

Dynamics of apatite and calcium silicate hydrate composites resorption in Tris-buffer

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Abstract. Composites based on several biocompatible inorganic compounds present oneself a class of promising materials that can be used to repair damaged areas of hard tissue. Development of these composites and their bioactivity establishing is paid a significant attention. Investigation of processes occurring at composite powder materials soaking based on poorly crystallized carbonate substituted hydroxyapatite and calcium silicate hydrate in Tris-buffer was performed in this work. Chemical composition analysis of solutions contacted with composites with varying calcium phosphate and calcium silicate contents was carried out. Examination of undissolved solid phases was performed by SEM, FTIR and LDA. The obtained results indicate that materials components gradually dissolve in Tris-buffer. Moreover, the samples resorption rate increases with the growth of calcium silicate hydrate content in materials composition. Precipitation of the apatite was repeatedly detected in experimental conditions; it designates a potentially high bioactivity of investigated synthetic mixtures.

1. Introduction

At designing the materials for restoration of damaged areas of human bones, a great attention is paid to the ability of synthetic compositions dissolution in the biological fluids surrounding the implantation area. This property provides the gradual replacement of an artificial object by newly formed bone tissue.

In laboratory conditions, the resorbability of materials with different composition is studied by obtaining kinetic data of their dissolution in aqueous solutions. The latter can be Tris-buffer with pH = 7.40 [1], 0.9 % NaCl isotonic with human blood plasma [2, 3, 4], HCl - acidic agent of gastric juice [5], acetate buffer [6] with pH ~ 4.5 (it is close to the osteoclast secretion acidity), human blood plasma prototype (simulated body fluid, SBF) [7] or distilled water [8]. Experiments are usually carried out at 25 or 37 °C in a closed system in static or dynamic conditions. The dissolution process is usually controlled by periodic measuring of calcium, phosphates or other ions concentrations from the samples composition, released into solution, as well as the system pH or samples weight loss. Fixed time dependencies of the parameters mentioned above are the source of information about velocity and mechanism of materials dissolution. New materials are compared with widely used in medicine ceramics based on hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) [4]. The compared parameters can be the initial or specific dissolution rate [4], the equilibrium or maximum concentrations of Ca^{2+} and / or PO_4^{3-} [2, 7, 8], the substances weight loss [3]. The ranging of the studied materials according to these characteristics values allows to identify compounds with high potential of bioactivity in *in vitro* conditions.

A wide range of substances, including calcium phosphates (CP) with various stoichiometric composition, as well as pyrophosphates, carbonates, sulphates, calcium silicates were investigated by



the described method. The experimental results indicate that none of the listed compounds possess resorption kinetics comparable to the rate of bone tissue formation *de novo*. Thus, HA, due to the small value of the solubility product ($SP = 10^{-117}$ [4]), changes extremely slowly, whereas, for example, $CaCO_3$ degrades too quickly. In this regard, attempts to create composite materials based on several compounds with different resorption rates are being made.

Calcium silicates (CS) can be effective dopants to CP due to the active participation of silicate ions in the bioapatite formation *in vivo*. In this regard, the synthesis methods of mixtures of these salts in crystalline and amorphous state are actively developed. The article [9] presents the results of poorly crystalline carbonate substituted hydroxyapatite (CHA) and calcium silicate hydrate (CSH) nanocomposites synthesis. The peculiarities of these materials dissolution in a weak alkaline aqueous medium should be investigated.

2. Task definition

The task of this work is a physicochemical investigation of the powder composite materials (poorly crystalline carbonate substituted hydroxyapatite and calcium silicate hydrate) resorption in Tris-buffer.

3. Materials and methods

Samples containing variable amounts of HA and CSH (Table 1) were obtained as reported in [9]. For dissolution experiments powders were sieved through laboratory sieves to obtain a fraction of particles with diameters from 40 to 70 μm . In all experiments the solid / liquid ratio was equal to 1/200 g/ml, $t = 22^\circ C$, the system was not stirred. After a certain period of time (from 1 day to 3 months), pH and Ca^{2+} , PO_4^{3-} and SiO_3^{2-} concentration in solutions were determined. At the end of experiments undissolved part of the powders was filtered, washed on the filter with distilled water, and dried at $90^\circ C$ to constant weight. The weight loss was calculated from the difference between the initial sample weight and weight of its part remained after 3 months of soaking in Tris-buffer. The solid phase was examined by scanning electron microscopy (SEM), FT-IR spectroscopy (FTIR) and laser diffraction analysis (LDA).

