Composition characteristics and regularities of dissolving of hydroxyapatite materials obtained in water solutions with varied content of silicate ions

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Abstract. Research aimed at developing new bioactive materials for the repair of defects in bone tissues, do not lose relevance due to the strengthening of the regenerative approach in medicine. From this point of view, materials based on calcium phosphates, including silicate ions, consider as one of the most promising group of substances. Methods of synthesis and properties of hydroxyapatite doped with various amounts of SiO₄²⁻ ions are described in literature. In the present work synthesis of a solid phase in the systems Ca(NO₃)₂ - (NH₄)₂HPO₄ - Na₂SiO₃ - NH₄OH - H₂O (C₀/Cₚ = 1.70) performed with a wide range of sodium silicate additive concentration (y = Cₛ/Cₚ = 0 ÷ 5). It is established that under the studied conditions at y ≥ 0.3 highly dispersed poorly crystallized apatite containing isomorphic impurities of CO₃²⁻ and SiO₄²⁻ precipitates in a mixture with calcium hydrosilicate and SiO₂. It is shown that the resulting composites can gradually dissolve in physiological solution and initiate passive formation of the mineral component of hard tissues.

1. Introduction
Investigation of human bone matrix have shown that the basic mineral matter of hard tissues in composition and structure is close to hydroxyapatite (HA, Ca₁₀(PO₄)₆(OH)₂) [1]. The similarity of biogenic and synthetic salts has caused widespread use of synthetic salts in medicine as a biocompatible material for the reconstruction of bone defects. Stoichiometric HA form and it various ion-substituted modifications found the application. The possibility of introducing metal ions (Na⁺, K⁺, Mg²⁺, Sr²⁺, etc.) and anions (Cl⁻, F⁻, CO₃²⁻, SiO₄²⁻, etc.) into apatite structure is being studied [1]. Special attention is paid to silicate ions because silicon plays an important role in the early stage of cartilage and bone growth, it has a positive effect on the synthesis of collagen type I by osteoblast cells, promotes an increase in the density of the mineral constituent of bone tissue by stimulating the precipitation of basic calcium phosphate [2]. In this connection, the synthesis of HA containing various amounts of SiO₄²⁻ ions is being actively investigated. It is also promising to create composites that include silicate-substituted apatite and silicon phase that initiates the active incorporation of synthetic material into the bone components formation process.

2. Problem formulation
The main method for the synthesis of ion-substituted HA is precipitation of this sparingly soluble salt from an aqueous solution. As a rule, the amount of modifying additives is small in comparison with the concentrations of the main precipitation-forming ions.
The task of this work is the synthesis and physicochemical investigation of solid phases formed in alkaline aqueous calcium-phosphate systems containing varying amounts of SiO$_2^4$ as well as studying the regularities of dissolution of the resulting powder materials in physiological solution.

3. Materials and methods

Synthesis was carried out in aqueous solutions Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-Na$_2$SiO$_3$-NH$_2$OH-H$_2$O at room temperature ($t = 20-22$ °C). Ca(NO$_3$)$_2$-4H$_2$O (chemically pure), (NH$_4$)$_2$HPO$_4$ (pure for analysis), Na$_2$SiO$_3$-5H$_2$O (pure), NH$_2$OH (pure for analysis), HNO$_3$ (pure for analysis) and distilled water were used as the reagents for the systems preparation. The ratio of the concentrations of the initial components was set equal to $x = C_{Ca}/C_P = 1.70$, and the fraction of the silicon agent varied in the range $y = C_{Si}/C_P = 0 \div 5$. For comparison, the solid phase was synthesized in the absence of phosphate ions, at $C_{Ca}/C_{Si} = 1$. The ionic strength of solutions in each series of experiments did not exceed 0.6 mol/l.

Reaction media were prepared by a dropwise addition of samples were weighed on analytical scales. Powder-like samples were examined using X-ray diffraction analysis (XRD, diffractometers «D8 Advance» (Bruker) and «XRD-7000» (Shimadzu)), Fourier transform spectroscopy (FTIR-spectrometer «FT-801» (SIMEX); powders were pressed in tablets with KBr).

