically not of the connected water, until a constant weight was achieved.

A study of the phase composition was conducted using the X-ray powder diffractometer D8 Advance (Bruker) under the following conditions: Cu-kα radiation (wavelength 0.15406 nm) scanning step – 0.05°, time of accumulation of a signal – 4 seconds/point, div. slit=0.5, voltage and filament current 40 kV and 40 mA, respectively; scan region 2θ – 5-80°. The transcripts of the diffractograms were obtained from the databases of powder diffraction ICDD PDF-2 in the program EVA (Bruker). IR spectra of the precipitates were recorded on a spectrophotometer "FSM-2202". The scanning range was 400-5000 cm^{-1}, the resolution was 8 cm^{-1}. The samples were prepared by compression into tablets with KBr. The thermal analysis was carried out on a STA-449 C synchronous thermal analyzer "NETZSCH". The samples were calcined in platinum crucibles in the atmosphere of the air from 25 to 1000 °C at the rate of 10 °C/min. The mass of the sample varied depending on the values of the recorded signals of the mass loss effect and the thermal effect. The mass was 10-20 mg. The quantitative data of mass loss during annealing were obtained using software Proteus 7.10. By obtained thermogravimetric (TG) and differential thermal (DTG) curves, the mass losses of the components of the samples were determined by heating. A differential gravimetric curve (DTA) was used for the characteristics of the observed thermal effects (changes in energy, °C/min). The specific surface areas of the samples were studied by the method of single-point nitrogen adsorption at 77.4 K on the adsorption apparatus "Sorbtometr", produced by Katakon LLC, Russia. The calculation of the obtained values of S_{BET} (m^2/g) was done using the BET method.

3. Results and Discussion

Using XRD and IR-spectroscopy, it was found that all precipitates are single-phase and are presented by CHA (figure 1a, b), regardless of the presence of organic substance in the initial model solution. On IR spectrums there are absorption of the bonds of inorganic groups and molecules: PO_4^{3-}, CO_3^{2-}, -OH и H_2O. The presence of modes of vibrations of the organic groups $-NH_2$, $-CH_n$, C=O, -N-H indicates that the glycine and albumin are in the composition of the precipitates.

However, according to data of the thermal analysis, the presence of organic substance in the initial medium reflects on the form of the curves TG, DTG and DTA (figure 2), which indicates different mass losses and thermal effects of these powders. Comparing the obtained thermal curves of the control sample (figure 1a) with the published data [14-20], we can distinguish five stages of thermo-transformation in the temperature range 25-1000 °C.

In the first stage (*I, 25-280 °C*, endothermic effect there is the removal of chemically free water and decomposition of volatile impurities (such as adsorbed CO_2).

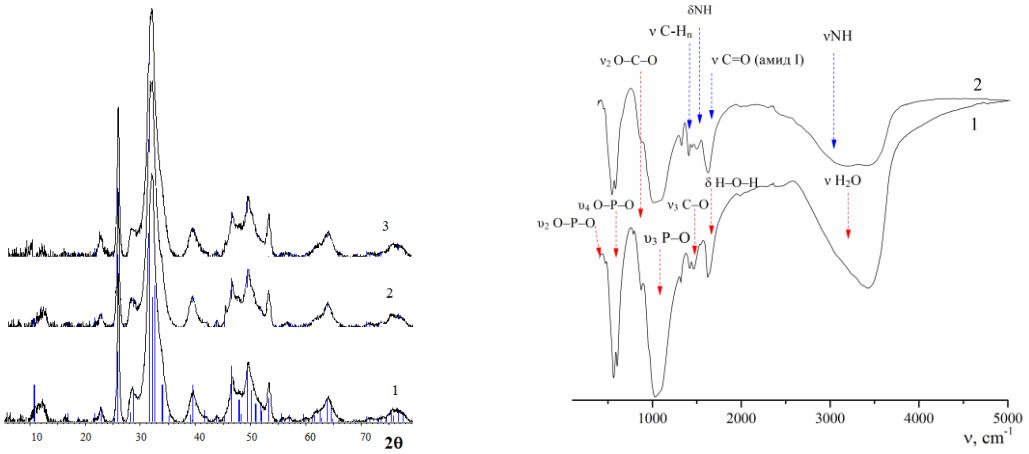


Figure 1. Diffractogram (a) and IR spectra (b) CHA: 1) pure; 2) 0.16 mol/l glycine; 3) 5 g/l albumin.