Table 1. Theoretical composition and samples designation

Designation	C10	C8	C6	C4	C2	C0
W_{HA} , wt. %	100	80	60	40	20	0
W_{CSH} , wt. %	0	20	40	60	80	100

The morphology of the solid phase particles was studied by SEM. Samples analysis was carried out by using a «JCM-5700» microscope (JEOL) equipped with an energy dispersive X-ray spectrometer «JED-2300» (JEOL) for elemental analysis in a high vacuum mode. The SpotSize parameter was selected as 10, 20 and 50, with the accelerating voltage value from 10 kV to 20 kV and magnification from $500\times$ to $10000\times$.

FTIR spectra were scanned on IR-spectrometer «FT-801» (Simex) using the KBr pellet technique for the range of $500 - 4000\text{ cm}^{-1}$ with 4 and 32 times scanning resolution. The program «ZaIR 3.5» was used to obtain and process of the spectra.

Curves of powders particle size distribution were obtained by LDA on a laser particle size analyzer «SALD-2300» (Shimadzu). The complex refractive index was equal to $1.65 \pm 0.00i$ in all measurements. Differential curves of powder particles size distribution and median diameter values were obtained using software «WingSALD II».

Ion concentrations in solutions were determined by photometric methods on spectrophotometer «UV-1200» (EcoView). Calcium was determined photometrically by reaction with Arsenazo III in an alkaline medium, phosphates were determined by UV-method with ammonium molybdate, silicates were determined as a yellow form of molybdosilicic acid. pH was measured by using the pH-meter «pH-150MI» equipped with a combined pH-electrode and thermal sensor.

4. Results

The Tris-buffer used in this study as a medium for dissolving of synthetic powders is an aqueous solution of tris(hydroxymethyl)aminomethane ((HOCH₂)₃CNH₂) that does not contain Ca²⁺, PO₄³⁻ and SiO₃²⁻ in the initial state. These ions appear in experimental systems as a result of interaction of solid and liquid phases (Fig. 1). At the initial stage of experiments (up to 3 – 7 days) Ca²⁺, PO₄³⁻ and SiO₃²⁻ concentrations in solutions increase and after that a gradual decrease of ions amounts in the systems is recorded. For all studied samples, calcium ions are found in the greatest amount in solutions. Moreover, the higher HA proportion in the powder, the greater current Ca²⁺ concentration in the solution at each time point (Fig. 1a). In the case of the control sample C10 dissolution, which contain only CHA, the phosphates content in the systems firstly increases up to 0.14 mmol/L and then gradually decreases to 0.1 mmol/L. In the media contacted with composite samples C8 – C2 and CSH powder (C0) PO₄³⁻ ions are presented in smaller quantities (Fig. 1b). Their accumulation in solutions, which occurs at the initial stage of experiment, is replaced by a sharp decrease of anions quantity and at $t > 7$ days their concentration does not exceed 20 μ mol/L. The silicates concentrations in solutions contacted with samples with different compositions have similar values (Fig. 1c). In addition to Ca²⁺, PO₄³⁻ and SiO₃²⁻ appearance in the Tris-buffer, the variation of solutions pH was recorded (Fig. 1d). Curves $\text{pH} = f(t)$, as well as $C_i = f(t)$, pass a maximum at $t = 7$ days, after which the pH value gradually decreases. The highest growth of pH was observed in the system with C0 powder ($\text{pH}_{\text{max}} = 8.53$). The recorded trends of Ca²⁺, PO₄³⁻ and SiO₃²⁻ concentrations and pH changes in time indicate that in experimental condition the process of repeatedly CP precipitation occur.