Regularities of samples dissolution in 0.9% NaCl, isotonic with human blood plasma, were studied. For kinetic measurements 0.2000 ± 0.0002 g of samples were placed in a plastic vessel with tight-fitting lid and 50.0 ml of 0.9% NaCl solution were added. The measurements were carried out at room temperature ($t = 20\div22$ °C). During the experiment, system’s pH in time changes were monitored (pH-meter «SevenEasy S20» with electrode for pH measurement «InLab®413» (Mettler Toledo)) and concentrations of calcium ions, phosphates and silicates passing into the liquid phase were controled. To determine the concentration of Ca$^{2+}$, PO$_4^{3-}$ and SiO$_2^{4}$, aliquots of solutions were withdrawn at predetermined intervals. A corresponding volume of fresh 0.9% NaCl was added to the system to maintain the constancy of its volume during the measurements.

4. Results

XRD analysis showed that precipitates, obtained from the systems Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-Na$_2$SiO$_3$-NH$_2$OH-H$_2$O in all range of variation of the silicon agent concentrations and treated at 300 °C, contain only HA crystalline phase (figure 1). A calculation using the Selyakov-Scherrer formula [3] showed that the effective diameter of the apatite particles in the samples is about 17 nm (table 1). For $y \geq 1$, the crystallite size decreases to 8 nm. The parameters of the unit cell of the obtained HA samples were calculated according to the diffraction data (table 1). An increase of the parameter $c$ relative to the reference value characteristic to Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ was noted, the variation of $a$ value in the interval 9.417-9.429 and increase of the cell volume with increasing $y$ was observed. These changes may be related with isomorphous substitutions in the anion apatite sublattice.
Figure 1. Diffractograms of samples obtained in systems with a variable amount of silicate ions.

Table 1. Characteristics of solid phases (XRD data)

| Sample | y   | Size of HA crystall, nm | Parameters of HA unit cell |
|--------|-----|------------------------|---------------------------|
|        |     |                        | a, Å^a                    | c, Å^a                     |
| N1     | 0   | 17.9 ± 0.3             | 9.427 ± 0.002             | 6.879 ± 0.002              |
| N2     | 0.1 | 19.1 ± 0.3             | 9.428 ± 0.002             | 6.883 ± 0.002              |
| N3     | 0.3 | 17.2 ± 0.4             | 9.427 ± 0.002             | 6.883 ± 0.002              |
| N4     | 0.5 | 17.0 ± 0.4             | 9.428 ± 0.002             | 6.885 ± 0.002              |
| N5     | 0.75| 17.6 ± 0.4             | 9.429 ± 0.002             | 6.889 ± 0.002              |
| N6     | 1   | 14.9 ± 0.3             | 9.425 ± 0.002             | 6.895 ± 0.002              |
| N7     | 2   | 13.3 ± 0.3             | 9.424 ± 0.002             | 6.898 ± 0.002              |
| N8     | 5   | 8.6 ± 0.2              | 9.417 ± 0.004             | 6.898 ± 0.004              |

^a The reference values for the stoichiometric HA: a = 9.419 Å, c = 6.883 Å [card N 55-0592 of the powder diffraction database ICDD PDF_2, version 2006].

Thus, FTIR results show the presence of CO$_3^{2-}$ and SiO$_4^{4-}$ ions in the precipitated apatite (figure 2). Absorption bands that appear in the IR spectra of the samples at 875 cm$^{-1}$ ($v_2$), 1420 and 1460 cm$^{-1}$ ($v_3$) indicate the partial replacement of PO$_4$-tetrahedra in HA structure by the groups CO$_3^{2-}$ which appear in the reaction media as a result of absorption CO$_2$ from air. The decrease of intensity of O-H absorption bands (630 and 3570 cm$^{-1}$, figure 2) could be the evidence of silicates incorporate into HA composition, which is observed as a result of the following mechanism of isomorphic substitution:

$$\text{PO}_4^{3-} + \text{OH}^- \rightleftharpoons \text{SiO}_4^{4-} + \square_{OH}$$

(1)