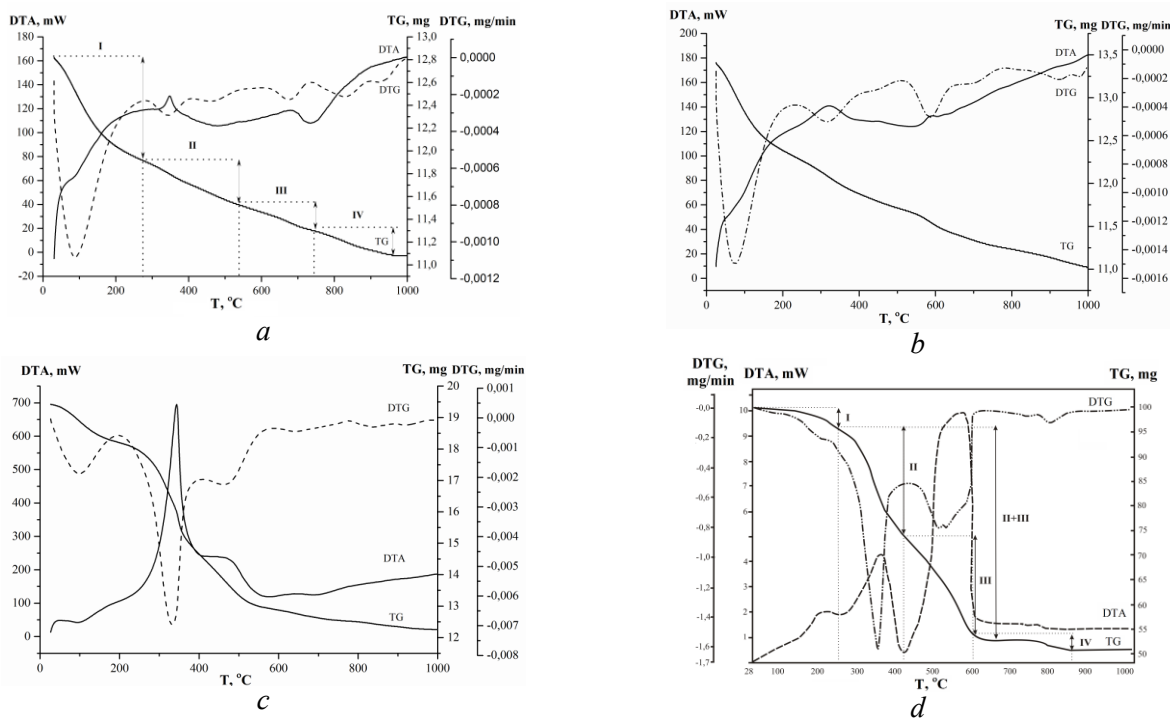


Figure 2. Thermograms CHA: a) pure; b) 0.04 mol/l glycine; c) 5 g/l albumin; d) human bone tissue «in the norm» [20].

The second and third stages (exothermic effects) are followed by the loss of water associated in various ways with the solid phase: physically adsorbed (*II, 280-470 °C*), chemisorbed and crystallization (*III, 470-750 °C*). Schematically the processes of water removal on the data can be represented as:

$[КГА \cdot nH_2O] \cdot mH_2O_{(sol.)} \rightarrow КГА \cdot nH_2O_{(sol.)} + mH_2O_{(g.)} + Q$; $КГА \cdot nH_2O_{(sol.)} \rightarrow КГА_{(sol.)} + H_2O_{(g.)} + Q$.

At higher temperatures, at the fourth and fifth stages (*IV and V, 750-900 °C and 900-1000 °C*) there are two endothermic peaks at 830 and 940 °C on DTA curves. In these temperature ranges, the transformation of CHA occurs, namely, the removal of carbonate ions from its structure in the form of CO_2 and transition of non-stoichiometric carbonated hydroxyapatite to the stoichiometric phase or β-$Ca_3(PO_4)_2$:

$$Ca_{10}(PO_4,CO_3)_6(OH)_{2(sol.)} \rightarrow Ca_{10}(PO_4)_6(OH)_{2(sol.)} + 6\ CO_{2(g.)} - Q;$$

$$Ca_9(PO_4)_{6-x-y}(HPO_4)_y(CO_3)_x(OH)_{2-y(sol.)} \xrightarrow{800\ °C} 3\ \beta\text{-}Ca_3(PO_4)_{2\ (sol.)} + x\ CO_{2(g.)} + 2\ H_2O_{(g.)} - Q\ [22].$$

In works [1, 23] it is marked that at a temperature of 850 °C, the transformation of GA hexagonal syngony into monoclinic form (hexagonal P63/m → monoclinic P21/b) is possible due to the reorientation of the dipolar groups OH^- in the triangular channels of Ca^{2+} ions of its crystal lattice. At 900 ^{0}C the formation of oxyhydroxyapatite from stoichiometric HA is possible according to the reaction [24]:

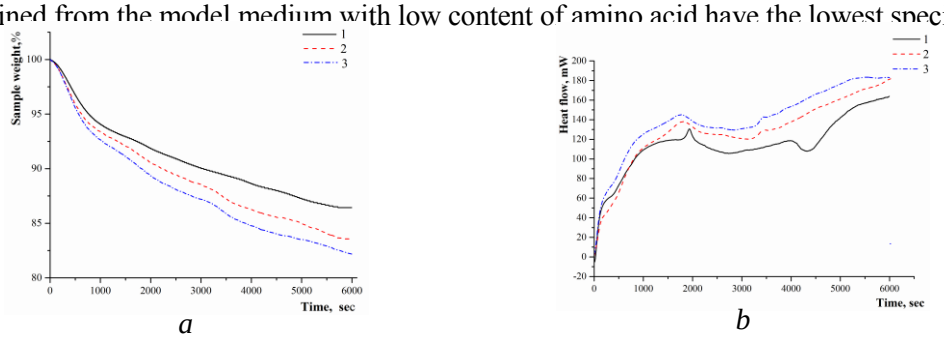
$$Ca_{10}(PO_4)_6(OH)_{2(sol.)} \rightarrow Ca_{10}(PO_4)_6(OH)_{2-2x}O_xn_{x(sol.)} + xH_2O_{(g.)},$$ где n_x – vacancy, x<1.

It was found that the powders, synthesized in the presence of an organic component, are characterized by the highest total losses of mass, and their transformation takes place at lower temperatures (I-IV, Table 1, figure 2 and 3a). Perhaps this is due to the presence of organic substance in the solid phase, which is consistent with the data of IR-spectroscopy.

Table 1. Temperatures of transformation and mass losses (Δm) of the components of powders.

Stages	T beginning of transformation (°C)			Δm (mg)							
					In the presence of glycine (mol/l)				In the presence of albumin (g/l)		
	Control sample	In the presence of glycine	In the presence of albumin	Control sample	0.04	0.08	0.16	0.24	5	7	10
I	25	25	25	7.098	7.367	7.300	8.352	10.647	6.282	5.381	5.430
II	280	240	200	2.340	3.302	3.408	3.728	4.095	18.280	18.130	20.440
III	470	450	400	2.496	2.964	3.015	3.057	3.744	9.166	9.195	12.449
IV	750	670	600	1.248	1.524	1.700	1.417	1.872	1.905	2.090	1.741
V	900	850	800	0.390	1.354	1.268	1.268	1.395	1.287	1.097	1.076
I+V		25-1000		13,572	16,511	16.733	17.822	21.753	35.920	35.893	41.136

It is known that thermal decomposition of aliphatic amino acids, to which belongs glycine, as well as peptides occurs at temperatures not exceeding 400 °C (T_m glycine = 232 - 262 °C; T_m albumin <90 °C.) [15, 16, 25]. Thus, the thermal degradation of glycine occurs in two steps. At the initial stage (fast stage, ΔH > 0) at 200-270 °C, oligopeptides and diketopiperazine (cyclic dipeptide) are formed. Methylamine, formamide, acetamide, propionamide, N-atsetilamid and acetic acid, water, carbon dioxide are by-products. Further, at 270-370 °C slow stage (ΔH < 0) of complete oxidation of degradation products proceeds. From data of Table 2 it follows that the thermal transformation of glycine, which is present in the obtained powders, occurs in stage III and corresponds at 2000 seconds to the exothermic peak on the DTA curve (321 °C). Exactly on the third stage with the increasing content of amino acids in the initial medium, the mass losses of glycine increase linearly. However, less energy is consumed for the destruction of the samples containing larger amounts of amino acids (figure 3*b*). Possibly at a lower concentration of glycine in an aqueous solution, components of zwitterions (NH_3^+- $C^{\alpha}H_2$ - COO^-) bond with the ions of calcium and PO_4^{3-} CHA more tightly and have a high adsorption activity in relation to their surface. This correlates well with the data of the specific surface area (Table 2). It is seen that the samples obtained from the model medium with low content of amino acid have the lowest specific surface.