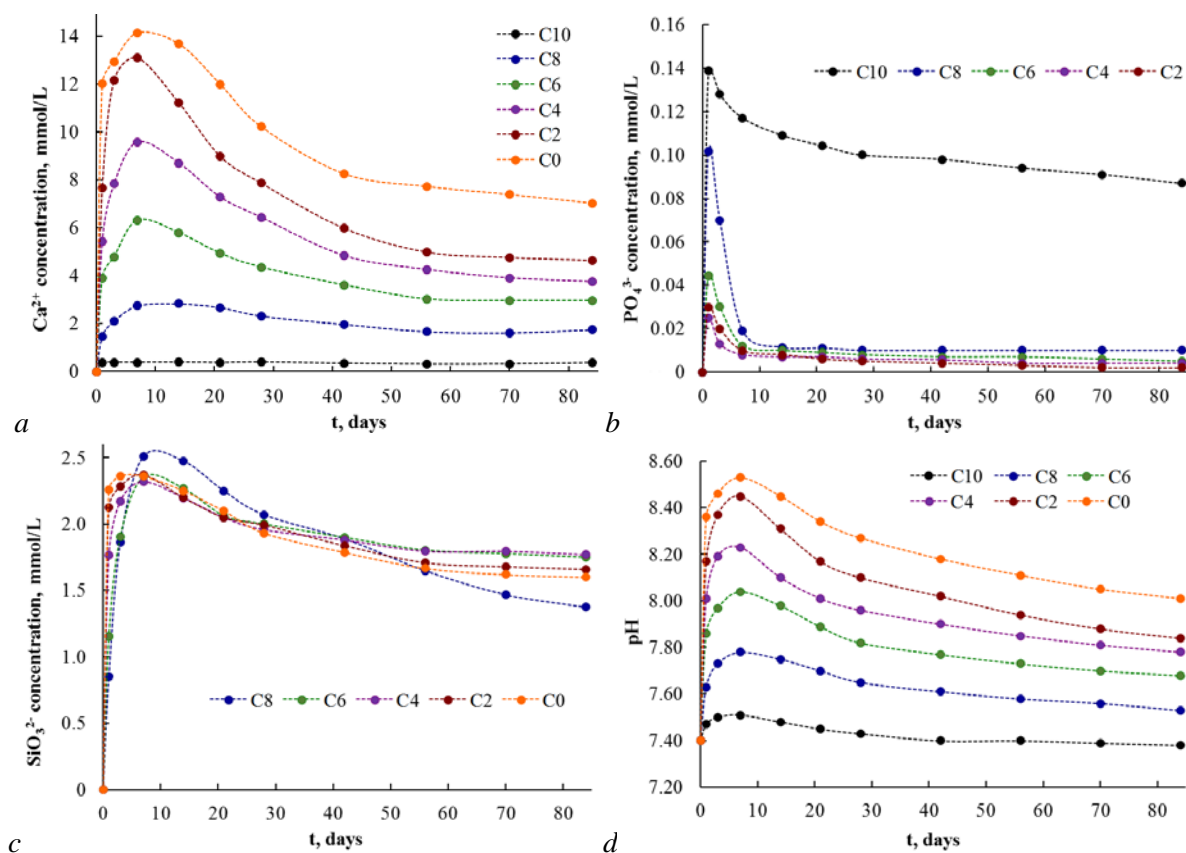


Figure 1. Dependences of Ca²⁺ (a), PO₄³⁻ (b), SiO₃²⁻ (c) concentrations and pH (d) from the soaking time of samples in Tris-buffer

As a result of Ca²⁺, PO₄³⁻ and SiO₃²⁻ transition into solution during the resorption process the

composition and weight of solid phases are changing. According to the data presented in table 2 the values of samples weight loss increase with increasing of CSH content in them. During the experiment, almost half of initial amount of C0 powder dissolves, while sample C10 loses only one tenth of weight. Calcium content in the materials also reduces. A decrease of phosphorus content in undissolved part of materials (relative to the initial value) is noted for powders C10 and C8. Quantity of this element in the composites C6 – C2 increases after soaking in Tris-buffer. It is associated with decreasing of calcium and silicon content in samples and PO_4^{3-} consumption in the process of CP precipitation. As a result of materials resorption, silicates content in composition of C8 – C4 composites undissolved part decreases relative to the initial value (Table 2). However, in samples C2 and C0, after soaking in Tris-buffer, silicon content exceeds the amount of this element in the initial samples. The reasons for this, obviously, are the higher rate of Ca^{2+} release into solution (compared to SiO_3^{2-}) and the impossibility of repeatedly CP precipitation (due to the absence of phosphate ions in the medium).

Table 2. Weight loss and changes of samples composition before and after soaking in Tris-buffer

Sample	Δm , wt. %	W_{Ca} , wt. %	W_{P} , wt. %	W_{Si} , wt. %
C10	12.8	40.2 / 35.2	18.6 / 13.7	—
C8	25.1	37.6 / 33.5	14.5 / 12.9	3.7 / 0.9
C6	34.2	35.3 / 30.8	9.9 / 11.2	8.8 / 4.7
C4	39.1	33.6 / 25.2	6.8 / 8.0	12.7 / 10.5
C2	40.4	31.4 / 19.6	3.3 / 4.6	17.0 / 17.2
C0	45.8	26.7 / 11.0	—	18.8 / 24.4

* To the left of the bar – element content in the initial sample, to the right of the bar – element content in the sample after soaking in Tris-buffer, Δm – sample weight after the experiment.

Evaluation of the particle sizes in undissolved part of the powders showed a decrease in particles median diameters (Fig. 2). This also indicates changes that occur during materials soaking in Tris-buffer.