Molar coefficients Ca/P and Ca/(P+Si) for obtained solid phases were calculated from results of chemical analysis (table 2). It is established that for all powders Ca/P value corresponds to non-stoichiometric apatite (nHA) with phosphorus deficiency, which arises as a result of isomorphic anion substitutions. The values of Ca/(P+Si) coefficients significantly decrease with the y growth, which is probably due to the appearance of a non-apatite component in the composition of the powders. This assumption was confirmed by FTIR data. In addition to the absorption bands of the groups included in HA structure, peaks of the silicate phase are fixed in the spectra of the solid phases deposited in systems Ca(NO$_3$)$_2$·(NH$_4$)$_2$HPO$_4$·Na$_2$SiO$_3$·NH$_2$OH·H$_2$O at y $\geq 0.3$. At 790-800 cm$^{-1}$ the mode of stretching vibrations of siloxane bridges (Si-O-Si) in the composition of silicon oxide [1, 4] and calcium silicate hydrate (CSH, rCaO·mSiO$_2$·nH$_2$O) is recorded in the spectra of the samples [5]. The shoulder at 1200 cm$^{-1}$ is due to stretching vibrations of Si-O bonds in SiO$_2$ [4] and CSH for which
the molar ratio Ca/Si ≤ 0.7 [6]. These reflexes become more pronounced as the percentage of silicate ions in the initial reaction system (y) increases, indicating an increase of the content of these components in solid phase.

Figure 2. FTIR spectra of the samples obtained at y = 0 (1), y = 0.3 (2), y = 0.75 (3), y = 2 (4), y = 5 (5), y = 0* Ca/Si = 1 (6).

Table 2. Samples composition

| Sample | y  | Ca/P | Ca/P + Si | PO$_4^{3-}$ (n) | SiO$_4^{4-}$ | Δ$^a$ |
|--------|----|------|----------|-----------------|-------------|------|
| N1     | 0  | 1.71 | 1.71     | 5.06            | 0           | 0.94 |
| N2     | 0.1| 1.73 | 1.67     | 5.04            | 0.16        | 0.96 |
| N3     | 0.3| 1.74 | 1.45     | 5.00            | 1.01        | 1.00 |
| N4     | 0.5| 1.74 | 1.25     | 4.99            | 1.97        | 1.01 |
| N5     | 0.75| 1.74 | 1.05     | 4.99            | 3.24        | 1.01 |
| N6     | 1  | 1.75 | 0.92     | 4.98            | 4.48        | 1.02 |
| N7     | 2  | 1.78 | 0.62     | 4.89            | 9.26        | 1.11 |
| N8     | 5  | 1.88 | 0.29     | 4.61            | 25.3        | 1.39 |
| N9     | 0* | -    | 0.49     | -               | -           | -    |

$^a$ Δ - the difference between the number of PO$_4^{3-}$ ions in stoichiometric HA and HA obtained in this work (Δ=6-n).

To study the bioactivity of synthetic materials, the solubility of powders in a weakly acid medium (pH = 6.20) was studied in a 0.9% solution of NaCl isotonic with human plasma. According to figure 3a, at the initial stage of dissolving of each material the pH rapidly grow from the initial acidity of the 0.9% NaCl solution (pH ≈ 6.20). The most intensive grow of pH happen during
the first day, when the solid particles interact with the unsaturated solution. The maximum pH jump in a given period is fixed for powders with high silicon content (N7-N9). For them pH reaches values of the order of 9÷10.5 units. When N1-N6 samples dissolve, a gradual increase in the alkalinity of the solutions is observed with increasing γ. However, the pH of the liquid phase does not exceed 7.1 ± 0.1. According to the current understanding of HA and calcium silicates degradation chemistry [7, 8], a sharp increase in pH at the initial stage of dissolution of the material occurs as a result of the absorption of protons and their binding to water molecules, dihydrogen phosphate ions and silanol groups. Obviously, the jump in pH is the stronger the more SiO<sub>4</sub><sup>4-</sup>, PO<sub>4</sub><sup>3-</sup> and OH<sup>-</sup> ions are contained in the substance. The maximum appearance on a curves pH = f(τ), which characterize the resorption of samples rich in silicon (N7-N9), is probably caused by secondary precipitation processes.

In the course of the experiments it was established that, depending on the materials composition, their behavior in the physiological solution is different. Thus, when the control samples N1 and N9 are resorbed, the Ca<sup>2+</sup> concentration in the liquid phase increases. In this case, the solubility of CSH powder (N9) exceeds threefold given characteristic of the sample composed of apatite (N1). The amounts of PO<sub>4</sub><sup>3-</sup> and SiO<sub>4</sub><sup>4-</sup>, passing into the solution, also increase in time. Calculation of the molar coefficients Ca/P and Ca/Si showed that in case of N1 degradation calcium and phosphate ions emerge into the solution in approximately equal amounts, whereas in the case of CSH (N9) resorption, the Ca/Si ratio is close to 2/3. As a result, Ca<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup> and SiO<sub>4</sub><sup>4-</sup> gradually accumulate in solution. It indicates the absence of parallel processes in which this ions could be consumed. Thus, powders based only on HA or CSH are able to dissolve in physiological solution, but they do not stimulate the subsequent precipitation of calcium phosphates.

