Bio resorbability of the modified hydroxyapatite in Tris-HCL buffer

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Abstract. The solubility of carbonated hydroxyapatite powders and granulated carbonated hydroxyapatite produced from the synovial biofluid model solution has been studied. The kinetic characteristics of dissolution were determined. It was found that the solubility of carbonated hydroxyapatite is higher as compared to that of hydroxyapatite. The impact of the organic matrix on the rate of sample dissolution was revealed. For HA-gelatin composites, as the gelatin concentration grows, the dissolution rate becomes greater, and a sample of 6.0 g / L concentration has higher resorbability. The results of the research can be used to study the kinetics of dissolution and the biocompatibility of ceramic materials for medicine, namely for reconstructive surgery, dentistry, and development of drug delivery systems.

1. Introduction
Currently, much attention is paid to studying and improving the bioactive properties of the materials produced based on calcium phosphates. Interest in these compounds is due to the fact that the human bone tissue is a biocomposite based on hydroxyapatite (HA) with an admixture of amorphous calcium phosphates, brushite, octacalcium phosphate [1,2] and high-protein collagen. Therefore, calcium phosphate-based materials are widely used in medicine for bone defect replacement [3].

However, widespread use of these compounds is hindered by some problems. The most significant of the problems is low dissolution (i.e. bioresorption) rate of calcium phosphate-based materials. A significant disadvantage of the HA-based material most frequently used for implantation is its low bioresorbability rate. This problem can be solved through chemical modification of calcium phosphate, since it is known that isomorphic impurities introduced into calcium phosphate may increase both its bioactive properties and solubility.

The resorption process may be enhanced through ionic substitution of phosphate and hydroxide anions in the apatite structure for carbonate, silicate and other groups [4]. The carbonate-substituted hydroxyapatites are similar to the mineral components of the natural bone in their composition and structure, and silicate ions in the HA composition play a bridging role in the physiological processes of growth and restoration of bone and cartilaginous tissues, etc. One of the most frequently used forms of phosphate calcium HA-based ceramics are granules, coatings and other forms, in which collagen, gelatin and chitosan are used as matrix-carriers. As is well-known, the human body contains buffer solutions, and the study of the resorption processes of the bioactive samples based on HA is one of the urgent issues to promote practical application of these products.

The most common buffer simulating the physiological medium of the human body is Tris-HCl buffer with, pH equal to 7.4. Since pH of this buffer is similar to the physiological values of the organism, this buffer was used as the solvent to simulate the medium of a living organism.
The study aims to estimate the kinetic parameters of the dissolution for samples based on modified HA in Tris-HCl buffer.

2. Materials and methods
Modified hydroxyapatite was synthesized from the model medium similar to the human synovial fluid in its ionic and electrolyte composition, pH and ionic strength [5]. To produce materials with properties similar to those of the bone apatite, carbonate ions (CO\textsubscript{3}\textsuperscript{2–}) were introduced in the synovial fluid model solutions, their concentration being varied in the range from 0 to 32 mmol/l. These concentration ranges correspond to the content of ions in the human intracellular, interstitial and synovial fluid [5]. Crystallization of the solid phase was carried out for 30 days.

To make granules of carbonated hydroxyapatite (CHA) the resulting powder with a weight of 0.2–1 g was mixed with 1.5–7.5 ml of the 5, 10 and 15 mass. % aqueous gelatin solution and stirred to obtain a homogeneous mass. The mixture was dripped through a capillary with a diameter of 1–2 mm in oil, cooled to −1−0 °C using a eutectic mixture of fine ice mixed with CaCl\textsubscript{2}. The resulting granules were washed successively with ethanol and acetone and dried at a temperature of 25–27 °C in air for 6–7 hours.

Dissolution of the produced samples was performed in Tris-HCl buffer at physiological pH equal to 7.4 under continuous stirring. Several experiments were performed with different initial sample mass of calcium phosphate (m = 0.100 and 0.200 g) and under different temperatures.

At regular intervals, the values of acidity and calcium ion concentration were recorded via direct potentiometry. The experimental data obtained were used to plot the graphic dependences in pCa = f(τ) coordinates to evaluate the solubility of the synthesized samples. To determine the dissolution rate for each of the samples, the kinetic curves were analyzed using the regression analysis [7]. After that, these curves were mathematically processed according to the algorithm suggested in [8]. The morphology of the samples was studied with the JEOL JSM 6610LV Scanning Electron Microscope.

3. Results and Discussion
As a result of the synthesis from the synovial fluid model solution under varying concentration of carbonate ions, carbonated hydroxyapatite was produced. The above techniques and algorithms were used to study the solubility of the synthesized HA and KGA in Tris-HCl buffer at physiological pH of the human synovial fluid.

The data in Fig. 1 (change in the calcium ion concentration over time) were used to obtain graphic dependences (Fig. 2) to evaluate the degree of dissolution of the synthesized calcium phosphates. The data in Fig. 2 were used to determine the dissolution orders for CHA and HA (Table 1).

After that, the solubility of HA and CHA was investigated at different temperatures to calculate the activation energy of the processes. The obtained kinetic curves are shown in Figure 3; the curves and formula 3 were used to determine the values of the activation energies.