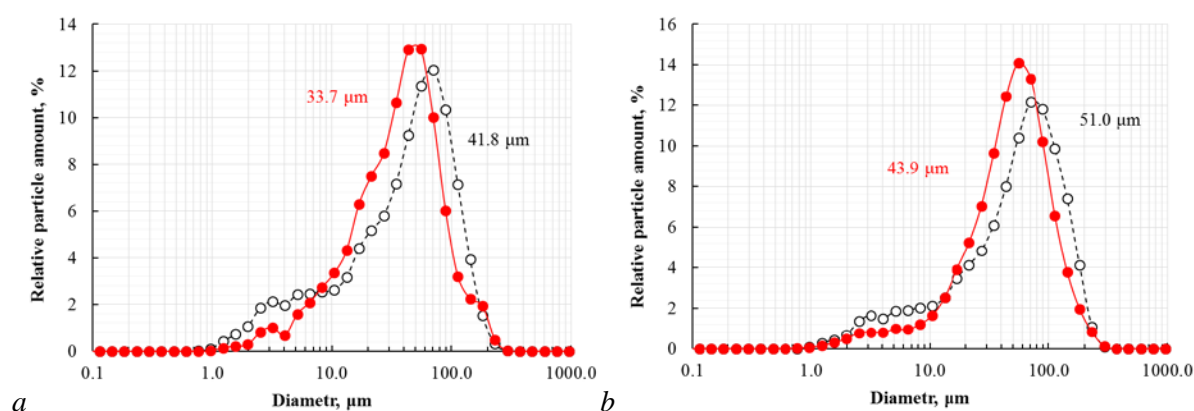


Figure 2. Differential curves of particle size distribution for composites C5 (a) and C0 (b) before and after soaking in Tris-buffer.

○ – initial powder, ● – powder after experiment; values of median diameters are given near with curves.

The method of FTIR spectroscopy is used to analyze the composition of solid phases separated from the solutions at the end of experiment. Fig. 3 shows the IR-spectra of samples before and after their soaking in Tris-buffer. The spectra comparison showed an intensity decreasing of the absorption bands at 565 and 603 cm^{-1} , caused by triply degenerate stretching vibrations of O–P–O bonds in HA [10].