![Graphs](image1.png)

**Figure 3.** Dependences of pH (a), concentration of calcium ions (b), silicates (c), phosphates (d) from the time of dissolution samples N1 (1), N3 (2), N5 (3), N7 (4), N8 (5), N9 (6) in 0.9% NaCl.

During degradation in the weakly acid medium of N2-N8 samples, a regular increase in the concentration of SiO<sub>4</sub><sup>4-</sup> in solutions is recorded. There is a positive correlation between the number of ions of this type released into the solution and the content of silicon in the solid phase. However, the expected accumulation of calcium as a result of the dissolution of solid phases (N2-N8) does not occur. The curves C(Ca<sup>2+</sup>) = f(τ) have the same descending character as C(PO<sub>4</sub><sup>3-</sup>) = f(τ). The amounts of metal ions and phosphates decrease synchronously with time (figure 3b-d). In this case, the higher
the silicon content in the sample the lesser amounts of Ca$^{2+}$ and PO$_4^{3-}$ are found in the solution. Obviously, the uptake of Ca$^{2+}$ and PO$_4^{3-}$ occurs as a result of the HA precipitation reaction:

$$10 \text{Ca}^{2+} + 6 \text{PO}_4^{3-} + 2 \text{OH}^- \rightarrow \text{Ca}_{10} \text{(PO}_4)_6 \text{(OH)}_2$$

(2)

A decrease of heterogeneous systems pH under 1 to 3 days study of samples N7, N8 interaction with physiological solution (figure 3a) may also be associated with the intensive progress of this process. The described processes cause the fact that over time and for silicon rich samples significant amounts of silicates are found in the solution. In general, ion concentrations in the systems (C$_i$) decrease in the following sequence: SiO$_4^{4-}$ > PO$_4^{3-}$ > Ca$^{2+}$. This indicates that silicates take active participation in the process of materials resorption.

5. Discussion

According to results of investigation in studied systems Ca(NO$_3$)$_2$ - (NH$_4$)$_2$HPO$_4$ - Na$_2$SiO$_3$ - NH$_4$OH - H$_2$O in all range of variation of the silicon agent concentrations highly dispersed poorly crystallized silicon-containing carbonated hydroxyapatite precipitates. The excess of silicate ions introduced into the system release as amorphous SiO$_2$ and CSH. Based on the results of experiments with 0.9% NaCl, it was found that silicate phase (CSH) predominantly dissolves in weakly acidic media. As a result concentrations of Ca$^{2+}$ and SiO$_4^{4-}$ ions in the liquid phase increased. The reaction of HA sedimentation passing under these conditions, recorded by the synchronous decrease in the amounts of calcium and phosphate ions in solutions, characterizes the synthetic samples as bioactive. The materials are capable to initiate a passive (without the participation of specialized cells) formation of hard tissues mineral component. The described patterns suggest that materials based on the developed compositions can be effective in restoring damaged areas of hard tissues in dentistry and bone tissue engineering.

6. Conclusion

Highly dispersed poorly crystallizedapatite containing CO$_3^{2-}$ and SiO$_4^{4-}$ isomorphic impurities forms in systems Ca(NO$_3$)$_2$ - (NH$_4$)$_2$HPO$_4$ - Na$_2$SiO$_3$ - NH$_4$OH - H$_2$O. At $y \geq 0.3$ silicate phase (CSH) and SiO$_2$ additionally deposit. In vitro experiments showed that material’s solubility increases as the content of silicate phase in the samples composition increases and exceeds this characteristic ofapatite. The Ca$_{10}$ (PO$_4$)$_6$ (OH)$_2$ precipitation, recorded by the synchronous decrease of calcium and phosphate ions amounts in 0.9% NaCl solution, characterizes synthetic mixtures as bioactive. The materials are capable to initiate a passive (without the participation of specialized cells) formation of hard tissues mineral component.

Materials based on the developed compositions can be effective in the restoration of damaged areas of hard tissues in dentistry and bone tissue engineering.

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