The analysis of the data in Table 1 showed that the process of CHA dissolution is characterized by lower activation energy if compared with that for HA. When comparing HA and CHA solubility (Figure 4), carbonated hydroxyapatite is found to be more soluble than hydroxyapatite. In our opinion, this may be due to different mechanisms of dissolution which is indicated by the change in the reaction order from n=1 to n=2 for CHA. A number of authors suggest that this may be caused by the predominance of internal diffusion processes over external ones [9].
Figure 1. Time dependence of HA (a) and CHA (b) dissolution

Figure 2. Kinetic curves of HA (a) and CHA (b) dissolution with different masses of the initial samples, (τ - time, min).

Table 1. Kinetic parameters of HA and CHA dissolution

| Phase             | HA   | CHA   |
|-------------------|------|-------|
| Order of the dissolution reaction, n | 1    | 2     |
| Activation energy (kJ/mol)         | 13.12| 9.92  |

Next, the dissolution process of CHA-gelatin granulated samples was explored. The pCa system changed significantly within the first 24 hours when the samples of granulated CHA-gelatin were dissolved in Tris buffer. Investigation of the sample solubility in the subsequent days showed insignificant change in pCa values. To determine the rate of sample dissolution, each of the kinetic curves was analyzed using the regression analysis (Fig.5). It was found that the time dependence of pCa in Tris-HCl buffer in the initial section may be approximated by a linear function.

To verify the determined patterns by the method of optical microscopy, the surface morphology of the considered samples before and after dissolution was investigated in the considered model media. Figure 6 shows the images of the granulated carbonated hydroxyapatite at 60-fold magnification.

The results of quantitative evaluations of the kinetic parameters are presented in Table. 2. The analysis of the kinetic curves showed that granule dissolution is a multi-stage process; at the initial stage (up to 1000–1200 min) the time dependence of the calcium ion concentration in the solution \(C(t)=-\lg C_{Ca}^{2+}\) may be approximated by a linear function. The dissolution decelerates with time, and kinetics obeys the exponential dependence. It is known that exponential dependence corresponds to kinetics of the first order reaction when the rate of change in calcium ion quantity is proportional to the number of "active regions" in the dissolved material at the given moment [10].
Figure 3. Solubility of HA and CHA at different temperatures

Figure 4. Comparison of HA and CHA solubility

Figure 5. Dependence of Ca\(^{2+}\) ions concentration on dissolution time of CHA-gelatin granules in Tris buffer (pH=7.4) with varying mass fraction of gelatin

Figure 6. Images of the granulated carbonated hydroxyapatite with gelatin mass fraction of 5% (a), 10% (b) and 15% (c) (mass) after dissolution in Tris buffer (pH=7.4) within an hour
The quantitative evaluation of the rate of change in the calcium ion concentration in the solution depending on gelatin concentration in granules is of great relevance. At the "exponential" stage of dissolution, the rate of change in the concentration decreases with time, therefore, the initial dissolution rate can be considered as a quantitative measure.

Table 2. Parameters of the granule dissolution depending on gelatin concentration in the Tris buffer solution

| C_{gel}, % | R^2    | Equation                  |
|-----------|--------|---------------------------|
| 5         | 0.8976 | pCa = 4.4982 + 0.1903e^{-0.086t} |
| 10        | 0.9719 | pCa = 4.3563 + 0.2917e^{-0.0025t} |
| 15        | 0.9937 | pCa = 4.3061 + 0.3800e^{-0.0017t} |

Based on the data presented in Table 2, decrease of the initial rate of sample dissolution is seen to cause increase in the content of the granulated gelatin which may be due to hindered diffusion of the solvent into the porous internal region of granules that correlates to the data obtained with the scanning electron microscopy (Fig. 7).

The analysis of the sample micrographs showed that as the gelatin concentration in the initial suspension of samples increases, the number of open pores decreases due to formation of a thin gelatin film on the surface of the granule mineral component.

![Figure 7. Surface morphology of CHA-gelatin composite: a, b, c are for \( \omega_{gelatin} = 5 \) mass%, 10 mass%, 15 mass%, respectively](image)

The kinetic patterns of dissolution can be explained based on the polyampholyte properties of gelatin: minimum swelling of gelatin is observed at pH approximately equal to 4.7, and pH of the maximum swelling is at pH close to 3.2. In the alkaline medium, the \( -\text{COOH} \) group dissociates to form the \( -\text{COO}^- \) group. The molecules of gelatin acquire a net negative charge. Change in the macromolecule charge leads to change in macromolecule conformations which results in the change in solution viscosity. This is due to the fact that the tendency of \( -\text{COOH} \) groups to dissociate is greater than the tendency of \( -\text{NH}_2 \) groups to protonate, that is proteins are stronger acids than bases. This means that to achieve the pI in the gelatin solution, the excess of acid (pH <7) is required to suppress ionization of carboxyl groups. Therefore, acid medium may be most favorable for rapid dissolution of the CHA-gelatin composite.
4. Conclusion
Thus, the study has shown that:
CHA solubility in Tris-buffer at physiological pH of the human synovial fluid is higher than that of HA.
Different patterns of the kinetic curves and impact of the organic matrix on the dissolution rate have been determined for granulated CHA samples.
For HA-gelatin composites, growth of the gelatin concentration results in the increase of the dissolution rate. The sample with concentration of 6.0 g/l in the Tris buffer solution has higher resorbability.
The results of the research can be used to study the biocompatibility and kinetics of dissolution of ceramic materials for medicine, namely for traumatology, reconstructive surgery, dentistry, and development of drug delivery systems.

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