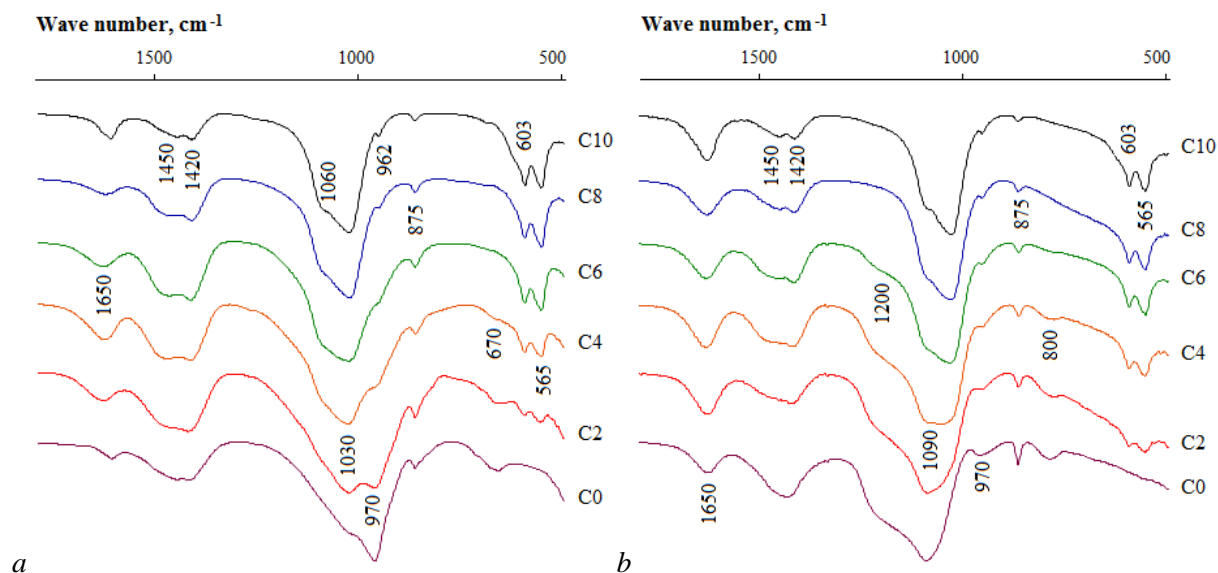


Figure 3. IR-spectra of samples before (a) and after (b) soaking in Tris-buffer

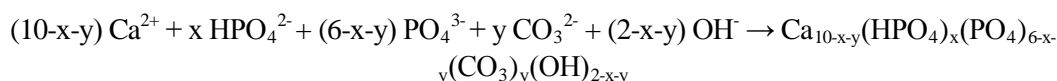
Shift of absorption maxima at 970 and 1030 cm^{-1} , corresponded to vibrations of Si–O bonds in CSH [11], towards large wave numbers, where a strong band with a maximum at about 1090 cm^{-1} and a shoulder at 1200 cm^{-1} caused by the valence asymmetric vibrations of siloxane group connecting silica chains in the composition of amorphous SiO_2 , appear is also recorded. These bands become the more pronounced the higher CSH content in the sample. This indicates the CSH transformation into amorphous silica gel ($\text{nSiO}_2 \cdot \text{mH}_2\text{O}$) during salt contact with an aqueous medium.

5. Discussion

It was found that Ca^{2+} , PO_4^{3-} and SiO_3^{2-} ions appear in aqueous systems when powdered composite materials based on poorly crystalline CHA and CSH interact with the solution. This fact, as well as a change of composition and particles size of the solid phases, which did not dissolve in Tris-buffer after 3 months, indicates that process of powders components resorption occur in experimental condition. Decreasing of phosphate absorption bands intensity in IR-spectra of samples after soaking in solution (Fig. 3) may indicate a reduction of CHA content in materials as a result of its partial dissolution. The silicate component of the powders is also actively resorbed in the studied conditions. It is indicated by increasing of samples weight loss at the end of experiment, current pH values and calcium concentration in the systems which occur with growth of CSH proportion in samples. As a result of powders interaction with the solution and due to the higher rate of Ca^{2+} release into liquid phase compared with SiO_3^{2-} , initial CSH transforms into amorphous silica gel ($\text{nSiO}_2 \cdot \text{mH}_2\text{O}$), the bands of which are recorded in IR-spectra of undissolved part of powders (Fig. 3).

The analysis results of chemical composition of solutions at different times of composites contact with Tris-buffer allow to conclude that several processes occur in the studied systems. Fig. 1 showed that at the initial stage of experiments (up to 3 – 7 days) pH and ions concentrations in solutions increase, then pH and Ca^{2+} , PO_4^{3-} and SiO_3^{2-} amounts decrease in systems. The growth of all indicators at first 3 – 7 days is caused, as noted earlier, by the dissolution of material components. Whereas the

subsequent reduction in pH and amounts of calcium, phosphates and silicates in systems is obviously related to the reaction of apatite repeatedly precipitation:



This process becomes possible after sufficient ions quantities accumulation in solution and level of system saturation of low soluble compound obtaining. Ca^{2+} , PO_4^{3-} and OH^- consumption in this reaction causes a downtrend of the curves $C(\text{Ca}^{2+}) = f(t)$, $C(\text{PO}_4^{3-}) = f(t)$ and $\text{pH} = f(t)$. At the same time, calcium concentration and pH value decrease slightly in comparison with changing of phosphates content in solutions. The reason of this may be a partial compensation of Ca^{2+} and OH^- ions amounts in systems due to CSH and CHA resorption, occurring parallelly with CP deposition. Rapid replenishment of phosphate ions amount in solutions under the experimental conditions is not observed, apparently due to low HA solubility. The observed reduction of silicates concentration in systems at $t > 7$ days and close values of this ions amount in solutions contacted with samples with different CSH content probably due to the fact that calcium silicate phase is blocked from the solution by CP and silica gel which form as a result of resorption and crystallization.

The experimentally recorded processes of composites components dissolution (CHA and CSH) and CP repeatedly precipitations in experimental systems allow to suggest that the studied mixtures have the bioactive property. Differences in rates of Ca^{2+} , PO_4^{3-} and SiO_3^{2-} release into solutions, recorded for samples with varying CHA and CSH contents, indicate the possibility of material resorption rate *in vivo* adjusting by changing of relative calcium phosphate and calcium silicate content in materials.

6. Conclusion

It was established that powder composite materials based on poorly crystalline carbonate substituted hydroxyapatite and calcium silicate hydrate can gradually dissolve in a weak alkaline aqueous solution. Silicate component resorption proceeds most actively. Over time, with Ca^{2+} and PO_4^{3-} accumulation in systems and pH increase, repeatedly precipitation of apatite becomes possible. The combination of dissolution processes and subsequent crystallization indicates a potentially high bioactivity of the studied synthetic mixtures.

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