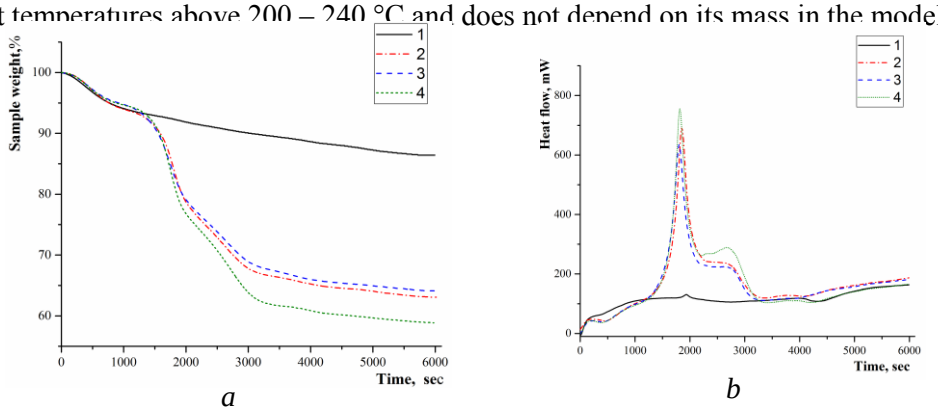
a *b*

Figure 3. TG curves (a) and DTA (b) CHA: 1) pure; 2) glycine 0.04 mol/l; 3) 0.12 mol/l.

Table 2. The specific surface area of the powders.

CHA	Control sample	In the presence of glycine (mol/l)			In the presence of albumin (g/l)		
		0.04	0.08	0.016	5	7	10
S_{BET}, m^2/g	130±7	70±1	52±2	75±3	44±2	48±2	25±2

Interesting results were obtained in the analysis of the thermograms of the samples synthesized in the presence of albumin (figure 4, Table 1). It was shown that the presence of protein leads to higher mass loss at temperatures above 200 – 240 °C and does not depend on its mass in the model solution.



a *b*

Figure 4. TG curves (a) and DTA (b) CHA: 1) pure; albumin – 2) 5 г/л; 3) 7 г/л; 4) 10 г/л.

We believe that, despite the low temperature of denaturation of pure albumin, the destruction of the polypeptide chains bounded with the surface of CHA occurs in the temperature interval 200-400 °C ... 450 (stage II, DTA maximum at 340 ... 250 °C and 2000 seconds figure 3a). We obtained similar results previously while studying the thermal curves of bone tissue (step II, figure 2d). In [21] it is shown that at this stage (DTA maximum/peak at 360 °C) there is a thermal oxidation of organic substances with less molecular mass than a collagen; for example, non-collagen proteins (albumin). In addition, high temperatures of thermal deterioration of albumin in the solid phase can be explained by a complex set of amino acids. Human and serum albumin are globular proteins, whose primary structure includes 585 and 582 residues respectively. These residues can be different amino acids: neutral, basic and acidic; their side radicals can be various functional groups (e.g., tryptophan, phenylalanine, tyrosine, etc.) [16]. It is known that the destruction of such amino acids may consist of four or five stages, and proceed at temperatures of 300 ... 400 °C and 600 ... 700 °C [26]. The presence of charged functional groups and zwitterions of amino acids provides a stronger interaction of albumin with the surface of CHA, which is in agreement with data from works about the chemisorption mechanism of their interaction [27]. The powders synthesized from the medium containing the protein, unlike the samples prepared in the presence of glycine, are characterized by the lowest specific surface area of powders. At the maximum content of albumin (10 g/l), the sample is formed, for which S_{micro} value is 5 times lower than the specific surface area of the control sample (Table 2).

In all samples, for which the precipitate was held in the presence of organic substance, a significant loss of mass at temperatures of more than 600 ... 670 °C (stage IV and V, Table 2) was recorded. At these temperatures, the removal of carbonate ions from CHA structure occurs. This fact indirectly indicates that in the crystalline grid of CHA of these precipitates, there are numerous carbonate ions; therefore, the samples with glycine characterized by low crystallinity and regularity of structure. At IR spectra of these powders, the reduction of intensity of mode υ_3(P–O) и υ_4(O–P–O) of phosphate groups (figure 1b) is indicated.

4. Conclusion

Thus, CHA powders were synthesized in the presence of albumin and glycine from prototypes of synovial fluid. It was shown that organic substance is present in the solid phase of the samples.

Investigated thermal effects and mass loss of the samples CHA obtained in the presence of glycine and albumin. It was found that the thermal decomposition of organic substance associated with surface area of CHA mainly takes place at temperatures of 200-400 ... 450°C. It was shown that with increasing content of amino acid in the initial medium, mass losses of glycine increased linearly. The mass loss for the powders obtained in albumin environment does not depend on its concentration in a model solution. For all samples obtained in the medium containing organic substance, unlike the control sample, the smallest specific surface area and crystallinity are characteristic.

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